ORGANIC EXPERIMENTS

Fourth Edition

LOUIS F. FIESER

KENNETH L. WILLIAMSON

SAFETY PRACTICES IN THE ORGANIC LABORATORY[1]

GENERAL: Never work in the laboratory alone. Perform no unauthorized experiments. Do not use mouth suction to fill pipettes. Confine long hair and loose clothes while working in the laboratory. Wear shoes. Learn the location of and correct use of the nearest fire extinguisher. Learn the location of the safety shower and first aid kit and be prepared to give help to others.

SAFETY GLASSES: Safety glasses should be worn **at all times** while in the laboratory, whether you actively engage in experimental work or not. Contact lenses should **never** be worn in the laboratory because they cannot be removed rapidly enough if reagents accidently splash in the eye.

FIRE: Avoid unnecessary flames. Check the area near you for volatile solvents before lighting a burner. Check the area near you for flames if you are about to begin working with a volatile solvent. Be particularly careful of the volatile solvents diethyl ether, petroleum ether (ligroine), benzene, methanol, ethanol, and acetone.

CHEMICALS: Handle every chemical with care. Avoid contact with skin and clothing. Wipe up spills immediately, especially near the balances and reagent shelf. Replace caps on bottles as soon as possible. Do not use an organic solvent to wash a chemical from the skin as this may actually *increase* the rate of absorption of the chemical through the skin. Avoid the inhalation of organic vapors, particularly aromatic solvents and chlorinated solvents. Use care in smelling chemicals and do not taste them unless instructed to do so. Drinking, eating, or smoking in the laboratory is forbidden.

CAUTION: It has been determined that several chemicals that are widely used in the organic laboratory (e.g., benzene and chloroform) cause cancer in test animals when administered in large doses. Where possible, the use of these chemicals is avoided in this book. In the few cases where suspected carcinogens are used, the precautions noted should be followed carefully.

IN CASE OF ACCIDENT[1]

In case of accident notify the laboratory instructor ***immediately.***

FIRE

Burning Clothing. Prevent the person from running and fanning the flames. Rolling the person on the floor will help extinguish the flames and prevent inhalation of the flames. If a safety shower is nearby hold the person under the shower until flames are extinguished and chemicals washed away. Do not use a fire blanket if a shower is nearby. The blanket does not cool and smouldering continues. Remove contaminated clothing. Wrap the person in a blanket to avoid shock. Get prompt medical attention.

Do not, under any circumstances, use a carbon tetrachloride (toxic) fire extinguisher and be very careful using a CO_2 extinguisher (the person may smother).

Burning Reagents. Extinguish all nearby burners and remove combustible material and solvents. Small fires in flasks and beakers can be extinguished by covering the container with an asbestos-wire gauze square, a big beaker, or a watch glass. Use a dry chemical or carbon dioxide fire extinguisher directed at the base of the flames. **Do not use water.**

Burns, either Thermal or Chemical. Flush the burned area with cold water for at least 15 min. Resume if pain returns. Wash off chemicals with a mild detergent and water. Current practice recommends that no neutralizing chemicals, unguents, creams, lotions, or salves be applied. If chemicals are spilled on a person over a large area quickly remove the contaminated clothing while under the safety shower. Seconds count and time should not be wasted because of modesty. Get prompt medical attention.

CHEMICALS IN THE EYE: Flush the eye with copious amounts of water for 15 min using an eye-wash fountain or bottle, or by placing the injured person face up on the floor and pouring water in the open eye. Hold the eye open to wash behind the eyelids. After 15 min of washing obtain prompt medical attention, regardless of the severity of the injury.

CUTS: Minor Cuts. This type of cut is most common in the organic laboratory and usually arises from broken glass. Wash the cut, remove any pieces of glass, and apply pressure to stop the bleeding. Get medical attention.

Major Cuts. If blood is spurting place a pad directly on the wound, apply firm pressure, wrap the injured to avoid shock, and get **immediate** medical attention. Never use a tourniquet.

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[1] Adapted from *Safety in Academic Chemistry Laboratories*, prepared by the American Chemical Society Committee on Chemical Safety, March 1974.

Organic Experiments

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Fourth Edition

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Preface

As in previous editions, the object of *Organic Experiments,* Fourth Edition, is the presentation of clear and concise directions for efficient laboratory work. The beginning student is introduced to the most modern techniques of organic chemistry and trained in accurate observation and deductive reasoning.

In this latest edition of *Organic Experiments,* the chapters that cover the fundamental operations of distillation, crystallization, and extraction have been rewritten to provide more detailed instructions. Concern for laboratory safety led us to employ cyclohexane and toluene, or water and ethanol mixtures, in the distillation experiment, rather than the widely used, mildly toxic, and carcinogenic benzene and chloroform. The application of Raoult's law to these distillations is discussed. The chapter on crystallization presents a clear, seven-step process for crystallization of any organic compound, as well as a section on solving crystallization problems (e.g., oils, handling very small quantities, and inducing crystallization). The distribution coefficient is covered in the chapter on extraction, where the application of acidity constants to the separation of acids and bases is also considered. In this section, the utility of a flow sheet to describe the extraction and isolation of a product from a reaction mixture is illustrated. These and later chapters include a large number of questions and problems to help the student focus on theory and on the most important aspects of each experiment.

Organic Experiments, Fourth Edition, emphasizes modern apparatus and techniques. Throughout this edition will be found illustrations of such things as the single pan electronic balance (which we strongly advocate), electrically heated melting point apparatus, ^{13}C nmr spectra, and demountable silver chloride IR cells. Chapter 60, Glass Blowing, acquaints the student with a specialized and extremely useful technique.

This edition continues and expands the use of spectroscopic methods in structure determination. The chapter on nmr spectroscopy now includes a correlation table of proton chemical shifts. In addition, a discussion of ^{13}C nmr has been introduced and a number of ^{13}C spectra have been added throughout the book. Each chapter on spectroscopy has a bibliography to aid the student in solving spectroscopic unknowns.

A few experiments have been eliminated to make room for new ones. The free radical chlorination of 1-chlorobutane has been added to the chapter on alkanes and alkenes as a typical reaction of saturated hydrocarbons. As an example of clinical laboratory work, the quantitative application of the Liebermann-Burchard test for cholesterol has been incorporated in the experiment entitled "Blood Cholesterol" in which students can analyze their own blood for this important steroid.

The nitration of benzene is replaced with the nitration of methyl benzoate. The reaction is rapid, the product is crystalline, and the necessity of working with mildly toxic and carcinogenic benzene is avoided. The synthesis of sulfanilamide, therefore, starts with nitrobenzene. The synthesis of dibenzalacetone illustrates the aldol condensation in an experiment easily completed in less than one laboratory period.

Hitherto, ferrocene has been a most difficult molecule to synthesize because of the necessity of working with metallic sodium in completely anhydrous solvents under a nitrogen atmosphere with stirring. We have devised a synthesis of this interesting metallocene that easily can be carried out in one laboratory period with standard apparatus. The aromatic character of this molecule is illustrated by the phosphoric acid—catalyzed acetylation to give acetylferrocene.

Finally, we have added a practical application of organic analysis and elementary biochemistry: the chemistry of winemaking. The student will find that with proper attention to technique—careful titration, clean apparatus, and close attention to details —it is possible to produce a wine from readily available grape concentrates that, while not great, does rise above the mediocre.

I wish to thank Milton Soffer of Smith College and David Todd of Worcester Polytechnic Institute for many helpful suggestions. I acknowledge the use of spectra from L. F. Johnson and W. C. Jankowski, *Carbon-13 NMR Spectra* (New York: John Wiley & Sons, Inc., Wiley Interscience Publications, 1972). My thanks, also, to Jay W. Ponder for his assistance in proofreading the text. Finally, I am deeply indebted to Mary Fieser for her unfailing attention to every detail of this revision in a period during which she lost a devoted husband and we, a great teacher, friend, and organic chemist, Louis F. Fieser.

KENNETH L. WILLIAMSON

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Pasteur pipette
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Aspirator and filter trap
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Calibrated dropping tubes
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APPARATUS AND TECHNIQUES

Welcome to the organic chemistry laboratory. A first assignment is to check your equipment. By referring to the drawings in this chapter, you can identify the pieces of equipment that are not now familiar to you.

We strongly recommend the use of standard-taper ground glass equipment of the types shown in the drawings, either the $^{19}/_{22}$ or the $^{14}/_{20}$ size (Fig. 1.1). (The numbers refer to the diameter and length of the ground joint in mm.) This equipment is easy to put together and also to take apart; when carefully greased it is vacuum tight. Do not leave it joined for long periods of time as the joints may become stuck. If so, consult your laboratory instructor for suggestions.

Check to see that your thermometer is correct (20°C = 68°F) and replace flasks having star-shaped cracks. Remember that porcelain apparatus and equipment with graduations and ground glass joints are expensive; Erlenmeyer flasks, beakers, and test tubes are, by comparison, fairly cheap. If ground glass equipment is not available in your laboratory, follow local instructions for making similar assemblies, which will be adequate for your work.

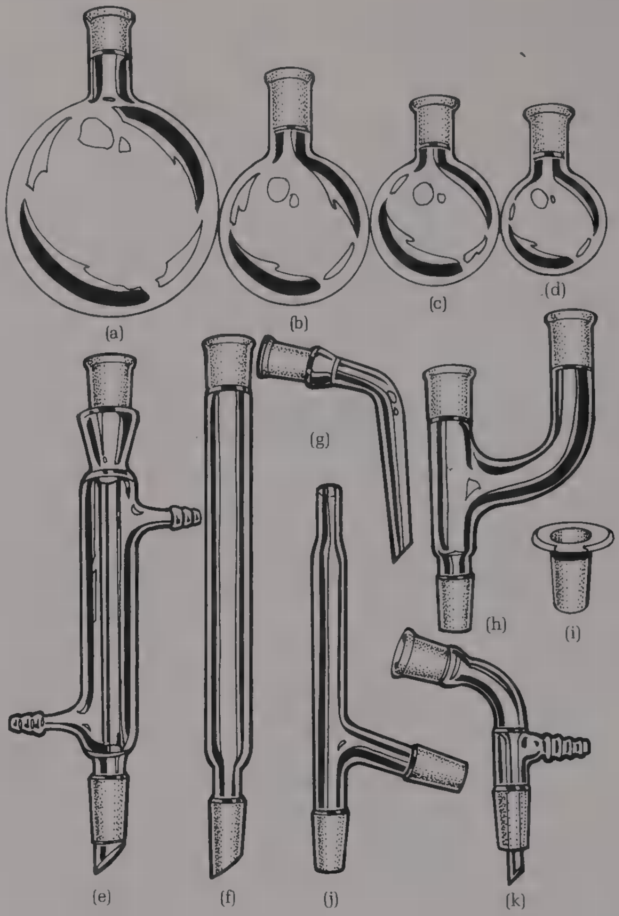


FIGURE 1.1 Standard taper ground glass apparatus (14/20 or 19/22). Round-bottomed flasks of (a) 250, (b) 100, (c) 50, and (d) 25 ml capacity; (e) condenser; (f) distilling column; (g) simple bent adapter; (h) Claisen distilling head; (i) stopper; (j) distilling head; and (k) vacuum adapter.

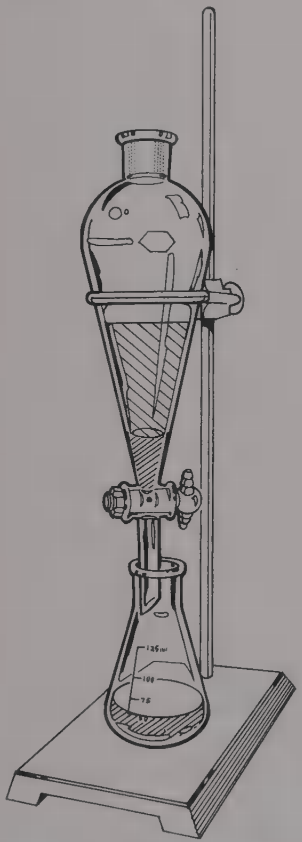


FIGURE 1.2 Separatory funnel with Teflon stopcock.

A separatory funnel with a Teflon stopcock is illustrated in Fig. 1.2. Teflon has a low coefficient of friction and is chemically inert, but it deforms under tension and the stopcock will stick in the glass bore of the funnel if not properly cared for. Remember to loosen the stopcock after each use. If the stopcock should become stuck, cool the end of the funnel in ice or Dry Ice. Usually the stopcock will shrink enough to be loosened.

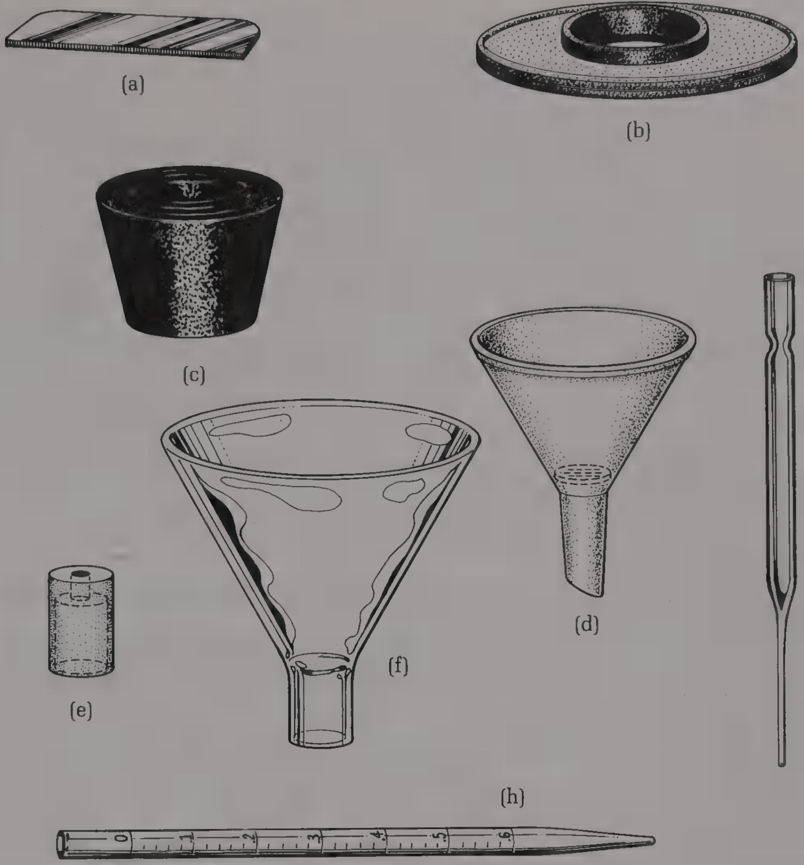
Other useful pieces of apparatus are illustrated in Fig. 1.3. Their usefulness will become apparent in later chapters.

1. Washing and Drying Laboratory Equipment

▼
Clean up as you go

Considerable time can be saved by cleaning each piece of equipment soon after use, for you will know then what contaminant is present and be able to select the proper method for removal. A tar will be easier to remove before it has dried and hardened. A small amount of organic tar usually can be dis-

FIGURE 1.3 Miscellaneous organic chemical apparatus: (a) glass scorer, (b) Filtervac, (c) set of neoprene adapters, (d) Hirsch funnel with perforated plate in place, (e) thermometer adapter, (f) powder funnel, (g) Pasteur pipette, (h) graduated 1-ml pipette.



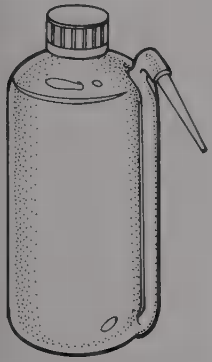


FIGURE 1.4 Polyethylene wash bottle.

solved with a few milliliters of an appropriate organic solvent. If the amount of tar is large, first try warm water and a detergent; let the vessel soak for a time and then see if the material can be dislodged with a test-tube brush. Acetone (bp 56.1°) has great solvent power and is often effective. It is miscible with water and vaporizes readily, and so is easy to remove from the vessel. Cleaning after one operation often can be carried out during the period in which a second experiment is in process.

▼
Wash bottles

A polyethylene bottle (shown in Fig. 1.4) is a convenient wash bottle for acetone. The Nalgene® 400-ml wash bottles are recommended for other common solvents. The name, symbol, or formula of a solvent can be written on a bottle with a Magic Marker or wax pencil. A volume up to 25 ml per squeeze can be delivered from the spout into a graduate; a large volume of solvent can be poured from the top. The dispensing spout gives an ultrafine stream; it can be enlarged to increase the stream. For crystallizations and for quick cleaning of apparatus, it is convenient to have available one bottle for each frequently used solvent.

Capillary dropping tubes (Pasteur pipettes, Fig. 1.5) are useful for transferring small quantities of liquid, adding reagents dropwise, and washing the insides of flasks.

▼
Use caution!

Sometimes a reaction flask will not be clean after washing with a detergent and with acetone. If so, try an abrasive household cleaner. Do not hesitate to bend the handle of your test-tube brush to reach the curved sides of the flask. As a last resort use a powerful oxidizing agent. In this process, rinse the flask with water, let it drain, and *carefully* add about 5 ml of concentrated sulfuric acid and 1 ml of concentrated nitric acid. Let the mixture remain in the flask for a time if there is a vigorous reaction, then heat on the steam bath. After the reaction is over, you will find that, on decantation of the acids and washing with water, the flask is clean.

To dry a piece of apparatus rapidly, rinse with a few milliliters of acetone and invert over a beaker to drain. *Do not use compressed air,* which contains droplets of oil, water, and particles of rust. Instead draw a slow stream of air through the apparatus using the suction of your water aspirator.

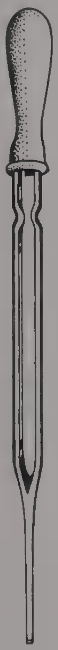


FIGURE 1.5 Capillary dropping tube (Pasteur pipette).

2. Using Rubber Stoppers and Boring Corks

Insertion of a glass tube into a rubber stopper is easy if the glass is lubricated with a small drop of glycerol. Grasp the tube very close to the end to be inserted; if it is grasped at a distance, especially at the bend, the pressure applied for insertion may break the tube and result in a serious cut. Stoppers of the type shown in Fig. 1.6 are safer and easier to use than those with a cylindrical bore. If a glass tube or thermometer should become stuck to a stopper, it can be removed by painting on glycerol and forcing the pointed tip of an 18-cm spatula between the rubber and glass. Another method is to select a cork borer that fits snugly over the glass tube, moisten it with glycerol, and slowly work it through the stopper. When the stuck object is valuable, such as a thermometer, the best policy is to cut the stopper with a sharp knife.

▼
Boring corks

In the construction of some apparatus, the necessity will arise for insertion of a large diameter glass tube into a flask or condenser. Since corks are much easier to bore than rubber stoppers, they are used in this case. Select a high quality cork that fits only 4–5 mm into the opening of the flask and soften it in a cork roller (Fig. 1.7a) or by rolling underfoot. The cork should then be flexible enough to go about half its length into the opening of the flask and will not split when being bored.

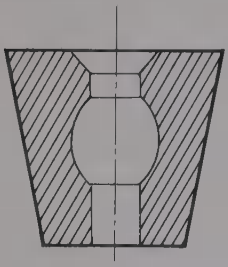


FIGURE 1.6 Cross section of the fusiform type of rubber stopper (Arthur H. Thomas Co.)

Select a sharp cork borer slightly smaller than the size of the hole required, hold it in the right hand and work it into the cork while held in the left hand. After each twist, grasp the cork at a new place and check the alignment. When the cork has been cut halfway through, withdraw the borer by twisting, push out the plug if present, and finish boring the hole from the other end of the cork (Fig. 1.7b). If these operations are done carefully, it will not be necessary to ream the hole with a rat-tail file. The secret to proper cork-boring technique is a sharp borer and 90% twisting motion and 10% pushing motion on the borer.

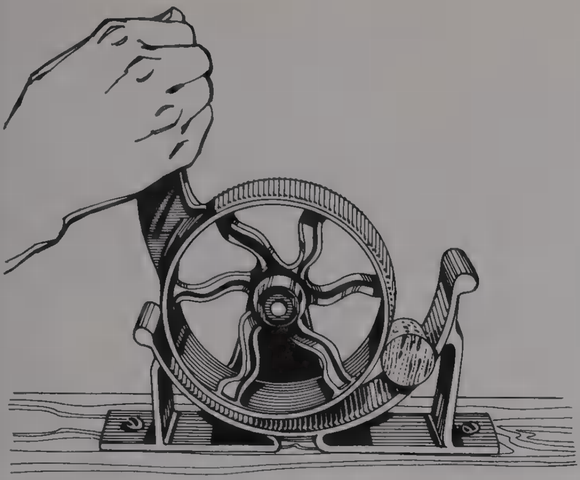


FIGURE 1.7(a) Cork roller used to soften cork stoppers.

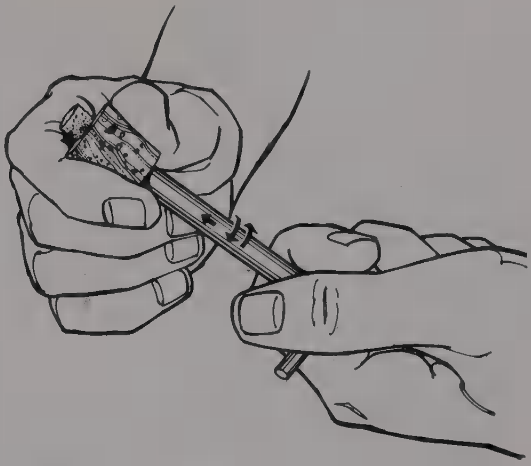


FIGURE 1.7(b) Boring a hole through a softened cork.

▼
Mostly a twisting motion

To sharpen the cork borer, insert the sharpener as far as it will go and turn the borer while pressing on the blade with your thumb (Fig. 1.8). A fine shaving of brass will come off, leaving a razor-sharp edge on the borer.

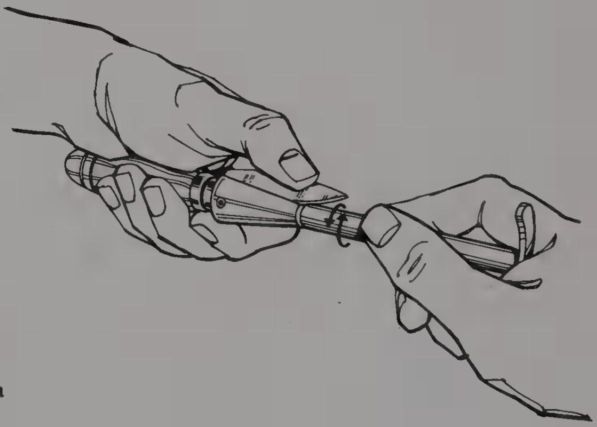


FIGURE 1.8 Sharpening a cork borer.

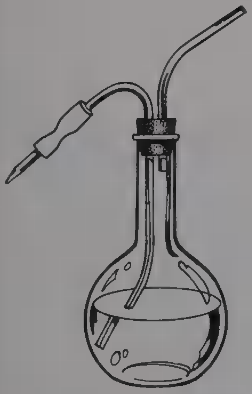


FIGURE 1.9 Water wash bottle (Florence flask).

3. Wash Bottles

A wash bottle of distilled water is indispensable in the laboratory, and the classical design shown in Fig. 1.9 is recommended. It is easily constructed from a 500-ml Pyrex Florence flask, 7-mm tubing, and a 2-hole, No. 5 rubber stopper—preferably of the fusiform type. One can direct the stream of water from the wash bottle by guiding the flexible tip with the forefinger. After an

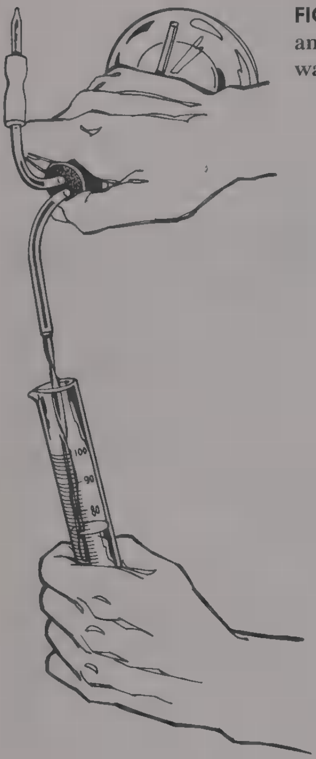


FIGURE 1.10 Delivering large amounts of water from wash bottle.

initial delivery, the few drops of water remaining in the tip can be forced out separately by a quick breath of air. A large volume of water, for example 25, 50, or 100 ml, can be measured rapidly into a graduate by inverting the wash bottle and delivering the liquid into the container through the mouthpiece (Fig. 1.10). Cut off the flow a little short of the required amount and then add water until the mark is reached, using the wash bottle in the normal fashion.

4. The Aspirator and Filter Trap

You will frequently use a vacuum to promote sublimation, to reduce the boiling points of liquids, to hasten drying of solids, to remove liquid from a crystalline product, and to speed up slow filtrations. Water flowing through the aspirator (which works on Bernoulli's principle) will produce a vacuum equal to the vapor pressure of the water (17 torr at 20°, 5 torr at 4°). Polypropylene aspirators are inexpensive and will not corrode as brass ones. A check valve is built into all aspirators, but when the water is turned off it may back into the vacuum system. For this reason a trap is always installed in the line (Fig. 1.11). A valve for ready release of vacuum is made by inserting a 6-cm long piece of thin-walled rubber tubing (5 mm dia. hole × 3 mm wall thickness) between the jaws of a wooden or plastic clothes clip and slipping the tubing a short distance over the upright glass tube of the flask.

▼
A convenient pressure gauge

The water passing through the aspirator should always be turned on full force. With proper adjustment of the clothes clip and with an efficient aspirator, the thin-walled rubber tubing will collapse and the clothes clip will bend, and thus act as an indicator (see Fig. 1.11). Thus, you will know if all connections in the system are tight. You will, in time, learn to hear the difference in the sound of an aspirator working on an open system and one working on a closed system. Figure 1.12 shows a filter trap made from a 250-ml gas bottle with a three-hole rubber stopper, set in a wooden block to provide stability.

5. Steam Baths and Hot Plates

Many operations in the organic laboratory are conducted on the steam bath because the solvents used often boil below 100° and are flammable. Figure 1.13 shows the correct way to heat solvents in a round-bottomed flask on a steam bath; an annular space of a few mm is left between the flask and the support ring (three wood matchsticks serve as spacers), and a *slow* trickle of steam is allowed to envelop the flask. When heating solvents in an Erlenmeyer flask, again let a slow trickle of steam rise around the flask by having the flask slightly off center on the rings (Fig. 1.14).

The 3-in., 70-watt hot plate shown in Fig. 1.15 is ideal as a heat source for crystallizations in small Erlenmeyer flasks. Since a single hot plate does not

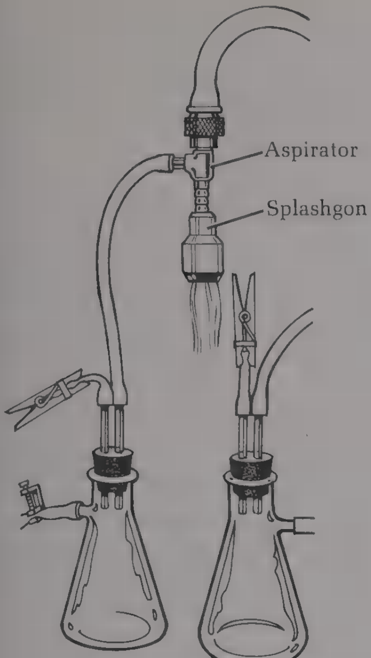


FIGURE 1.11 Filter traps functioning as pressure gauges, (*left*) under vacuum, and (*right*) at atmospheric pressure.

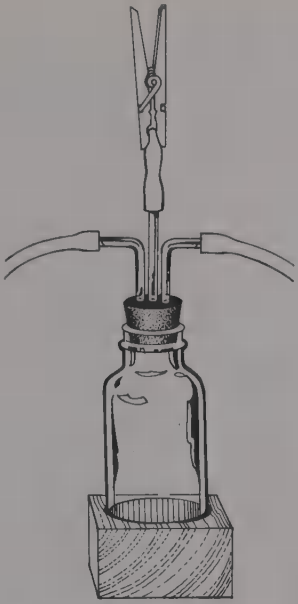


FIGURE 1.12 Filter trap made with a gas bottle.

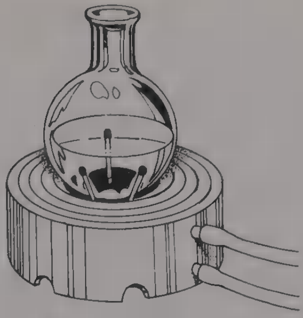


FIGURE 1.13 Heating a round-bottomed flask on the steam bath. Matchstick spacers allow steam to come up around flask for more efficient heating.

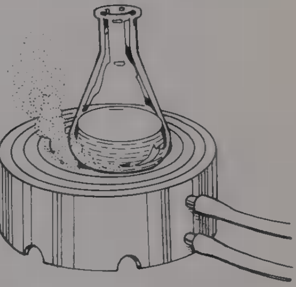


FIGURE 1.14 Heating an Erlenmeyer flask on the steam bath. Placing flask slightly off center allows steam to rise around flask.

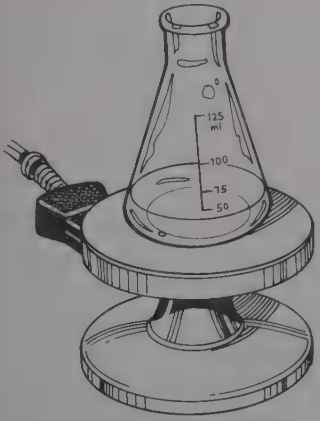


FIGURE 1.15 70-Watt hot plate (Wilkens-Anderson 40635).

draw much current, several plates can be plugged in on the same bench without risk of blowing a fuse. The hot plate is free from fire hazard; at full heat the element does not glow, and ether dropped on it does not ignite. Because it is slow to heat you should plug in the hot plate at the beginning of the period before use.

The steam bath is useful for *controlled* heating. After prolonged heating, a thermometer in a flask of water heated on a steam bath will register 90° as the maximum temperature, significantly below the boiling point of water. The hot plate will heat 150 ml of Dow silicone fluid to the much higher temperature of 172° after prolonged heating. [Silicone fluid (bp $> 300°$) gives excellent service in melting-point baths but is more expensive than di-*n*-butyl phthalate (bp 340°), the fluid recommended for student use.]

The time-temperature curve in Fig. 1.16 shows that the hot plate will heat 50 ml of water to boiling in 5 min; the steam bath does not heat as rapidly. Ten ml of methanol can be brought to the boiling point in 36 seconds on the hot plate, and, as seen in Fig. 1.17, there is enough space on it for two or three 25-ml Erlenmeyer flasks.

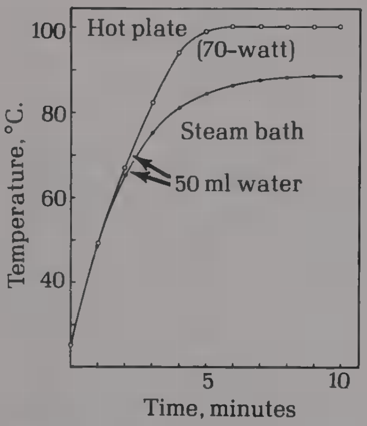


FIGURE 1.16 Time-temperature curve for heating 50 ml of water on the hot plate and on a steam bath.

FIGURE 1.17 Hot plate being used in crystallization.

6. Swirling and Dissolving Solids

Heat alone will not bring a solid into solution rapidly. Lumps of solid should first be crushed with the flattened end of a stirring rod, and then the hot mixture swirled by grasping the top of the flask and moving the bottom in a circular motion (Fig. 1.18). Alternatively, the flask can be rotated while inclined in the steam bath. A motor-driven stirrer is essential when stirring must be conducted over a long period of time or in large-scale operations (Fig. 1.19).

FIGURE 1.18 Swirling a liquid in a flask. The bottom of the flask is given a circular motion, the top remains stationary.

7. Transfer of a Solid

The proper technique for transfer of a solid to a reaction vessel or a crystallization flask is shown in Fig. 1.20. The sample is placed on a creased filter paper (or, better, smooth glazed weighing paper) and the near end of the crease rested over the mouth of the flask. A stainless steel spatula is used to scrape the product into the flask. Toward the end of the operation, the creased paper should be raised until nearly vertical and tapped on both sides with the spatula to dislodge adhering solid. The same technique serves for transfer of a small sample of a solid to a specimen vial, except that the vial should be placed on a piece of clean paper in case of spillage.

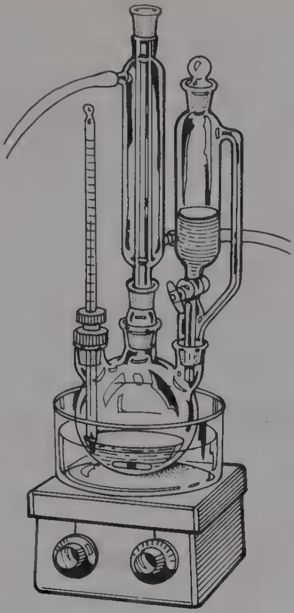


FIGURE 1.19 A reaction mixture being heated in an oil bath on a hot plate and stirred with a Teflon-covered stirring bar. Apparatus of this type is used in research for large-scale reactions.

FIGURE 1.20 A technique for transferring solids.

WEIGHTS AND MEASURES

Figure 1.21 shows that equal weights of solid materials may occupy considerably different volumes; estimation of weight by inspection can be very misleading. In performing laboratory experiments you must sometimes weigh reagents very carefully. At other times a rough estimate will suffice. Use good judgment since careful weighing is time-consuming. The new single-pan

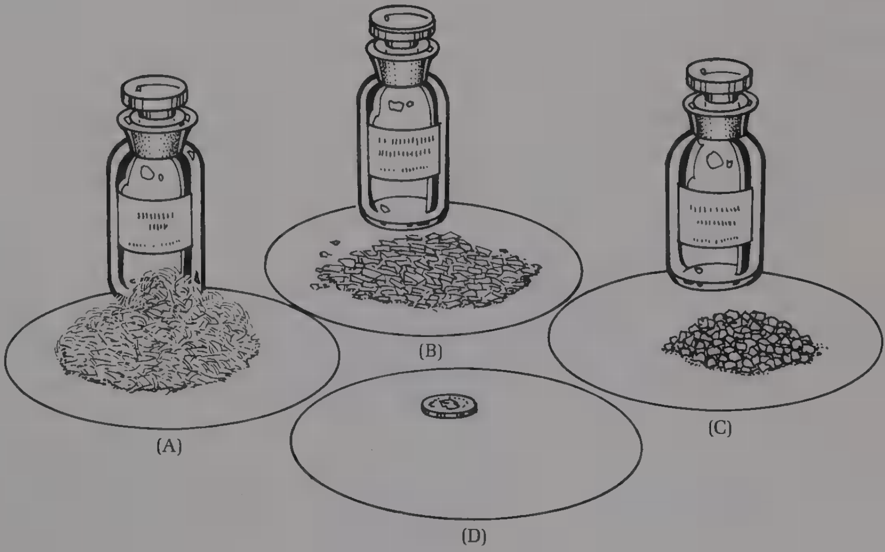


FIGURE 1.21 Equal weights (6.1 g): (a) benzoic acid, (b) 2,3-dimethylnaphthalene, (c) *dl*-hydrobenzoin, and (d) a quarter-dollar coin.

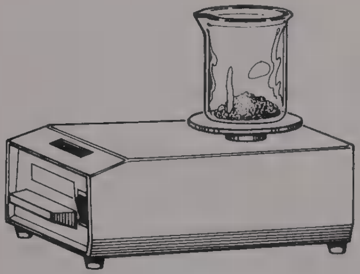


FIGURE 1.22 Single-pan electronic balance with automatic zeroing and digital readout.

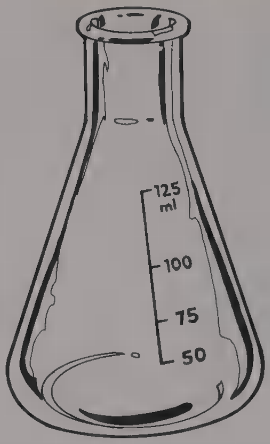


FIGURE 1.23 Erlenmeyer flask with approximate volumes graduations.

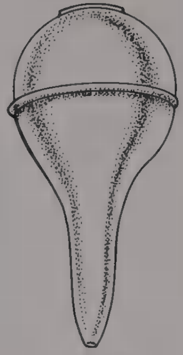


FIGURE 1.24 1-oz. ear syringe.

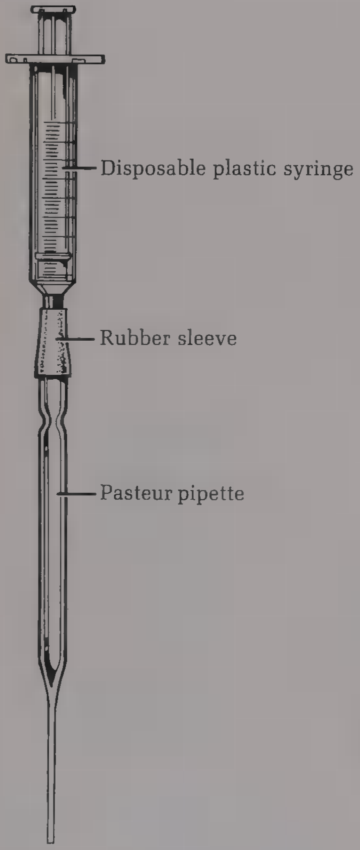


FIGURE 1.25 A calibrated Pasteur pipette.

electronic balances (Fig. 1.22) make weighing a pleasure. A container such as a beaker is placed on the pan. At the touch of a button the digital readout becomes zero and the desired quantity of reagent can be added to the beaker as the weight is registered periodically to the nearest hundredth of a gram.

It is often convenient to weigh reagents on glossy weighing paper and then transfer the chemical to the reaction container (Fig. 1.20). The success of a given experiment often depends on using starting materials and reagents in definite amounts. Inexperienced workers often believe that if one milliliter of a reagent will do the job then two milliliters will do the job twice as well. Such assumptions are often erroneous.

Liquids can be measured by either volume or weight according to the relationship

$$\text{Volume (ml)} = \frac{\text{weight (g)}}{\text{density (g/ml)}}$$

Modern Erlenmeyer flasks and beakers have approximate volume calibrations fused into the glass (Fig. 1.23). More accurate volumetric measurements are made in the 10- and 100-ml graduated cylinders. For volumes less than about 4 ml use a graduated pipette. *Never* apply suction to a pipette by mouth. The smaller pipettes can be filled with a small rubber bulb, the larger ones with an ear syringe or similar bulb (Fig. 1.24). A Pasteur pipette can be converted into a calibrated pipette with the addition of a plastic syringe body (Fig. 1.25).

THE LABORATORY NOTEBOOK

A complete, accurate record is an essential part of laboratory work. Failure to keep such a record means laboratory labor lost. An adequate record includes the procedure (what was done), observations (what happened?) and conclusions (what the results mean).

Use a lined, paperbound, $8\frac{1}{2} \times 11$ in. notebook and record all data in ink. Allow space at the front for a table of contents, number the pages throughout, and date each page as you use it. Reserve the left-hand page for calculations and numerical data, and use the right-hand page for notes. Never record *anything* on scraps of paper to be recorded later in the notebook. Do not erase, remove, or obliterate notes; simply draw a single line through incorrect entries.

The notebook should contain a statement or title for each experiment followed by balanced equations for all principal and side reactions, and where relevant, mechanisms of the reactions. Consult your textbook for supplementary information on the class of compounds or type of reaction involved. Give a reference to the procedure used; do not copy verbatim the procedure in the laboratory manual.

Before coming to the lab to do preparative experiments prepare a table (in your notebook) of reagents to be used and the products expected, with their physical properties. (An illustrative table appears with the first preparative experiment, the preparation of *n*-butyl bromide, Chapter 13.) From your table, use the molar ratios of reactants and determine the limiting reagent and calculate the theoretical yield (in grams) of the desired product. Enter all data in your notebook (left-hand page).

Include an outline of the method of purification of the product by means of a flow sheet, which lists all possible products, by-products, unused reagents, solvents, etc., that appear in the crude reaction mixture. On the flow sheet diagram indicate how each of these is removed, e.g., by extraction, various washing procedures, distillation, or crystallization. With this information entered in the notebook before coming to the laboratory you will be ready to carry out the experiments with the utmost efficiency. Plan your time before the laboratory period. Often two and three experiments can be run simultaneously.

▼
The Laboratory Notebook
What you did.
How you did it.
What you observed.
Your conclusions.

When working in the laboratory, record everything you do and everything you observe *as it happens*. The recorded observations constitute the most important part of the laboratory record as they form the basis for the conclusions you will draw at the end of each experiment. Record the physical properties of the product, the yield in grams, and the percentage yield. When your record of an experiment is complete, another chemist should be able to read the account with complete understanding and to determine what you did, how you did it, and what conclusions you reached. In other words, from the information in your notebook a chemist should be able to repeat your work.

2

Simple and Fractional Distillation

KEYWORDS

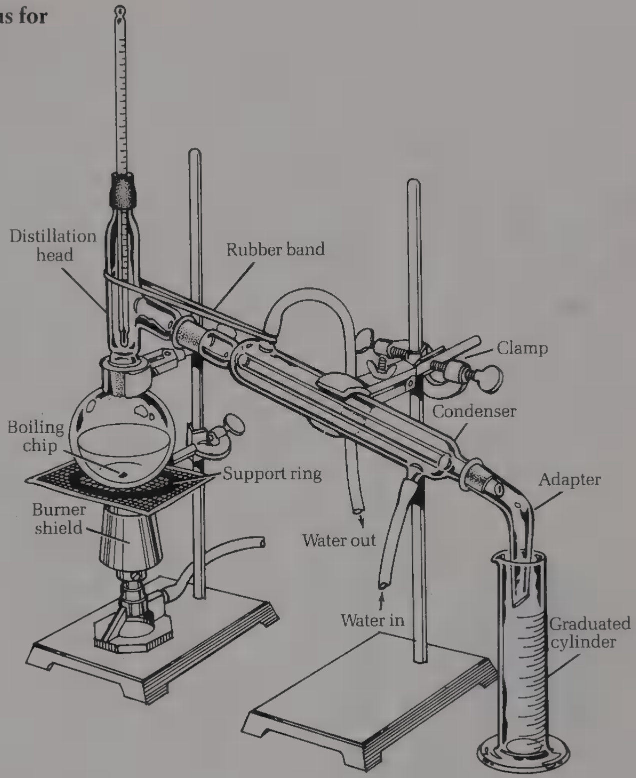
Simple distillation
Fractional distillation
Liquid–vapor equilibrium
Fractionating column, packing
Azeotrope
Thermometer calibration
Boiling stone

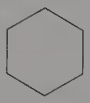
The separation of organic compounds is one of the most important tasks of the organic chemist. Organic compounds seldom occur in pure form in nature or as products of a laboratory synthesis. The most commonly used method for purification of liquids is distillation, a process by which one liquid can be separated from another liquid, or a liquid from a nonvolatile solid.

▼
The distillation process

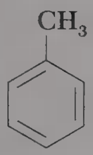
When water is heated with a flame in a simple distillation apparatus (Fig. 2.1), the vapor pressure of the liquid, or the tendency of molecules to escape from the surface, increases until it becomes equal to the atmospheric pressure, at which point the liquid begins to boil. Addition of more heat will supply the heat of vaporization required for conversion of the liquid water to gas (steam), which rises in the apparatus, warms the distillation head and thermometer, and flows down the condenser. The cool walls of the condenser remove heat from the vapor and the vapor condenses to the liquid form. Distillation should be conducted slowly and steadily and at a rate such that the thermometer bulb always carries a drop of condensate and is bathed in a flow of vapor. Liquid and vapor are then in equilibrium, and the temperature recorded is the true boiling point. If excessive heat is applied to the walls of the distilling flask above the liquid level, the vapor can become superheated, the drop will disappear from the thermometer, the liquid-vapor equilibrium is upset, and the temperature of the vapor rises above the boiling point.

FIGURE 2.1 Apparatus for simple distillation.





Cyclohexane
bp 81.4°,
den 0.78, MW 84.16



Toluene
bp 110.8°,
den 0.87, MW 92.13

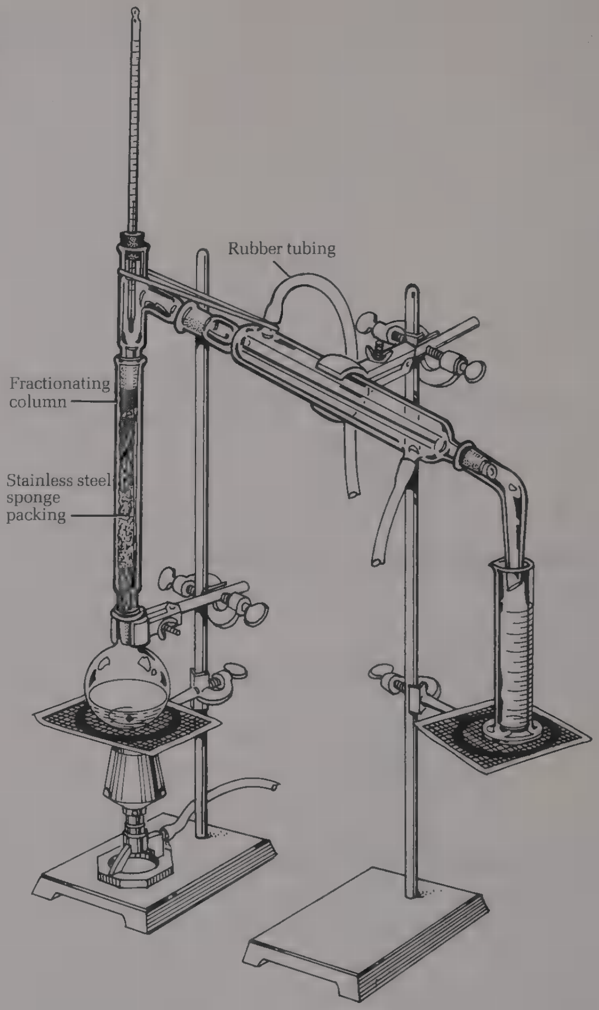
▼
Simple distillation

Consider the separation of cyclohexane and toluene. When distilled in a simple distilling apparatus a mixture of these two miscible liquids starts to distil somewhat above the boiling point of cyclohexane and stops distilling somewhat below the boiling point of toluene. All fractions of the distillate are mixtures and little separation of the two components is achieved. A better separation could be obtained by redistillation of each fraction. If redistillation is repeated often enough, the two components of the mixture will eventually be separated. Fortunately this series of condensations and redistillations is done automatically in a fractionating column.

▼
Fractionating column packed with a stainless steel sponge

The fractionating column shown in Fig. 2.2 contains a stainless steel scouring sponge (Fig. 2.3), which forms a porous packing for equilibration of vapor and condensate. At the start of the distillation of a mixture of cyclohexane and toluene the mixture boils and the vapors condense in the lowest part of the fractionating column. The composition of this condensate is similar to that of the first fraction collected in a simple distillation—richer in cyclohexane than in toluene, but by no means pure cyclohexane. Then, as more cyclohexane and toluene boil, the temperature of these vapors is higher than that of the first portion of the mixture, because this portion contains less cyclohexane and more toluene. These hot vapors contact the liquid already in the fractionating column from the first part of the distillation and a heat transfer takes place, which causes the less volatile component (cyclohexane) to boil from that liquid.

FIGURE 2.2 Apparatus for fractional distillation. The position of the thermometer bulb is critical.



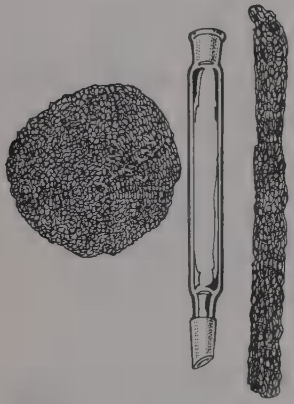
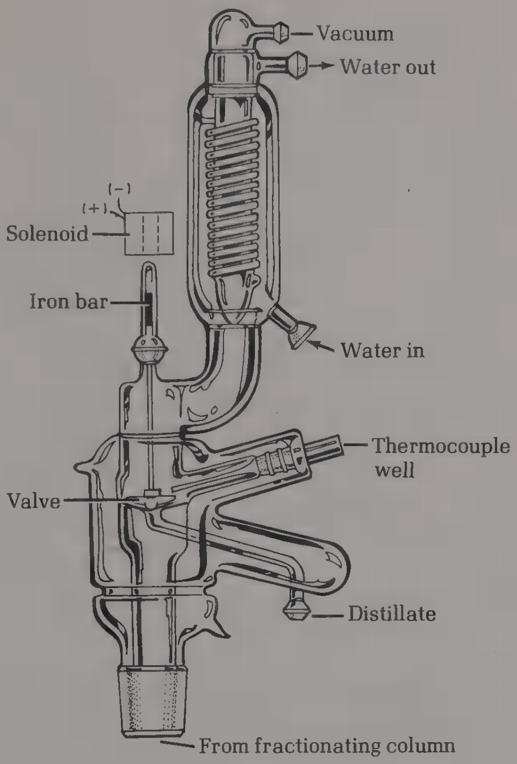


FIGURE 2.3 Fractionating column and packing.

▼
Equilibration between ascending vapor and descending condensate

A succession of these condensations and redistillations occur throughout the column. The efficiency of a column is rated by the number of simple distillations that take place within the column. After cyclohexane–toluene vapor has warmed the entire length of the column, the less volatile part condenses and trickles down over the surface of the packing, while fresh vapor from the flask forces its way through the descending condensate with attendant heat interchange. A number of equilibrations between ascending vapor and descending condensate take place throughout the column. The vapor that eventually passes into the receiver is highly enriched in the more volatile cyclohexane, whereas the condensate that continually drops back into the flask is depleted of the volatile component and enriched with the less volatile toluene. The packing is used in the column to increase the vapor-liquid contact area. Since equilibration is fairly slow, slow distillation effects better separation.

FIGURE 2.4 Total reflux fractionating head, vacuum jacketed.



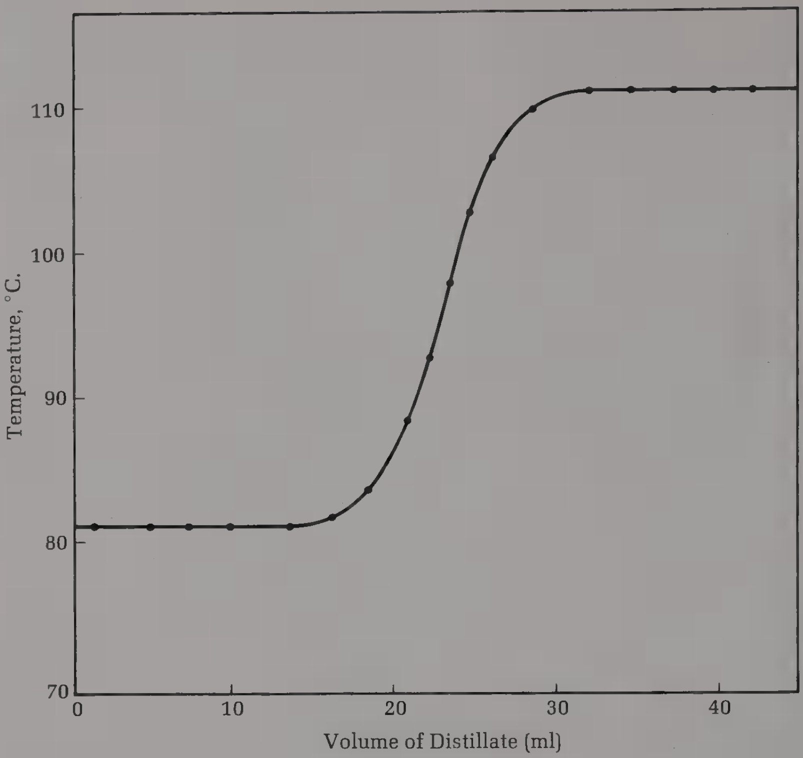
In research, fractionating heads are frequently used at the top of a distillation column (Fig. 2.4). These heads are designed so that all the vapor boiled from the flask can be condensed and returned to the column (total reflux). A solenoid lifts the small iron bar and opens the valve about once a minute to release one drop at a time to a receiver; thus, there is time for complete equilibration to take place in the column between drops. With an efficient column and this type apparatus, liquids with boiling points differing by only one or two degrees can be separated. The stainless steel sponge-packed column illustrated and specified for these experiments achieves a fairly sharp separation (see the distillation curve of Fig. 2.5), since the boiling points of cyclohexane and toluene differ by 30°.

Raoult's law states that the vapor pressure of one component (e.g., cyclohexane) above a multicomponent mixture (e.g., cyclohexane and toluene) is equal to the product of the vapor pressure of pure cyclohexane and the mole fraction of cyclohexane in the liquid mixture:

$$P_c = P_c^\circ N_c$$

where P_c is the partial pressure of cyclohexane, P_c° is the vapor pressure of pure cyclohexane at the given temperature, and N_c is the mole fraction of cyclo-

FIGURE 2.5 Fractionation graph of a mixture of cyclohexane and toluene.



hexane in the mixture. If toluene were the other component in a two-component mixture then similarly

$$P_t = P_t^\circ N_t$$

and the total vapor pressure above the solution, P_{Tot}, would be given by the sum of the partial pressures due to cyclohexane and toluene:

$$P_{Tot} = P_c + P_t$$

Figure 2.6 is a temperature–composition diagram of the cyclohexane–toluene system. From the curve labeled "liquid", the boiling point of any mixture of cyclohexane and toluene can be determined. A mixture of 25 mole percent cyclohexane and 75 mole percent toluene has a boiling point of 100° indicated by *A*. At this temperature cyclohexane has a vapor pressure of 433 mm and toluene a vapor pressure of 327 mm. Since the mixture is at the boiling point, the sum of these two partial pressures equals 760 mm. If a bit of the vapor in equilibrium with this boiling mixture were condensed and analyzed it would be found to be 433:760 or 57 mole percent cyclohexane (*B*). This is the greatest separation that can be achieved on simple distillation of this mixture. As the simple distillation proceeds, the boiling point of the mixture moves toward 110° along the line from *A*, and the vapor composition becomes richer

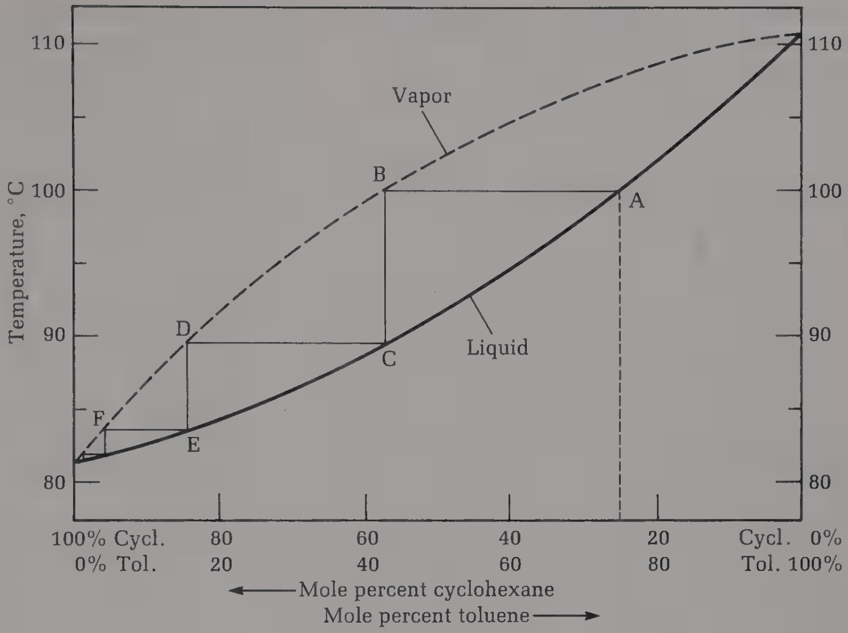


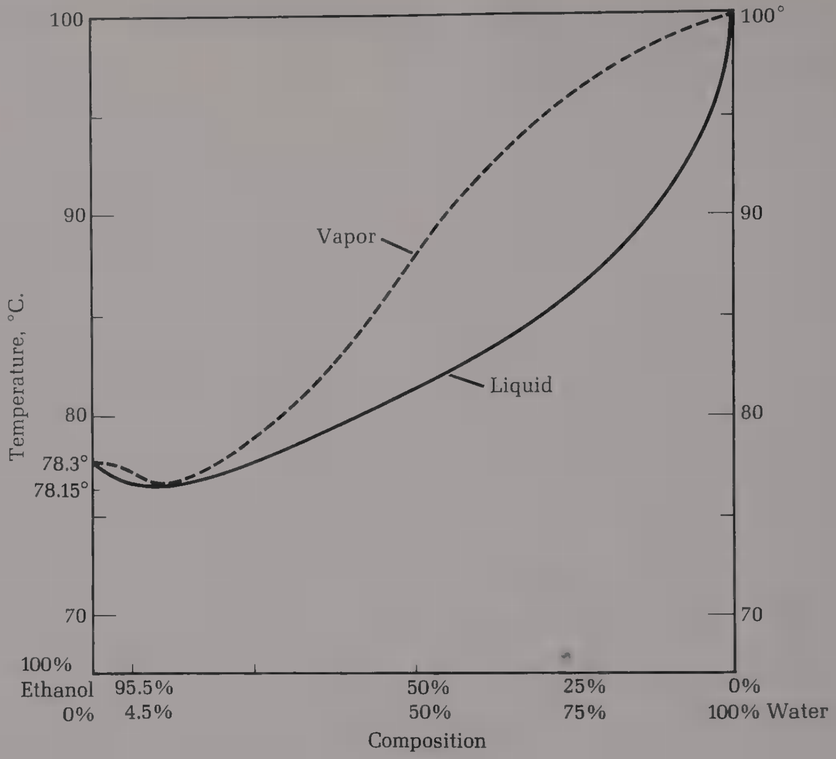
FIGURE 2.6 **Boiling point–composition curves for cyclohexane–toluene mixtures.**

in toluene as it moves from *B* to 110°. In order to obtain pure cyclohexane a number of simple distillations would be necessary. The vapor of composition *B* condenses to give a small amount of liquid of composition *C*. This liquid, which boils at 90°, can be redistilled to give a vapor containing 85 mole percent cyclohexane (*D*), which can be condensed to a liquid (*E*), which can be distilled to give (*F*), etc. This repeated simple distillation would be a very laborious process, but fortunately this combination of condensation and boiling occurs automatically and repeatedly inside a fractionating column.

Not all liquids form ideal solutions and conform to Raoult's law. Ethanol and water are such liquids. Because of molecular interaction, a mixture of 95.5% (by weight) of ethanol and 4.5% of water boils *below* (78.15°) the boiling point of pure ethanol (78.3°). Thus, no matter how efficient the distilling apparatus, 100% ethanol cannot be obtained by distillation of a mixture of 75% water and 25% ethanol. A mixture of liquids of a certain definite composition that distils at a constant temperature without change in composition is called an **azeotrope**; 95% ethanol is such an azeotrope. The boiling point–composition curve for the ethanol–water mixture is seen in Fig. 2.7. To prepare 100% ethanol the water can be removed chemically (reaction with calcium oxide) or by removal of the water as an azeotrope with still another liquid. An azeotrope mixture of 32.4% ethanol and 67.6% benzene (bp 80.1°) boils at 68.2°. A ternary azeotrope (bp 64.9°) contains 74.1% benzene, 18.5% ethanol, and 7.4% water. Absolute alcohol (100% ethanol) is made by addition of benzene to 95% alcohol and removal of the water in the volatile benzene–water–alcohol azeotrope.

▼
95% Ethanol, a low-boiling azeotrope

FIGURE 2.7 Boiling point–composition curves for ethanol–water mixtures.



▼ *Azeotropes*

A pure liquid has a constant boiling point. A change in boiling point during distillation is an indication of impurity. The converse proposition, however, is not always true, and constancy of a boiling point does not necessarily mean that the liquid consists of only one compound. For instance, two miscible liquids of similar chemical structure and which boil at the same temperature individually will have nearly the same boiling point as a mixture. And, as noted previously, azeotropes have constant boiling points which can be either above or below the boiling points of the individual components.

▼ *Nonvolatile solute*

When a solution of sugar in water is distilled, the boiling point recorded on a thermometer located in the vapor phase is 100° (at 760 torr) throughout the distillation, whereas the temperature of the boiling sugar solution itself is initially somewhat above 100° and continues to rise as the concentration of sugar in the remaining solution increases. The vapor pressure of the solution is dependent upon the number of water molecules present in a given volume, and hence with increasing concentration of nonvolatile sugar molecules and decreasing concentration of water, the vapor pressure at a given temperature decreases and a higher temperature is required for boiling. However, sugar molecules do not leave the solution, and the drop clinging to the thermometer bulb is pure water in equilibrium with pure water vapor.

When a distillation is carried out in a system open to the air and the boiling point is thus dependent on existing air pressure, the prevailing barometric pressure should be noted and allowance made for appreciable deviations from the accepted bp temperature (see Table 2.1). Distillation can also be done at the lower pressures that can be achieved by an oil pump or an aspirator with substantial reduction of boiling point (see Chapter 59).

Table 2.1 Variation in Boiling Point with Pressure

Pressure, mm	Water, °C	Benzene, °C
780	100.7	81.2
770	100.4	80.8
760	100.0	80.1
750	99.6	79.9
740	99.2	79.5
584*	92.8	71.2

*Instituto de Quimica, Mexico City, altitude 7700 ft (2310 meters).

EXPERIMENTS

1. Calibration of Thermometer

Test the 0° point of your thermometer with a well-stirred mixture of crushed ice and distilled water. To check the 100° point put 10 ml of water in a 25 × 150-mm test tube, clamp the tube in a vertical position, add one carborundum boiling stone or porcelain chip to prevent bumping, and boil gently with the thermometer in the vapor from the boiling water. Then immerse the bulb in the liquid and see if you can observe superheating. Check the atmospheric pressure to determine the true boiling point of the water.

2. Simple Distillation

Apparatus. Assemble the apparatus for simple distillation shown in Fig. 2.1, starting with the burner and followed by the support ring and a wire gauze placed just above the top of the burner shield. Place the flask with the liquid to be distilled in contact with the gauze. One or two boiling stones are put in the flask to promote even boiling. Each ground joint is greased by putting three or four stripes of grease lengthwise around the male joint and pressing the joint firmly into the other without twisting. The air is thus eliminated and the joint will appear almost transparent. (Do not use excess grease as it will contaminate the product.) Water enters the condenser at the tublature nearest the receiver. Because of the large heat capacity of water only a very small stream (3 mm dia.) is needed; too much water pressure will cause the tubing to pop off. A heavy rubber band can be used to hold the condenser to the distillation head. Note that the bulb of the thermometer is below the opening into the side arm of the distillation head.

(A) Simple Distillation of a Cyclohexane–Toluene Mixture. Place a mixture of 30 ml of cyclohexane and 30 ml of toluene and a boiling chip in a

dry 100-ml round-bottomed flask and assemble the apparatus for simple distillation. After making sure all connections are tight, heat the flask strongly until boiling starts. Then adjust the flame until the distillate drops at a regular rate of about one drop per second. Record both the temperature and the volume of distillate at regular intervals. After 50 ml of distillate are collected, discontinue the distillation. Record the barometric pressure, make any thermometer correction necessary, and plot boiling point versus volume of distillate. Save the distillate for fractional distillation (Experiment 3, below).

(B) Simple Distillation of an Ethanol–Water Mixture. In a 500-ml round-bottomed flask place 200 ml of a 20% aqueous solution of ethanol. Follow the procedure (above) for the distillation of a cyclohexane–toluene mixture. Discontinue the distillation after 50 ml of distillate have been collected. In the hood place three drops of distillate on a Pyrex watch glass and try to ignite it with the blue cone of a microburner flame. Does it burn? Is any unburned residue observed?

3. Fractional Distillation

Apparatus. Assemble the apparatus shown in Fig. 2.3. The fractionating column is packed with one-fourth to one-third of a stainless steel sponge. The column should be perfectly vertical and it should be insulated with glass wool covered with aluminum foil with the shiny side in. However, in order to observe what is taking place within the column, insulation is omitted for this experiment.

(A) Fractional Distillation of a Cyclohexane–Toluene Mixture. After the flask from the simple distillation experiment has cooled, pour the 50 ml of distillate back into the distilling flask, add one or two new boiling chips, and assemble the apparatus for fractional distillation. The stillhead delivers into a short condenser fitted with a bent adapter leading into a 10-ml graduated cylinder. Quickly bring the mixture to a boil. As soon as boiling starts, turn the flame to the smallest, just nonluminous, flame that will stay lit (cut the air supply to the burner). Heat slowly at first. A ring of condensate will rise slowly through the column; if you cannot at first see this ring, located it by touching the column with the fingers. The rise should be very gradual, in order that the column can acquire a uniform temperature gradient. Do not apply more heat until you are sure that the ring of condensate has stopped rising, then increase the heat gradually. In a properly conducted operation, the vapor-condensate mixture reaches the top of the column only after several minutes. Once distillation has commenced, it should continue steadily without any drop in temperature at a rate not greater than 1 ml in 1.5–2 min. Observe the flow and keep it steady by slight increases in heat as required. Protect the column from drafts. Record the temperature as each ml of distillate collects, and make more frequent readings when the temperature starts to rise abruptly. Each time the

▼
Best fractionation by slow and steady heating

graduated cylinder fills, quickly empty it into a series of labeled 25-ml Erlenmeyer flasks. Stop the distillation when a second constant temperature is reached. Plot a distillation curve and record what you observed inside the column in the course of the fractionation. Combine the fractions which you think are pure and turn in the product in a bottle labeled with your name, desk number, the name of the product, the bp range, and the weight.

(B) Fractional Distillation of an Ethanol–Water Mixture. Place the 50 ml of distillate from the simple distillation experiment in a 100-ml round-bottomed flask, add one or two boiling chips, and assemble the apparatus for fractional distillation. Follow the procedure (above) for the fractional distillation of a cyclohexane–toluene mixture. Repeat the ignition test. Is any difference noted?

4. Unknowns[1]

▼
Note to the instructor

You will be supplied with an unknown, prepared by the instructor, that is a mixture of two solvents from those listed in Table 2.2. The solvents are mutually soluble and differ in boiling point by more than 20°. Fractionate the unknown, identify the components from the boiling points, and estimate the composition of the original mixture from the distillation curve.

Table 2.2 Some Properties of Common Solvents

Solvent	Boiling Point	Latent Heat of Vaporization[a]	Surface Tension[b] (20°)
Acetone	56.5°	125.3	23.7
Methanol	64.7°	261.7	22.6
Hexane	68.7°	79.2	18.4
1-Butanol	117.2°		26.2
2-Methyl-2-propanol	82.2°		20.7
Water	100.0°	536.6	72.7
Toluene	110.6°	86.8	28.4

[a] Calories per gram at the bp
[b] Dynes per centimeter

QUESTIONS

1. In the simple distillation experiment (2a or 2b) can you account for the boiling point of your product in terms of the known boiling points of the pure components of your mixture?
2. From the plot of bp vs. volume of distillate in the simple distillation experiment, what can you conclude about the purity of your product?

---

[1] Because of the large holdup of the column relative to the small volume distilled, the unknown should contain at least 10 ml of the less volatile component. Methanol and toluene form an azeotrope bp 63.8° (69% methanol).

3. From the bp vs. volume of distillate plot in the fractional distillation of the cyclohexane–toluene mixture (3a), what conclusion can you draw about the homogeneity of the distillate?
4. From the bp vs. volume of distillate in the fractional distillation of the ethanol–water mixture (3b), what conclusion can you draw about the homogeneity of the distillate? Does it have a constant boiling point? Is it a pure substance since it has a constant boiling point?
5. What is the effect on the boiling point of a solution produced by a soluble nonvolatile substance (e.g., sodium chloride)? What is the effect of an insoluble substance such as sand or charcoal?
6. Calculate the weight of toluene vapor required to fill half of the distillation flask and the fractionation column and distillation head (assume a volume of 70 ml and a temperature of 110°, the bp of toluene).
7. In the distillation of a pure substance (e.g., water), why does not all the water vaporize at once when the boiling point is reached?
8. In fractional distillation, liquid can be seen running from the bottom of the distillation column back into the distilling flask. What effect does this returning condensate have on the fractional distillation?
9. Why is it dangerous to attempt to carry out a distillation in a completely closed apparatus, one with no vent to the atmosphere?
10. Why is better separation of two liquids achieved by slow rather than fast distillation?
11. What is the effect of a) salt and b) ethanol on the boiling point of water? On the freezing point of water?

3

Melting Points

KEYWORDS

- Melting point capillary tube
- Depression of melting point
- Mixed melting point
- Stem correction
- Rast molecular weight determination
- Molality
- Evacuating and sealing a capillary tube

A pure nonionic crystalline organic compound usually has a sharp and characteristic melting point. A mixture of very small amounts of miscible impurities will produce a depression of the melting point and an increase in the melting point range (0.5° to 1° for a pure compound). Consequently the melting point of a compound is a criterion for purity as well as for identification.

The melting point of an organic solid can be determined by introducing a tiny amount into a small capillary tube (Fig. 3.1), attaching this to the stem of a thermometer centered in a heating bath, heating the bath slowly, and observing the temperatures at which melting begins and is complete. Pure samples usually have sharp melting points, for example 149.5–150° or 189–190°; impure samples of the same compounds melt at lower temperatures and over a wider range, for example 145–148° or 187–189°. The contaminant that depresses the melting point and extends the melting range may be an indefinitely characterized resinous material, or it may be a trace of a second chemical entity of melting point either higher or lower than that of the major

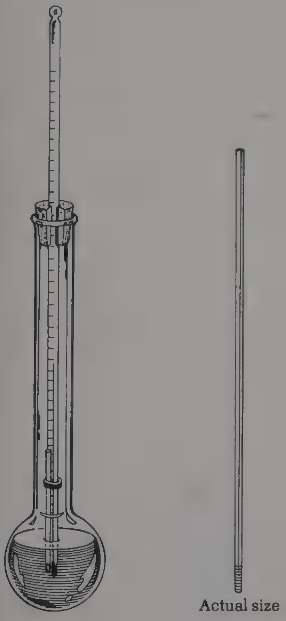


FIGURE 3.1 Melting point flask and capillary tube. The Pyrex flask is 22 cm long, the neck 18 mm o.d., the bulb 55 mm o.d.

component. Under equilibrium conditions (no super-cooling) the temperature at which a pure solid melts is identical with that at which the molten substance solidifies or freezes. Just as salt lowers the freezing point of water, so one compound (A) depresses the melting point of another (B) with which it is mixed. If pure A melts at 150–151° and pure B at 120–121°, mixtures of A with small amounts of B will melt unsharply at temperatures below 150° and mixtures of B containing A will melt below 120°. Both the temperature and sharpness of melting are useful criteria of purity.

▼
Mixed melting point

A third substance (C) may have exactly the same melting point as A, namely 150–151°, but if A and C are mixed and the melting point of the mixture is observed, the one substance will be found to depress the melting point of the other. Depression, or nondepression, of melting point is invaluable in the identification of unknowns. An unknown D found to melt at 150–151° can be suspected of being identical with one or the other known substances A and C; observation that the mixture AD shows a melting point depression would exclude identity with A, and failure of C to depress the melting point of D would prove C and D identical.

EXPERIMENTS

Apparatus. A variety of different apparatus is available for use in melting point determinations. A very simple one is illustrated in Fig. 3.2. The thermometer is fitted through a cork, a section of which is cut away for pressure release and so that the scale is visible (Fig. 3.1). A single-edge razor blade is convenient for cutting, and the cut can be smoothed or deepened with a triangular file. The curvature of the walls of the flask causes convection currents in the heating liquid to rise evenly along the walls and then descend and converge at the center; hence, center the thermometer in the flask. The long neck prevents spilling and fuming and minimizes error due to stem exposure. The bulb of the flask (dry!) is three-quarters filled with di-*n*-butyl phthalate; if it darkens in use the liquid should be replaced. The heating flask is mounted as shown in Fig. 3.2, with the bulb close to the chimney of a microburner. Careful control of heat input required in taking a melting point is accomplished both by regulating the gas supply with the screw pinchclamp and by raising or lowering the heating bath.

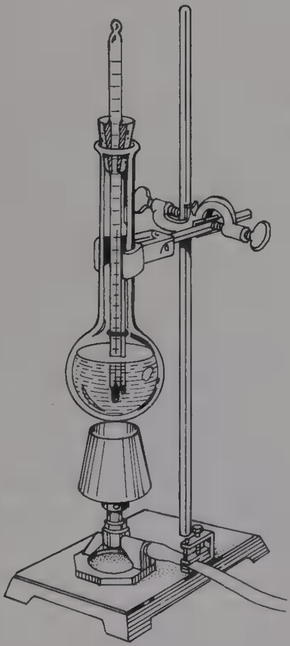


FIGURE 3.2 Apparatus for melting point determination.

Two commercial melting point devices are illustrated in Figs. 3.3 and 3.4. The Mel-Temp apparatus (Fig. 3.3) consists of an electrically heated aluminum block that accommodates three capillaries. The sample is illuminated through the lower port and observed through a 6-power lens through the upper port. The heating rate can be controlled, and with a special thermometer the apparatus can be used up to 500°.

The Thomas-Hoover Uni-Melt apparatus (Fig. 3.4) will accommodate seven capillaries in a small beaker of high-boiling silicone oil which is stirred and heated electrically. The heating rate is controlled with a variable transformer. The rising mercury column of the thermometer can be observed with a traveling periscope device so the eye need not move away from the capillary. For industrial analytical and control work there is even an apparatus (Mettler)

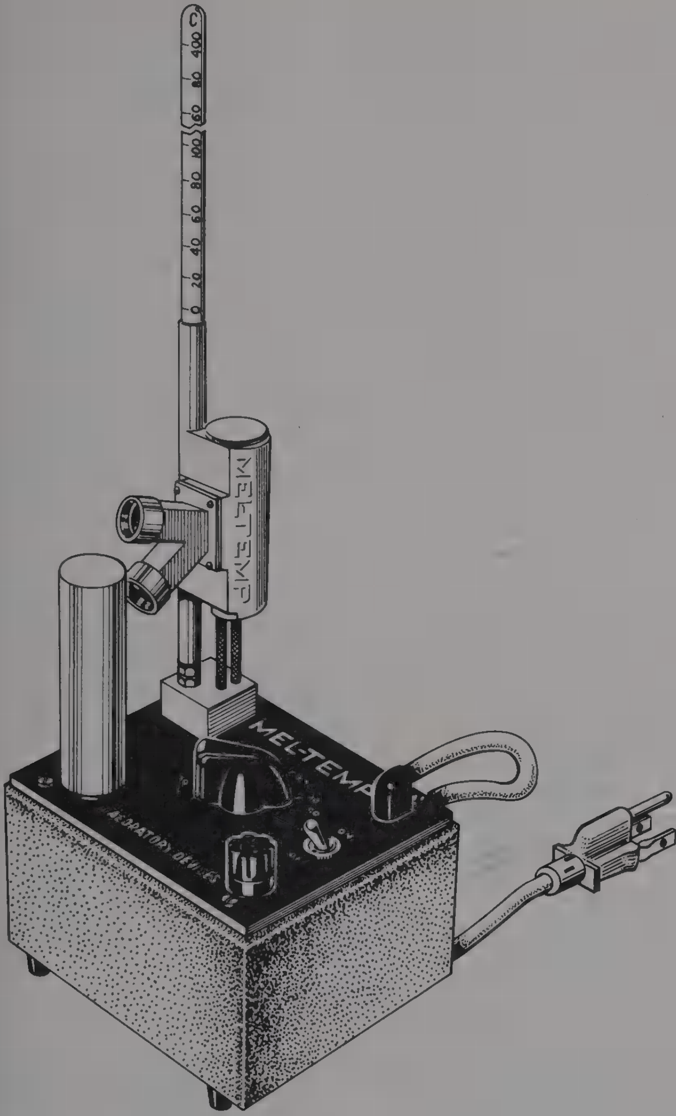


FIGURE 3.3 Mel-Temp melting point apparatus.

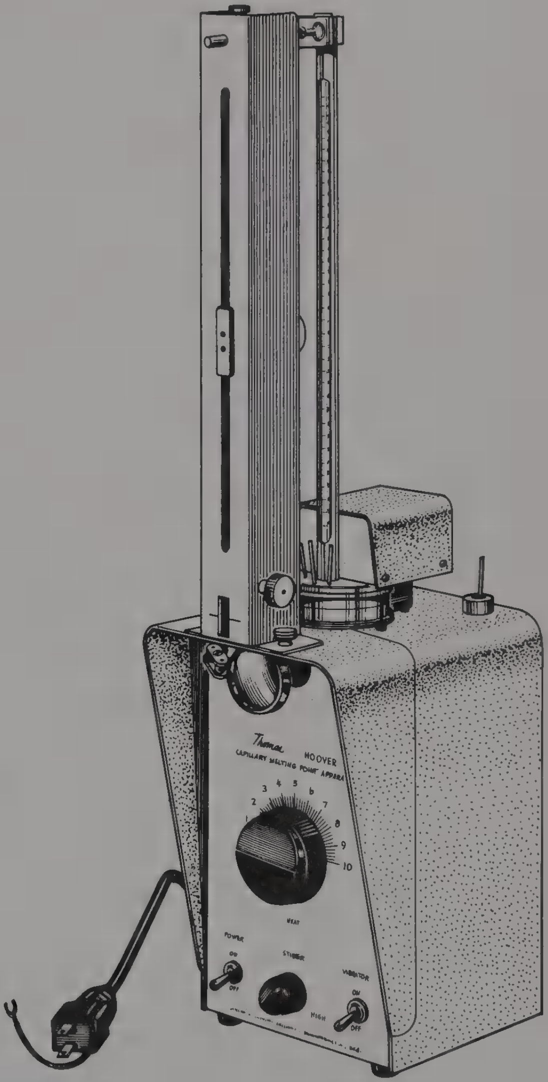


FIGURE 3.4 Thomas-Hoover Uni-Melt melting point apparatus.

that automatically determines the melting point and displays the result in digital form.

Capillary melting point tubes can be obtained commercially or can be made by drawing out 12-mm soft-glass tubing. The 12-mm tubing is rotated in the hottest part of the Bunsen burner flame until it is very soft and begins to sag. It should not be drawn out during the heating. Remove from the flame, hesitate

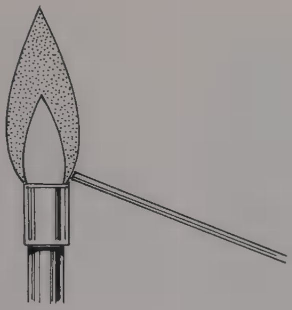


FIGURE 3.5 Sealing a melting point capillary tube.

for a second or two, and then draw it out steadily but not too rapidly to arm's length. With some practice it is possible to produce 10–15 good tubes in a single drawing. The long capillary tube can be cut into sections with the sharp edge of a scorer. Alternatively, bring a sharp file or glass scorer resting on your forefinger underneath the tube, put your thumb over the place to be cut, and gently draw in the file or scorer so as to produce a slight scratch. On applying gentle pressure with the thumb the tube will now break cleanly. The tube is sealed by rotating the end in the edge of a small flame (Fig. 3.5). The actual size of a satisfactory capillary tube is shown in Fig. 3.1.

Regardless of the type of melting point apparatus used, the thermometer must be calibrated. Most thermometers encountered in the organic laboratory are of the 76-mm immersion variety and are so marked. These thermometers should read more or less correctly when immersed to a depth of 76 mm in the fluid whose temperature is being measured. However, the thermometer should be calibrated by determination of the melting points of substances of known melting points.

EXPERIMENTS

1. Calibration of Thermometer

If the thermometer has not previously been calibrated at 0° and 100° (Experiment 2.1), it should now be done. In addition determine the melting point of standard substances (Table 3.1) over the temperature range of interest.

Table 3.1 Melting Point Standards

Compound	Melting Point	Compound	Melting Point
Naphthalene	80–82°	4-Toluic Acid	180–182°
Urea	132.5–133°	Anthracene	214–217°
Sulfanilamide	164–165°	Caffeine	236–237.5°

2. Melting Points of Pure Urea and Cinnamic Acid

Using a metal spatula crush the sample to a fine powder on a hard surface such as a watch glass. Push a melting point capillary into the powder and force the powder down in the capillary by tapping the capillary or by dropping it through a long glass tube held vertically and resting on a hard surface. The column of solid should be no more than 2–3 mm in height and it should be tightly packed.

The melting point capillary is held to the thermometer by a rubber ligature[1] or a rubber band made by cutting a thin slice off the end of a piece of 5 mm rubber tubing with scissors; the band may require replacement from time to time. Insertion of a fresh tube under the rubber band is facilitated by leaving the used tube in place. The sample should be close to and on a level with the center of the thermometer bulb, which must be fully submerged and centered in the heating flask. If the approximate melting temperature is known, the

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[1] S. S. White Dental Mfg. Co., Philadelphia, Pa., small rubber ligatures, No. 241, 225 parts per box.

bath can be heated rapidly until the temperature is about 20° below this point, but the heating during the last 15–20° must be slow and regular. It is important that the temperature rise no more than 2° per minute while the sample is melting. As the melting point is approached the sample may shrink because of crystal structure changes. However, the melting process begins when the first drops of liquid are seen in the capillary and it ends when the last trace of solid disappears. For a pure compound this whole process may occur over a range of only 0.5°, hence the necessity of having the temperature rise slowly during the determination.

If determinations are to be done on two or three samples that differ in melting point by as much as 10°, two or three capillaries can be secured to the thermometer together and the melting points observed in succession without removal of the thermometer from the bath. As a precaution against interchange of tubes while they are being attached, use some system of identification, such as one, two, and three dots made with a marking pencil.

Determine the melting point of either urea (mp 132.5–133°) or cinnamic acid (mp 132.5–133°). Repeat the determination and if the two determinations do not check within 1°, do a third one.

3. Melting Points of Urea–Cinnamic Acid Mixtures

Make mixtures of urea and cinnamic acid in the approximate proportions 1:4, 1:1, and 4:1 by putting side by side the correct number of equal sized small piles of the two substances and then mixing them. Grind the mixture thoroughly for at least a minute on a watch glass using a metal spatula. Note the ranges of melting of the three mixtures and use the temperatures of complete liquefaction to construct a rough diagram of mp versus composition.

4. Unknown

Determine the melting point of one of the following unknowns selected by the instructor:

Maleic anhydride, mp 54–56°
Naphthalene, mp 80–82°
Acetanilide, mp 113.5–114°
Benzoic acid, mp 121.5–122°
Cinnamic acid, mp 132.5–133°
4-Nitroaniline, mp 148–149°
Salicylic acid, mp 158.5–159°
Sulfanilamide, mp 165–166°
Succinic acid, mp 184.5–185°
p-Terphenyl, mp 210–211°

5. Molecular Weight Determination by Melting Point Depression. The Rast Method

The molecular weight of an unknown organic compound is most accurately determined by mass spectroscopy. However, it is possible to determine the molecular weight of a sample with very simple equipment to an accuracy (± 10%) sufficient for most purposes. The method depends on the fact that a 1 molal solution (1 mole of compound per 1000 g of solvent) in camphor will

▼
Molality = g of solute 1000 g of solvent

depress the melting point of camphor by 37.7°. Camphor is used for this purpose because of its exceptionally large molal freezing point depression constant and its ability to dissolve a wide range of organic compounds. The molecular weight can be calculated from the expression

$$\mathrm{MW} = \frac{(\text{g sample})\,(1000\text{ g camphor})\,(37.7°/\text{mole})}{(\text{g camphor})\,(\Delta T)}$$

where ΔT is the observed melting point depression.

The melting behavior of camphor is peculiar, since even the purest material melts over a considerable temperature range: the solid softens, then it turns to a glassy semisolid, then a clear liquid appears in the upper part of the tube, and finally a skeleton of crystals remains in the lower part and slowly decreases in size until it eventually disappears. The point of disappearance of the last crystal, the melting point, can be determined with accuracy. If the bath liquid is allowed to cool very slowly, an equally accurate determination can be made of the freezing point, the temperature at which the first tiny crystals of camphor separate. That the freezing point is usually found to be about 2° below the melting point is probably attributable to supercooling of the unstirred solution. Dilute solid solutions (1–6%) of solid organic compounds in camphor behave similarly; the transition temperatures are lower, the melting ranges wider, and the difference between melting and freezing point somewhat greater. High accuracy in determining the extent of depression is attainable with the apparatus of Fig. 3.1 by attaching to the thermometer one tube containing pure camphor and another containing a mixture of camphor and the substance under investigation. Errors due to inaccuracy of the thermometer or to stem exposure are thus cancelled. Very slow heating and cooling and very careful observation are required. It is necessary also to seal off the capillary at a point that is below the level of the heating fluid (Fig. 3.6), for if there is any vapor space above this level, camphor will sublime into the cooler area and leave a residual melt enriched in the solute. The capillary is evacuated prior to sealing to facilitate formation of a firm seal without increasing the fragility of the tube. The technique of sealing is generally useful, since some compounds undergo air oxidation when heated in open capillary tubes and true melting points are obtainable only when the determinations are made in evacuated tubes.

6. Identification of Unknowns

Suppose you have two unknowns, A and B. One unknown contains *x* grams of acetanilide (C_8H_9ON) per 100 g of camphor and the other contains *x* grams of benzophenone ($C_{13}H_{19}O$) per 100 g of camphor.[2] The problem is to deter-

▼
Note for the instructor

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[2] Weigh $x/10$ g of substance and 10 g of camphor in a test tube, stopper loosely, melt without undue overheating, pour the melt into a mortar, swirl until solid, then at once dislodge the solid and while it is still hot grind it lightly and not too long. Concentrations of 1–6% are satisfactory.

mine the melting and freezing points of the two unknowns and of camphor simultaneously, establish which unknown is acetanilide and which is benzophenone, and calculate the value of x, common to both samples. Care should be taken when filling, sealing, and mounting the tubes, for once this is done properly the determinations can be repeated until you can obtain checking results with confidence.

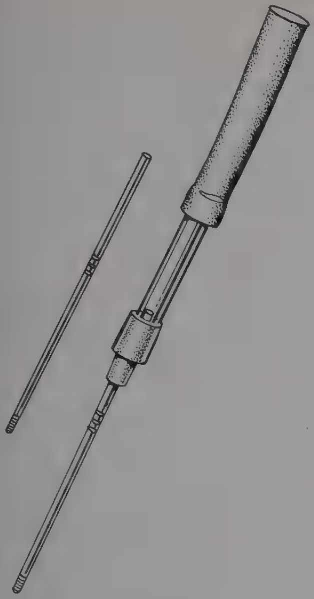

FIGURE 3.6 Evacuation of a capillary tube prior to sealing.

Push the open ends of three melting point capillary tubes into powdered unknowns A and B and camphor (contained in a short specimen tube) and tamp down the solid in each tube until you have a column about 5 mm long. Mark each tube and record the contents. Each tube, in turn, is connected to the suction pump tubing by an adapter (Fig. 3.6) consisting of a glass tube capped with a rubber vaccine stopper[3] with a hole pierced through it with an awl or red-hot sewing needle. Lubricate the end of the hole extending toward the wide part of the stopper, grasp the capillary very close to the sealed end, and thrust it through nearly to the full length. Connect to the suction pump, restore the tube markings if necessary, and make a guiding mark 1.5 cm from the closed end where a seal is to be formed. Grasp the end of the tube with one hand and the adapter with the other and, with suction pump operating, hold the point of sealing near the small flame of a microburner, steady your hands on the bench so that neither will move much when the tube is melted, and then hold the tube in the flame until the walls collapse to form a flat seal and remove it at once. Take the tube out of the adapter and if the two straight parts of the tube are not in line, reheat to soften the seal and correct the alignment.

Mount tubes of A, B, and camphor side by side on the thermometer under a rubber band, insert the thermometer, and make sure that all the seals are completely submerged. The bath may be heated rapidly to about 130°, but from this point on the temperature rise should be as slow as possible. At the outset, a very small flame about 7 cm below the flask should produce a slow, gradual rise of 1° in 30–40 sec. Determine the temperature of disappearance of the last crystal for each sample in succession and then continue heating until the bath is about 2° above the melting point of camphor. Lower the flame by small increments until the temperature starts to fall and then adjust the heating so that the temperature falls 1° in 30–40 sec. Determine each freezing point in turn and then keep the bath at about 140° while calculating the depressions in melting and freezing points. If the two depressions are very divergent, or if the difference between mp and fp for either A or B is much greater than that for camphor, repeat all the determinations.

In order to assess the data, calculate the value of x for each separate determination from the equation:

$$x = \frac{\Delta t \times \mathrm{MW}}{377}$$

Tabulate the results, put the more divergent values in parentheses, and average the others. When the true value of x is disclosed, calculate the molecular

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[3] Rubber stoppers, serum with sleeve, small size, R 7950, Scientific Glass Co., Bloomfield, N.J.

weights based on your average Δt values and estimate the limit of accuracy of the method. On the estimate that a 3-mm column of camphor in a capillary tube weighs 1 mg, calculate the weights of acetanilide and benzophenone used in your determinations.

Since the melting point apparatus is required for the next experiment it may be put away in assembled form. When parts are required for other purposes, the heating flask should be allowed to cool, stoppered tightly with a cork, and stored.

QUESTIONS

1. What effect would poor circulation of the melting point bath liquid have on the observed melting point?
2. What is the effect of an insoluble impurity, such as charcoal, on the observed melting point of a compound?
3. Three test tubes, labeled A, B, and C, contain substances with approximately the same melting points. How could you prove the test tubes contain three different chemical compounds?
4. One of the most common causes of inaccurate melting points is too rapid heating of the melting point bath. Under these circumstances how will the observed melting point compare with the true melting point?

4

Crystallization

KEYWORDS

- Solute
- Solvent
- Filtrate
- Mother liquor
- Seed crystals
- Decolorizing charcoal (Norit, Darco)
- "Like dissolves like"
- Solvent pairs
- Stemless funnels
- Hirsch funnel
- Neoprene adapter
- Filter block
- Clarification
- Swirling

▼
The correct quantity of solvent

A solid substance can be purified very effectively by dissolving it in a suitable solvent at the boiling point, filtering the hot solution by gravity to remove any suspended insoluble particles, and letting crystallization proceed. Separation of crystals before the hot solution has all passed through the filter paper can be largely prevented by determining the minimum volume of solvent required to effect solution and then adding a deliberate excess (ca. 20%). A little more fresh hot solvent is required to wash the paper clean. The total filtrate is then evaporated to the original volume and let stand undisturbed at room temperature until the solution has cooled to the same temperature and crystals have ceased to increase in number or size. The flask is then chilled in an ice bath to promote further crystallization. The crystals that have separated in this first crop are collected by suction filtration and washed free of mother liquor with a little fresh, chilled solvent. If it is deemed worthwhile, the combined mother liquor and washings can be concentrated to a small volume and let stand for separation of a second crop. The quality of each crop is ascertained by the melting point.[1]

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[1] An audio-visual program detailing all aspects of crystallization is available from H. A-V Co., 43 Woodbridge St., South Hadley, Ma. 01075.

The specification that the hot solution saturated with solute at the boiling point be let stand undisturbed means that crystallization is allowed to proceed without subsequently disturbing the flask.

For estimation of the temperature, the flask should be touched very lightly between the thumb and a finger without any movement of the flask. Crystallization does not happen at once. It usually starts after an induction period, ranging from several minutes to an hour or two, even though the temperature has dropped well below that at which the solution is saturated with solute.

Experiment 1. Crystallization of Pure Phthalic Acid, $C_6H_4(COOH)_2$

The process of crystallization can be observed readily using phthalic acid. In a reference book such as *The Handbook of Chemistry and Physics*, in the table "Physical Constants of Organic Compounds," the entry for phthalic acid gives the following solubility data (in grams of solute per 100 ml of solvent) where the superscripts refer to temperature in °C:

Water	Alcohol	Ether, etc.
0.54^{14}	11.7^{18}	0.69^{15} eth., i. chl.
18^{99}		

The large difference in solubility in water as a function of temperature suggests this as the solvent of choice. The solubility in alcohol is high at room temperature. Ether is difficult to use because it is so volatile and the compound is insoluble in chloroform.

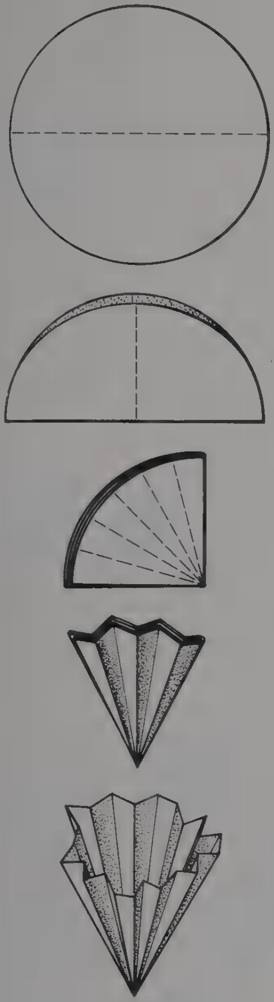
5.5ml 9

Crystallize 1.0 g of phthalic acid from the minimum volume of water, using the above data to calculate the required volume. Add the solid to the smallest practical Erlenmeyer flask and then, using a Pasteur pipette, add water dropwise from a full 10-ml graduated cylinder. After a portion of the water has been added, heat the solution to boiling on a hot plate or over a Bunsen burner. Continue to add water dropwise until all the solid just dissolves. Place the flask on the laboratory bench and allow it to cool undisturbed to room temperature, during which time the crystallization process can be observed. Then cool the flask in an ice bath, decant (pour off) the mother liquor (the liquid remaining with the crystals), and remove the last traces of liquid with a Pasteur pipette. Scrape the crystals onto a filter paper using a stainless steel spatula, squeeze the crystals between sheets of filter paper to remove traces of moisture, and allow the crystals to dry. Compare the calculated volume of water with the volume of water actually used to dissolve the acid and calculate the percent recovery of dry, recrystallized phthalic acid.

Decolorizing Charcoal and Filtration

Frequently a sample to be purified contains a soluble impurity that gives rise to solutions and crystals that are slightly colored when they should be colorless, or have an off color rather than a pure color. Such samples are treated with decolorizing (activated) charcoal. The fine carbon particles present a large active surface (300 to 2000 square meters per gram) for adsorption of dissolved substances, particularly the polymeric, resinous by-products that appear in traces in most organic reaction mixtures. Decolorizing charcoal is added to the hot solution prior to filtration, and the solution is kept hot for a brief period, shaken to wet the carbon, and filtered. No more charcoal should be used than is actually needed, for an excess may adsorb some of the desired compound.

FIGURE 4.1 Fluting a filter paper.



When using decolorizing charcoal be particularly careful not to add the charcoal to a solution at the boiling point; the charcoal particles function like thousands of boiling chips and will cause the solution to boil over. Stop heating the solution for a few seconds, add the charcoal, and cautiously heat the solution to near the boiling point. After a minute or two, filter the hot solution through a fluted filter paper held in a previously heated stemless funnel.

Filter paper may be fluted in a number of ways. One method is shown in Fig. 4.1. The object of fluting is to decrease the area of contact of the filter paper with the funnel and so speed the filtration. If filtration is slow, the solution will cool and could deposit crystals on the filter paper. The funnel should be heated, most conveniently by inverting it over a steam bath. It should be wiped dry before use. The fluted paper should always be below the rim of the funnel. Proper sizes of funnels, filter paper, and flasks are shown in Fig. 4.2.

Experiment 2. Decolorization of Brown Sugar (Sucrose, $C_{12}H_{22}O_{11}$)

Raw sugar is refined commercially with the aid of decolorizing charcoal. The clarified solution is seeded generously with small sugar crystals, and excess water removed under vacuum to facilitate crystallization. The pure white crystalline product is collected by centrifugation. Brown sugar is partially refined sugar and can be decolorized easily using charcoal.

Dissolve 15 g of dark brown sugar in 30 ml of water in a 50-ml Erlenmeyer flask by heating and stirring. Pour half of the solution into another 50-ml flask.

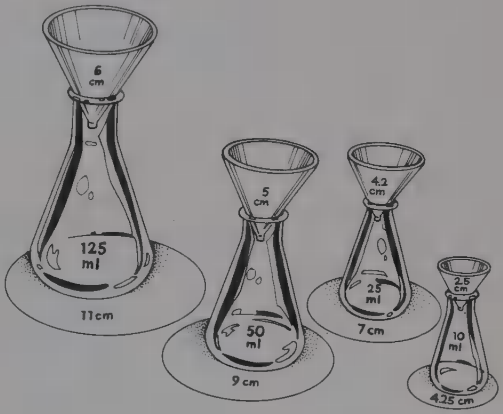


FIGURE 4.2 Assemblies for gravity filtration. Stemless funnels have diameters of 2.5, 4.2, 5.0, and 6.0 cm.

Heat one of the solutions nearly to the boiling point, allow it to cool slightly, and add to it 250 mg (0.25 g) of decolorizing charcoal. Bring the solution back to near the boiling point for two minutes, then filter the hot solution into an Erlenmeyer flask through a fluted filter paper held in a previously heated funnel. Treat the other half of the sugar solution in exactly the same way but use only 50 mg of decolorizing charcoal. In collaboration with a fellow student try heating the solutions for only 15 sec after addition of the charcoal. Compare your results.

Sugars as a class are notoriously difficult to crystallize; sucrose is probably the easiest sugar to work with and even it cannot readily be crystallized in the laboratory. You can try by adding an equal volume of 95% ethanol to your decolorized filtrates and a number of seed crystals (granulated sugar), and setting the stoppered and labeled flasks in a refrigerator until the next laboratory period.

Choosing a Solvent and Solvent Pairs

The proper choice of solvent is an important part of the art of crystallization. The ideal solvent should be chemically inert toward the solute, and should dissolve the solute readily at the boiling point of the solvent but sparingly at low temperature (0° to 25°). It should dissolve impurities either very easily or not at all, be highly volatile so that it can readily be evaporated from the crystals, and be of low flammability, low cost, and low toxicity. No one solvent meets all these specifications. The properties of solvents commonly used for crystallization are listed in Table 4.1.

In general "like dissolves like", that is, hydrocarbon solvents will dissolve hydrocarbons and hydroxylic solvents such as methanol (CH_3OH), ethanol (C_2H_5OH), and acetic acid (CH_3COOH) will dissolve polar compounds. Hydroxylic solvents are all miscible with one another and with water, and they resemble one another in that they are able to dissolve substances of specific types, particularly hydroxylic compounds, and are less effective solvents for structurally dissimilar compounds such as hydrocarbons.

Solvent power usually increases with increasing boiling point. For example, ethanol dissolves about twice as much of a given solute as methanol. On the other hand, ethanol is more costly than methanol and so may not be the solvent of choice when working on a large scale. The chlorinated hydrocarbons methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and carbon tetrachloride (CCl_4) are particularly expensive, and the latter two are toxic as well. Ligroin is a mixture of aliphatic hydrocarbons including the isomeric hexanes and heptanes. It is comparable to petroleum ether (a mixture of isomeric pentanes and hexanes) but has greater solvent power and a higher boiling point. A substance that dissolves in petroleum ether or ligroin is almost invariably insoluble in water. Acetone is similar in solvent action to ethanol, and ether is similar to benzene. Most commonly used solvents for crystallization of organic compounds are flammable. Check to see that no flames are nearby when carrying out crystallization with these solvents. In addition, benzene is

Table 4.1 Properties of Some Common Crystallization Solvents

Solvent	Boiling Point	Freezing Point	Density	Miscibility with Water	Flammability
Water	100°	0°	1.0	+	0
Methanol	64.7°	<0°	0.79	+	+
95% Ethanol	78.1°	<0°	0.81	+	++
Acetic acid	118°	16.7°	1.05	+	+
Acetone	56.5°	<0°	0.79	+	+++
Ether	34.6°	<0°	0.71	–	++++
Petroleum ether	35–65°	<0°	0.63	–	++++
Ligroin (normal, low bp)	65–75°	<0°	0.68	–	+++
Ligroin (high bp)	100–115°	<0°	0.70	–	++
Benzene	80.1°	5°	0.88	–	++++
Toluene	110.6°	<0°	0.87	–	++
Dichloromethane (methylene chloride)	41°	<0°	1.34	–	0
Carbon tetrachloride	76.6°	<0°	1.59	–	0
Chloroform	61.2°	<0°	1.48	–	0

toxic and a mild carcinogen and should be used only with adequate ventilation, preferably in the hood. Toluene, although more difficult to remove from crystals because of its higher boiling point, is not a carcinogen and much less toxic than benzene.

Two miscible solvents of different solvent power constitute a solvent pair. The hydrocarbon naphthalene is insoluble in water but so soluble in methanol that it crystallizes from this solvent only if the solution is highly concentrated and the temperature is low. It can be crystallized efficiently from a suitably proportioned methanol–water mixture; the hydrocarbon is dissolved in excess methanol and then water is added little by little at the boiling point until the solution is saturated. See Experiment 3.

Other useful solvent pairs are listed in Table 4.2. If solubility tests conducted with 20-mg samples of material to be crystallized show that it is readily soluble in one member of a solvent pair and sparingly soluble in the other, a solution of the sample in the first solvent can be adjusted to conditions suitable for crystallization by dilution with the second solvent. Methylene chloride (bp 41°) has more solvent power for most compounds than methanol (bp 65°)

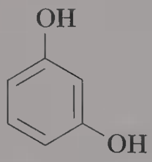
Table 4.2 Solvent Pairs

Methanol–Water
Ethanol–Water
Acetic acid–Water
Acetone–Water
Ether–Methanol
Ether–Acetone
Ether–Petroleum ether
Benzene–Ligroin
Methylene chloride–Methanol

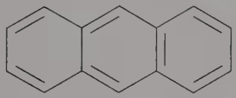
and is more volatile; hence crystallization is accomplished by dissolving the solid in methylene chloride, filtering, adding methanol, and boiling down the filtrate until the product begins to crystallize.

Experiment 3. Solubility Tests

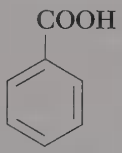
▼
Test Compounds:



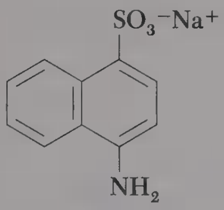
Resorcinol



Anthracene



Benzoic acid



Sodium naphthionate

To test the solubility of a solid, transfer an amount roughly estimated to be about 20 mg with a small spatula into a 10 × 75-mm test tube and add about 0.5 ml of solvent estimated as a 1-cm column in the small test tube. Stir with a fire-polished stirring rod (4-mm), break up any lumps, and determine if the solid is readily soluble at room temperature. If the substance is readily soluble in either methanol, ethanol, acetone, or acetic acid at room temperature, add a few drops of water from a wash bottle to see if a solid precipitates. If it does, heat the mixture, adjust the composition of the solvent pair to produce a hot solution saturated at the boiling point, let the solution stand undisturbed, and note the character of the crystals that form. If the substance fails to dissolve in a given solvent at room temperature, heat the suspension and see if solution occurs. If the solvent is flammable, heat the test tube on the steam bath or in a small beaker of water kept warm on the steam bath or a hot plate. If the solid completely dissolves, it can be declared readily soluble in the hot solvent; if some but not all dissolves, it is said to be moderately soluble, and further small amounts of solvent should then be added until solution is complete. When a substance has been dissolved in hot solvent, cool the solution by holding the flask under the tap and, if necessary, induce crystallization by rubbing the walls of the flask with a stirring rod to make sure that the concentration permits crystallization. Then reheat to dissolve the solid, let the solution stand undisturbed, and inspect the character of the ultimate crystals.

Make solubility tests on the test compounds shown to the left in each of the solvents listed. Note the degree of solubility in the solvents, cold and hot, and suggest suitable solvents, solvent-pairs, or other expedients for crystallization of each substance. Record the crystal form, at least to the extent of distinguishing between needles (pointed crystals), plates (flat and thin), and prisms. How do your observations conform to the generalization that like dissolves like?

Solvents:

Water—hydroxylic, ionic
Toluene—an aromatic hydrocarbon
Ligroin—a mixture of aliphatic hydrocarbons

Crystallization of a Typical Organic Compound

Apparatus

The Erlenmeyer flask (Pyrex) is particularly well adapted to crystallization. The flask can be conveniently held at the neck with the thumb and finger and for dissolving the solid the flask can be put directly on a steam bath or hot

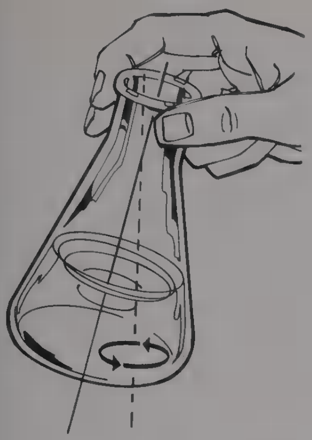


FIGURE 4.3 Swirling of a solution.

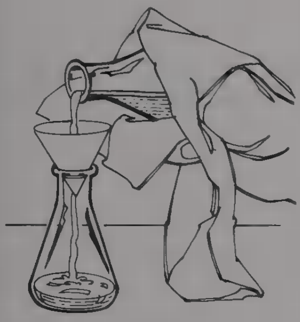


FIGURE 4.4 Pouring a hot liquid.

plate or, if the solvent is water, over a free flame. The contents can be agitated by swirling, that is, imparting a circulatory motion to the contents by moving the bottom of the flask in a circle as the top remains stationary (Fig. 4.3); the conical shape of the flask prevents spillage. *Never use a beaker for crystallization.* If lumps or large crystals of solid are slow in dissolving, the process can be hastened by crushing the material against the bottom of the flask with a flattened stirring rod made by heating the end of a piece of 4-mm, soft glass rod to redness and pressing it onto a flat surface, such as a coin). To pour hot liquid cover the hand with a folded towel and grasp the flask (Fig. 4.4), or use a clamp to handle the flask.

The funnels used in crystallization should be stemless (to avoid crystallization in the stem) and supported merely by resting them in the mouth of the receiving flask. Proper matching sizes of flasks, funnels, and papers are indicated in Fig. 4.5.

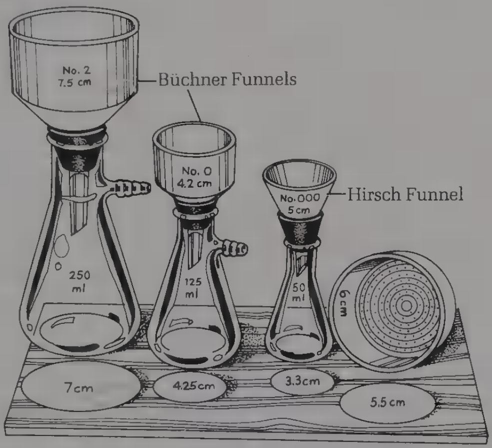


FIGURE 4.5 Matching filter assemblies. The 6.0-cm polypropylene Büchner funnel (*right*) resists breakage and can be disassembled for cleaning.

After a product has crystallized at room temperature, the Erlenmeyer is usually let stand for a time in an ice-water bath to effect maximum separation of crystals from the solution. The crystals are then collected by suction filtration on a Büchner or Hirsch funnel which is placed in a neoprene adapter atop a filter flask (Fig. 4.6). The filter flask is connected through a filter trap (Figs. 1.11 and 1.12) to the water aspirator by thick-walled tubing which will not collapse under vacuum. This tubing is so heavy that the 50-ml and 125-ml filter flasks will tip over unless provided with a support, such as a filter block (Fig. 4.6), or clamped to a ring stand. Larger filter flasks have adequate stability and require no support.

The perforated plate of the funnel is covered by a disc of filter paper of appropriate size, centered in the funnel, and moistened with the crystallization solvent. Suction is applied and the liquid and solid mixture is poured in. As soon as all the solvent has been pulled through the crystals the suction is broken, clean cold wash solvent added to the funnel, and suction reapplied. After the crystals have been washed and while suction is maintained, the

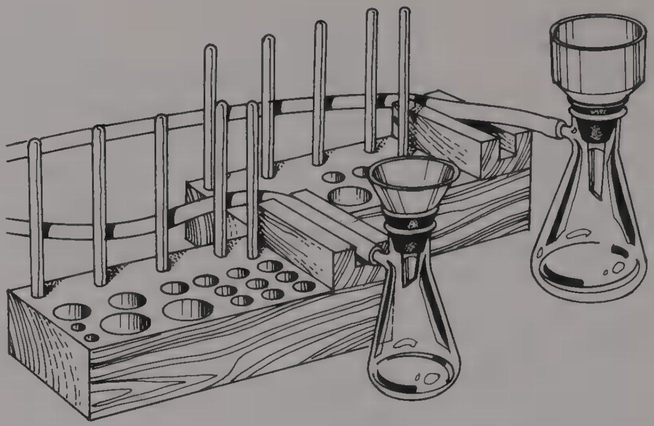


FIGURE 4.6 Suction filter assemblies with filter blocks for firm support.

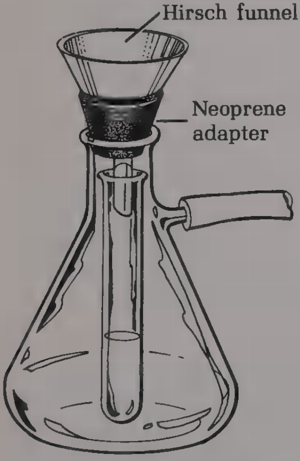


FIGURE 4.7 Suction filter assembly for small quantities of filtrates.

crystals are pressed on the filter disc with a clean cork to eliminate traces of solvent. A technique for collecting a small quantity of filtrate is illustrated in Fig. 4.7.

Once collected and washed the crystals must be dried. If the solvent is fairly volatile merely pulling air through the filter cake will effect drying. Small quantities of crystals can be given a preliminary drying by spreading the substance out on a filter paper, folding the filter paper over the solid, and then squeezing excess solvent into the filter paper.

A solid that has been collected by suction filtration may dry slowly at atmospheric conditions, particularly if the solvent is water and the solid noncrystalline. For rapid drying, place the moist solid in a tared flask of appropriate size (a tared flask is one that has been weighed and the weight recorded) and attach a large one-hole rubber stopper by means of a 6-mm dia glass tube to the suction tubing leading to the aspirator. Atmospheric pressure will hold the flask to the stopper when the aspirator starts working (Fig. 4.8). Flasks from 10 ml to 125 ml can be evacuated in this manner and used to dry a solid. Turn on the aspirator to full force, apply steam and observe the pressure gauge. When the pointer reaches a position of maximum declination, kink the vacuum tube and see whether the pointer moves any farther; if so, all connections are not tight. After about 15 min of evacuation on the steam bath, disconnect the flask, wipe it, and weigh. Repeat the operation until the weight is constant.

FIGURE 4.8 Drying a solid by reduced air pressure.

Experiment 4. Recrystallization of a Typical Organic Compound, Naphthalene

Add 2.0 g of impure naphthalene[2] to a 50-ml Erlenmeyer flask along with 3 ml of methanol. Heat the mixture to boiling over a steam bath or hot plate and then add methanol dropwise until the naphthalene just dissolves when

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[2] A mixture of 100 g of naphthalene, 1 g of decolorizing charcoal, and 0.3 g of a dye such as congo red.

the solvent is boiling. The total volume of methanol should be 4 ml. Remove the flask from the heat and cool it rapidly in an ice bath. Note that the contents of the flask set to a solid mass, which would be impossible to handle. Add enough methanol to bring the total volume to 15 ml, heat the solution to the boiling point, remove the flask from the heat, allow it to cool slightly, and add 30 mg of decolorizing charcoal to remove the colored impurity in the solution. This amount of charcoal is a pile 6 mm ($\frac{1}{4}$ in.) in diameter and can be picked up on the end of a stainless steel spatula. Heat the solution to the boiling point for two minutes, then filter through a fluted filter paper in a previously warmed stemless funnel into a 50-ml Erlenmeyer flask.

Pour the solution first onto the upper part of the section of paper that is reinforced by the extra folds, for this minimizes passage of carbon particles through the paper. In case the first few drops of filtrate do contain carbon, watch for the point where the filtrate becomes clear, change to a clean receiving flask, and reheat and refilter the first filtrate that contains carbon. Sometimes filtration is slow because the funnel fits so snugly into the mouth of the flask that a back pressure develops. If you note that raising the funnel increases the flow of filtrate, fold a small strip of paper two or three times and insert it between the funnel and flask. Wash the used flask with 2 ml of hot methanol and use this liquid to wash the filter paper, transferring the solvent with a Pasteur pipette in a succession of drops around the upper rim of the filter paper.

Since the filtrate is far from being saturated with naphthalene at this point it will not yield crystals on cooling; however, the solubility of naphthalene in methanol can be greatly reduced by addition of water. Heat the solution to the boiling point and add water dropwise from a 10-ml graduated cylinder, using a Pasteur pipette (or use a precalibrated pipette). After each addition of water the solution will turn cloudy for an instant. Swirl the contents of the flask and heat to redissolve any precipitated naphthalene. After the addition of 3.5 ml of water the solution will be almost saturated with naphthalene at the boiling point of the solvent. Remove the flask from the heat and place it on a cork ring or other insulating surface to cool, without being disturbed, to room temperature. Immerse the flask in an ice bath along with another flask containing methanol and water in the ratio of 15:3.5. This cold solvent will be used for washing the crystals. The cold crystallization mixture is collected on a small Büchner funnel (50-mm). In collecting the product by suction filtration use a spatula to dislodge crystals and ease them out of the flask. If crystals still remain in the flask, some filtrate can be poured back into the crystallization flask as a rinse for washing as often as desired, since it is saturated with solute. To free the crystals from contaminating mother liquor, break the suction and pour a few milliliters of the fresh cold solvent mixture into the Büchner funnel and immediately reapply suction. Repeat this process until the crystals and the filtrate are free of color. Press the crystals with a clean cork to eliminate excess solvent, pull air through the filter cake for a few minutes, and then put the large flat platelike crystals out on a filter paper to dry. The yield of pure white crystalline naphthalene should be about 1.6 g. The mother liquor contains about 0.25 g and about 0.15 g is retained in the charcoal and on the filter paper.

Experiment 5. Purification of an Unknown

Bearing in mind the seven-step crystallization procedure:

(1) choosing the solvent
(2) dissolving the solute
(3) decolorizing the solution
(4) filtering suspended solids
(5) crystallizing the solute
(6) collecting and washing the crystals
(7) drying the product

You are to purify 2.0 g of a crude unknown. Conduct tests for solubility and crystallizability in several organic solvents, solvent pairs, and water. Conserve your unknown by using very small quantities for solubility tests. If only a drop or two of solvent is used the solvent can be evaporated by heating the test tube on the steam bath and the residue used for another test. Submit as much pure product as possible with evidence of its purity.

Crystallization Problems and Their Solutions

Crystallization of Very Small Quantities

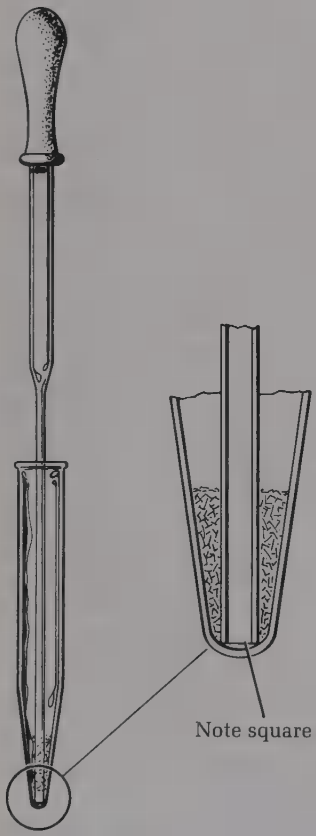


FIGURE 4.9 **Micro-scale crystallization in a centrifuge tube.**

Often only enough of a pure compound is desired to obtain an accurate melting point. The characterization of unknown organic compounds, for example, is often done by preparing small amounts of crystalline derivatives of the unknown (see Chapter 58). In this case, place the crude material in a conical-bottomed centrifuge tube or a very small test tube. Save a seed crystal. Add the appropriate solvent or solvent mixture, bring the sample into solution by heating, seed if necessary to induce crystallization, allow the tube to cool undisturbed to room temperature, and then cool it in an ice bath. Instead of trying to scrape this small sample out of the tube and onto a Hirsch funnel, remove the mother liquor using a Pasteur pipette equipped with a rubber bulb. Check to see that the tip of the pipette is perfectly square, then push the tip through the crystals toward the bottom of the tube, expel crystals that may have been forced into the tip, seat the tip of the pipette squarely on the bottom of the tube and withdraw the mother liquor slowly into the pipette with the rubber bulb (Fig. 4.9). The small clearance between the square tip of the pipette and the bottom of the tube will filter all but the finest of crystals. The remaining crystals are easily washed by adding a few drops of cold solvent, which is then withdrawn from the crystals in the same way as the mother liquor while immersing the tube in an ice bath. In this manner a very small sample can be recrystallized and washed several times in the course of a few minutes. To dry the sample, simply connect the tube to the aspirator. If the sample is known to melt above 100°, the last traces of solvent can be removed by heating the evacuated tube on the steam bath. A melting point capillary can be filled by inverting it and forcing it into the crystals.

Induction of Crystallization

Occasionally a sample will not crystallize from solution on cooling, even though the solution is saturated with the solute at elevated temperature. The easiest method for inducing crystallization is to add to the supersaturated solution a seed crystal that has been saved from the crude material (if it was crystalline before recrystallization was attempted). The probably apocryphal tale is told that the great sugar chemist Emil Fischer merely had to wave his beard over a recalcitrant solution and the appropriate seed crystals would drop out, causing crystallization to occur. In the absence of seed crystals, crystallization can often be induced by scratching the inside of the flask with a stirring rod at the air–liquid interface. One theory holds that part of the freshly scratched glass surface has angles and planes corresponding to the crystal structure, and crystals start growing on these spots. Often crystallization is very slow to begin and placing the sample in a refrigerator overnight will bring success. Other expedients are to change the solvent (usually to a poorer one) and to place the sample in an open container where slow evaporation and dust from the air may help induce crystallization.

Oils and ''Oiling Out''

Some saturated solutions on cooling deposit not crystals but small droplets referred to as oils. Should these droplets subsequently crystallize and be collected they will be found to be rather impure. Should the temperature of the saturated solution be above the melting point of the solute when it starts to come out of solution the solute will, of necessity, be deposited as an oil. Similarly, the melting point of the desired compound may be depressed to a point such that a low-melting eutectic mixture of the solute and the solvent comes out of solution. The simplest remedy for this latter problem is to lower the temperature at which the solution becomes saturated with the solute by simply adding more solvent. In extreme cases it may be necessary to lower this temperature well below room temperature, such that cooling of the solution with Dry Ice becomes necessary.

QUESTIONS

1. An unknown sample was crystallized from benzene. On cooling to 0° in an ice bath the amount of crystalline material seen in the flask was quite large, but much of it seemed to disappear when an attempt was made to collect it by suction filtration. Explain.
2. A sample of naphthalene, which should be pure white, was found to have a greyish color after the usual purification procedure. The melting point was correct and the melting point range small. Explain the grey color.
3. Why is gravity filtration and not suction filtration used to remove suspended impurities and charcoal from a hot solution?
4. How many milliliters of boiling water are required to dissolve 25 g of phthalic acid? If the solution were cooled to 14°, how many grams of phthalic acid would crystallize out?

5

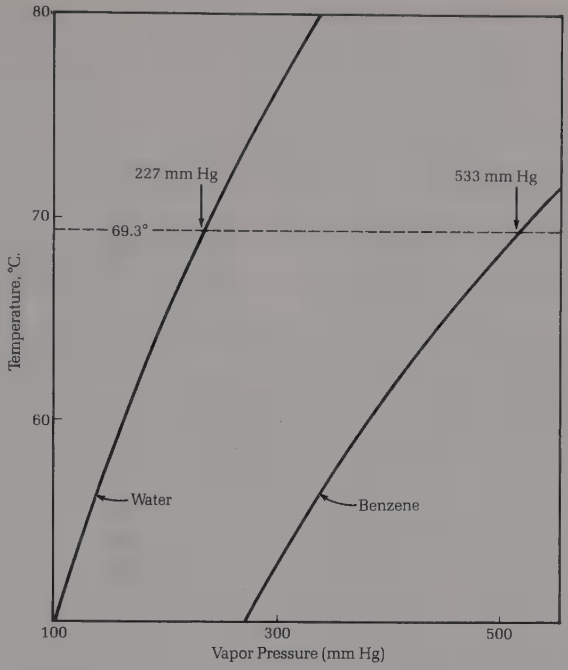
Steam Distillation

KEYWORDS

Immiscible liquids
Partial pressure
Vapor pressure
Boiling point
Dalton's law
Water trap
Isolation of citral

When a mixture of cyclohexane and toluene are distilled (Experiment 2.2 and 2.3) the boiling point of these two miscible liquids is *between* the boiling points of each of the pure components. By contrast, if a mixture of benzene and water (immiscible liquids) is distilled, the boiling point of the mixture will be found *below* the boiling point of each pure component. Since the two liquids are essentially insoluble in each other, the benzene molecules in a droplet of benzene are not diluted by water molecules from nearby water droplets, and hence the vapor pressure exerted by the benzene is the same as that of benzene alone at the existing temperature. The same is true of the water present. Because they are immiscible, the two liquids independently exert pressures against the common external pressure, and when the sum of the two partial pressures equals the external pressure boiling occurs. Benzene has a vapor pressure of 760 torr at 80.1°, and if it is mixed with water the combined vapor pressure must equal 760 torr at some temperature below 80.1°. This temperature, the boiling point of the mixture, can be calculated from known values of the vapor pressures of the separate liquids at that temperature. Vapor pressures found for water and benzene in the range 50–80° are plotted in Fig. 5.1. The dotted line cuts the two curves at points where the sum of the vapor pressures is 760 torr; hence this temperature is the boiling point of the mixture (69.3°).

FIGURE 5.1 Vapor pressure vs. temperature curves for water and benzene.



Practical use can sometimes be made of the fact that many water-insoluble liquids and solids behave as benzene does when mixed with water, volatilizing at temperatures below their boiling points. Thus, naphthalene, a solid, boils at 218° but distils with water at a temperature below 100°. Since naphthalene is not very volatile, considerable water is required to entrain it and the conventional way of conducting the distillation is to pass steam into a boiling flask containing naphthalene and water. The process is called *steam distillation.* With more volatile compounds, or with a small amount of material, the substance can be heated with water in a simple distillation flask and the steam generate *in situ.*

▼
Used for isolation of perfume and flavor oils

Some high-boiling substances decompose before the boiling point is reached and, if impure, cannot be purified by ordinary distillation. However, they can be freed from contaminating substances by steam distillation at a lower temperature at which they are stable. Steam distillation also offers the advantage of selectivity, since some water-insoluble substances are volatile with steam and others are not, and some volatilize so very slowly that sharp separation is possible. The technique is useful in processing natural oils and resins, which can be separated into steam-volatile and nonsteam-volatile fractions. It is useful for recovery of a nonsteam-volatile solid from its solution in a high-boiling solvent such as nitrobenzene, bp 210°; all traces of the solvent can be eliminated and the temperature can be kept low.

The boiling point remains constant during a steam distillation so long as adequate amounts of both water and the organic component are present to saturate the vapor space. Determination of the boiling point and correction for any deviation from normal atmospheric pressure permits calculation of the amount of water required for distillation of a given amount of organic substance. According to Dalton's law the molecular proportion of the two components in the distillate is equal to the ratio of their vapor pressures (p) in the boiling mixture; the more volatile component contributes the greater number of molecules to the vapor phase. Thus,

$$\frac{\text{Moles of water}}{\text{Moles of substance}} = \frac{p_{\text{water}}}{p_{\text{substance}}}$$

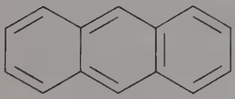
The vapor pressure of water (p_{water}) at the boiling temperature in question can be found by interpolation of the data of Table 5.1 and that of the organic substance is, of course, equal to $760 - p_{\text{water}}$. Hence, the weight of water required per gram of substance is given by the expression

$$\begin{matrix}\text{Wt. of water per} \\ \text{g of substance}\end{matrix} = \frac{18 \times p_{\text{water}}}{\text{MW of substance} \times (760 - p_{\text{water}})}$$

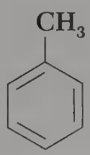
From the data given in Fig. 5.1 for benzene–water, and the molecular weight 78.11 for benzene, the water required for steam distillation of 1 g of benzene is only $227 \times {}^{18}/_{533} \times {}^{1}/_{78} = 0.10$ g. Nitrobenzene (bp 210°, MW 123.11) steam distils at 99° and requires 4.0 g of water per gram. The low molecular weight of water makes water a favorable liquid for two-phase distillation of organic compounds.

EXPERIMENTS

Apparatus In the assembly shown in Fig. 5.2, steam is passed into a 250-ml, round-bottomed flask through a section of 6-mm glass tubing fitted into a stillhead with a piece of 5 mm rubber tubing connected to a trap, which in turn is connected to the steam line. The trap serves two purposes: it allows water, which is in the steam line, to be removed before it reaches the round-bottomed flask, and adjustment of the clamp on the hose at the bottom of the trap allows precise control of the steam flow. The stopper in the trap should be wired on, as shown, as a precaution. A bent adapter attached to a *long* condenser delivers the condensate into a 250-ml Erlenmeyer flask.



Anthracene
mp 216°



Toluene
bp 111°,
den 0.866

1. Recovery of a Dissolved Substance

Measure 50 ml of a 0.2% solution of anthracene in toluene[1] into the 250-ml round-bottomed flask and add 100 ml of water. For an initial distillation to determine the boiling point and composition of the toluene–water azeotrope, fit the stillhead with a thermometer instead of the steam-inlet tube. Heat the mixture with a microburner, distil about 50 ml of the azeotrope and record a

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Note for the instructor

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[1] Pure, fluorescent anthracene should be used. Preparation: Chapter 39.

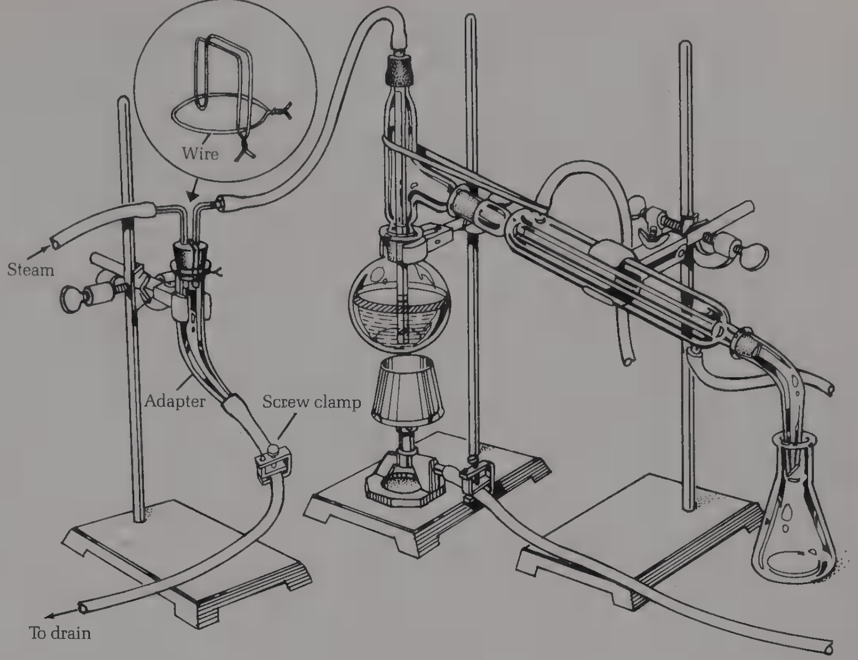


FIGURE 5.2 Steam distillation apparatus.

value for the boiling point. After removing the flame, pour the distillate into a graduate and measure the volumes of toluene and water. Calculate the weight of water per gram of toluene and compare the result with the theoretical value calculated from the vapor pressure of water at the observed boiling point (see Table 5.1).

Replace the thermometer with the steam-inlet tube. To start the steam distillation, heat the flask containing the mixture with a small flame to prevent water from condensing in the flask to the point where water and product splash over into the receiver. Then turn on the steam valve, making sure the screw clamp on the bottom of the trap is open. Slowly close the clamp, and allow

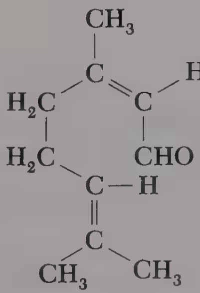
Table 5.1 Vapor Pressure of Water in mm of Mercury

$t°$	p	$t°$	p	$t°$	p	$t°$	p
60	149.3	70	233.7	80	355.1	90	525.8
61	156.4	71	243.9	81	369.7	91	546.0
62	163.8	72	254.6	82	384.9	92	567.0
63	171.4	73	265.7	83	400.6	93	588.6
64	179.3	74	277.2	84	416.8	94	610.9
65	187.5	75	289.1	85	433.6	95	633.9
66	196.1	76	301.4	86	450.9	96	657.6
67	205.0	77	314.1	87	468.7	97	682.1
68	214.2	78	327.3	88	487.1	98	707.3
69	223.7	79	341.0	89	506.1	99	733.2

steam to pass into the flask. Unlike ordinary distillations, steam distillations are usually run as fast as possible, with proper care to avoid having material splash into the receiver and to avoid having steam escape uncondensed.

Continue distillation by passing in steam until the distillate is clear and then until fluorescence appearing in the stillhead indicates that a trace of anthracene is beginning to distil. Stop the steam distillation by opening the clamp at the bottom of the trap and then turning off the steam valve. Grasp the round-bottomed flask with a towel when disconnecting it and, using the clamp to support it, cool it under the tap. The bulk of the anthracene can be dislodged from the flask walls and collected on a small suction filter. To recover any remaining anthracene, add a little acetone to the flask, warm on the steam bath to dissolve the material, add water to precipitate it, and collect the precipitate on the same suction filter. About 80% of the hydrocarbon in the original toluene solution should be recoverable. When dry, crystallize the material from about 1 ml of benzene and observe that the crystals are more intensely fluorescent than the solution or the amorphous solid. The characteristic fluorescence is quenched by mere traces of impurities.

2. Isolation of a Natural Product



Citral
den 0.89

Citral, a fragrant terpene aldehyde made up of two isoprene units, is the main component of the steam-volatile fraction of lemon grass oil and is used in a commercial synthesis of vitamin A. Lemon grass tea is a popular drink in Mexico.

Using a graduate, measure out 10 ml of lemon grass oil (**not** lemon oil) into the 250-ml boiling flask. Rinse the remaining contents of the graduate into the flask with a little ether. Add 100 ml of water, make connections as in Fig. 5.2, heat the flask with a small flame, and pass in steam. Distil as rapidly as the cooling facilities allow and continue until droplets of oil no longer appear at the tip of the condenser (about 250 ml of distillate).

Pour 50 ml of ether into a 125-ml separatory funnel, cool the distillate if necessary, and pour a part of it into the funnel. Shake, let the layers separate, discard the lower layer, add another portion of distillate and repeat. When the last portion of distillate has been added, rinse the flask with a little ether to recover adhering citral. Use the techniques described in Chapter 6 for drying, filtering, and evaporating the ether. Take the tare of a 1-g tincture bottle, transfer the citral to it with a capillary dropping tube, and determine the weight and the yield from the lemon grass oil. Label the bottle and store (in the dark) for later testing for the presence of functional groups.

QUESTIONS

1. A mixture of ethyl iodide (C_2H_5I, bp 72.3°) and water boils at 63.7°. What weight of ethyl iodide would be carried over by 1 g of steam during steam distillation?
2. Iodobenzene (C_6H_5I, bp 188°) steam distils at a temperature of 98.2°. How

many molecules of water are required to carry over one molecule of iodobenzene? How many grams per gram of iodobenzene?

3. The condensate from a steam distillation contains 8 g of an unknown compound and 18 g of water. The mixture steam distilled at 98°. What is the molecular weight of the unknown?

6

Extraction

KEYWORDS

- Use of ether
- Equilibration
- Drying ether with saturated salt (NaCl) solution
- Distribution ratio
- Soxhlet extractor
- Separatory funnel
- Separation of acidic, basic, and neutral substances
- Use of drying agents

Extraction is one of man's oldest chemical operations. The preparation of a cup of coffee or tea involves the extraction of flavor and odor components from dried vegetable matter with hot water. Similarly perfume ingredients and many drugs are isolated by extraction into organic solvents. Subsequent evaporation of the solvent leaves the desired components. Extraction is used to isolate the products of many organic reactions. For example, the ketone cyclohexanone, a liquid of bp 157°, is obtained by oxidation of the alcohol cyclohexanol with dichromate in aqueous acetic acid. The product, which is somewhat soluble in water, is isolated from a mixture of water and acetic acid by extraction into diethyl ether, $CH_3CH_2OCH_2CH_3$, a solvent that is immiscible with water and will dissolve cyclohexanone much more readily than water. The extraction of cyclohexanone is accomplished by shaking the aqueous solution with an equal volume of ether in a separatory funnel. A liquid–liquid extraction of this type involves the distribution of the solute, cyclohexanone, between the two immiscible solvents, water and ether.

The solvent used for extraction should have many properties of a satisfactory recrystallization solvent. It should readily dissolve the substance to be extracted, it should have a low boiling point so that it can readily be removed, it should not react with the solute or the other solvent, it should not be flammable or toxic, and it should be relatively inexpensive. In addition it should not be miscible with water (the usual second phase). No solvent meets all these criteria, but several come close. Diethyl ether, usually referred to simply as ether, is probably the most common solvent used for extraction, but it is extremely flammable.

Ether has high solvent power for hydrocarbons and for oxygen-containing compounds and is so highly volatile (bp 34.6°) that it is easily removed from an extract at a temperature so low that even highly sensitive compounds are not likely to decompose.

Ether is useful for isolation of natural products that occur in animal and plant tissues having high water content. Although often preferred for research work because of these properties, ether is avoided in industrial processes because of the fire hazard, high solubility in water, losses in solvent recovery incident to volatility, and the oxidation on long exposure to air to a peroxide, which in a dry state may explode. Alternative water-immiscible solvents sometimes preferred, even though they do not match all the favorable properties of ether, are: petroleum ether, ligroin, benzene, carbon tetrachloride, chloroform, methylene chloride, ethylene dichloride (1,2-dichloroethane), 1-butanol. The chlorinated hydrocarbon solvents are heavier than water rather than lighter and hence, after equilibration of the aqueous and nonaqueous phases, the heavier lower layer is drawn off into a second separatory funnel for washing and the upper aqueous layer is extracted further and discarded. Chlorinated hydrocarbon solvents have the advantage of freedom from fire hazard, but their higher cost militates against their general use.

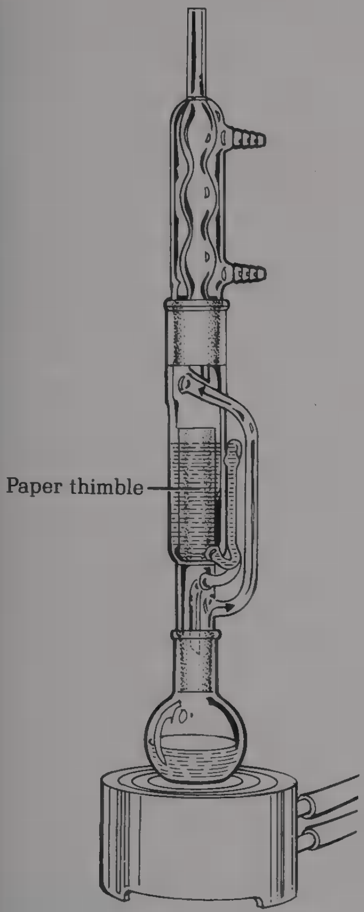


FIGURE 6.1 Soxhlet extractor.

For exhaustive extraction of solid mixtures, and even of dried leaves or seeds, the solid is packed into a filter paper thimble placed in a Soxhlet extractor (Fig. 6.1). Solvent vapor rises in the large diameter tube on the right, and condensed solvent drops onto the solid contained in a filter paper thimble, leaches out soluble material and, after initiating an automatic siphon, carries it to the boiling flask where nonvolatile extracted material accumulates. The same solvent is repeatedly used, and even substances of very slight solubility can be extracted by prolonged operation.

Distribution Coefficient

There is a quantitative relationship governing the distribution of a solute between two solvents, expressed by the distribution equation. This equation states that the ratio of the concentration of the solute, cyclohexanone (C), in one solvent to the concentration in the other solvent is a constant, k. To a good approximation the *concentration* of the solute in each solvent can be correlated with the *solubility* of the solute in the pure solvent, a figure that can be found readily in tables of solubility in reference books.

$$k = \frac{\text{concentration of C in ether}}{\text{concentration of C in water}} \cong \frac{\text{solubility of C in ether (g/100 ml)}}{\text{solubility of C in water (g/100 ml)}}$$

Consider a compound, A, which dissolves in ether to the extent of 12 g/100 ml and dissolves in water to the extent of 6 g/100 ml.

$$k = \frac{12\text{ g}/100\text{ ml ether}}{6\text{ g}/100\text{ ml water}} = 2$$

If a solution of 6 g of A in 100 ml of water is shaken with 100 ml of ether then

$$k = \frac{(x\text{ grams of A}/100\text{ ml ether})}{(6 - x\text{ grams of A}/100\text{ ml water})} = 2$$

from which

$$\begin{aligned} x &= 4.0\text{ g of A in the ether layer} \\ 6 - x &= 2.0\text{ g left in the water layer} \end{aligned}$$

It is, however, more efficient to extract the 100 ml of aqueous solution twice with 50-ml portions of ether rather than once with a 100-ml portion.

$$k = \frac{(x\text{ g of A}/50\text{ ml})}{(6 - x\text{ g of A}/100\text{ ml})} = 2$$

from which

$$\begin{aligned} x &= 3.0\text{ g in ether layer} \\ 6 - x &= 3.0\text{ g in water layer} \end{aligned}$$

If this 3.0 g/100 ml of water is extracted once more with 50 ml of ether we can calculate that 1.5 g will be in the ether layer, leaving 1.5 g in the water layer. So two extractions with 50-ml portions of ether will extract 3.0 g + 1.5 g = 4.5 g of A, while one extraction with a 100-ml portion of ether removes only 4.0 g of A. Three extractions with $33\frac{1}{3}$-ml portions of ether would extract 4.7 g. Obviously there is a point at which the increased amount of A extracted does not repay the effort of multiple extractions, but keep in mind that several small-scale extractions are more effective than one large-scale extraction.

The extraction of an organic substance from an aqueous solution is aided by saturation of the aqueous layer with inorganic salts like sodium chloride or potassium carbonate, which greatly decreases the solubility of the organic substance in the aqueous layer. This phenomenon is called "salting out" and is utilized in several experiments in this text (for example, in the isolation of cyclohexanone).

At room temperature water dissolves 7.5% of ether by weight and ether dissolves 1.5% of water. Shaking together 100 ml each of ether and water gives an upper ether layer of 90 ml and a lower aqueous layer of 105 ml. However, ether is virtually insoluble in water saturated with sodium chloride (36.7 g/100 ml). If ether that contains dissolved water is shaken with a saturated aqueous solution of sodium chloride, water will be transferred from the ether to the aqueous layer. So, strange as it may seem, ethereal extracts routinely are dried by shaking them with saturated sodium chloride solution. The final traces of water are removed from the ether layer with a chemical drying agent such as anhydrous sodium sulfate. In this way evaporation of the ether will leave the desired organic compound, uncontaminated with residual water.

Separation of Acids and Bases by Extraction

An ether solution of a carboxylic acid, a phenol, a neutral compound, and an organic base such as an amine can be separated by extraction. The solubility properties of these acids and bases and their salts allow them to be separated from each other and from neutral compounds. Carboxylic acids ($pK_a \cong 5$) are stronger acids than phenols ($pK_a \cong 10$). As a consequence carboxylic acids can be converted to their salts by interaction with a weak base such as bicarbonate ion

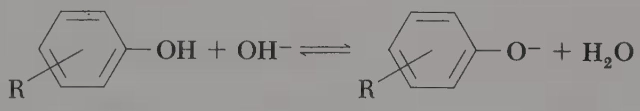
$$\underset{\text{carboxyl group}}{RCOOH} + HCO_3^- \rightleftharpoons \underset{\text{carboxylate anion}}{RCOO^-} + H_2O + CO_2$$

but bicarbonate will have no effect on phenols. This follows from the acidity constants of benzoic acid, phenol, and carbonic acid (Table 6.1).

Table 6.1 Acidity Constants

		K_a
Benzoic acid	$C_6H_5COOH + H_2O \rightleftharpoons C_6H_5COO^- + H_3O^+$	6×10^{-5}
Phenol	$C_6H_5OH + H_2O \rightleftharpoons C_6H_5O^- + H_3O^+$	1×10^{-10}
Carbonic acid	$H_2CO_3 + H_2O \rightleftharpoons HCO_3^- + H_3O^+$	3×10^{-7}

To convert the weakly acidic phenols to their corresponding salts a strong base (hydroxide ion) must be employed.



a phenol a phenolate anion

Amines, being bases, are converted to salts with acid.

$$\underset{\text{amine}}{R_3N} + H_3O^+ \rightleftharpoons \underset{\text{ammonium salt}}{R_3NH^+} + H_2O$$

In each of these three reactions a covalent organic compound, soluble in organic solvents like ether, is converted to an ionic compound, soluble in water.

Consider the separation of an ether solution of benzoic acid, *p*-chlorophenol, aniline, and naphthalene. This mixture could be separated by adding an aqueous solution of sodium bicarbonate to the ether solution, which would convert benzoic acid to water-soluble benzoate.

$$C_6H_5COOH + NaHCO_3 \longrightarrow C_6H_5COO^-Na^+ + CO_2 + H_2O$$

When the layers are separated the aqueous layer could be acidified to regenerate benzoic acid which, being crystalline, could be removed by filtration.

$$C_6H_5COO^-Na^+ + HCl \longrightarrow C_6H_5COOH + Na^+Cl^-$$

The addition of an excess of aqueous sodium hydroxide solution to the ether solution of the three remaining compounds would convert the *p*-chlorophenol to a water-soluble anion.

$$p\text{-}ClC_6H_4OH + NaOH \longrightarrow p\text{-}ClC_6H_4O^-Na^+ + H_2O$$

After separation of the layers, acidification of the aqueous layer would regenerate *p*-chlorophenol which could be filtered from the solution.

$$p\text{-}ClC_6H_5O^-Na^+ + HCl \longrightarrow p\text{-}ClC_6H_5OH + Na^+Cl^-$$

If the ether solution were now to be shaken with 5% HCl the aniline would be converted to the water soluble hydrochloride.

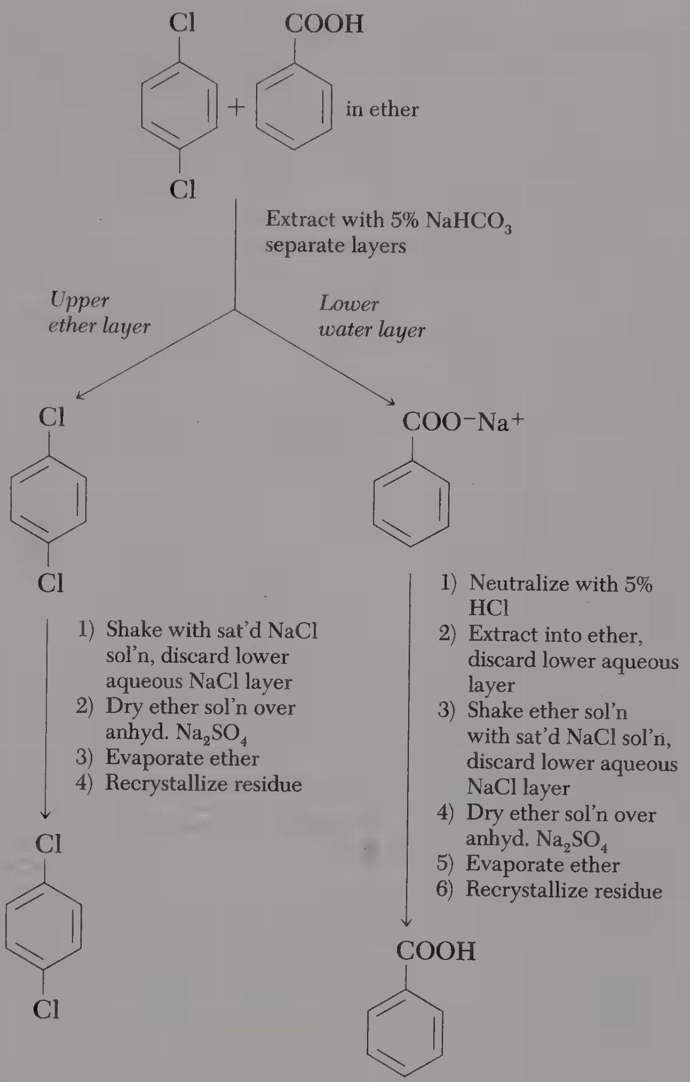
$$C_6H_5NH_2 + HCl \longrightarrow C_6H_5NH_3{}^+Cl^-$$

Separation of the aqueous layer from the ether layer followed by neutralization of the aqueous layer with sodium hydroxide solution would regenerate aniline.

$$C_6H_5NH_3{}^+Cl^- + NaOH \longrightarrow C_6H_5NH_2 + Na^+Cl^- + H_2O$$

The aniline, being a liquid and slightly soluble in water, is best isolated from the neutral aqueous layer by extraction into ether. After separation of the ether from the aqueous layer, the ether layer is dried (sat'd NaCl, anhyd. Na_2SO_4) and evaporated. The original ether solution now contains naphthalene plus water from the various extractions with acids and bases. The water is eliminated by shaking the ether solution with saturated aqueous sodium chloride solution, separating the layers, and drying the ether layer over anhydrous sodium sulfate. Evaporation of the ether should leave crystalline naphthalene.

A simpler separation problem would be to separate benzoic acid from the neutral compound *p*-dichlorobenzene. This separation involves the principles described above. A convenient method for describing the isolation process is in the form of a flow sheet:



Extract with 5% $NaHCO_3$ separate layers

1) Shake with sat'd NaCl sol'n, discard lower aqueous NaCl layer
2) Dry ether sol'n over anhyd. Na_2SO_4
3) Evaporate ether
4) Recrystallize residue

1) Neutralize with 5% HCl
2) Extract into ether, discard lower aqueous layer
3) Shake ether sol'n with sat'd NaCl sol'n, discard lower aqueous NaCl layer
4) Dry ether sol'n over anhyd. Na_2SO_4
5) Evaporate ether
6) Recrystallize residue

FIGURE 6.2 Correct positions for holding a separatory funnel when shaking.

▼
Extinguish all flames when working with ether!

Apparatus and Operations

As indicated above with the example of cyclohexanone prepared by the oxidation of cyclohexanol, a frequently used method of working up a reaction mixture is to dilute the mixture with water and extract with ether in a separatory funnel (Fig. 6.2). When the stoppered funnel is shaken to distribute the components between the immiscible solvents ether and water, pressure always develops through volatilization of ether from the heat of the hands, and liberation of a gas (CO_2) will increase the pressure. Consequently, the funnel is so grasped that the stopper is held in place by one hand and the stopcock by the other, as illustrated. After a brief shake or two the funnel is held in the inverted position shown and the stopcock opened cautiously (with the funnel stem pointed away from nearby people) to release pressure. The mixture can then be shaken more vigorously and pressure released as necessary. When equilibration is judged to be complete, the slight, constant terminal pressure due to ether is released, the stopper is rinsed with a few drops of ether delivered by a capillary dropping tube, and the layers are allowed to separate. The organic reaction product is distributed wholly or largely into the upper ether layer, whereas inorganic salts, acids, and bases pass into the water layer, which can be drawn off and discarded. If the reaction was conducted in alcohol or acetone solution the bulk of the solvent is removed in the water layer and the rest can be eliminated in two or three washings with 1–2 volumes of water conducted with the techniques used in the first equilibration. The separatory funnel should be supported in a ring stand as shown in Figure 6.3.

▼
Acids

Acetic acid is also distributed largely into the aqueous phase, but if the reaction product is a neutral substance the residual acetic acid in the ether can be removed by one washing with excess 5% sodium bicarbonate solution. If the reaction product is a higher MW acid, for example benzoic acid (C_6H_5COOH), it will stay in the ether layer, while acetic acid is being removed by repeated washing with water; the benzoic acid can then be separated from neutral by-products by extraction with sodium bicarbonate or sodium hydroxide solution and acidification of the extract. Acids of high molecular weight are extracted only slowly by sodium bicarbonate and sodium carbonate is used in its place; however, carbonate is more prone than bicarbonate to produce emulsions. Sometimes an emulsion in the lower layer can be settled by twirling the stem of the funnel. An emulsion in the upper layer can be broken by grasping the funnel by the neck and swirling it. Since the tendency to emulsify increases with removal of electrolytes and solvents, a little sodium chloride or hydrochloric acid solution is added with each portion of wash water. If the layers are largely clear but an emulsion persists at the interface, the clear part of the water layer can be drawn off and the emulsion run into a second funnel and shaken with fresh ether. Very persistent emulsions can sometimes be broken by vacuum filtration using a Büchner funnel.

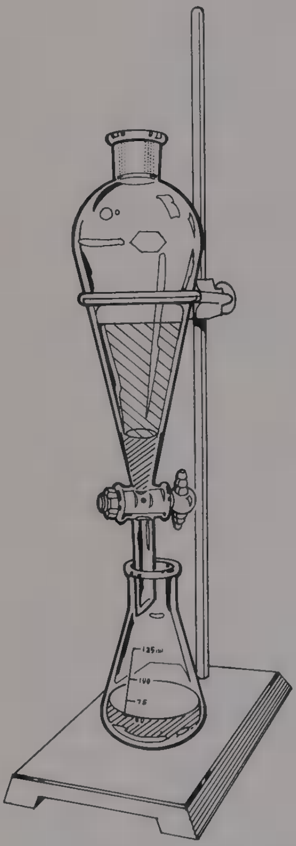


FIGURE 6.3 Separatory funnel with Teflon stopcock.

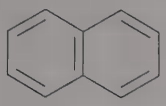
Before adding a liquid to the separatory funnel, check the stopcock. If it is glass see that it is properly greased, bearing in mind that too much grease will clog the hole in the stopcock and also contaminate the extract. If the stopcock is Teflon see that it is adjusted for a tight fit in the bore. Store the separatory funnel with the Teflon stopcock loosened to prevent sticking. Since Teflon has a much larger temperature coefficient of expansion than glass, a stuck stopcock can be loosened by cooling the stopcock in ice or Dry Ice. Do not store liquids in the separatory funnel; they often leak or cause the stopper or stopcock to freeze. In order to have sufficient room for mixing the layers, fill the separatory funnel no more than three-fourths full. Withdraw the lower layer from the separatory funnel through the stopcock and pour the upper layer out through the neck.

All too often the inexperienced chemist discards the wrong layer when using a separatory funnel. Through incomplete neutralization a desired component may still remain in the aqueous layer or the densities of the layers may change. Cautious workers save all layers until the desired product has been isolated. The organic layer is not always the top layer. If in doubt, test the layers by adding a few drops of each to water in a test tube.

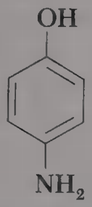
EXPERIMENTS

$pH = -\log [H^+]$
pK_a = *acidity constant*
pK_b = *basicity constant*

1. Separation of a Neutral and Basic Substance



Naphthalene
mp 80°, neutral



***p*-Aminophenol**
mp 186°, pK_a 8.2, pK_b 8.8
(Hydrochloride, mp 302°, dec)

A mixture of naphthalene, a neutral hydrocarbon, and *p*-aminophenol, an amphoteric compound, is to be separated by extraction from ether with dilute hydrochloric acid. *p*-Aminophenol darkens on storage due to air oxidation, but this colored impurity is removed in the extraction process. Note the detailed directions for extraction carefully. In the next experiment you are to work out your own extraction procedure.

To a 125-ml separatory funnel add 30 ml of water, 30 ml of ether, 1 ml of conc'd hydrochloric acid, and 2 g of a mixture of equal parts of naphthalene and practical grade *p*-aminophenol (dark brown). Stopper the funnel and shake thoroughly to distribute the hydrocarbon into the ether phase and *p*-aminophenol into the aqueous phase as the hydrochloride. Each time you remove the stopper, rinse it and the mouth of the funnel with ether. When you think equilibrium has been reached, add a small spatulaful of stannous chloride crystals (to reduce impurities in the practical grade *p*-aminophenol) and shake vigorously for a few minutes. If the brown color is not largely discharged from the aqueous layer, add another small portion of stannous chloride (avoid excess) and shake again. Draw off the nearly colorless aqueous solution of the hydrochloride into a 125-ml Erlenmeyer. Extract again with 10 ml of water and 1 ml of conc'd hydrochloric acid. To recover more of the hydrochloride, rinse the walls of the funnel by directing a stream of water from a wash bottle around the mouth of the funnel (a few milliliters is enough). Drain off the water layer into the Erlenmeyer and put the flask on the steam bath to drive off the dissolved ether.

▼
Technique of drying

To dry the ether layer add about 10 ml of a saturated aqueous sodium chloride solution to the separatory funnel, shake the funnel vigorously, allow the layers to separate, and then draw off the lower aqueous layer. This procedure should always be employed to remove water that dissolves in ether during an extraction.

In this particular experiment the ethereal layer is usually dark and contains suspended scum, but it can be clarified easily. To do so, add about 3 small spatulasful of decolorizing charcoal and shake the funnel vigorously for a minute or two. Then pour the ethereal solution out of the neck of the funnel into a 50-ml Erlenmeyer flask. Rinse the separatory funnel with ether. Add about 5 g of anhydrous sodium sulfate to the flask. (A 10 × 75-mm test tube holds 5 g of the drying agent.) Swirl the flask from time to time. After 5 min remove the drying agent by filtration through a filter paper held in a stemless funnel placed in the neck of a tared (previously weighed) 50-ml Erlenmeyer flask. Add a boiling chip and evaporate the extract on the steam bath under an aspirator tube. Ether (bp 34.6°) is volatile and highly flammable, but a *small* quantity can be evaporated on the steam bath with safety by removal of the flammable vapor with the aspirator tube (Fig. 6.4). Since you have the tare weight of the flask, you can then evacuate it to constant weight (see Fig. 4.8) after all the solvent seems to have evaporated and been carried away through the aspirator tube. It is convenient to scratch an identifying mark on the outside of flasks frequently used and to keep a list of their tare weights in your notebook.

▼
Extinguish all nearby flames when working with ether!

After removal of the last traces of ether the molten naphthalene should solidify readily on cooling; yield 0.8–0.9 g. Dissolve the crude product in a small volume of methanol with heating (steam bath) and, if the solution is colored, add excess solvent, clarify with decolorizing charcoal, and either concentrate the solution to the point of saturation or add water by drops to produce a saturated solution. Naphthalene crystallizes in large white plates, which should be washed with chilled methanol.

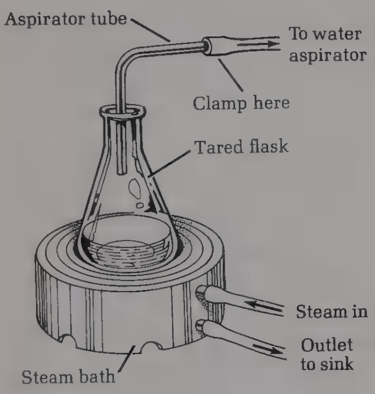


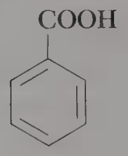
FIGURE 6.4 Aspirator tube in use.

If the hot aqueous solution containing *p*-aminophenol hydrochloride and stannous chloride is not clear and colorless, add one small spatulaful of decolorizing charcoal and filter the hot solution into an Erlenmeyer having a side arm; rinse and wash as usual. Then add 10 ml of conc'd hydrochloric acid, close the flask with a rubber stopper, clamp it within the rings of a steam bath, and wrap a towel around it for efficient heating. Connect the side arm to an efficient suction pump and turn it on full force. The solution is to be concentrated until crystals of *p*-aminophenol hydrochloride begin to separate (10–15 min) and then cooled, or else taken to dryness. In either case, rub the crystals with acetone and use this solvent for transferring the solid to a suction filter; yield 0.7–0.8 g. The quality of this salt can be judged better from the color than from the mp. Dissolve a small sample in water and add sodium bicarbonate (solid or aqueous solution) and compare the color of the free aminophenol with that of the starting material.

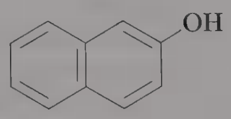
2. Separation of Acidic and Neutral Substances

▼
pK_a = acidity constant

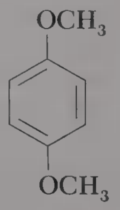
A mixture of equal parts of the following substances is to be separated by extraction[3]:



Benzoic acid
mp 123°, pK_a 4.17



2-Naphthol
mp 123°, pK_a 9.5



Hydroquinone dimethyl ether
mp 57°

▼
Note for the Instructor

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[3] Heat a mixture of the components on the steam bath and stir until a homogeneous melt results, then cool with stirring and break up the warm solid with a spatula.

As can be inferred from the pK_a values, benzoic acid reacts with sodium bicarbonate to form a water-soluble sodium salt, whereas 2-naphthol does not; 2-naphthol, in turn, is soluble in aqueous sodium hydroxide solution and hydroquinone dimethyl ether is insoluble in aqueous alkali. The solubility of benzoic acid in 100 g of cold water is 0.2 g; of 2-naphthol, 0.1 g.

You are to plan a procedure for separating 3.0 g of the mixture into the components and to have the plan checked by the instructor before proceeding. A flow sheet is a convenient way to present the plan. In each case select a solvent for crystallization on the basis of solubility tests.

QUESTIONS

1. Suppose a reaction mixture, when diluted with water, afforded 300 ml of an aqueous solution of 30 g of the reaction product malononitrile, $CH_2(CN)_2$, which is to be isolated by extraction with ether. The solubility of malononitrile in ether at room temperature is 20.0 g per 100 ml and that in water is 13.3 g per 100 ml. What weight of malononitrile would be recovered by extraction with (a) three 100-ml portions of ether; (b) one 300-ml portion of ether? *Suggestion:* For each extraction let x equal the weight extracted into the ether layer. In case (a) the concentration in the ether layer is $x/100$ and that in the water layer $(30 - x)/300$; the ratio of these quantities is equal to $k = 20/13.3$.
2. Draw a flow sheet illustrating the separation of benzoic acid, *p*-chlorophenol, aniline, and naphthalene.
3. Why is it necessary to remove the stopper from a separatory funnel when liquid is being drained from it through the stopcock?
4. The pK_a of *p*-nitrophenol is 7.15. Would you expect this to dissolve in sodium bicarbonate solution? The pK_a of 3,6-dinitrophenol is 5.15. Will it dissolve in bicarbonate solution?
5. The distribution coefficient, $k =$ (conc. in ligroin/conc. in water), between ligroin and water for solute A is 7.5 What weight of A would be removed from a solution of 10 g of A in 100 ml of water by a single extraction with 100 ml of ligroin? What weight of A would be removed by four successive extractions with 25-ml portions of ligroin? How much ligroin would be required to remove 98.5% of A in a single extraction?

7

Infrared Spectroscopy

KEYWORDS

Wavenumber
Reciprocal cm, microns
Band spectra
Transmittance, absorbance
Double beam spectrometer
Molecular vibrations
Stretching, bending
Group frequencies
Fingerprint region
Correlation tables
Neat solutions, mulls, KBr discs
Demountable and solution cells
Nujol, paraffin oil
Polystyrene calibration film
Carbonyl frequencies

The presence and also the environment of functional groups in organic molecules can be identified by infrared spectroscopy. Like nuclear magnetic resonance and ultraviolet spectroscopy, infrared spectroscopy is nondestructive. Moreover, the small quantity of sample needed, the speed with which a spectrum can be obtained, the relatively small cost of the spectrometer, and the wide applicability of the method combine to make infrared spectroscopy one of the most useful tools available to the organic chemist.

An infrared spectrum is a record of the absorption of electromagnetic radiation by the sample in the region 4000 to 667 wave-numbers (reciprocal cm, cm^{-1}), which is equal to the wavelength region of 2.5 to 15 microns (μ) ($1\mu = 1 \times 10^{-4}$ cm).

$$\text{cm}^{-1} = \frac{10{,}000}{\mu}$$

▼ $A = \log\left(\frac{1}{T}\right)$

Spectra are often presented with an abscissa linear in cm^{-1} and an ordinate linear in percentage transmittance with 100% at the top. The ordinate is also often presented in absorbance, where absorbance, A, is the logarithm of the reciprocal of the transmittance (T). Solution spectra consist of very closely

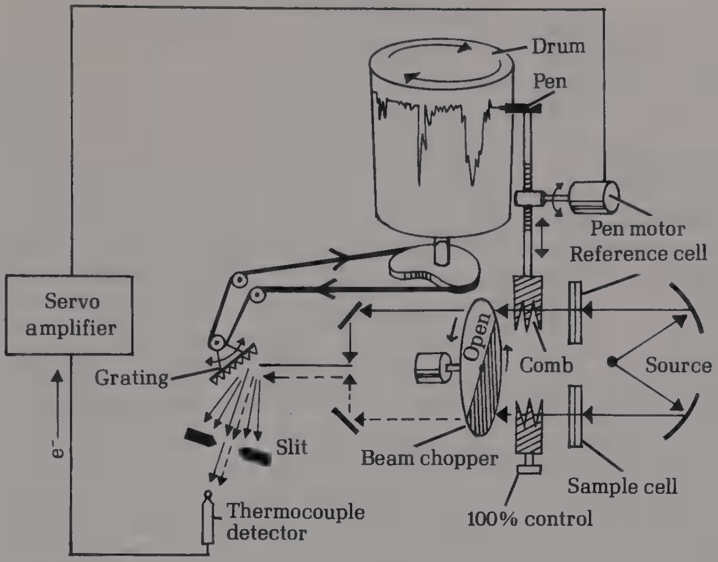

FIGURE 7.1 Schematic diagram of a double-beam, optical null, infrared spectrometer.

spaced unresolved lines resulting in a *band* spectrum. By comparison with nmr and ultraviolet spectra, infrared absorption bands are upside down.

▼
The infrared spectrometer

Figure 7.1 is a schematic representation of a typical double beam, optical null, infrared spectrometer. An electrically heated metal rod serves as the radiation source; the radiation passes through both the sample cell and reference cell, through combs and a beam chopper to the dispersion grating. In some instruments the radiation is dispersed by a prism made of sodium chloride, which is transparent to infrared radiation. Of the electromagnetic radiation frequencies spread out by the grating (or prism) only a small range of frequencies is allowed to pass through the slit to the detector, which is a thermocouple with a very rapid response time.

The spectrometer works on the optical null principle. The detector senses infrared light coming alternately through the substances in the sample and reference cells. If the amount of light is the same from both beams the detector produces a direct current and nothing happens, but if less light comes through the sample beam than the reference beam (because of absorption of radiation by the sample molecules), then the detector senses an alternating current which alternates at the rate the chopper is turning. This current is amplified in the servo amplifier which activates the pen motor. The pen motor moves the pen down the paper drawing an absorption band and at the same time drives a comb into the reference beam just far enough so that the detector will again sense a null, i.e., no alternating current. The motion of the drum holding the paper is linked to the grating so that as the drum moves the grating moves with it to scan the entire range of frequencies.

Absorption of infrared radiation results in changes in the amplitude of vibration of various parts of an organic molecule. Each band in the spectrum results from the absorption of radiation of a specific frequency which excites a particular bond or group of bonds in the molecule causing the atoms to vibrate with respect to each other. Most of the important peaks in infrared spectra result from changes in the frequencies of bond stretching or bending.

▼ *Characteristic group frequencies*

Although it is possible to calculate the exact frequencies of vibrations of all the atoms in extremely simple molecules, the principal usefulness of infrared spectroscopy lies in empirical correlations of thousands of spectra of complex molecules. Certain group frequencies are well characterized and are known to appear in very narrow frequency ranges in the spectrum. The C—H bond stretching vibrations, for example, occur in the 3600 to 2700 cm^{-1} range, with different types of C—H bonds at specific frequencies within this range. For instance, saturated tertiary carbon-proton bonds absorb at 2890 cm^{-1}, isolated carbon-vinyl proton bonds at 3020 cm^{-1}, aromatic carbon-proton bonds at 3070 cm^{-1}, and acetylenic bonds at 3300 cm^{-1}.

Absorption of infrared radiation requires that a bond undergo a change in dipole moment upon excitation. The C=C stretching mode of ethylene shows up as an exceedingly weak peak near 1650 cm^{-1} because stretching the C=C bond does not change the dipole moment of the molecule. Some of the most intense (and most useful) bands in an infrared spectrum come from the carbonyl group. This is because the carbon and the oxygen atoms are of different electronegativity, bound by a double bond, and the change in dipole moment is large when the bond stretches; consequently, the infrared absorption is intense. In general, the intensities of infrared bands are proportional to the change in dipole moment causing the absorption band and not to the number of atoms causing the absorption, as in nmr.

The amount of energy (and hence the frequency of the radiation) required to excite the stretching and bending modes depends upon the strength of the bond and the masses of the atoms attached to the bond; the amount of energy required decreases with decreasing bond order ($C\equiv C > C=C > C-C$) and decreases with increasing mass (e.g., $C-F > C-Cl > C-Br$). An infrared spectrum is conveniently divided into two regions; the region from 4000 to ca. 1550 cm^{-1}, which includes vibrational frequencies characteristic of particular functional groups (e.g., O—H, N—H, C—H at 3600 to 2700 cm^{-1}; O—H · · · · X hydrogen bonds at 3300 to 2500 cm^{-1}; C≡C, C≡N at 2400 to 2000 cm^{-1}; and C=C, C=O at 1850 to 1550 cm^{-1}). The region from 1550 to 650 cm^{-1} is characteristic of the complex vibrations for the whole molecule. Bands in this region are difficult to interpret, but nevertheless are highly characteristic of the molecule; hence this region is designated the "fingerprint region." If two samples give identical infrared spectra, including the entire fingerprint region, they can be presumed to be identical. Infrared spectroscopy is more useful than melting points, for example, as proof that two substances are identical.

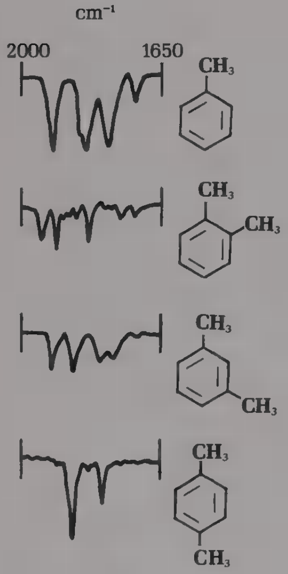

FIGURE 7.2 Band patterns of toluene and *o*-, *m*-, and *p*-xylene.

Extensive correlation tables and discussions of characteristic group frequencies can be found in specialized references.[1] As one example, consider the band patterns of toluene, and of *o*-, *m*-, and *p*-xylene, which appear in the

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[1] See list of references at end of chapter.

frequency range 2000 to 1650 cm^{-1} (Fig. 7.2). These band patterns are due to changes in the dipole moment accompanying changes in vibrational modes of the aromatic ring and are surprisingly similar to those for monosubstituted and other *o*-, *m*-, and *p*-disubstituted benzenes.

▼
Sample, solvents, and equipment ***must be dry***

Infrared spectra can be determined on neat (undiluted) liquids, on solutions with an appropriate solvent, and on solids as mulls and KBr pellets. Glass is opaque to infrared radiation; therefore, the sample and reference cells used in infrared spectroscopy are sodium chloride plates. The sodium chloride plates are fragile and can be attacked by moisture. *Handle only by the edges.*

Spectra of Neat Liquids

To run a spectrum of a neat liquid (free of water!) remove a demountable cell (Fig. 7.3) from the desiccator and place a drop of the liquid between the salt plates, press the plates together to remove any air bubbles and add the top rubber gasket and metal top plate. Next, put on all four of the nuts and *gently* tighten them to apply an even pressure to the top plate. Place the cell in the sample compartment (nearest the front of the spectrometer) and run the spectrum.

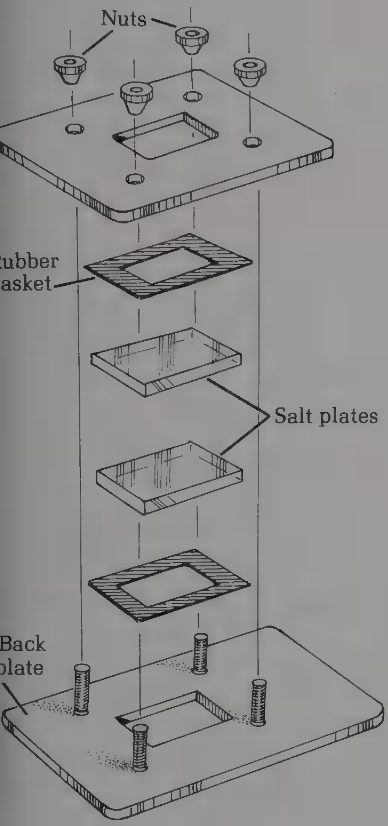


FIGURE 7.3 Exploded view of a demountable salt cell for analyzing the infrared spectra of neat liquids.

Although running a spectrum on a neat liquid is convenient and results in no extraneous bands to interpret, it is not possible to control the path length of the light through the liquid in a demountable cell. A low viscosity liquid when squeezed between the salt plates may be so thin that the short path length gives peaks that are too weak. A viscous liquid, on the other hand, may give peaks that are too intense. A properly run spectrum will have the most intense peak with an absorbance of about 1.0. Unlike the nmr spectrometer, the infrared spectrometer does not usually have a control to adjust the peak intensities; this control is possible by adjusting the sample concentration.

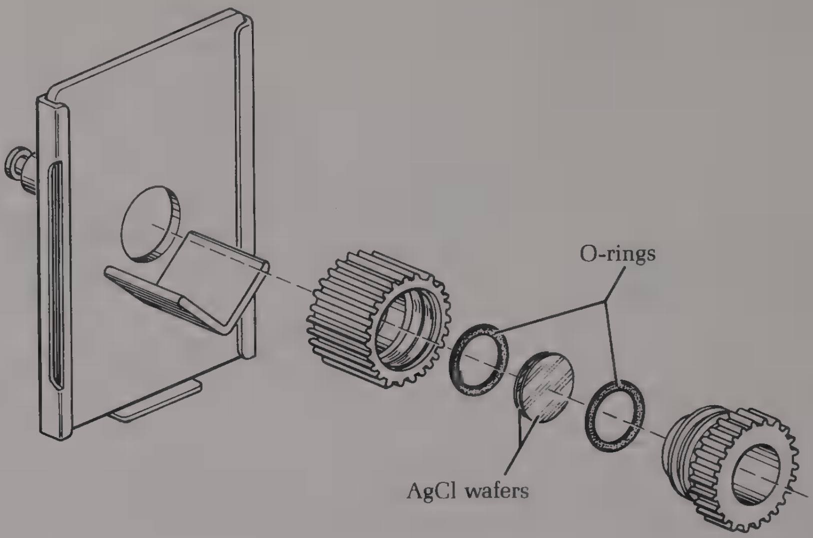
Another demountable cell is pictured in Fig. 7.4. The plates are thin wafers of silver chloride, which is transparent to infrared radiation. This cell has advantages over the salt cell in that the silver chloride discs are more resistant to breakage than NaCl plates, they are less expensive, and they are not affected by water. Because silver chloride is photosensitive the wafers must be stored in the dark to prevent them from turning black. Since one side of each wafer is recessed, the thickness of the sample can be varied according to the manner in which the cell is assembled. The discs are cleaned by rinsing them with an organic solvent such as acetone or ethanol and wiping dry with an adsorbent paper towel.

Spectra of Solutions

▼
Solvents: $CHCl_3$, CS_2, CCl_4

The most widely applicable method of running spectra of solutions involves dissolving an amount of the liquid or solid sample in an appropriate solvent to give a 10% solution. Just as in nmr spectroscopy, the best solvents to use are carbon disulfide and carbon tetrachloride, but since these compounds are not polar enough to dissolve many substances, chloroform is used as a compromise.

FIGURE 7.4 Demountable silver chloride cell.



Unlike nmr solvents, no solvent suitable in infrared spectroscopy is entirely free of absorption bands in the frequency range of interest (Figs. 7.5a and b). In chloroform, for instance, no light passes through the cell between 650 and 800 cm^{-1}. As can be seen from the figures, spectra obtained using carbon disulfide and chloroform cover the entire infrared frequency range. In practice, a base line is run with the same solvent in both cells to ascertain if the cells are clean and matched (Fig. 7.5c). Often it is necessary to obtain only one spectrum employing one solvent, depending on which region of the spectrum you need to use.

Three large drops of solution will fill the usual sealed infrared cell (Fig. 7.6). A 10% solution of a liquid sample can be approximated by dilution of one drop of the liquid sample with nine of the solvent. Since weights are more difficult to estimate, solid samples should be weighed to obtain a 10% solution.

▼
Filling the cell

The infrared cell is filled by inclining it slightly and placing about three drops of the solution in the lower hypodermic port with a capillary dropper. The liquid can be seen rising between the salt plates through the window. In the most common sealed cell, the salt plates are spaced 0.1 mm apart. Make sure that the cell is filled past the window and that no air bubbles are present. Then place the Teflon stopper lightly but firmly in the hypodermic port. Be particularly careful not to spill any of the sample on the outside of the cell windows.

Fill the reference cell from a clean hypodermic syringe in the same manner as the sample cell and place both cells in the spectrometer, with the sample cell toward the front of the instrument. After running the spectrum, force clean solvent through the sample cell, using a syringe attached to the top port of the cell (Fig. 7.7). Finally, with the syringe pull the last bit of solvent from both cells, blow clean dry compressed air through the cells to dry them, and store them in a desiccator.

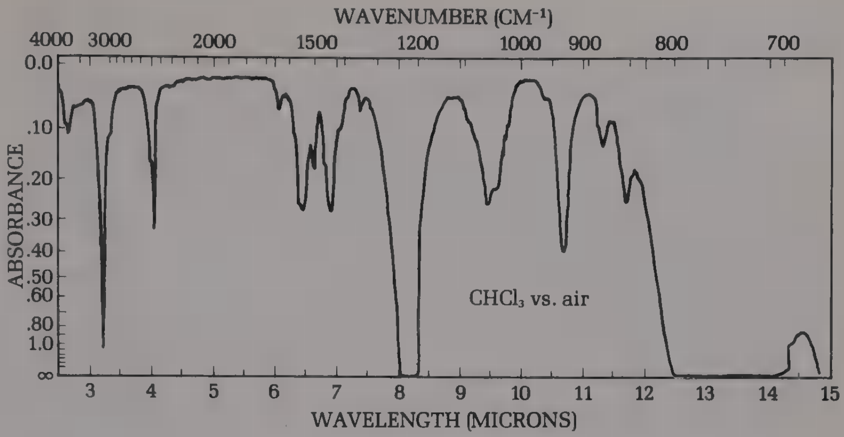


FIGURE 7.5a Spectrum of chloroform in sample cell, air in reference cell. No infrared light passes through chloroform between 1200 and 1250 cm^{-1}, and between 650 and 800 cm^{-1}, therefore no information about sample absorption in those regions can be obtained.

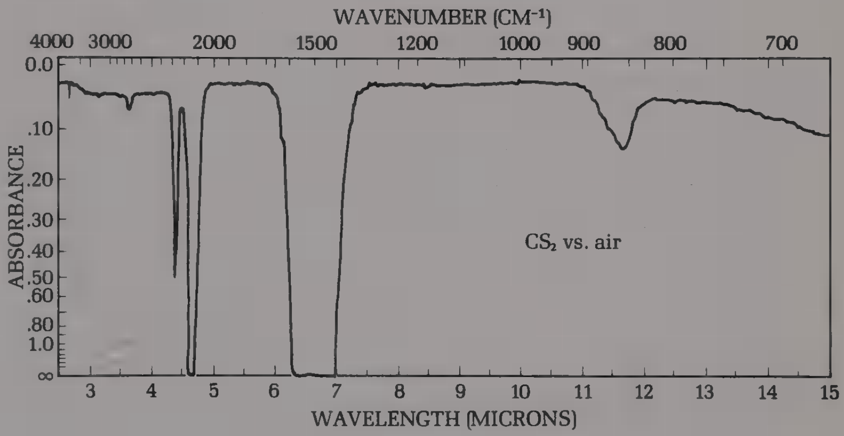


FIGURE 7.5b Spectrum of carbon disulfide in sample cell, air in reference cell. No infrared light passes through carbon disulfide between 1430 and 1550 cm^{-1}.

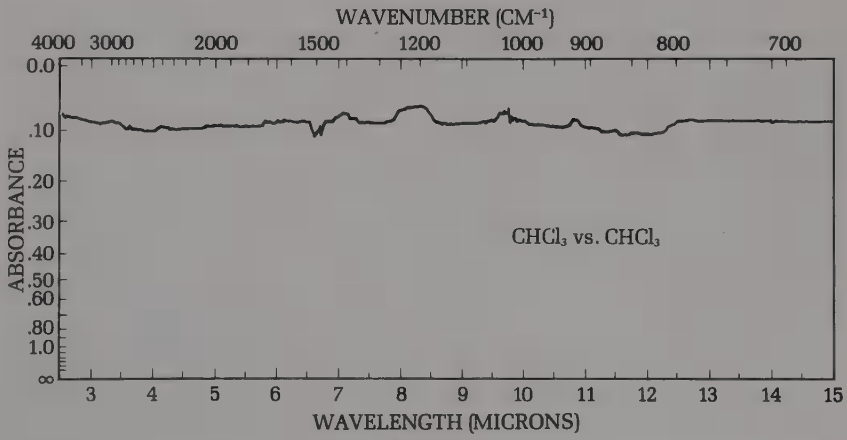


FIGURE 7.5c Spectrum of chloroform in both sample and reference cells. A typical baseline.

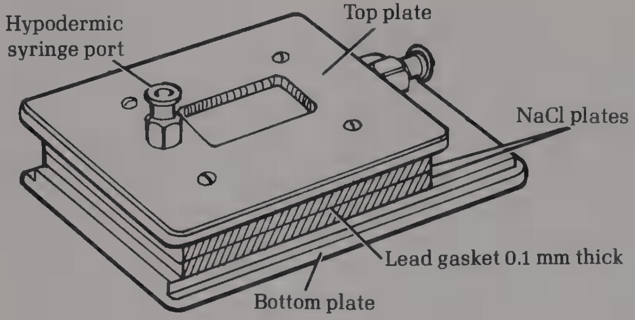


FIGURE 7.6 Sealed infrared sample cell.

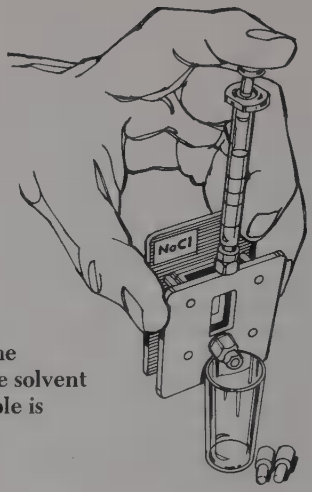


FIGURE 7.7 Flushing the infrared sample cell. The solvent used to dissolve the sample is used in this process.

Mulls and KBr Discs

Solids insoluble in the usual solvents can be run as mulls or KBr discs. In preparing the mull, the sample is ground to a particle size less than that of the wavelength of light going through the sample (2.5 microns), in order to avoid scattering the light. About 15 to 20 mg of the sample is ground for 3 to 10 minutes in an agate mortar until it is spread over the entire inner surface of the mortar and has a caked and glassy appearance. Then, to make a mull, 1 or 2 drops of paraffin oil (Nujol) (Fig. 7.8) is added, and the sample ground two to five more minutes. The mull is transferred to the bottom salt plate of a demountable cell (Fig. 7.3) using a rubber policeman, the top plate added and twisted to distribute the sample evenly and to eliminate all air pockets, and the spectrum run. Since the bands from Nujol obscure certain frequency

▼
Sample must be finely ground

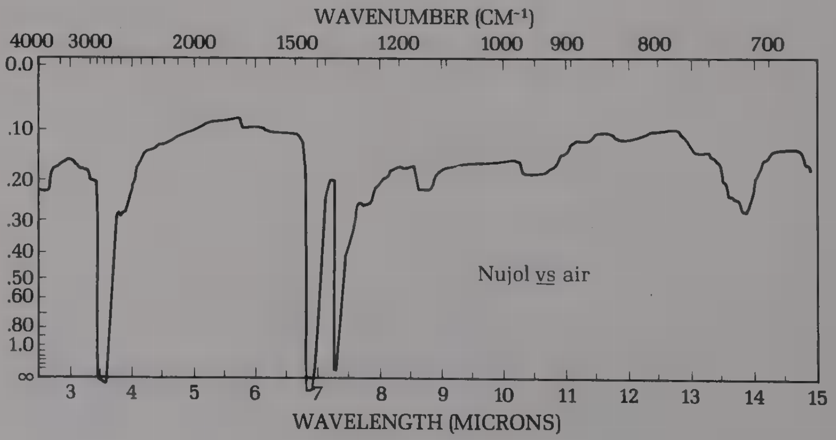


FIGURE 7.8 Infrared spectrum of Nujol (paraffin oil).

regions, running another mull using Fluorolube as the mulling agent will allow the entire infrared spectral region to be covered. If the sample has not been ground sufficiently fine, there will be marked loss of transmittance at the short-wavelength end of the spectrum. After running the spectrum, the salt plates are wiped clean with a cloth saturated with an appropriate solvent.

▼
Handle salt plates with care

The spectrum of a solid sample can also be run by incorporating the sample in a KBr disc. This procedure needs only one disc to cover the entire spectral range, since KBr is completely transparent to infrared radiation. Although very little sample is required, making the disc calls for special equipment and time to prepare it. Since KBr is hygroscopic, water is a problem. The sample is first ground as for a mull and 1.5 mg of this is added to 300 mg of spectroscopic grade KBr (previously dried in an oven and stored in a desiccator). The two are gently mixed (not ground) and quickly placed in a 13-mm die and subject to 14,000–16,000 lb/sq in. pressure for 3–6 min while under vacuum in a specially constructed hydraulic press. A transparent disc is produced which is removed from the die with tweezers and placed in a special holder, prior to running the spectrum.

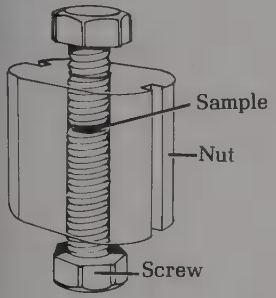


FIGURE 7.9 KBr disc die. Pressure is applied by tightening the machine screws with a wrench.

A simple low cost small press is illustrated by Fig. 7.9. The press consists of a large nut and two machine screws. The sample is placed between the two machine screws (which have polished faces), and the machine screws are tightened with a wrench with the nut held in a vise. The screws are then loosened and removed. The KBr disc is left in the nut, which is then mounted in the spectrometer to run the spectrum.

Running the Spectrum

Satisfactory spectra are easily obtained with the lower cost spectrometers, even those which have only a few controls and require few adjustments. To run a spectrum the paper must be positioned accurately, the pen set between 90% and 100% transmittance with the 100% control (0.0 and 0.05 absorbance), and the speed control set for a fast scan (usually one of about three minutes). The calibration of a given spectrum can be checked by backing up the drum and superimposing a spectrum of a thin polystyrene film. This film, mounted in a cardboard holder which has the frequencies of important peaks printed on it, will be found near most spectrometers. The film is held in the sample beam and parts of the spectrum to be calibrated are rerun. The spectrometer gain (amplification) should be checked frequently and adjusted when necessary. To check the gain, put the pen on the 90% transmittance line with the 100% control. Place your finger in the sample beam so that the pen goes down to 70% T. Then, quickly remove your finger. The pen should overshoot the 90% T line by 2%.

▼
Calibration with polystyrene film

Throughout the remainder of this book representative infrared spectra of starting materials and products will be presented and the important bands in each spectrum identified.

EXPERIMENT

Unknown Carbonyl Compound

Run the infrared spectrum of an unknown carbonyl compound obtained from the laboratory instructor. Be particularly careful that all apparatus and solvents are completely free of water, which will damage the sodium chloride cell plates. Immediately after running the spectrum, position the spectrometer pen at a wavelength of about 6.2 μ without disturbing the paper, and rerun the spectrum in the region from 6.2 to 6.4 μ while holding the polystyrene calibration film in the sample beam. This will superimpose a sharp calibration peak at 6.246 μ (1601 cm^{-1}) and a less intense peak at 6.317 μ (1583 cm^{-1}) on the spectrum. Determine the frequency of the carbonyl peak and list the possible types of compounds which could correspond to this frequency (Table 7.1).

Table 7.1 Characteristic Infrared Carbonyl Stretching Frequencies (chloroform solutions)

	Wavenumber cm^{-1}	Wavelength μ
Aliphatic ketones	1725–1705	5.80–5.87
Acid chlorides	1815–1785	5.51–5.60
α,β-Unsaturated ketones	1685–1666	5.93–6.00
Aryl ketones	1700–1680	5.88–5.95
Cyclobutanones	1775	5.64
Cyclopentanones	1750–1740	5.72–5.75
Cyclohexanones	1725–1705	5.80–5.87
β-Diketones	1640–1540	6.10–6.50
Aliphatic aldehydes	1740–1720	5.75–5.82
α,β-Unsaturated aldehydes	1705–1685	5.80–5.88
Aryl aldehydes	1715–1695	5.83–5.90
Aliphatic acids	1725–1700	5.80–5.88
α,β-Unsaturated acids	1700–1680	5.88–5.95
Aryl acids	1700–1680	5.88–5.95
Aliphatic esters	1740	5.75
α,β-Unsaturated esters	1730–1715	5.78–5.83
Aryl esters	1730–1715	5.78–5.83
Formate esters	1730–1715	5.78–5.83
Vinyl and phenyl acetate	1776	5.63
δ-Lactones	1740	5.75
γ-Lactones	1770	5.65
Acyclic anhydrides (two peaks)	1840–1800 1780–1740	5.44–5.56 5.62–5.75
Primary amides	1694–1650	5.90–6.06
Secondary amides	1700–1670	5.88–6.01
Tertiary amides	1670–1630	5.99–6.14

REFERENCES

1. L. J. Bellamy, *The Infrared Spectra of Complex Organic Molecules*, John Wiley, New York, 3rd Ed., 1975.
2. R. T. Conley, *Infrared Spectroscopy*, Allyn and Bacon, Boston, 2nd Ed., 1972.
3. K. Nakanishi and P. H. Solomon, *Infrared Absorption Spectroscopy*, Holden-Day, San Francisco, 1977.
4. R. M. Silverstein, C. G. Bassler, and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, John Wiley, New York, 3rd Ed., 1974. (Includes ir, uv, and nmr.)
5. H. A. Szymanski and R. E. Erickson, *Infrared Band Handbook*, Plenum, New York, 2nd Ed., 1970.
6. C. J. Pouchert, *The Aldrich Library of Infrared Spectra*, Aldrich Chemical Co., Milwaukee, 1970.

8

Nuclear Magnetic Resonance Spectroscopy

KEYWORDS

Integral
Downfield, upfield
TMS (tetramethylsilane)
Coupling constant
Chemical shift
Shift reagents [$Eu(dpm)_3$]
Nmr solvents (CS_2, $CDCl_3$)
Phase
Spinning side bands
Saturation
Magnetic impurities, removal
Homogeneity

▼
NMR—Determination of the number, kind, and relative locations of hydrogen atoms (protons) in a molecule

Nuclear magnetic resonance (nmr) spectroscopy is a means of determining the number, kind, and relative locations of certain atoms, principally hydrogen, in molecules. Experimentally, the sample, 0.3 ml of a 20% solution in a 5-mm o.d. glass tube, is placed in the probe of the spectrometer between the faces of a powerful (14,000 gauss) permanent or electromagnet and irradiated with radiofrequency energy (60 MHz; 60,000,000 cps for protons). The absorption of radiofrequency energy versus magnetic field strength is plotted by the spectrometer to give a spectrum.

In a typical ^1H spectrum (Fig. 8.1, ethyl iodide) the relative numbers of hydrogen atoms (protons) in the molecule are determined from the *integral*, the stair-step line over the peaks. The height of the step is proportional to the area under the nmr peak, and in nmr spectroscopy (contrasted with infrared, for instance) the area of each group of peaks is directly proportional to the number of hydrogen atoms causing the peaks. Integrators are part of all nmr spectrometers, and running the integral takes no more time than running the spectrum. The different kinds of protons are indicated by their *chemical shifts*.[1] For ethyl iodide the two protons adjacent to the electro-

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[1] Chemical shift is a measure of a peak's position relative to the peak of a standard substance (e.g., TMS, tetramethylsilane), which is assigned a chemical shift value of 0.0.

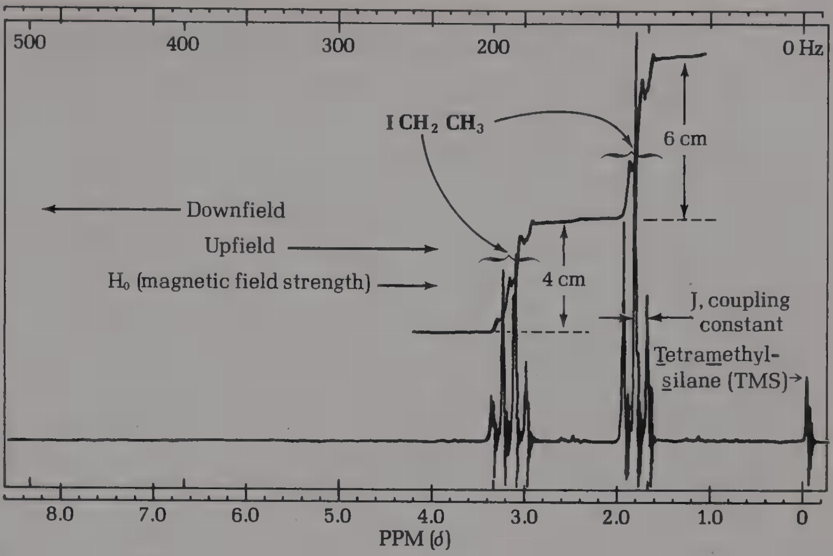

FIGURE 8.1 Proton nmr spectrum of ethyl iodide. The staircase-like line is the integral. In the integral mode of operation the recorder pen moves from left to right and moves vertically a distance proportional to the areas of the peaks over which it passes. Hence, the relative area of the quartet of peaks at 3.20 ppm to the triplet of peaks at 1.83 ppm is given by the relative heights of the integral (4 cm is to 6 m as 2 is to 3). The relative numbers of hydrogen atoms are proportional to the peak areas (2H and 3H).

negative iodine atom are *downfield* (at lower magnetic field strength) ($\delta = 3.20$ ppm) from the three methyl protons at $\delta = 1.83$ ppm. Tables of chemical shifts for protons in various environments can be found in reference books,[2] and are given graphically in Fig. 8.2.

The relative locations of the five protons in ethyl iodide are indicated by the pattern of peaks on the spectrum. The three peaks indicate methyl protons adjacent to two protons; four peaks indicate methylene protons adjacent to three methyl protons. In general, in molecules of this type, a given set of protons will appear as $n + 1$ peaks if they are adjacent to n protons. The distance between adjacent peaks in the quartet and triplet is the *coupling constant, J.*

Not all nmr spectra are so easily analyzed as the spectrum for ethyl iodide. Consider the one for 3-hexanol (Fig. 8.3). Twelve protons give rise to an unintelligible group of peaks between 1.0 and 2.2 ppm. It is not clear from a 3-hexanol spectrum which of the two low-field peaks (on the left-hand side of the spectrum) should be assigned to the hydroxyl proton and which should be assigned to the proton on C-3.

Deuterium atoms give no peaks in the nmr spectrum; thus the peak in the 3-hexanol spectrum for the proton on C-3 will be evident if the spectrum of the alcohol is one in which they hydroxyl proton of the alcohol has been replaced by deuterium. The hydroxyl proton of 3-hexanol is acidic and will exchange rapidly with the deuterium of D_2O.

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[2] Suggested references are L. M. Jackman and S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed., Pergamon Press, New York, 1969 and R. J. Abraham and P. Loftus, *Proton and Carbon-13 Spectroscopy, An Integrated Approach*, Heyden and Son, Philadelphia, 1978. See also K. L. Williamson, *Basic NMR Spectroscopy*, Prentice Hall Media, 150 White Plains Road, Tarrytown, N.Y. This is an audio-visual program in six parts (The NMR phenomenon, Chemical shifts, Coupling constants, Preparing the sample, Running the spectrum, and Applications).

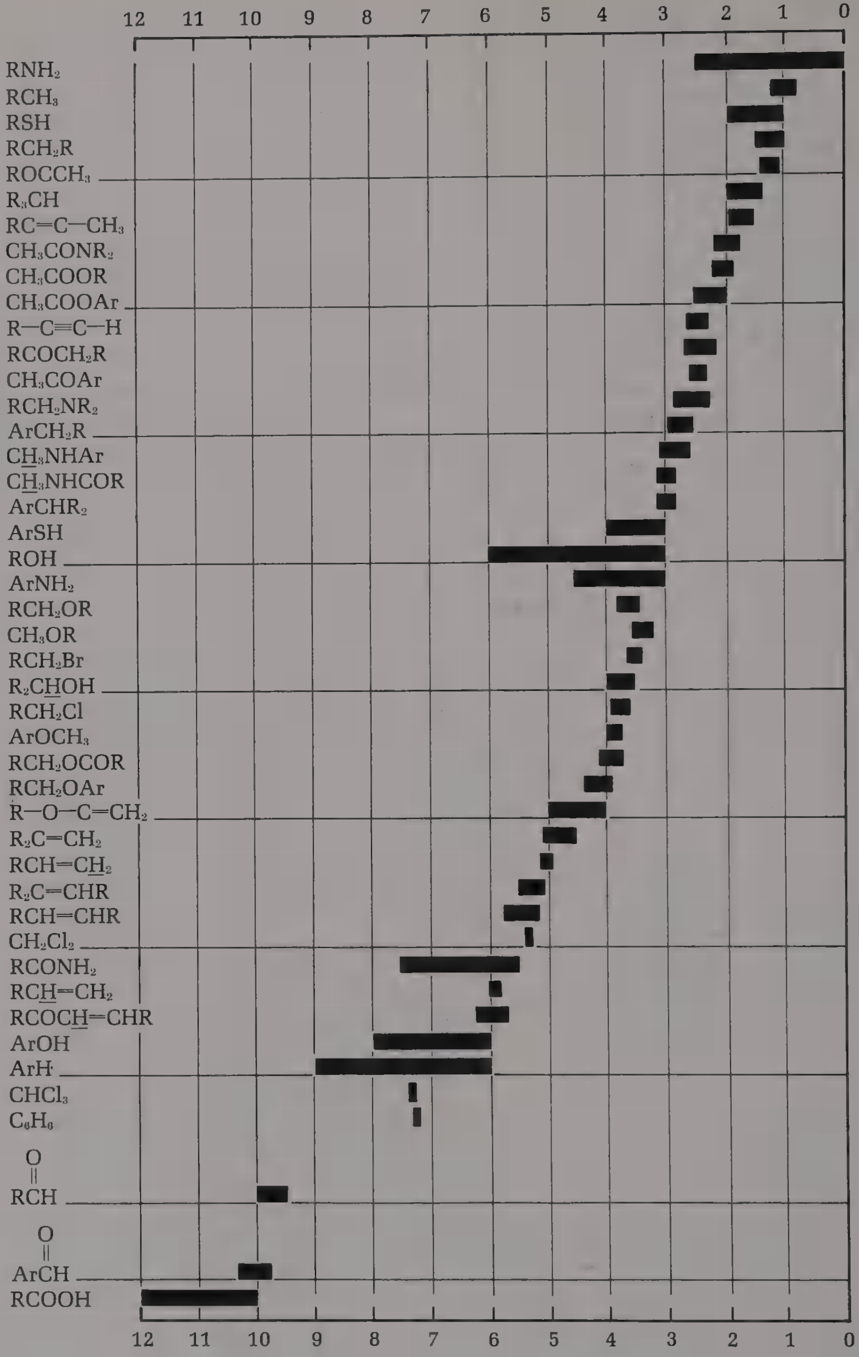
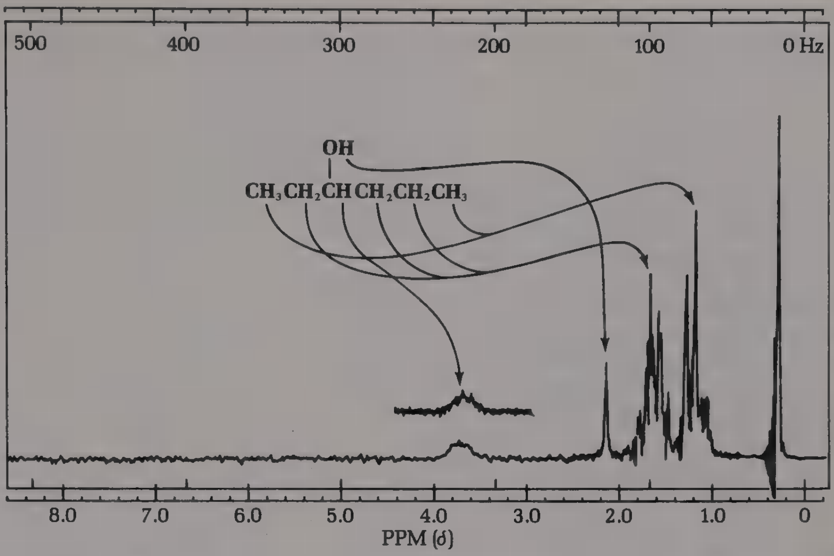


FIGURE 8.2 ^{1}H Chemical shifts, ppm from TMS.

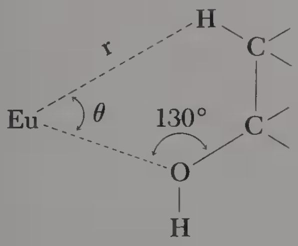
FIGURE 8.3 Proton nmr spectrum of 3-hexanol.



Addition of a few milligrams of a hexacoordinate complex of europium {tris(dipivaloylmethanato)europium(**III**), [Eu(dpm)$_3$]} to an nmr sample, which contains a Lewis base center (an amine or basic oxygen, such as a hydroxyl group), has a dramatic effect on the spectrum. This lanthanide (soluble in CS_2 and $CDCl_3$) causes large shifts in the positions of peaks arising from the protons near the metal atom in this molecule and is therefore referred to as a shift reagent. It produces the shifts by complexing with the unshared electrons of the hydroxyl oxygen, the amine nitrogen, or other Lewis base centers. As part of the complex the europium atom exerts a large dipolar effect on nearby hydrogens, with resulting changes in the nmr spectrum.

With no shift reagent present, the nmr spectrum of 2-methyl-3-pentanol (Fig. 8.4 A) is not readily analyzed. Addition of about 10 mg of Eu(dpm)$_3$ (the shift reagent) to the sample (0.5 ml of a 0.4 *M* solution) causes very large downfield shifts of peaks owing to protons near the coordination site (Fig. 8.4 B).[3] Further additions of 10-mg portions of the shift reagent cause further downfield shifts (Figs. 8.4 D, 8.4 E, and 8.4 F), so that in Fig. 8.4 G only the methyl peaks appear within 500 Hz of TMS. Finally, when the mole ratio of Eu(dpm)$_3$ to alcohol is 1:1 we find the spectrum shown in Fig. 8.5. The two protons on C-4 and the two methyls on C-2 are magnetically nonequivalent because they are adjacent to a chiral center (an asymmetric carbon atom, C-3), and therefore each gives a separate set of peaks. With shift reagent added, this spectrum can be analyzed by inspection.

Quantitative information about molecular geometry can be obtained from shifted spectra. The shift induced by the shift reagent, $\Delta H/H$, is related to the distance (r) and the angle (θ) which the proton bears to the europium atom.



$$\frac{\Delta H}{H} = \frac{3\cos^2\theta - 1}{r^3}$$

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[3] See also K. L. Williamson, D. R. Clutter, R. Emch, M. Alexander, A. E. Burroughs, C. Chua, and M. E. Bogel, *J. Am. Chem. Soc.*, 96, 1471 (1974).

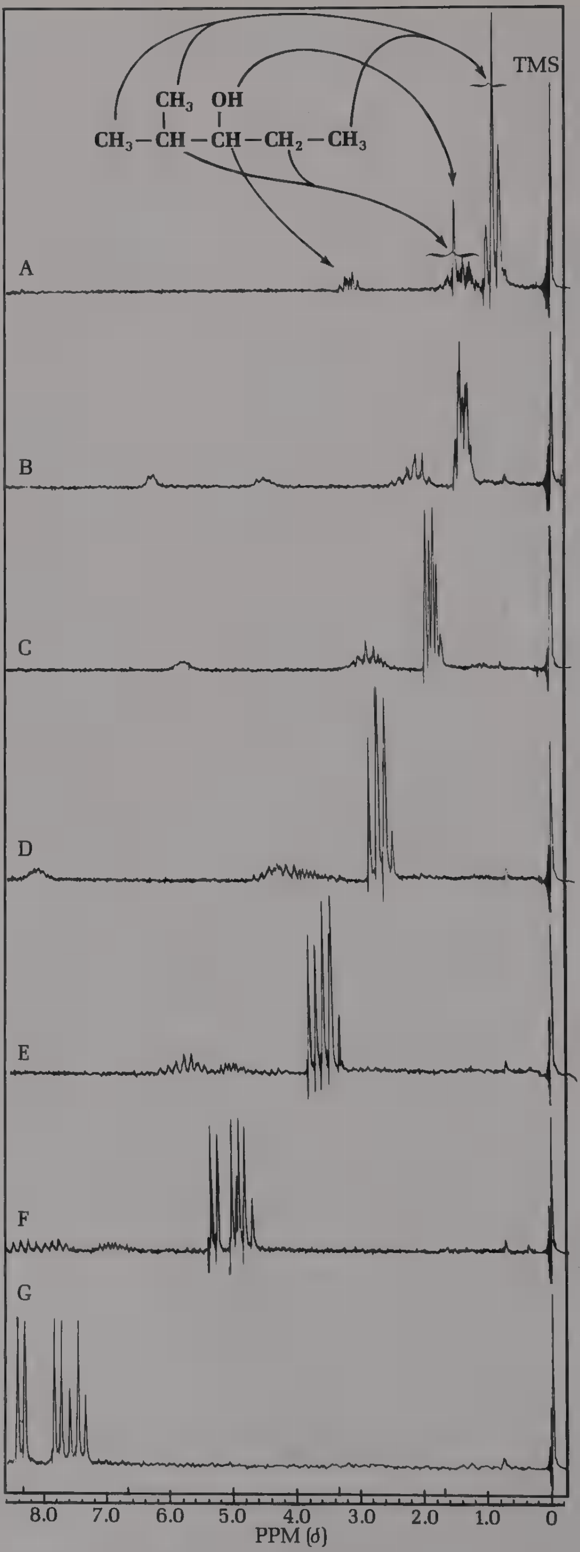


FIGURE 8.4 The ^{1}H nmr spectrum of 2-methyl-3-pentanol (0.4M in CS_2) with various amounts of shift reagent present. (A) No shift reagent present. All methyl peaks are superimposed. The peak for the proton adjacent to the hydroxyl group is downfield from the others because it is adjacent to the electronegative oxygen atom. (B) 2-Methyl-3-pentanol + Eu(dpm)$_3$. Mole ratio of Eu(dpm)$_3$ to alcohol = 0.05. The hydroxyl proton peak at 1.6 ppm in spectrum A appears at 6.2 ppm in spectrum B because it is closest to the Eu in the complex formed between Eu(dpm)$_3$ and the alcohol. The next closest proton, the one on the hydroxyl bearing carbon atom, gives a peak at 4.4 ppm. Peaks due to the three different methyl groups at 1.1–1.5 ppm begin to differentiate. (C) Mole ratio of Eu(dpm)$_3$ to alcohol = 0.1. The hydroxyl proton does not appear in this spectrum because its chemical shift is greater than 8.6 ppm with this much shift reagent present. (D) Mole ratio of Eu(dpm)$_3$ to alcohol = 0.25. Further differentiation of methyl peaks (2.3–3.0 ppm) is evident. (E) Mole ratio of Eu(dpm)$_3$ to alcohol = 0.5. Separate groups of peaks begin to appear in the region 4.2–6.2 ppm. (F) Mole ratio of Eu(dpm)$_3$ to alcohol = 0.7. Three groups of peaks (at 6.8, 7.7, and 8.1 ppm) due to the protons on C-2 and C-4 are evident, and three different methyls are now apparent. (G) Mole ratio of Eu(dpm)$_3$ to alcohol = 0.9. Only the methyl peaks appear on the spectrum. The two doublets come from the methyls attached to C-2 and the triplet comes from the terminal methyl at C-5.

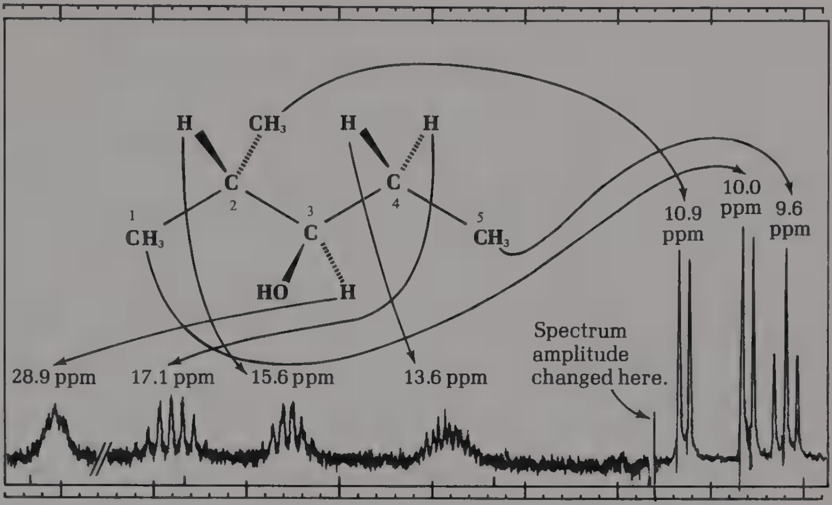
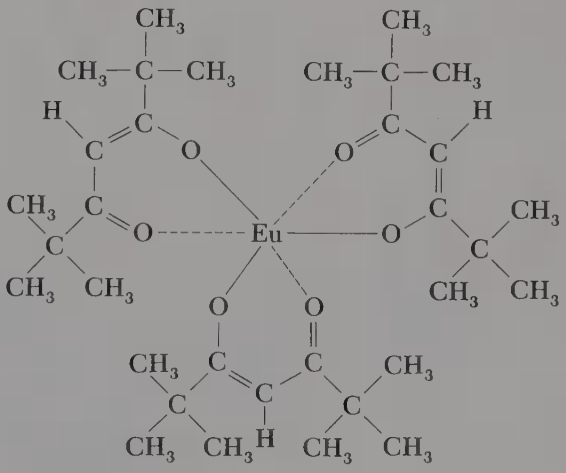


FIGURE 8.5 **Proton nmr spectrum of 2-methyl-3-pentanol containing Eu(dpm)$_3$. Mole ratio of Eu(dpm)$_3$ to alcohol = 1.0. Compare this spectrum to those shown in Fig. 8.4. Protons nearest the hydroxyl group are shifted most. Methyl groups are recorded at reduced spectrum amplitude. Note the large chemical shift difference between the two protons on C-4. The average conformation of the molecule is the one shown and was calculated from the equation on p. 71.**

Eu(dpm)$_3$ is a metal chelate (*chele,* Greek, claw). The β-diketone dipivaloylmethane (2,2,6,6-tetramethylheptane-3,5-dione) is a ligand, in this case a bidentate ligand, attached to the europium at two places. Since Eu^{3+} is hexacoordinate, three dipivaloylmethane molecules cluster about this metal atom. However, when a molecule with a basic group like an amine or an alcohol is in solution with Eu(dpm)$_3$ the europium will expand its coordination sphere to complex with this additional molecule. Such a complex is weak and so its nmr spectrum is an average of the complexed and uncomplexed molecule.



Tris(dipivaloylmethanato)europiumIII, Eu(dpm)$_3$
MW 701.78, mp 188–189°

Carbon-13 Spectra

Although the most common and least expensive nmr spectrometers are those capable of observing protons, spectrometers capable of observing carbon atoms of mass 13 are becoming prevalent. In many cases ^{13}C spectra are much simpler and easier to interpret than ^{1}H spectra because the spectrum, as usually presented, consists of a single line for each chemically and magnetically distinct carbon atom. The element carbon consists of 98.9% of carbon with mass 12 and spin 0 (nmr inactive) and only 1.1% of ^{13}C with spin $\frac{1}{2}$ (nmr active). Carbon, with such a low concentration of spin $\frac{1}{2}$ nuclei, gives such a small signal in a conventional nmr spectrometer that special means must be employed to obtain an observable spectrum. In a CW (continuous wave) spectrometer, proton spectra are produced by sweeping the radiofrequency through the spectrum while holding the magnetic field constant, a process that requires anywhere from one to five minutes. If one tries to obtain a ^{13}C spectrum under these conditions the signal is so small it cannot be distinguished from the random noise in the background. In order to increase the signal-to-noise ratio a number of spectra are averaged in a small computer built into the spectrometer. Since noise is random and the signal coherent, the signal will increase in size and the noise decrease as many spectra are averaged together. Spectra are accumulated rapidly by applying a very short pulse of radiofrequency energy to the sample and then storing the resulting free induction decay signal in digital form in the computer. The free induction decay signal (FID), which takes just 0.6 sec to acquire, contains frequency information about all the signals in the spectrum. In a few minutes several hundred FID's can be obtained and averaged in the computer. The FID is converted to a spectrum of conventional appearance by carrying out a Fourier transform computation on the signal using the spectrometer's computer. These principles are illustrated for very dilute proton spectra in Fig. 8.6.

Since only one in a hundred carbon atoms have mass 13 the chances of a molecule having two ^{13}C atoms adjacent to one another are small. Consequently, coupling of one carbon with another is not observed. Coupling of the ^{13}C atoms with ^{1}H atoms leads to excessively complex spectra, so this is ordinarily eliminated by noise decoupling the protons. This decoupling distorts the peaks such that peak areas of carbon spectra are not proportional to the number of carbon atoms present. Unlike proton spectra, which are observed over a range of approximately 15 ppm, carbon spectra occur over a 200-ppm range and peaks from magnetically distinct carbons rarely overlap. For example, each of the twelve carbons of sucrose gives a separate line (Fig. 8.7). The same factors that control proton chemical shifts are operative for carbon atoms. Carbons of high electron density (e.g., methyl groups) appear upfield near the carbon atoms of tetramethylsilane, the zero of reference, while carbon atoms bearing electron withdrawing groups or atoms appear downfield. It should not be surprising that carbonyl carbons are found furthest downfield, between 160 and 220 ppm. Figure 8.8 gives the chemical shift ranges for carbon atoms.

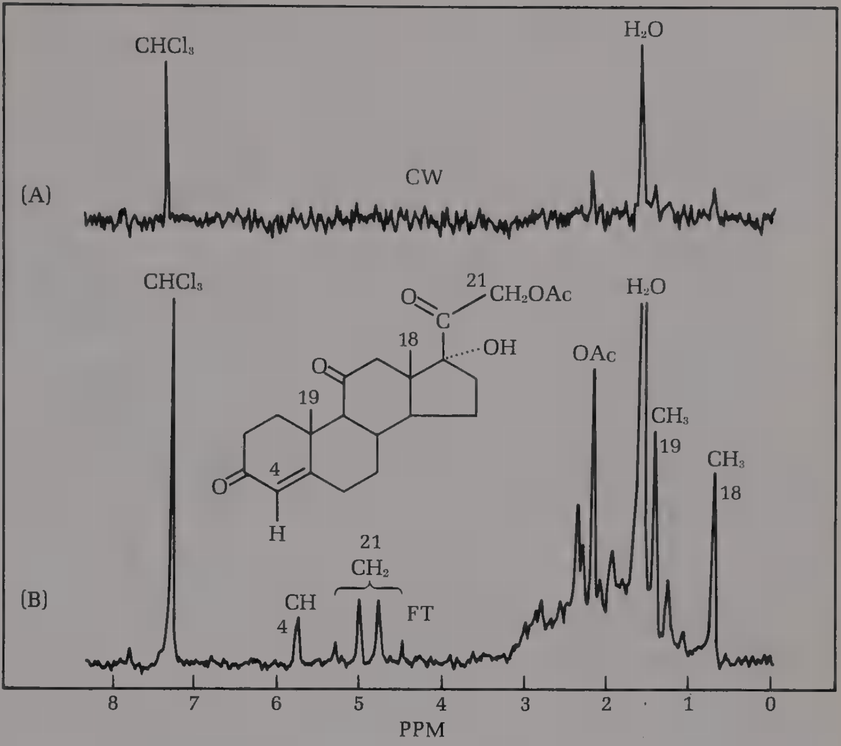


FIGURE 8.6 ^{1}H nmr spectra of cortisone acetate, 300 μg/0.3 ml, in $CDCl_3$. (A) Continuous wave (CW) spectrum, 500 sec scan time. (B) Fourier transform (FT) spectrum of the same sample, 250 scans (500 sec). The H_2O and $CHCl_3$ are contaminants.

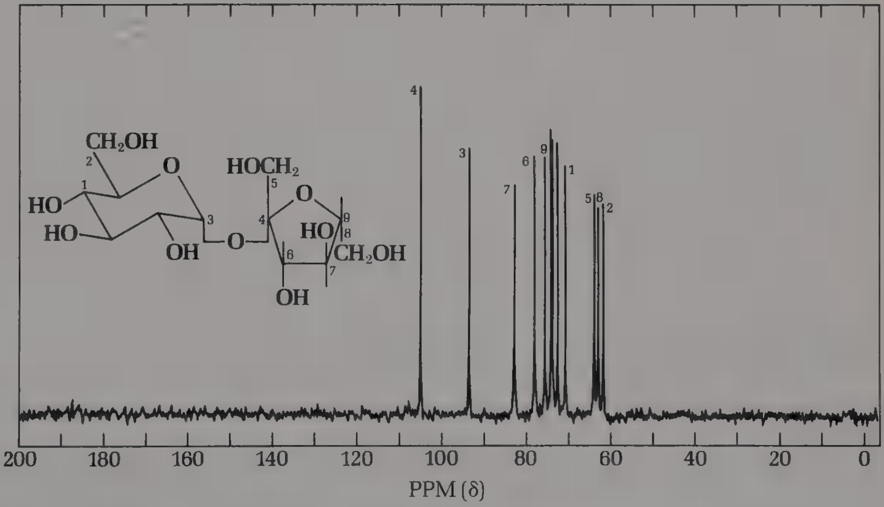
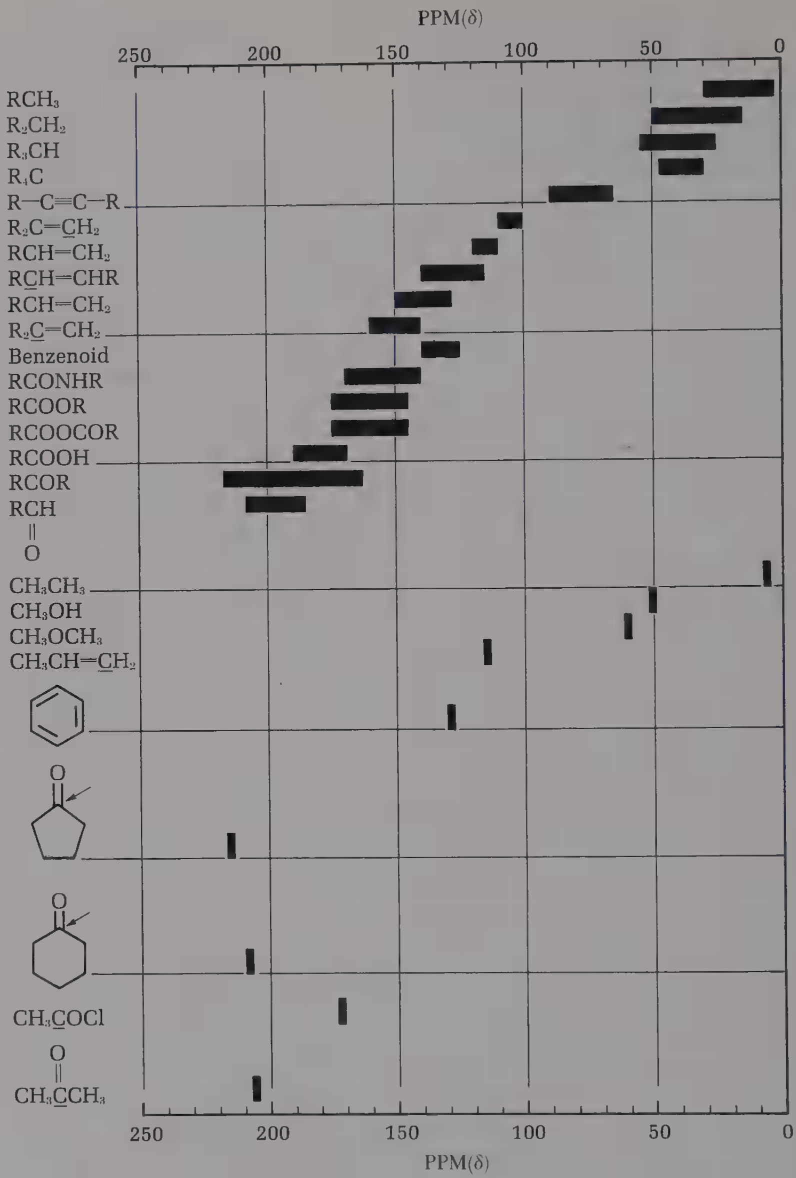


FIGURE 8.7 ^{13}C nmr spectrum of sucrose. Not all lines have been assigned to individual carbon atoms.

FIGURE 8.8 ^{13}C chemical shifts, ppm from TMS.



EXPERIMENT

▼

Sample size: 0.3 ml of 10–20% solution plus TMS

Procedure

A typical ^1H nmr sample is 0.3 to 0.5 ml of a 10–20% solution of a nonviscous liquid or a solid in a proton-free solvent contained in a 5-mm dia glass tube. The sample tube must be of uniform outside and inside diameter with uniform wall thickness. Test a sample tube by rolling it down a very slightly inclined piece of plate glass. Reject all tubes that roll unevenly.

The ideal solvent, from the nmr standpoint, is carbon tetrachloride. It is proton-free and nonpolar but unfortunately a poor solvent. Carbon disulfide is an excellent compromise. It will, however, react with amines.

▼
CS_2, the solvent of choice

Deuteriochloroform ($CDCl_3$) is one of the most widely used nmr solvents. Although more expensive than nondeuteriated solvents, it will dissolve a wider range of samples than carbon disulfide or carbon tetrachloride. Residual protons in the $CDCl_3$ will always give a peak at 7.27 ppm. Chemical shifts of protons are measured relative to the sharp peak of the protons in tetramethylsilane (taken as 0.0 ppm). Stock solutions of 3 to 5 percent tetramethylsilane in carbon disulfide and in deuteriochloroform are useful for preparing routine samples.

A wide variety of completely deuteriated solvents are commercially available, e.g., deuteroacetone (CD_3COCD_3), deuterodimethylsulfoxide (CD_3SOCD_3), deuterobenzene (C_6D_6), although they are expensive. For highly polar samples a mixture of the expensive deuterodimethylsulfoxide with the less expensive deuteriochloroform will often be satisfactory. Water soluble samples are dissolved in deuteriated water containing a water soluble salt [DSS, $(CH_3)_3SiCH_2CH_2CH_2SO_3^-Na^+$] as a reference substance. The protons on the three methyl groups bound to the silicon in this salt absorb at 0.0 ppm.

▼
Erratic spectra from ferromagnetic impurities; remove by filtration

Solid impurities in nmr samples will cause very erratic spectra. If two successive spectra taken within minutes of each other are not identical, suspect solid impurities, especially ferromagnetic ones. These can be removed by filtration of the sample through a tightly packed wad of glass wool in a capillary pipet (Fig. 8.9). If very high resolution spectra (all lines very sharp) are desired, oxygen, a paramagnetic impurity, must be removed by bubbling a fine stream of pure nitrogen through the sample for 60 sec. Routine samples do not require this treatment.

The usual nmr sample has a volume of 0.3 ml to 0.5 ml, even though the volume sensed by the spectrometer receiver coils (referred to as the active volume) is much smaller (Fig. 8.10). To average the magnetic fields produced by the spectrometer within the sample, the tube is spun by an air turbine at thirty to forty revolutions per second while taking the spectrum. Too rapid spinning or an insufficient amount of sample will cause the vortex produced by the spinning to penetrate the active volume, giving erratic nonreproducible

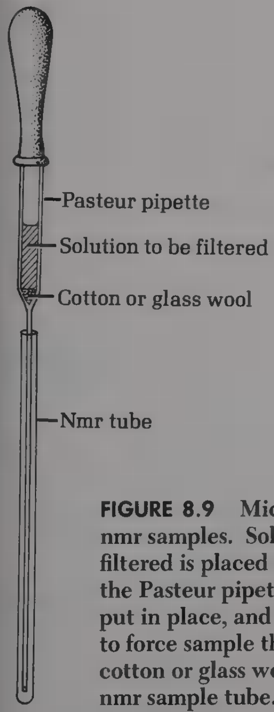


FIGURE 8.9 Micro filter for nmr samples. Solution to be filtered is placed in the top of the Pasteur pipette, rubber bulb put in place, and pressure applied to force sample through the cotton or glass wool into an nmr sample tube.

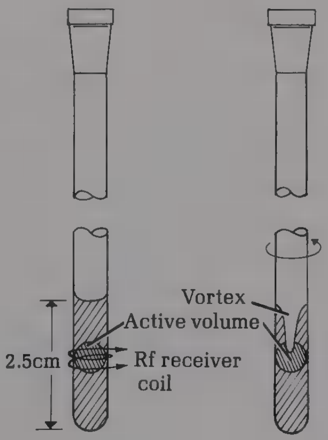


FIGURE 8.10 Effect of too rapid spinning or insufficient sample. The active volume is the only part of the sample detected by the spectrometer.

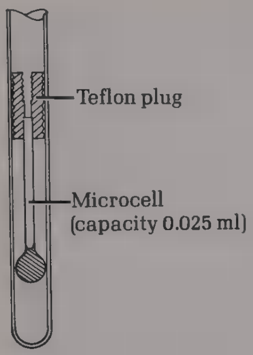


FIGURE 8.11 Nmr microcell positioned in an nmr tube. The Teflon lug supports the microcell and allows the sample to be centered in the active volume of the spectrometer.

spectra. A variety of microcells are available for holding and proper positioning of small samples with respect to the receiver coils of the spectrometer (Fig. 8.11). The vertical positioning of these cells in the spectrometer is critical. If microcells are used, only one or two mg of the sample are needed to give satisfactory spectra, in contrast to the 20–30 mg usually needed for a CW spectrum.

▼
Sweep zero

▼
Resolution, sharp, narrow peaks with good ringing = high resolution

Adjusting the Spectrometer To be certain the spectrometer is correctly adjusted and working properly, record the spectrum of the standard sample of chloroform and tetramethylsilane (TMS) usually found with the spectrometer. While recording the spectrum from left to right, the $CHCl_3$ peak should be brought to 7.27 ppm and the TMS peak to 0.0 ppm with the sweep zero control. The most important adjustment, the resolution control (also called homogeneity, or Y-control), should be adjusted for each sample so the TMS peak is as high and narrow as possible, with good ringing (Fig. 8.12). The signal (the peak traced by the spectrometer) should also be properly phased; it will then have the same appearance in both forward and backward scans (Fig. 8.12).

Small peaks symmetrically placed on each side of a principal peak are artifacts called spinning side bands (Fig. 8.13). They are recognized as such by changing the spin speed (see again Fig. 8.13), which causes the spinning side bands to change positions. Two controls on the spectrometer determine the height of a signal as it is recorded on the paper. One, the spectrum amplitude control, increases the size of the signal as well as the baseline noise (the jitter of the pen when no signal is present). The other, the radiofrequency

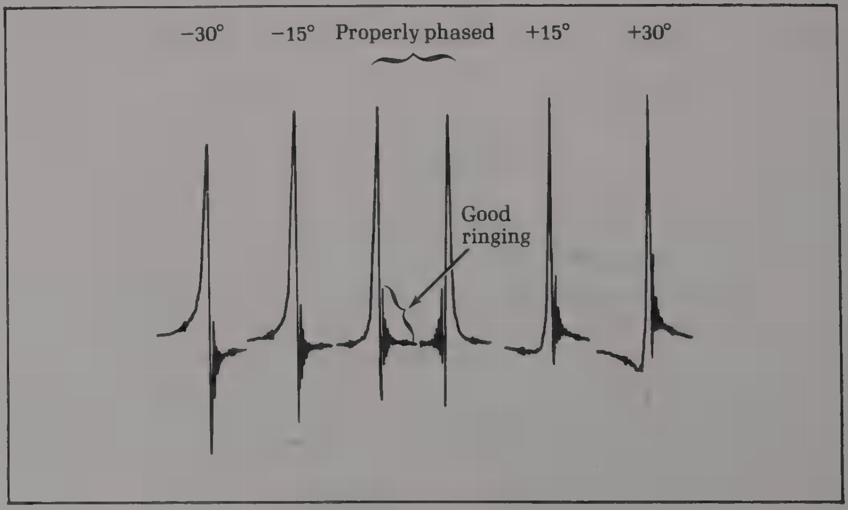


FIGURE 8.12 Effect of phasing on signal shape. The TMS peaks on both forward and backward scans are quite high and narrow, with good ringing and perfect symmetry.

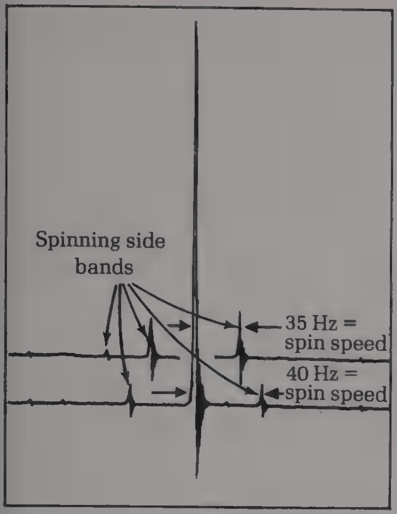


FIGURE 8.13 Spinning side bands.

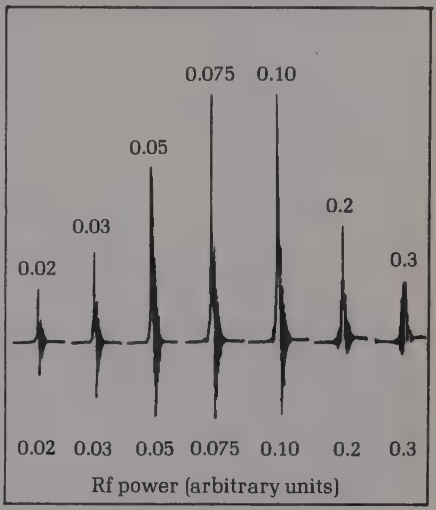


FIGURE 8.14 Saturation of the nmr signal for chloroform. At the optimum rf power level (0.075–0.10) the signal reaches maximum intensity. Further increase of power causes the signal to become smaller and broader, with poor ringing—saturation.

▼
Saturation

(rf) power control, increases the size of the signal alone, but only to a point, after which saturation occurs (Fig. 8.14). Applying more than the optimum rf power will cause the peak to become distorted and of low intensity.

Identification of Unknown Alcohol or Amine by ^{1}H NMR

Using a stock solution of 4% TMS in carbon disulfide, prepare 0.5 ml of a 0.4 *M* (or 10%) solution of an unknown alcohol or amine. Filter the solution, if necessary, into a clean, *dry* nmr tube. Set the TMS peak at 0.0 ppm, check the phasing, maximize the resolution, and run a spectrum over a 500-Hz range using a 250-sec sweep time. To the unknown solution add about 5 mg of $Eu(dpm)_3$ (the shift reagent), shake thoroughly to dissolve, and run another spectrum. Continue adding $Eu(dpm)_3$ in 10-mg portions until the spectrum is shifted enough for easy analysis. Integrate peaks and groups of peaks if in doubt about their relative areas. To protect the $Eu(dpm)_3$ from moisture store in a desiccator.

REFERENCES

Proton Spectroscopy

1. L. M. Jackman and S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon Press, Elmsford, N.Y., 2nd Ed., 1969.
2. D. W. Mathieson, *Nuclear Magnetic Resonance for Organic Chemists*, Academic Press, New York, 1967.
3. R. H. Bible, *Interpretation of NMR Spectra; An Empirical Approach*, Plenum Press, New York, 1965.
4. F. Bovey, *Nuclear Magnetic Resonance Spectroscopy*, Academic Press, New York, 1969.

5. E. D. Becker, *High Resolution NMR; Theory and Chemical Applications*, Academic Press, New York, 1969.

6. *High Resolution NMR Spectra Catalogs*, Varian Associates, Palo Alto, Vol. 1, 1962, Vol. 2, 1963.

Carbon Spectroscopy

7. J. B. Stothers, *Carbon-13 NMR Spectroscopy*, Academic Press, New York, 1972.

8. G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*, Wiley Interscience, New York, 1972.

9. L. F. Johnson and W. C. Jankowski, *Carbon-13 NMR Spectra*, Wiley Interscience, New York, 1972.

10. R. J. Abraham and P. Loftus, *Proton and Carbon-13 NMR Spectroscopy*, Heyden and Son, Philadelphia, 1978.

QUESTIONS

1. Propose a structure or structures consistent with the proton nmr spectrum of Fig. 8.15. Numbers adjacent to groups of peaks refer to relative peak areas. Account for missing lines.
2. Propose a structure or structures consistent with the proton nmr spectrum of Fig. 8.16. Numbers adjacent to groups of peaks refer to relative peak areas.
3. Propose a structure or structures consistent with the proton nmr spectrum of Fig. 8.17.
4. Propose structures for a, b, and c consistent with the carbon-13 nmr spectra of Figs. 8.18, 8.19, and 8.20. These are isomeric alcohols with the empirical formula $C_4H_{10}O$.

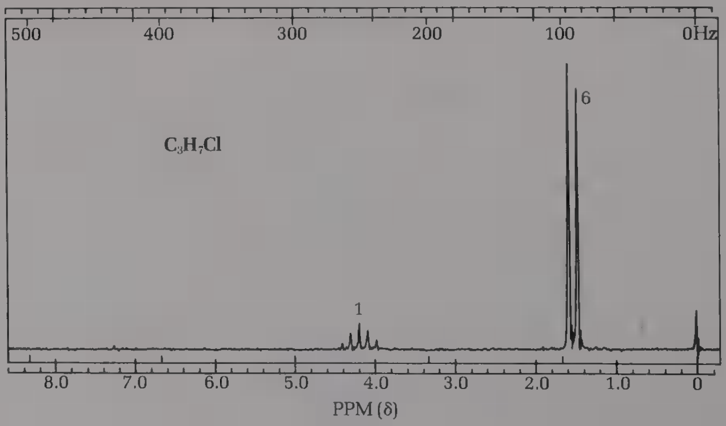


FIGURE 8.15 Proton nmr spectrum, Problem 1.

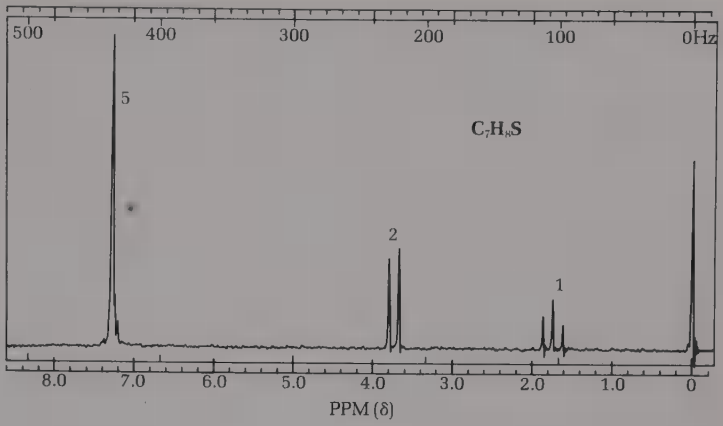


FIGURE 8.16 Proton nmr spectrum, Problem 2.

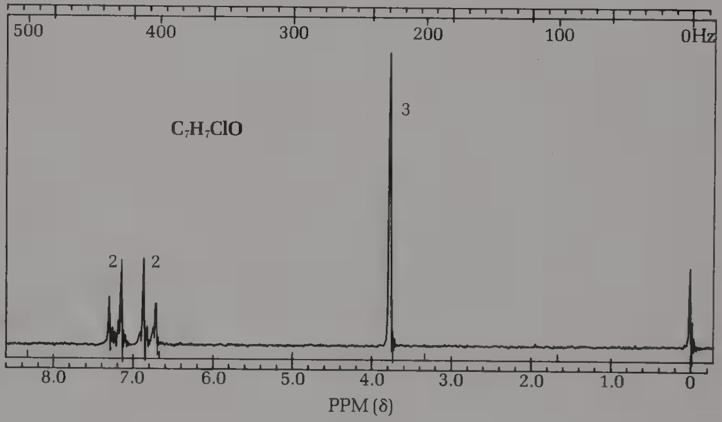


FIGURE 8.17 Proton nmr spectrum, Problem 3.

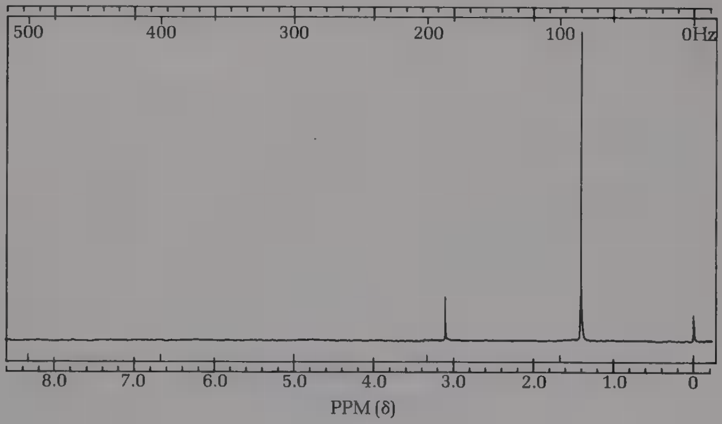


FIGURE 8.18 ^1H nmr spectrum of $C_4H_{10}O$, Problem 4a.

FIGURE 8.19 ^{13}C nmr spectrum of $C_4H_{10}O$, Problem 4b.

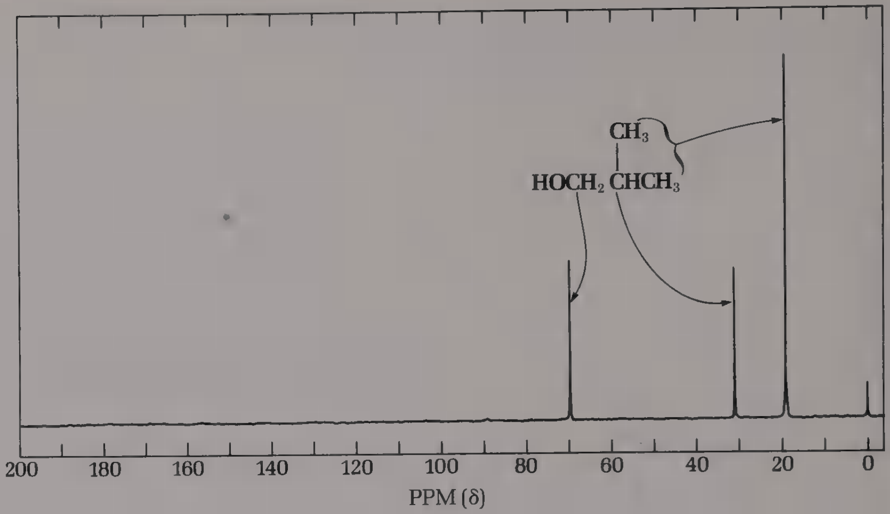
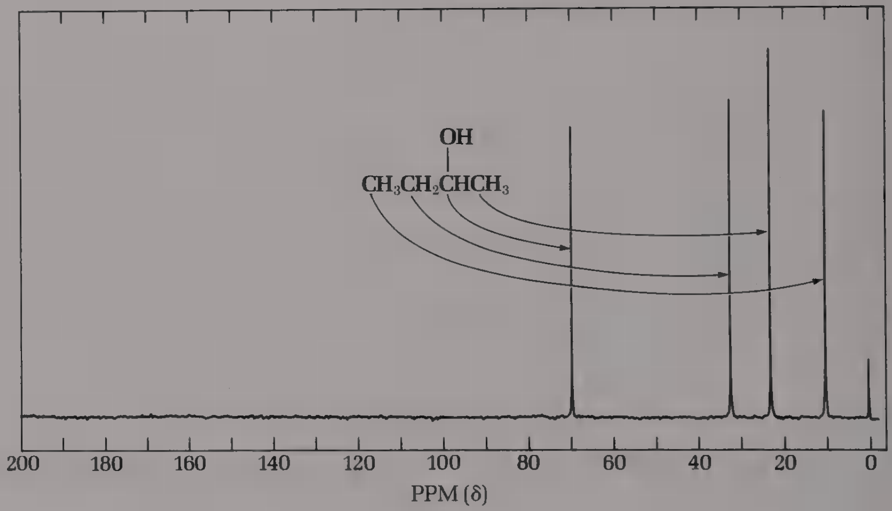


FIGURE 8.20 ^{13}C nmr spectrum of $C_4H_{10}O$, Problem 4c.



9

Ultraviolet Spectroscopy

KEYWORDS

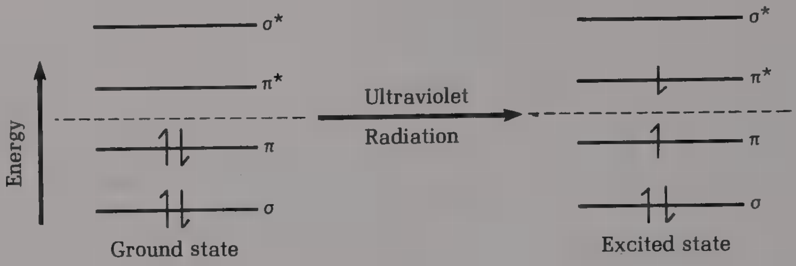
Electronic transitions
π-Electrons
Nanometers, nm
Molecular orbitals, bonding and antibonding
Band spectrum
Energy levels
Quantitative analysis
Molar extinction coefficient, ϵ
Beer-Lambert law
Absorbance
Wavelength of maximum absorption, λ_{max}
Spectro grade solvents
Woodward rules—dienes and polyenes
Fieser rules—enones and dienones
Exocyclic double bond
Homoannular diene
Solvent correction

Ultraviolet spectroscopy gives information about electronic transitions within molecules. Whereas absorption of low energy infrared radiation causes bonds in a molecule to stretch and bend, the absorption of short wavelength, high energy ultraviolet radiation causes electrons to move from one energy level to another with energies that are often capable of breaking chemical bonds.

We shall be most concerned with transitions of π-electrons in conjugated and aromatic ring systems. These transitions occur in the wavelength region 200 to 800 nm (nanometers, 10^{-9} meters, formerly known as mμ, millimicrons). Most common ultraviolet spectrometers cover the region 200 to 400 nm as well as the visible spectral region 400 to 800 nm. Below 200 nm air (oxygen) absorbs uv radiation; spectra in that region must therefore be obtained in a vacuum or in an atmosphere of pure nitrogen.

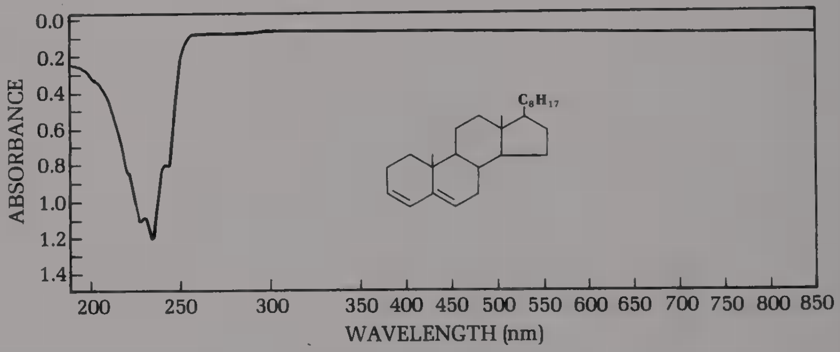
Consider ethylene, even though it absorbs uv radiation in the normally inaccessible region at 163 nm. The double bond in ethylene has two *s* electrons in a σ-molecular orbital and two, less tightly held, *p* electrons in a π-molecular orbital. Two unoccupied, high energy level, antibonding orbitals are associated with these orbitals. When ethylene absorbs uv radiation, one electron moves up from the bonding π-molecular orbital to the antibonding π^*-molecular orbital (Fig. 9.1). As the diagram indicates, this change requires less energy than the excitation of an electron from the σ to the σ^* orbital.

FIGURE 9.1 Electronic energy levels of ethylene.



By comparison with infrared spectra and nmr spectra, uv spectra are fairly featureless (Fig. 9.2). This condition results as molecules in a number of different vibrational states undergo the same electronic transition, to produce a band spectrum instead of a line spectrum.

FIGURE 9.2 The ultraviolet spectrum of cholesta-3,5-diene in ethanol.



Unlike ir spectroscopy, ultraviolet spectroscopy lends itself to precise quantitative analysis of substances. The intensity of an absorption band is usually given by the molar extinction coefficient, ϵ, which according to the Beer-Lambert Law is equal to the absorbance, A, divided by the product of the molar concentration, c, and the path length, l, in centimeters.

$$\epsilon = \frac{A}{cl}$$

The wavelength of maximum absorption (the tip of the peak) is given by λ_{max}. Since uv spectra are so featureless it is common practice to describe a spectrum like that of cholesta-3,5-diene (Fig. 18.2) as λ_{max} 234 nm ($\epsilon = 20{,}000$), and not bother to reproduce the actual spectrum.

The extinction coefficients of conjugated dienes and enones are in the range 10,000–20,000, so only very dilute solutions are needed for spectra. In the example of Fig. 9.2 the absorbance at the tip of the peak, *A*, is 1.2, and the path length is the usual 1 cm; so the molar concentration needed for this spectrum is 6×10^{-5} mole per liter.

$$c = \frac{A}{l\epsilon} = \frac{1.2}{20{,}000} = 6 \times 10^{-5} \text{ mole per liter}$$

which is 0.221 mg per 10 ml of solvent.

The usual solvents for uv spectroscopy are 95% ethanol, methanol, water, and saturated hydrocarbons such as hexane, trimethylpentane, and isooctane; the three hydrocarbons are often specially purified to remove impurities that absorb in the uv region. Any transparent solvent can be used for spectra in the visible region.

▼
Spectro grade solvents

Sample cells for spectra in the visible region are made of glass, but uv cells must be of the more expensive fused quartz, since glass absorbs uv radiation. The cells and solvents must be clean and pure, since very little of a substance produces a uv spectrum. A single fingerprint will give a spectrum!

Ethylene has λ_{max} 163 nm ($\epsilon = 15{,}000$) and butadiene has λ_{max} 217 nm ($\epsilon = 20{,}900$). As the conjugated system is extended, the wavelength of maximum absorption moves to longer wavelengths (toward the visible region): for example, lycopene with 11 conjugated double bonds has λ_{max} 470 nm ($\epsilon = 185{,}000$), Fig. 9.3. Since lycopene absorbs blue visible light at 470 nm the substance appears bright red. It is responsible for the color of tomatoes; its isolation is described in Chapter 49.

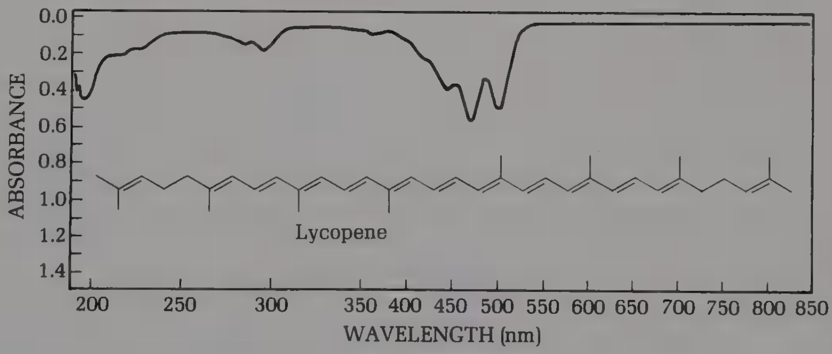


FIGURE 9.3 **The ultraviolet-visible spectrum of lycopene in isooctane.**

The wavelengths of maximum absorption of conjugated dienes and polyenes and conjugated enones and dienones are given by the Woodward and Fieser Rules, Tables 9.1 and 9.2.

The application of the rules in the above tables is demonstrated by the spectra of pulegone (1) and carvone (2), Fig. 9.4, with the calculations given in Tables 9.4 and 9.5.

Table 9.1 Rules for the Prediction of λ_{max} for Conjugated Dienes and Polyenes

	Increment (nm)
Parent heteroannular diene	214
Parent homoannular diene	253
Double bond extending the conjugation	30
Alkyl substituent or ring residue	5
Exocyclic location of double bond to any ring	5
Groups: OAc, OR	0
Solvent correction	0
λ_{max} = Total	

Table 9.2 Rules for the Prediction of λ_{max} for Conjugated Enones and Dienones

		Increment (nm)
Parent α,β-unsaturated system		215
Double bond extending the conjugation		30
R (alkyl or ring residue), OR, $OCOCH_3$	α	10
	β	12
	γ, and higher	18
α-Hydroxyl, enolic		35
α-Cl		15
α-Br		23
exo-Location of double bond to any ring		5
Homoannular diene component		39
Solvent correction, see Table 15.3		..
	λ_{max}^{EtOH} = Total	

Table 9.3 Solvent Correction

Solvent	Factor for Correction to ethanol
Hexane	+11
Ether	+7
Dioxane	+5
Chloroform	+1
Methanol	0
Ethanol	0
Water	−8

FIGURE 9.4 Ultraviolet spectra of (1) pulegone and (2) carvone in hexane.

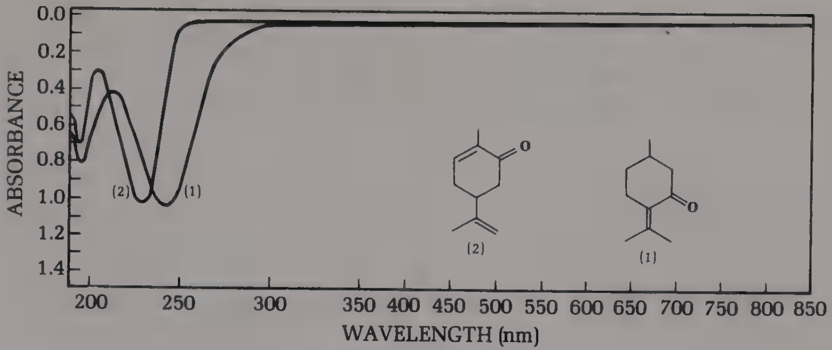


Table 9.4 Calculation of λ_{max} for Pulegone

Parent α,β-unsaturated system	215 nm
α-Ring residue, R	10
β-Alkyl group (two methyls)	24
Exocyclic double bond	5
Solvent correction (hexane)	−11
	Calc'd λ_{max} 243 nm; found 244 nm

Table 9.5 Calculation of λ_{max} for Carvone

Parent α,β-unsaturated system	215 nm
α-Alkyl group (methyl)	10
β-Ring residue	12
Solvent correction (hexane)	−11
	Calc'd λ_{max} 226 nm; found 229 nm

These rules will be applied in the next experiment in which cholesterol is converted into an α,β- unsaturated ketone.

No simple rules exist for calculation of aromatic ring spectra, but several generalizations can be made. From Fig. 9.5 it is obvious that as polynuclear aromatic rings are extended linearly, λ_{max} shifts to longer wavelengths.

As alkyl groups are added to benzene, λ_{max} shifts from 255 nm for benzene to 261 nm for toluene to 272 nm for hexamethylbenzene. Substituents bearing nonbonding electrons also cause shifts of λ_{max} to longer wavelengths, e.g., from 255 nm for benzene to 257 nm for chlorobenzene, 270 nm for phenol, and 280 nm for aniline (ϵ = 6,200–8,600). That these effects are the result of interaction of the π-electron system with the nonbonded electrons is seen dramatically in the spectra of vanillin and the derived anion (Fig. 9.6). Addition of two more nonbonding electrons in the anion causes λ_{max} to shift from 279 nm to 351 nm and ϵ to increase. Removing the electrons from the nitrogen of aniline by making the anilinium cation causes λ_{max} to decrease from 280 nm to 254 nm (Fig. 9.7). These changes of λ_{max} as a function of pH have obvious analytical applications.

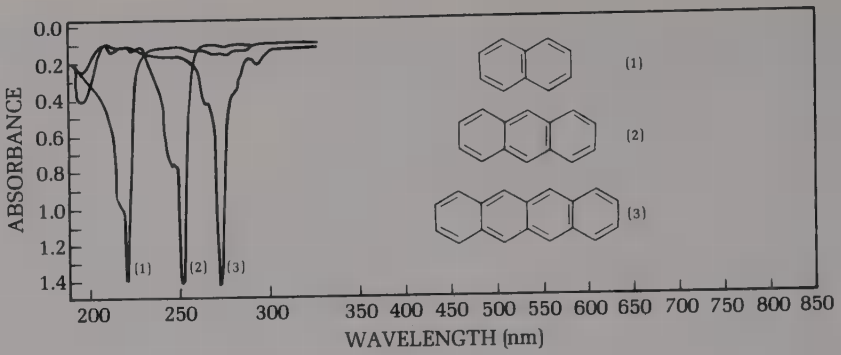


FIGURE 9.5 The ultraviolet spectra of (1) naphthalene, (2) anthracene, and (3) tetracene.

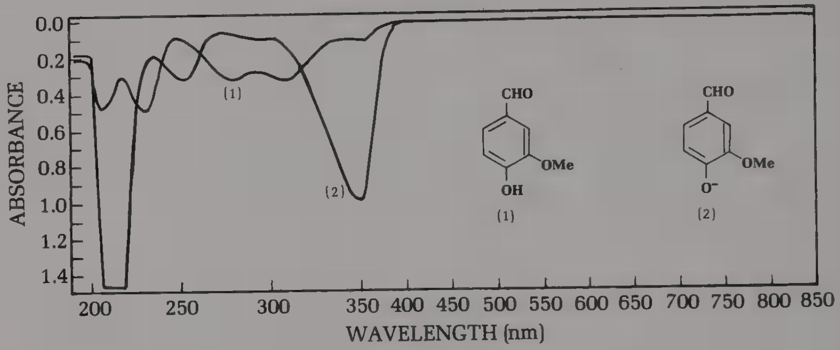


FIGURE 9.6 Ultraviolet spectrum of (1) neutral vanillin and (2) the anion of vanillin.

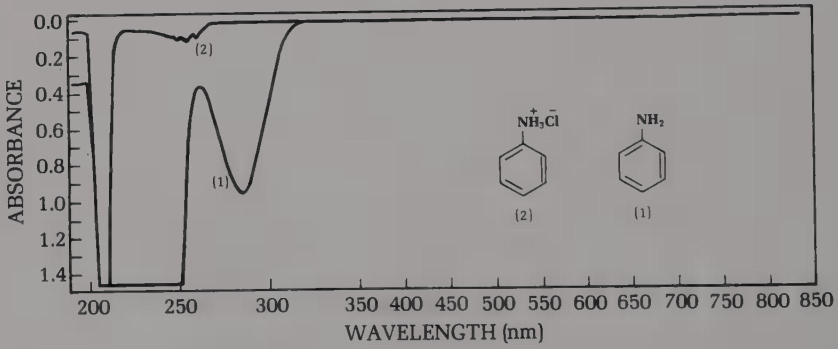


FIGURE 9.7 Ultraviolet spectrum of (1) aniline and (2) aniline hydrochloride.

Intense bands result from π-π conjugation of double bonds and carbonyl groups with the aromatic ring. Styrene, for example, has λ_{max} 244 nm (ϵ = 12,000) and benzaldehyde λ_{max} 244 nm (ϵ = 15,000).

EXPERIMENT

Ultraviolet Spectrum of Unknown Acid, Base, or Neutral Compound

Determine whether an unknown compound obtained from the instructor is acidic, basic, or neutral from the ultraviolet spectra in the presence of acid and base as well as in neutral media.

REFERENCES

1. A. E. Gillam and E. S. Stern, *An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, Edward Arnold, London, 1967.
2. C. N. R. Rao, *Ultraviolet and Visible Spectroscopy*, Butterworths, London, 2nd Ed., 1967.
3. H. H. Jaffe and M. Orchin, *Theory and Application of Ultraviolet Spectroscopy*, John Wiley, New York, 1962.
4. R. M. Silverstein, C. G. Bassler, and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, John Wiley, New York, 3rd Ed., 1974. (Includes ir, uv and nmr).

10

Gas Chromatography

KEYWORDS

Gas, vapor phase, and gas-liquid chromatography
Injector
Carrier gas, helium
Thermal detector
Retention time
Stationary phase, silicone oil, Carbowax
Column

▼
Separation of mixtures of volatile samples

▼
High-boiling liquid phase

Gas chromatography (gc), also called vapor phase chromatography (vpc) and gas-liquid chromatography (glc), is a means of separating volatile mixtures, the components of which may differ in boiling points by only a few tenths of a degree. The gc process is similar to fractional distillation, but instead of a glass column 25 cm long packed with a stainless-steel sponge the gc column used is a 3–10 m long coiled metal tube (dia 6 mm), packed with ground firebrick. The firebrick serves as an inert support for a very high-boiling liquid (essentially nonvolatile), such as silicone oil and low molecular weight polymers like Carbowax. These are the *liquids* of gas-*liquid* chromatography and are referred to as the stationary phase. The sample (1–25 *micro*liters) is injected through a silicone rubber septum into the column, which is being swept with a current of helium (ca. 200 ml/min). The sample first dissolves in the high-boiling liquid phase and then the more volatile components of the sample evaporate from the liquid and pass into the gas phase. Helium, the carrier gas, carries these components along the column a short distance where they again dissolve in the liquid phase before reevaporation (Fig. 10.1).

▼
The thermal conductivity detector

Eventually the carrier gas, which is a very good thermal conductor, and the sample reach the detector, an electrically heated tungsten wire. As long as pure helium is flowing over the detector the temperature of the wire is rather low and the wire has a high resistance to the flow of electric current.

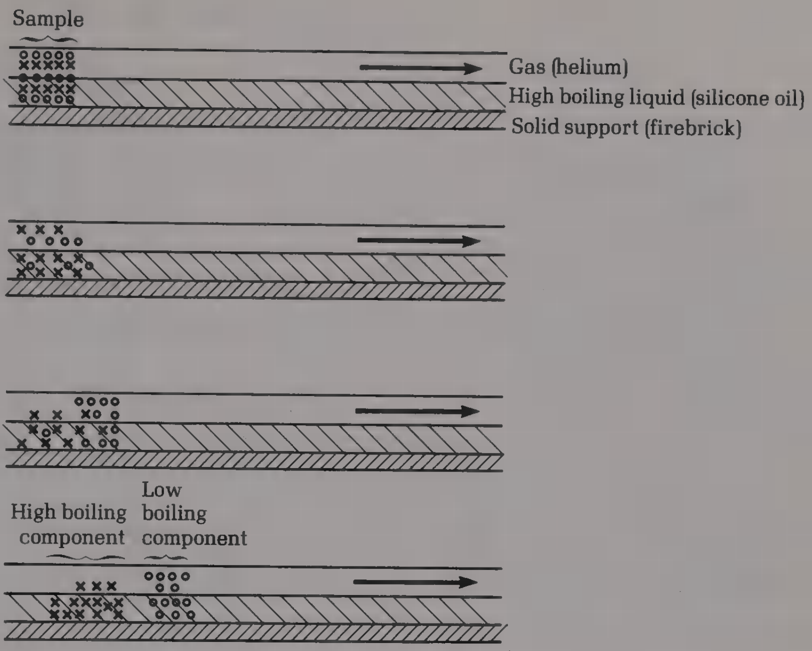


FIGURE 10.1 Gas chromatography: Diagrammatic partition between gas and liquid phases.

Organic molecules have lower heat capacities than helium. Hence, when a mixture of helium and an organic sample flows over the detector wire it is cooled less efficiently and heats up. When hot the electrical resistance of the wire becomes lower and offers less resistance to the flow of current. The detector wire actually is one leg of a Wheatstone bridge, connected to a chart recorder which records, as a peak, the amount of current necessary to again balance the bridge. The record produced by the recorder is called a **chromatogram.**

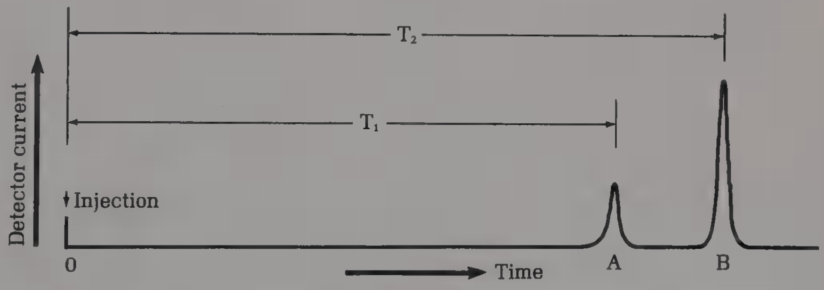
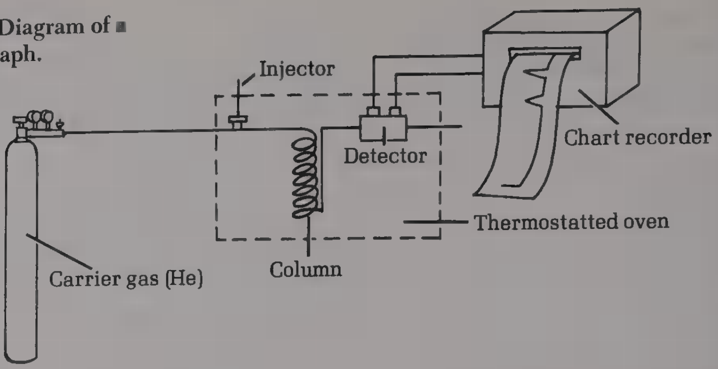


FIGURE 10.2 Gas chromatogram.

▼
The number and relative amounts of components in a mixture

A gas chromatogram is simply a recording of current versus time (which is equivalent to a certain volume of helium) (Fig. 10.2). In the illustration the smaller peak from component A has the shorter retention time, T_1, and so is a more volatile substance than component B (if the stationary phase is an inert liquid such as silicone oil). The areas under the two peaks are directly proportional to the amounts of A and B in the mixture. The retention time of a given

FIGURE 10.3 Diagram of a gas chromatograph.



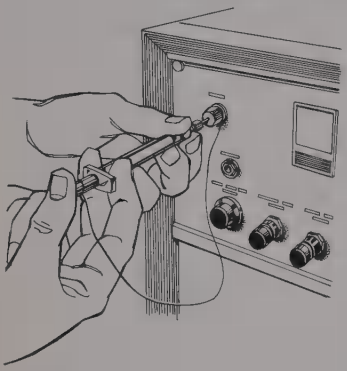


FIGURE 10.4 One of the commercially available chromatographs.

component is a function of the column temperature, the helium flow rate, and the nature of the stationary phase. Hundreds of stationary phases are available; picking the correct one to carry out a given analysis is somewhat of an art, but widely-used stationary phases are silicone oil and silicone rubber, both of which can be used at temperatures up to 300° and separate mixtures on the basis of boiling point differences of the mixture's components. More specialized stationary phases will, for instance, allow alkanes to pass through readily (short retention time) while holding back (long retention time) alcohols by hydrogen bonding to the liquid phase.

▼
The gas chromatograph

A diagram of a typical gas chromatograph is shown in Fig. 10.3. The carrier gas, usually helium, enters the chromatograph at ca. 60 lb/sq in. The sample (1 to 25 microliters) is injected through a rubber septum using a small hypodermic syringe (Fig. 10.4). The sample immediately passes through the column and then the detector. Injector, column, and detector are all enclosed in a thermostatted oven, which can be maintained at any temperature up to 300°. In this way samples which would not volatilize enough at room temperature can be analyzed.

Gas chromatography determines the number of components and their relative amounts in a very small sample. The small sample size is an advantage in many cases, but it precludes isolating the separated components. Some specialized chromatographs can separate samples as large as 0.5 ml per injection and automatically collect each fraction in a separate container. At the other extreme gas chromatographs equipped with flame ionization detectors can detect micrograms of sample and are used to analyze for traces of pesticides in food or traces of drugs in blood and urine. Clearly a gas chromatogram gives little information about the chemical nature of the sample being detected. However, it is sometimes possible to collect enough sample at the exit port of the chromatograph to obtain an infrared spectrum. As the peak for the compound of interest appears on the chart paper, a 2-mm dia glass tube, 3 in. long and packed with glass wool, is inserted into the rubber septum at the exit port. The sample, if it is not too volatile, will condense in the cold glass tube. Subsequently, the sample is washed out with a drop or two of solvent and an infrared spectrum obtained.

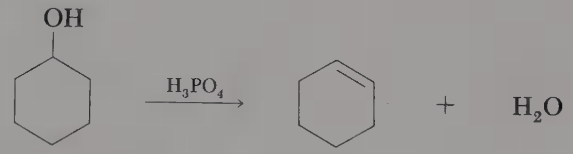
▼
Collecting a sample for an infrared spectrum

11

Olefins from Alcohols; Analysis of a Mixture by Gas Chromatography

KEYWORDS

Dehydration
Pyrolysis
Alumina; silica gel
Phosphoric acid
Cyclohexanol, cyclohexene
Chaser solvent
Xylene
2-Methyl-2-butanol (*t*-amyl alcohol)
2-Methyl-1-butene
2-Methyl-2-butene
Fractional distillation
Anhydrous sodium sulfate
Integration

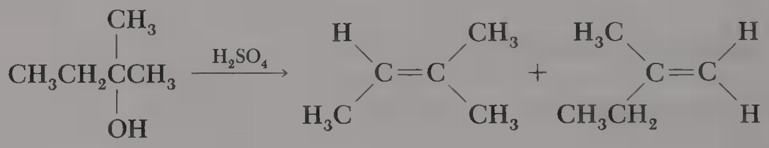


Cyclohexanol
mp 25°, bp 161°,
den 0.96, MW 100.16

Cyclohexene
bp 83°,
den 0.81, MW 82.14

Dehydration of cyclohexanol to cyclohexene can be accomplished by pyrolysis of the cyclic secondary alcohol with an acid catalyst at a moderate temperature or by distillation over alumina or silica gel. The procedure selected for this experiment involves catalysis by phosphoric acid; sulfuric acid is no more efficient, causes charring, and gives rise to sulfur dioxide. When a mixture of cyclohexanol and phosphoric acid is heated in a flask equipped with a fractionating column, the formation of water is soon evident. On further heating, the water and the cyclohexene formed distil together by the principle of steam distillation, and any high-boiling cyclohexanol that may volatilize is returned to the flask. However, after dehydration is complete and the bulk of the product has distilled, the column remains saturated with water–cyclohexene that merely refluxes and does not distil. Hence, for recovery of otherwise lost reaction product, a chaser solvent is added and distillation is continued. A suitable chaser solvent is water-immiscible technical xylene, bp about 140°; as it steam distils it carries over the more volatile cyclohexene. When the total water-insoluble layer is separated, dried, and redistilled through the dried column the chaser again drives the cyclohexene from the column; the difference in boiling points is such that a sharp separation is possible. The holdup in the metal sponge-packed column is so great that if a chaser solvent is not used in the procedure the yield will be only about one-third that reported in the literature.

An alternative experiment is the dehydration of 2-methyl-2-butanol (*t*-amyl alcohol) with dilute sulfuric acid, to a mixture of 2-methyl-1-butene and 2-methyl-2-butene, which can be analyzed by gas chromatography.



2-Methyl-2-butanol
bp 102°, den 0.805,
MW 88.15

2-Methyl-2-butene
bp 38.57°, den 0.662,
MW 70.14

2-Methyl-1-butene
bp 31.16°, den 0.662,
MW 70.14

EXPERIMENTS

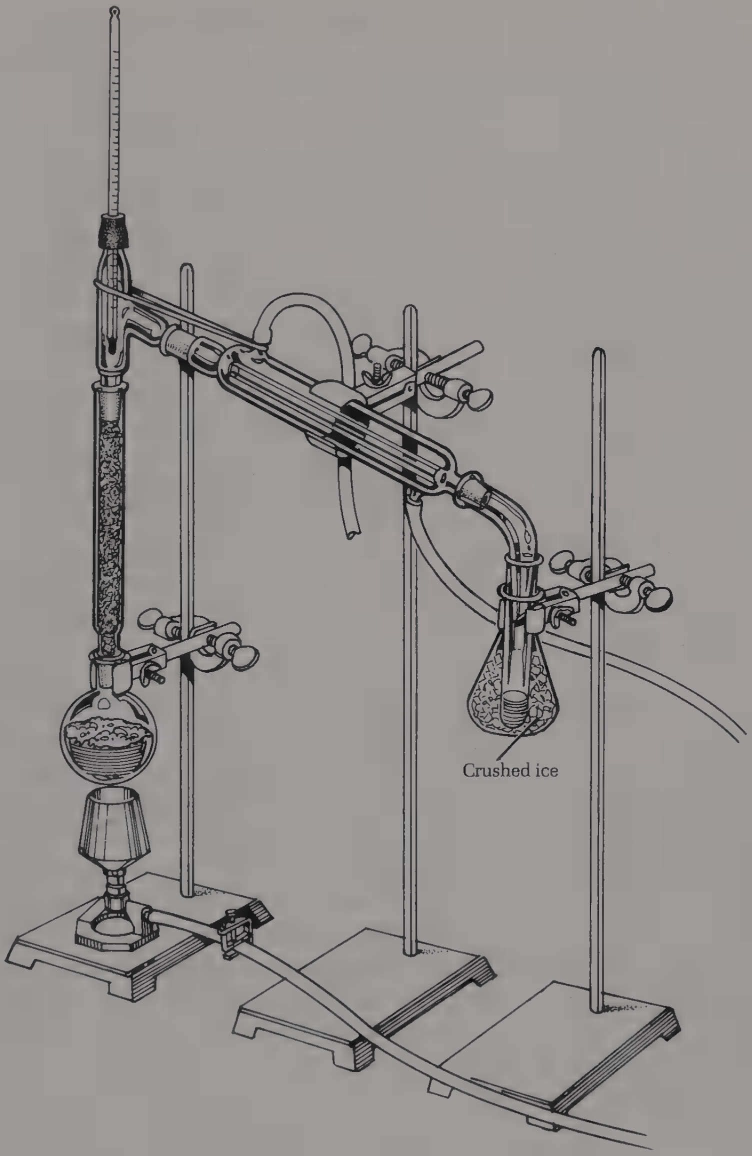
1. Preparation of Cyclohexene

Introduce 20.0 g of cyclohexanol (technical grade), 5 ml of 85% phosphoric acid, and a boiling stone into a 100-ml round-bottomed flask and shake to mix the layers. Use the arrangement for fractional distillation shown in Fig. 2.2 but modified by use of a bent adapter delivering into an ice-cooled test tube in a 125-ml Erlenmeyer receiver, as shown in Fig. 11.1.

▼
Use of a chaser

Note the initial effect of heating the mixture, and then distil until the residue in the flask has a volume of 5–10 ml and very little distillate is being formed; note the temperature range. Then let the assembly cool a little, remove the thermometer briefly, and pour 20 ml of technical xylene (the chaser solvent) into the top of the column through a long-stemmed funnel. Note the amount of the upper layer in the boiling flask and distil again until the volume of the layer has been reduced by about half. Pour the contents of the test tube into a small separatory funnel and rinse with a little chaser solvent; use this solvent for rinsing in subsequent operations. Wash the mixture with an equal volume of saturated sodium chloride solution, separate the water layer, run the upper layer into a clean flask, and add 5 g of anhydrous sodium sulfate (10 × 75-mm test tube-full) to dry it. Before the final distillation note the barometric pressure, apply any thermometer corrections necessary, and determine the reading expected for a boiling point of 83°. Dry the boiling flask,

FIGURE 11.1 Fractionation into an ice-cooled receiver.



column, and condenser, decant the dried liquid into the flask through a stemless funnel plugged with a bit of cotton, and fractionally distil, with all precautions against evaporation losses. The rim of condensate should rise very slowly as it approaches the top of the fractionating column in order that the thermometer may record the true boiling point soon after distillation starts. Record both the corrected boiling point of the bulk of the cyclohexene fraction and the temperature range, which should not be more than 2°. Typical yield, 13.2 g.

2. 2-Methyl-1-butene and 2-Methyl-2-butene

Look up the boiling points[1] and calculate the molecular weights[2] of the starting materials and products.

Pour 36 ml of water into a 250-ml round-bottomed flask, cool in an ice-water bath while **slowly** pouring in 18 ml of concentrated sulfuric acid. Cool this "1:2" acid further with swirling while slowly pouring in 36 ml (30 g) of *t*-amyl alcohol. Shake the mixture thoroughly and then mount the flask for distillation over a microburner as in Fig. 11.1 with the arrangement for ice cooling of the distillate, since the olefin to be produced is very volatile. Use a long condenser and a rapid stream of cooling water. Heat the flask with a small flame of a microburner until distillation of the hydrocarbon is complete. Transfer the distillate to a separatory funnel and shake with about 10 ml of 10% sodium hydroxide solution to remove any traces of sulfurous acid. The aqueous solution sinks to the bottom and is drawn off and discarded. Dry the hydrocarbon layer by filtering it through a cone of anhydrous sodium sulfate and distil the dried product through a fractionating column from a clean, dry flask, taking the same precautions as before to avoid evaporation losses. Collect in a tared (previously weighed) bottle the portion boiling at 37–43°. The yield reported in the literature is 84%; the average student yield is about 50%.

Inject a few microliters of product into a gas chromatograph maintained at room temperature and equipped with a 6 mm dia × 3 m column packed with 10% SE-30 silicone rubber on Chromosorb-W or a similar inert packing. Mark the chart paper at the time of injection. In a few minutes two peaks should appear. From your knowledge of the mechanism of dehydration of secondary alcohols which olefin should predominate? Does this agree with the boiling points? (In general, the compound with the shorter retention time has the lower boiling point.) Measure the relative areas under the two peaks. One way to perform this integration is to cut out the peaks with scissors and weigh the two pieces of paper separately on an analytical balance. Although time-consuming, this method gives very precise results. If the peaks are symmetrical, their areas can be approximated by simply multiplying the height of the peak by its width at half-height.

---

[1] First try the *Handbook of Chemistry and Physics*, CRC Press, West Palm Beach, Florida, or Lange's *Handbook of Chemistry*, Handbook Publishers, Inc., Sandusky, Ohio. If you cannot find the compounds in either of these, try Heilbron's *Dictionary of Organic Compounds*, Oxford University Press, New York, 4th edition, 1965–1973, or Rodd's *Chemistry of Carbon Compounds*, 2nd edition, edited by S. Coffey, Elsevier, Amsterdam, 1964–1976.

[2] See the back end-papers of this book for a convenient table for calculation of molecular weights.

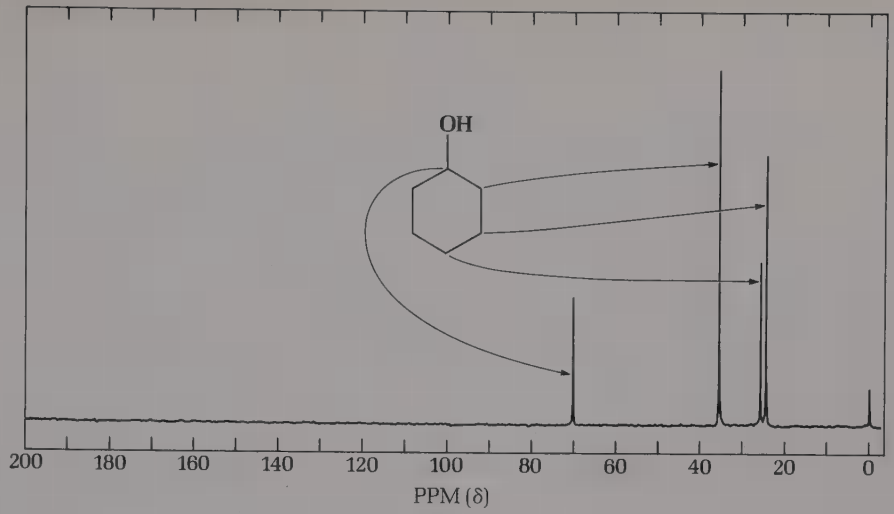


FIGURE 11.2 ^{13}C nmr spectrum of cyclohexanol.

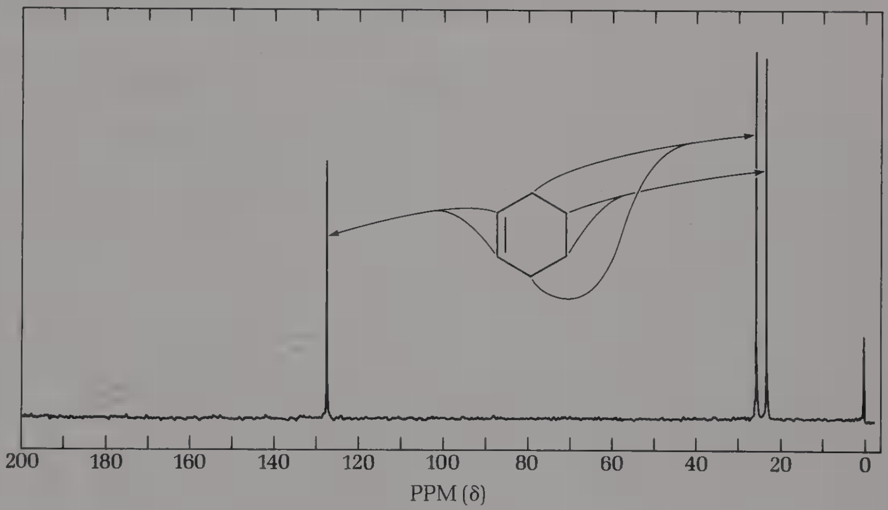


FIGURE 11.3 ^{13}C nmr spectrum of cyclohexene.

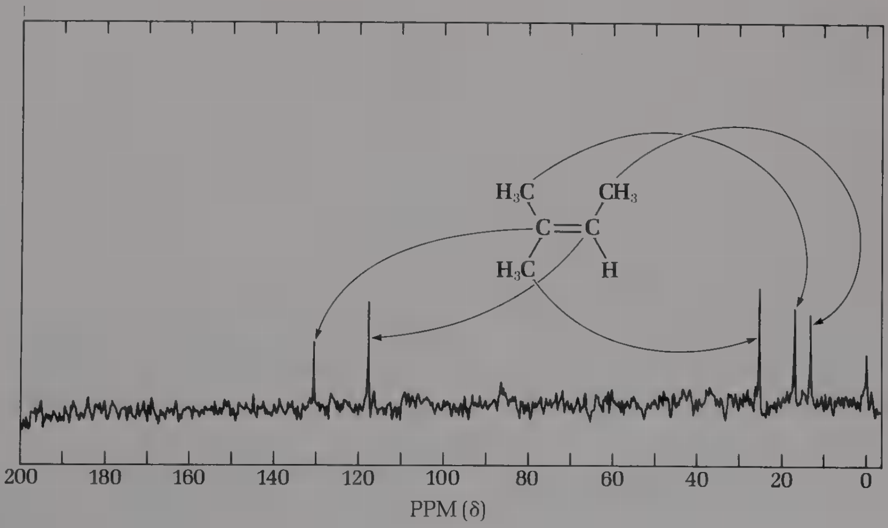


FIGURE 11.4 ^{13}C nmr spectrum of 2-methyl-2-butene.

12

Alkanes and Alkenes

KEYWORDS

- Halogenation
- Free radical chain reaction
- Initiation, propagation, termination
- 2,2′-Azobis-(2-methylpropionitrile), AIBN, initiator
- Sulfuryl chloride
- 1-Chlorobutane
- Gas trap
- Ligroin (saturated alkane)
- Cyclohexene
- Bromine water
- Bromine in CCl_4
- Acid permanganate
- Pyridinium hydrobromide perbromide
- N-Bromosuccinimide
- Bromohydrin
- Pinene
- Rubber

The alkanes are inert compounds that undergo only two important chemical reactions: halogenation and combustion. Combustion of alkanes is the world's leading source of energy and is familiar to everyone. Halogenation, in particular chlorination, is of importance because the products of halogenation are important solvents (chloroform, carbon tetrachloride, dichloromethane) as well as reactive intermediates from which other organic compounds can be derived.

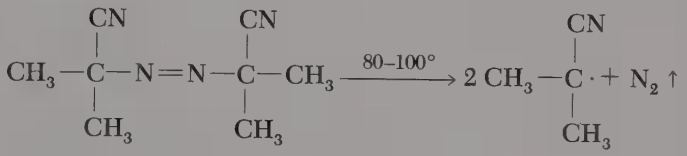
Chlorination is a free-radical chain reaction that proceeds by three sets of steps: initiation, propagation, and termination.

$$\begin{array}{ll}
\mathrm{Cl_2} \xrightarrow[\text{initiator}]{\text{heat, light, or}} 2\,\mathrm{Cl}\cdot & \textit{Chain Initiation} \\
\left.\begin{array}{l}
\mathrm{Cl}\cdot + \mathrm{R{-}H} \longrightarrow \mathrm{H{-}Cl} + \mathrm{R}\cdot \\
\mathrm{R}\cdot + \mathrm{Cl_2} \longrightarrow \mathrm{R{-}Cl} + \mathrm{Cl}\cdot
\end{array}\right\} & \textit{Chain Propagation Steps} \\
\left.\begin{array}{l}
\mathrm{Cl}\cdot + \mathrm{Cl}\cdot \longrightarrow \mathrm{Cl_2} \\
\mathrm{R}\cdot + \mathrm{R}\cdot \longrightarrow \mathrm{R{-}R} \\
\mathrm{R}\cdot + \mathrm{Cl}\cdot \longrightarrow \mathrm{R{-}Cl}
\end{array}\right] & \textit{Chain Termination Steps}
\end{array}$$

Commercially heat (200° to 300°) or ultraviolet light and chlorine gas are used to chlorinate alkanes. In the laboratory it is more convenient to use liquid sulfuryl chloride as the source of chlorine atoms:

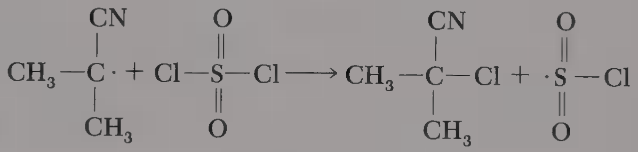
$$SO_2Cl_2 \longrightarrow \cdot SO_2Cl + Cl\cdot$$
$$\cdot SO_2Cl \longrightarrow SO_2 + Cl\cdot$$

and to start the reaction with an initiator, a molecule that easily forms free radicals. We shall use 2,2′-azobis-(2-methylpropionitrile), which on mild heating gives two free radicals and a molecule of nitrogen. These radicals combine with sulfuryl chloride to give a chlorosulfuryl radical that splits into sulfur dioxide and a chlorine radical. These three steps constitute the initiation steps in this method of chlorination:



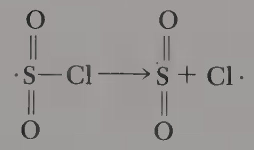
$$CH_3-\underset{CH_3}{\overset{CN}{C}}-N{=}N-\underset{CH_3}{\overset{CN}{C}}-CH_3 \xrightarrow{80\text{–}100^\circ} 2\,CH_3-\underset{CH_3}{\overset{CN}{C}}\cdot + N_2\uparrow$$

2,2′-Azobis-(2-methylpropionitrile)
MW 164.21, mp 102–103° (dec.)



$$CH_3-\underset{CH_3}{\overset{CN}{C}}\cdot + Cl-\underset{O}{\overset{O}{S}}-Cl \longrightarrow CH_3-\underset{CH_3}{\overset{CN}{C}}-Cl + \cdot\underset{O}{\overset{O}{S}}-Cl$$

Sulfuryl chloride
MW 134.97, den 1.67, bp 69°

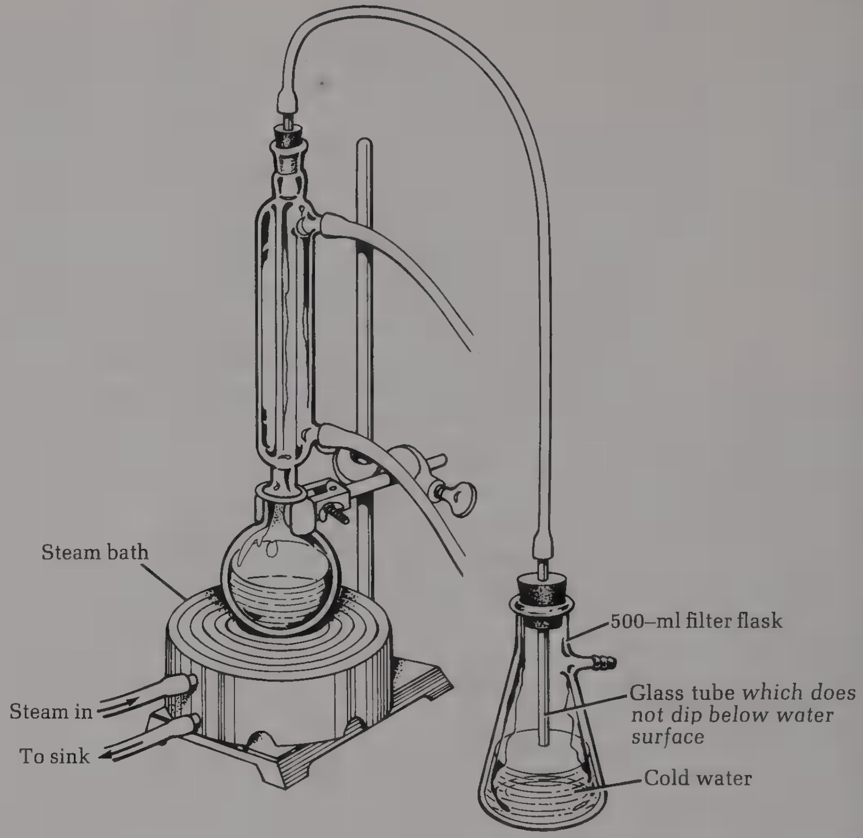


$$\cdot\underset{O}{\overset{O}{S}}-Cl \longrightarrow \underset{O}{\overset{O}{S}} + Cl\cdot$$

The composition of the products from the monochlorination of butane is determined by the probability factor (butane has 4 secondary hydrogens vs. 6 primary hydrogens) and by the relative reactivities of the hydrogens (2° > 1°). In the present experiment 1-chlorobutane is chlorinated to give the four possible products: 1,1-, 1,2-, 1,3- and 1,4-dichlorobutane. The relative amounts of these products depends on the probability factor as well as the relative reactivities of the hydrogens as they are now affected by the chlorine atom on C-1.

For each mole of 1-chlorobutane that is chlorinated a mole of sulfur dioxide gas and hydrochloric acid gas is given off. To render the laboratory habitable these noxious gases must be trapped. There are a number of ways of doing

FIGURE 12.1 Apparatus for chlorination of 1-chlorobutane with SO_2 and HCl gas trap.



this; all depend on dissolving the gases in water. A simple trap is shown in Fig. 12.1. Great care must be taken, no matter which type of trap is used, to insure that water does not get sucked back into the reaction flask.

EXPERIMENT

1. Free Radical Chlorination of 1-Chlorobutane

To a 100-ml round-bottomed flask equipped with a water-cooled reflux condenser and gas trap add 1-chlorobutane (25 ml, 21.6 g, 0.23 mole), sulfuryl chloride (8 ml, 13.5 g, 0.10 mole), 2,2′-azobis-(2-methylpropionitrile) (0.1 g) and a boiling chip. Heat the mixture to gentle reflux on the steam bath for 20 min. Remove the flask from the steam bath, allow it to cool somewhat and then *quickly*, to minimize the escape of sulfur dioxide and hydrochloric acid, lift the condenser from the flask and add a second 0.1-g portion of the initiator. Heat the reaction mixture for an additional 10 min, remove the flask and condenser from the steam bath, and cool the flask in a beaker of water. Pour the contents of the flask through a funnel into about 50 ml of water in a small separatory funnel, shake the mixture, and separate the two phases. Wash the organic phase with two 20-ml portions of 5% sodium bicarbonate solution, once with a 20-ml portion of water, and then dry the organic layer over anhydrous calcium chloride (about 4 g) in a dry Erlenmeyer flask. The mixture can be analyzed by gas chromatography at this point or the unreacted 1-chlorobutane can be removed by fractional distillation (up to bp 85°) and the pot residue analyzed by gas chromatography.

Gas chromatography

A Carbowax column works best, although any other nonpolar phase such as silicone rubber should work as well. Suggested conditions are: 5-mm × 2-m column, 35 ml/min flow rate, column temperature 60°. The order of elution will be first 1-chlorobutane, then 1,1-dichlorobutane, 1,2-dichlorobutane, 1,3-dichlorobutane, and finally 1,4-dichlorobutane.

From the percent yields of the dichlorobutanes calculate the relative reactivities for each hydrogen atom on C-1, C-2, C-3, and C-4 in 1-chlorobutane. How do these results compare with butane where the secondary hydrogens are 3.6 times as reactive as the primary hydrogens?

DISTINGUISHING BETWEEN ALKANES AND ALKENES

The following tests demonstrate properties characteristic of saturated and unsaturated hydrocarbons, provide means of distinguishing between compounds of the two types, and distinguish between pure and impure alkanes. Use your own preparation of cyclohexene as a typical alkene, purified 66–75° ligroin (Eastman Organic Chemicals No. 513) as a typical alkane mixture (the bp of hexane is 69°), and unpurified ligroin (Eastman No. P513) as an impure alkane. Write equations for all positive tests.

2. Bromine Water

Measure 3 ml of a 3% aqueous solution of bromine into each of three 13 × 100-mm test tubes, add 1-ml portions of purified ligroin to two of the tubes and 1 ml of cyclohexene to the third. Shake each tube and record the initial results. Put one of the ligroin-containing tubes in the desk out of the light and expose the other to bright sunlight or hold it close to a light bulb. When a change is noted compare the appearance with that of the mixture kept in the dark.

3. Bromine in Nonaqueous Solution

▼
Carbon tetrachloride is a carcinogen and toxic. Handle with care. Conduct this test in an efficient hood.

Treat 1-ml samples of purified ligroin, unpurified ligroin, and cyclohexene with 5–6 drops of a 3% solution of bromine in carbon tetrachloride. In case decolorization occurs, breathe across the mouth of the tube to see if hydrogen bromide can be detected. If the bromine color persists illuminate the solution and, if a reaction occurs, test as before for hydrogen bromide.

4. Acid Permanganate Test

To 1-ml portions of purified ligroin, unpurified ligroin, and cyclohexene add a drop of an aqueous solution containing 1% potassium permanganate and 10% sulfuric acid and shake. If the initial portion of reagent is decolorized, add further portions.

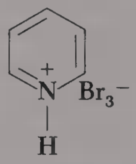
5. Sulfuric Acid

▼
Caution: student-prepared cyclohexene may be wet

Cool 1-ml portions of purified ligroin and cyclohexene in ice, treat each with 3 ml of conc'd sulfuric acid, and shake. Observe and interpret the results. Is any reaction apparent? Any warming? If the mixture separates into two layers, identify them.

6. Bromination with Pyridinium Hydrobromide Perbromide ($C_5H_5\overset{+}{N}HBr_3^-$)[1]

This substance is a crystalline, nonvolatile, odorless complex of high molecular weight (319.84), which, in the presence of a bromine acceptor such as an alkene, dissociates to liberate one mole of bromine. For small-scale experiments it is much more convenient and agreeable to measure and use than free bromine.



Pyridinium hydrobromide perbromide, MW 319.84

Weigh one millimole (320 mg) of the reagent as accurately as possible, put it into a 10-ml Erlenmeyer flask, and add 2 ml of acetic acid. Swirl the mixture and note that the solid is sparingly soluble. Add one millimole of cyclohexene to the suspension of reagent. Swirl, crush any remaining crystals with a flattened stirring rod, and if after a time the amount of cyclohexene appears insufficient to exhaust the reagent, add a little more. When the solid is all

▼
Note for the instructor

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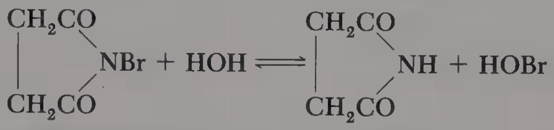
[1] Crystalline material suitable for small-scale experiments is supplied by Aldrich Chemical Co. Massive crystals commercially available should be recrystallized from acetic acid (4 ml per g). Preparation: Mix 15 ml of pyridine with 30 ml of 48% hydrobromic acid and cool; add 25 g of bromine gradually with swirling, cool, collect the product with use of acetic acid for rinsing and washing. Without drying the solid, crystallize it from 100 ml. of acetic acid. Yield of orange needles, 33 g (69%).

dissolved, dilute with water and note the character of the product. By what property can you be sure that it is the reaction product and not starting material?

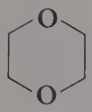
7. Formation of a Bromohydrin

N-Bromosuccinimide in an aqueous solution is in equilibrium with hypobromous acid and may be used to effect addition of this reagent:



N-Bromosuccinimide
MW 178.00

Succinimide



Dioxane
Bp 101.5°, den 1.04
(Dissolves organic compounds, miscible with water.)

▼

Dioxane has been reported to be a carcinogen. Conduct this test in a hood.

Weigh 178 mg of N-bromosuccinimide, put it into a 13 × 100-mm test tube and add 0.5 ml of dioxane and 1 millimole of cyclohexene. In another tube chill 0.2 ml of water and add to it 1 millimole of conc'd sulfuric acid. Transfer the cold dilute solution to the first tube with the capillary dropper. Note the result and the nature of the product that separates on dilution with water.

8. Tests for Unsaturation

Determine which of the following hydrocarbons are saturated and which are unsaturated or contain unsaturated material. Use any of the above tests that seem appropriate.

Pinene, the principal constituent of turpentine oil
Paraffin oil, a purified petroleum product
Gasoline produced by cracking
Cyclohexane
Rubber (The adhesive Grippit and other rubber cements are solutions of unvulcanized rubber. Squeeze a drop of it onto a stirring rod and dissolve it in toluene. For tests with permanganate or with bromine in carbon tetrachloride use only a drop of the former and just enough of the latter to produce coloration.)

13

n-Butyl Bromide

KEYWORDS

Preparative experiment
Stoichiometry
Laboratory notebook
Hydrobromic acid

n-Butyl alcohol
1-Butene
Dibutyl ether

Anhydrous calcium chloride
Washing in separatory funnel
Decant (pour off)

$$CH_3CH_2CH_2CH_2OH \xrightarrow{NaBr,\ H_2SO_4} CH_3CH_2CH_2CH_2Br + NaHSO_4 + H_2O$$

n-Butyl alcohol
bp 118°
den 0.810,
MW 74.12

n-Butyl bromide
bp 101.6°
den 1.275,
MW 137.03

▼
Choice of reagents

A primary alkyl bromide can be prepared by heating the corresponding alcohol with (a) constant-boiling hydrobromic acid (47% HBr); (b) an aqueous solution of sodium bromide and excess sulfuric acid, which is an equilibrium mixture containing hydrobromic acid; or (c) with a solution of hydrobromic acid produced by bubbling sulfur dioxide into a suspension of bromine in water. Reagents (b) and (c) contain sulfuric acid at a concentration high enough to dehydrate secondary and tertiary alcohols to undesirable by-products (alkenes and ethers) and hence the HBr method (a) is preferred for preparation of halides of the types R_2CHBr and R_3CBr. Primary alcohols are more resistant to dehydration and can be converted efficiently to the bromides by the more economical methods (b) and (c), unless they are of such high molecular weight as to lack adequate solubility in the aqueous mixtures. The NaBr-H_2SO_4

method is preferred to the Br_2-SO_2 method because of the unpleasant, choking property of sulfur dioxide. The overall equation is given above, along with key properties of the starting material and principal product.

▼
The laboratory notebook

The procedure that follows specifies a certain proportion of *n*-butyl alcohol, sodium bromide, sulfuric acid, and water; defines the reaction temperature and time; and describes operations to be performed in working up the reaction mixture. The prescription of quantities is based upon considerations of stoichiometry as modified by the results of experimentation. Before undertaking a preparative experiment you should analyze the directions and calculate the molecular properties of the reagents. Construction of tables (see below) of properties of starting material, reagents, products, and by-products provides guidance in regulation of temperature and in separation and purification of the product and should be entered in the laboratory notebook.

Reagents

Reagent	MW	Den	Bp	Wt used (g)	Moles	
					Theory	*Used*
n-C_4H_9OH	74.12	0.810	118°	16.2	0.22	0.22
NaBr	102.91	—	—	27.0	.22	.26
H_2SO_4	98.08	1.84	—	42.3	.22	.44

Product and By-Products

Compound	MW	Den	Bp		Yield	
			Given	*Found*	*Theory*	*Found*
n-C_4H_9Br	137.03	1.275	101.6°		30.1 g(100%)	__g__%
$CH_3CH_2CH{=}CH_2$			−6.3°			
$C_4H_9OC_4H_9$			141°			

One mole of *n*-butyl alcohol theoretically requires one mole each of sodium bromide and sulfuric acid, but the procedure calls for use of a slight excess of bromide and twice the theoretical amount of acid. Excess acid is used to shift the equilibrium in favor of a high concentration of hydrobromic acid. The amount of sodium bromide taken, arbitrarily set at 1.2 times the theory as an insurance measure, is calculated as follows:

$$\frac{16.2\ (\text{g of } C_4H_9OH)}{74.12\ (\text{MW of } C_4H_9OH)} \times 102.91\ (\text{MW of NaBr}) \times 1.2 = 27.0\ \text{g of NaBr}$$

The theoretical yield is 0.22 mole of product, corresponding to the 0.22 mole of butyl alcohol taken; the maximal weight of product is calculated thus:

$$0.22\ (\text{mole of alcohol}) \times 137.03\ (\text{MW of product}) = 30.1\ \text{g butyl bromide}$$

The probable by-products are 1-butene, dibutyl ether, and the starting alcohol. The alkene is easily separable by distillation, but the other substances are in the same boiling point range as the product. However, all three possible by-products can be eliminated by extraction with conc'd sulfuric acid.

EXPERIMENT

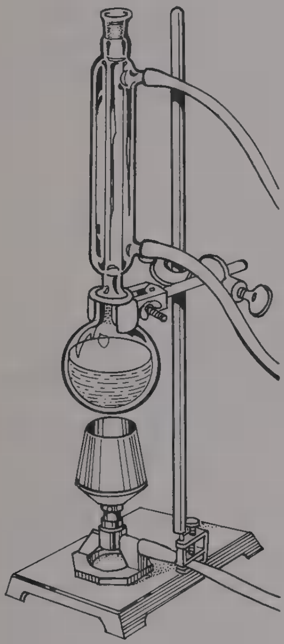


FIGURE 13.1 Refluxing a reaction mixture.

Put 27.0 g of sodium bromide, 30 ml of water, and 20 ml of *n*-butyl alcohol in a 250-ml round-bottomed flask, cool the mixture in an ice-water bath, and slowly add 23 ml of concentrated sulfuric acid with swirling and cooling. Mount the flask over a microburner and fit it with a short condenser for reflux condensation (Fig. 13.1). Heat to the boiling point, note the time, and adjust the flame for brisk, steady refluxing. The upper layer that soon separates is the alkyl bromide, since the aqueous solution of inorganic salts has a greater density. Reflux for 30 minutes, remove the flame, and let the condenser drain for a few minutes (extension of the reaction period to 1 hr increases the yield by only 1–2%). Remove the condenser, mount a stillhead in the flask, and set the condenser for downward distillation through a bent adapter into a 125-ml Erlenmeyer. Distil the mixture, make frequent readings of the temperature, and distil until no more water-insoluble droplets come over, by which time the temperature should have reached 115° (collect a few drops of distillate in a test tube and see if it is water soluble). The increasing boiling point is due to azeotropic distillation of *n*-butyl bromide with water containing increasing amounts of sulfuric acid, which raises the boiling point.

Pour the distillate into a separatory funnel, shake with about 20 ml of water, and note that *n*-butyl bromide now forms the lower layer. A pink coloration in this layer due to a trace of bromine can be discharged by adding a pinch of sodium bisulfite and shaking again. Drain the lower layer of *n*-butyl bromide into a clean flask, clean and dry the separatory funnel, and return the *n*-butyl bromide to it. Then cool 20 ml of conc'd sulfuric acid thoroughly in an ice bath and add the acid to the funnel, shake well, and allow 5 min for separation of the layers. The relative densities given in the tables presented in the introduction of this experiment identify the two layers; an empirical method of telling the layers apart is to draw off a few drops of the lower layer into a test tube and see whether the material is soluble in water (H_2SO_4) or insoluble in water (butyl bromide). Separate the layers, allow 5 min for further drainage, and separate again. Then wash the *n*-butyl bromide with 20 ml of 10% sodium hydroxide solution to remove traces of acid, separate, and be careful to save the proper layer.

▼
Calcium chloride: removes water and alcohol from a solution

Dry the cloudy *n*-butyl bromide by adding 2 g of anhydrous calcium chloride and warming the mixture gently on the steam bath with swirling until the liquid clears. Decant the dried liquid into a 50-ml flask through a funnel fitted with a small loose plug of cotton, add a boiling stone, distil, and collect material boiling in the range 99–103°. A typical student yield is in the range 21–25 g. Note the approximate volumes of forerun and residue.

▼
A proper label is important

Put the sample in a narrow-mouth bottle of appropriate size; make a neatly printed label giving the name and formula of the product and your name. Press the label onto the bottle under a piece of filter paper and make sure that it is secure. After all the time spent on the preparation, the final product should be worthy of a carefully executed and secured label.

QUESTIONS

1. What experimental method would you recommend for the preparation of *n*-octyl bromide? *t*-Butyl bromide?
2. Explain why the crude product is apt to contain certain definite organic impurities.
3. How does each of these impurities react with sulfuric acid when the crude *n*-butyl bromide is shaken with this reagent?

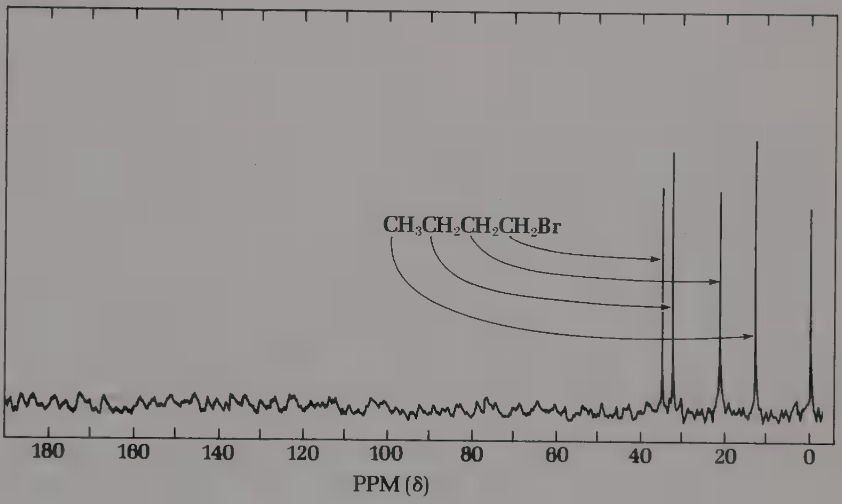


FIGURE 13.2 ^{13}C nmr spectrum of *n*-butyl bromide.

14

Aldehydes and Ketones

KEYWORDS

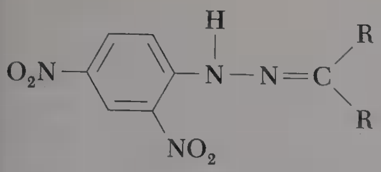
Phenylhydrazine
Phenylhydrazones
Semicarbazide
Semicarbazones
Bisulfite addition product
Iodoform test
Fluorene, fluorenone
Acid dichromate oxidation
Water-soluble carbonyl derivative
Tollens test
Girard reagent
Trimethylaminoacetohydrazide chloride
NMR and IR spectra

Sections 1–5 of this chapter present orientation experiments in preparation for the identification of unknowns (Section 6). First a test is described for a carbonyl compound by its reaction with phenylhydrazine in the presence of acetic acid as catalyst. All aldehydes and nearly all ketones rapidly form phenylhydrazones.

$$R_2C{=}O + \underset{\text{Phenylhydrazine}}{H_2NNHC_6H_5} \longrightarrow \underset{\text{Phenylhydrazone}}{R_2C{=}NNHC_6H_5} + H_2O$$

The increase in molecular weight of 90 units attending the conversion renders the derivative so much less soluble than the starting material that it precipitates or crystallizes and thus gives evidence that a reaction has occurred. The phenylhydrazine test to be carried out later is a generally applicable method of distinguishing aldehydes and ketones from substances of other classes.

Many phenylhydrazones are crystalline compounds suitable for characterization by melting point and mixed melting point determination, but some are oils and some are unstable. 2,4-Dinitrophenylhydrazones, prepared with 2,4-dinitrophenylhydrazine, $(NO_2)_2C_6H_3NHNH_2$, are often preferable for



2,4-Dinitrophenylhydrazone of a ketone

characterization because they are higher melting, less soluble, and more stable than the corresponding phenylhydrazones; the molecular weight change on conversion to the derivative is 180 units. Semicarbazones have comparable advantageous properties.

$$\underset{\textbf{Semicarbazide}}{R_2C{=}O + H_2NNHCONH_2} \longrightarrow \underset{\textbf{Semicarbazone}}{R_2C{=}NNHCONH_2} + H_2O$$

Oximes are less useful for characterization, for many are liquids at room temperature and those that are solids are often obtainable in crystalline form only with difficulty (the preparation of an oxime is described in Chapter 32). If a substance is to be compared with a compound that is known but not available for mixed melting point determination, probable identity can be established by showing that several derivatives of the substance all correspond in melting point with known derivatives. After the experience of preparing the phenylhydrazone, 2,4-dinitrophenylhydrazone, and semicarbazone derivatives of known compounds, you may find similar derivatives useful in characterization of your unknowns.

Nearly all aldehydes, unhindered cyclic ketones, and most methyl ketones afford solid, water-soluble bisulfite addition compounds, and the iodoform test distinguishes a methyl ketone from all aldehydes but acetaldehyde. Methyl aryl ketones such as acetophenone, $C_6H_5COCH_3$, fail to give bisulfite addition compounds but are recognizable by the iodoform test.

Aldehydes are very easily oxidized to carboxylic acids. The most general method for distinguishing between aldehydes and ketones depends on this oxidation/reduction reaction. In the Tollens test a silver complex is reduced to a metallic silver mirror, in the Fehling and Benedict tests a copper(II) complex is reduced to a red copper(I) oxide precipitate. Since many sugars, such as glucose, contain aldehyde groups these tests are often applied to this group of compounds (see Chapter 29).

EXPERIMENTS

1. 2,4-Dinitrophenylhydrazones

▼
Reagent: $(NO_2)_2C_6H_3NHNH_2$
MW 198.14, mp 197°

2,4-Dinitrophenylhydrazine[1] is a red solid of high melting point that has low solubility in ethanol. The yellow phosphate salt of this hydrazine (a base) is more soluble than the hydrazine itself, and a 0.1 *M* solution of the hydrazine in phosphoric acid-ethanol[2] is used in the following experiment and in the investigation of unknowns.

▼
Notes for the instructor

---

[1] The reagent is available commercially but expensive. *Preparation.* Dissolve 100 g of 2,4-dinitrochlorobenzene (mp 50–52°) in 200 ml of triethylene glycol, stir mechanically in a salt-ice bath to 15° (or until crystallization starts), and add 28 ml of 64% hydrazine solution (*Caution! Carcinogen*) by drops at a temperature of 20 ± 3° (25 min). When the strongly exothermal reaction is over, digest the paste on the steam bath, add 100 ml of methanol and digest further, then cool, collect, and wash with methanol. Yield 98 g (100%), mp 190–192°.

[2] Dissolve 2.0 g of 2,4-dinitrophenylhydrazine in 50 ml of 85% phosphoric acid by heating, cool, add 50 ml of 95% ethanol, cool again, and clarify by suction filtration from a trace of residue.

▼
Procedure for unknowns

To 10 ml (1 millimole) of the 0.1 *M* solution of 2,4-dinitrophenylhydrazine in phosphoric acid add 1 millimole of diethyl ketone, warm for a few minutes, and let crystallization proceed. Collect the product by suction filtration, wash the crystals with water to remove phosphoric acid, press the product as dry as possible and crystallize from ethanol. The melting point reported is 156°; note the contrast between this derivative and the phenylhydrazone of the same ketone.

A second experiment illustrates an alternative procedure, applicable where the 2,4-dinitrophenylhydrazone is known to be sparingly soluble in ethanol. The test substance is cinnamaldehyde ($C_6H_5CH{=}CHCHO$, MW 132.15), an α,β-unsaturated aldehyde. Measure 1 millimole of crystalline 2,4-dinitrophenylhydrazine into a 125-ml Erlenmeyer flask, add 30 ml of 95% ethanol, digest on the steam bath until all particles of solid are dissolved, and then add 1 millimole of cinnamaldehyde and continue warming. If there is no immediate change, add, from a Pasteur pipette, 6–8 drops of concentrated hydrochloric acid as catalyst and note the result. Warm for a few minutes, then cool and collect the product. Note that the derivative is more intensely colored than that of diethyl ketone; this is because it contains an α,β-double bond conjugated with the carbon-nitrogen double bond.

The alternative procedure strikingly demonstrates the catalytic effect of hydrochloric acid, but it is not applicable to a substance like diethyl ketone, whose 2,4-dinitrophenylhydrazone is much too soluble to crystallize from the large volume of ethanol. The first procedure is obviously the one to use for an unknown.

2. Phenylhydrazones

▼
Reagent: $C_6H_5NHNH_2$
MW 108.14, den 1.10

To prepare a stock solution of the phenylhydrazine reagent, measure 1.0 ml of phenylhydrazine with a calibrated pipette into a 25-ml Erlenmeyer flask, add 3 ml of acetic acid from a 10-ml graduate, swirl, and note the heat of neutralization; dilute the solution with 4–5 ml of water, pour it into a 10-ml graduate, and make up the volume to 10.0 ml. Return the solution to the Erlenmeyer flask for storage. The molecular weight and specific gravity of phenylhydrazine are such that 1 ml of the stock solution contains 1 millimole of phenylhydrazine acetate, the amine salt of acetic acid. Excess acetic acid is used both as condensation catalyst and as solvent for the reagent.

▼
Phenylhydrazones do not crystallize as readily as 2,4-dinitrophenylhydrazones.

A substance to be tested for the presence of a carbonyl group should first be examined to see whether or not it is soluble in water; if 8–10 micro drops dissolve in 1 ml of water at room temperature, the test is conducted by dissolving one millimole of sample in 1 ml of water and adding 1 ml of the stock solution (which contains 1 millimole of phenylhydrazine acetate); separation of an oil or solid constitutes a positive indication that the substance has a carbonyl group. Test acetone (5 drops) in this way.

▼
(a) Water-soluble substances

Diethyl ketone (MW 86.13) is soluble to the extent of about 4 drops (47 mg) in 1 ml of water. Measure 1 millimole of the ketone into 1 ml of water in a small flask or test tube, and note that the first few drops dissolve but that an oily layer eventually appears. Add a few drops of methanol to just bring the oil into solution and then add 1 ml of stock phenylhydrazine solution. Rub the oil against the flask with a stirring rod briefly to see if it will crystallize easily and if not, discard it.

▼

(b) Water-insoluble sample

A typical water-insoluble liquid ketone is mesityl oxide, $(CH_3)_2C{=}CH{-}COCH_3$, MW 98.14. Dissolve 1 millimole of the ketone in 1 ml of methanol, and add 1 ml of phenylhydrazine solution. Let the mixture stand for 5 min, collect the product, and see whether the melting point corresponds with the value 142° reported for the pure derivative. A water-insoluble solid is likewise tested in methanol solution.

Save the residual stock solution for testing unknowns.

▼

Reagent: $H_2NCONHNH_3^+Cl^-$
MW 111.54

3. Semicarbazones

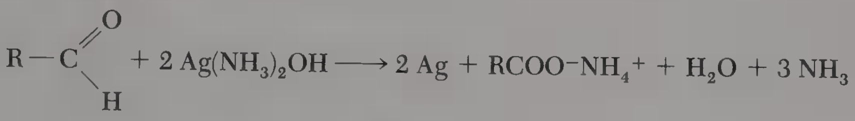
Semicarbazide (mp 96°) is not very stable in the free form and is used as the crystalline hydrochloride (mp 173°). Since this salt is insoluble in methanol or ethanol and does not react readily with typical carbonyl compounds in alcohol-water mixtures, a basic reagent is added to liberate free semicarbazide. In one procedure a suspension of semicarbazide hydrochloride and sodium carbonate in methanol is digested, filtered from the sodium chloride formed, and the carbonyl compound added to the filtrate. In another procedure a mixture of semicarbazide hydrochloride and sodium acetate in methanol-water is employed. The procedure that follows utilizes the aromatic amine pyridine as the basic reagent.

Procedure

Prepare a stock solution by dissolving 1.11 g of semicarbazide hydrochloride in 5 ml of water; 0.5 ml of this solution contains 1 millimole of reagent. To 0.5 ml of the stock solution add 1 millimole of acetophenone and enough methanol (1 ml) to produce a clear solution; then add 10 drops of pyridine (a two-fold excess) and warm the solution gently on the steam bath for a few minutes until crystals begin to separate. The melting point of the pure product is 198°.

Repeat the reaction with cinnamaldehyde instead of acetophenone and see if you can observe a difference. Cinnamaldehyde semicarbazone melts at 215°.

4. Tollens Test



$$\mathrm{R{-}C}\begin{array}{l}\diagup\!\!\!\diagup \mathrm{O}\\ \diagdown \mathrm{H}\end{array} + 2\,\mathrm{Ag(NH_3)_2OH} \longrightarrow 2\,\mathrm{Ag} + \mathrm{RCOO^-NH_4^+} + \mathrm{H_2O} + 3\,\mathrm{NH_3}$$

Tollens reagent, a solution of silver ammonium hydroxide and sodium hydroxide, is reduced by aldehydes, with deposition of metallic silver partly in the form of a mirror.

Add a few ml of 10% sodium hydroxide to each of the five marked test tubes and heat the tubes in the water bath (for thorough cleaning) while preparing a sufficient amount of Tollens reagent for five tests. Measure 2 ml of 5% silver nitrate solution and 1 ml of 10% sodium hydroxide into a test tube, and make a dilute solution of ammonia by mixing 1 ml of concentrated ammonia solution with 10 ml of distilled water. Add 0.5 ml of this solution to the precipitated silver oxide, stopper the tube, and shake. Repeat the process until almost all of the precipitate dissolves (3 ml, avoid an excess) and then dilute the solution to a volume of 10 ml. Empty the five tubes, rinse them with distilled water, and into each tube put one drop of a 0.1 *M* solution of glucose, a drop of cinnamaldehyde, acetone, acetophenone, and benzaldehyde. Add 1 ml of Tollens reagent to each tube and let the reactions proceed at room temperature at first. Watch closely and try to define the order of reactivity as measured not by the color of a solution but by the time of appearance of the first precipitate of metal. Silver can be removed from the test tubes with nitric acid.

5. Iodoform Test

The reagent contains iodine in potassium iodide solution[3] at a concentration such that 2 ml of solution, on reaction with excess methyl ketone, will yield 174 mg of iodoform. If the substance to be tested is water-soluble, dissolve 4 drops of a liquid or an estimated 50 mg of a solid in 2 ml of water in a 20 × 150-mm test tube; add 2 ml of 10% sodium hydroxide and then slowly add 3 ml of the iodine solution. In a positive test the brown color of the reagent disappears and yellow iodoform separates. If the substance to be tested is insoluble in water, dissolve it in 2 ml of dioxane, proceed as above, and at the end dilute with 10 ml of water.

▼
Caution! Dioxane and chloroform are mild carcinogens. Carry out these tests in a hood. Avoid all contact with the solvents.

Test hexane-2,5-dione (water-soluble), *n*-butyraldehyde (water-soluble), and acetophenone (water-insoluble).

Iodoform can be recognized by its odor and yellow color and, more securely, from the melting point (119°). The substance can be isolated by suction filtration of the test suspension or by adding 2 ml of chloroform, shaking the stoppered test tube to extract the iodoform into the small lower layer, withdrawing the clear part of this layer with a capillary dropping tube, and evaporating it in a small tube on the steam bath. The crude solid is crystallized from methanol-water.

6. Bisulfite Test

Prepare a stock solution from 5 g of sodium bisulfite dissolved in 20 ml of water with brief swirling. Put 1 ml of the solution into each of five 13 × 100-mm test tubes and to each tube add 5 drops of the following substances:

▼
Note to Instructor

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[3] Dissolve 25 g of iodine in a solution of 50 g of potassium iodide in 200 ml of water.

benzaldehyde, 4-methylpentan-2-one, cinnamaldehyde, diethyl ketone, and acetophenone. Shake each tube occasionally during the next 10 min and note the results.

If the bisulfite test is applied to a liquid or solid that is very sparingly soluble in water, formation of the addition product is facilitated by adding a small amount of methanol before the addition of the bisulfite solution.

7. NMR Spectroscopy

Only the acidic protons of carboxylic acids, phenols, and enols appear farther downfield than aldehydic protons, which appear at *ca.* 9.6–10 ppm, and are thus easily recognized.

8. Infrared Spectroscopy

The carbon-oxygen stretching vibration of the carbonyl group provides one of the most intense and useful bands in infrared spectroscopy. Aldehydes generally absorb at 1725 cm^{-1} and aliphatic ketones at 1715 cm^{-1}, too close to each other to distinguish these functional groups, but an aldehydic C–H stretch at 2720 cm^{-1} and 2820 cm^{-1} is definite. Aryl alkyl ketones, such as acetophenone, can absorb anywhere between 1680 and 1700 cm^{-1}, depending on the nature of substituents on the aromatic ring. α,β-Unsaturated ketones fall in this same frequency range.

Open chain, saturated ketones have carbonyl stretching frequencies near 1715 cm^{-1}, such as six-membered ring ketones have. As the ring size decreases, the frequency changes to 1740 cm^{-1} for five-membered ring ketones and 1770 cm^{-1} for four-membered ring ketones.

9. Unknowns

Your unknown may be any of the aldehydes or ketones listed in Table 14.1 or it may be a noncarbonyl compound.[4] Hence, the first test should be that with phenylhydrazine reagent; if this test is negative, report accordingly and proceed to another unknown. At least one derivative of the unknown is to be submitted to the instructor, but if you first do the bisulfite and iodoform characterizing tests, the results may suggest derivatives whose melting points will be particularly revealing.

▼
Note for the instructor

10. Citral

Prepare the 2,4-dinitrophenylhydrazone of the material you isolated from lemon grass oil (Chapter 5, Section 3) and so extend your previous characterization of the functional groups present.

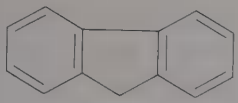
---

[4] See Tables 58.3 and 58.14 in Chapter 58 for additional aldehydes and ketones.

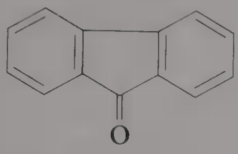
Table 14.1 Melting Points of Derivatives of Some Aldehydes and Ketones

					Melting Points		
Compound	Formula	MW	Den	Water solubility	Phenyl-hydrazone	2,4-DNP	Semi-carbazone
Acetone	CH_3COCH_3	58.08	0.79		42	126	187
n-Butanal	$CH_3CH_2CH_2CHO$	72.10	0.82	4 g/100 g	Oil	123	95(106)[a]
Diethyl ketone	$CH_3CH_2COCH_2CH_3$	86.13	0.81	4.7 g/100 g	Oil	156	138
Furfural	$C_4H_3O \cdot CHO$	96.08	1.16	9 g/100 g	97	212(230)[a]	202
Benzaldehyde	C_6H_5CHO	106.12	1.05	Insol.	158	237	222
Hexane-2,5-dione	$CH_3COCH_2CH_2COCH_3$	114.14	0.97	∞	120[b]	257[b]	224[b]
2-Heptanone	$CH_3(CH_2)_4COCH_3$	114.18	0.83	Insol.	Oil	89	123
3-Heptanone	$CH_3(CH_2)_3COCH_2CH_3$	114.18		Insol.	Oil	81	101
n-Heptanal	$n\text{-}C_6H_{13}CHO$	114.18	0.82	Insol.	Oil	108	109
Acetophenone	$C_{16}H_5COCH_3$	120.66	1.03	Insol.	105	238	198
Acetophenone	$C_6H_5COCH_3$	120.66	1.03	Insol.	105	238	198
2-Octanone	$CH_3(CH_2)_5COCH_3$	128.21	0.82	Insol.	Oil	58	122
Cinnamaldehyde	$C_6H_5CH{=}CHCHO$	132.15	1.10	Insol.	168	255	215
Propiophenone	$C_6H_5COCH_2CH_3$	134.17	1.01	Insol.	about 48°	191	182

[a] Both mps have been found, depending on crystalline form of derivative.
[b] Derivative.



Fluorene
Mp 114°, MW 166.22



Fluorenone
Mp 83°, MW 180.21

11. Preparation of a Ketone-Hydrocarbon Mixture

This mixture of a ketone and a hydrocarbon (fluorenone and fluorene) is to be used in the separation experiment employing Girard's reagent (next section) and also in the separation by chromatography experiment (Chapter 16). The hydrocarbon component, fluorene, is inexpensive, but the yellow ketone derived from it by dichromate oxidation is very costly because of difficulty of complete oxidation. Hence, you are to prepare your own mixture by brief, partial oxidation of fluorene.

▼
One half hour unattended heating

In a 250-ml Erlenmeyer flask dissolve 5.0 g of practical grade fluorene in 25 ml of acetic acid by heating on the steam bath with occasional swirling. In a 125-ml Erlenmeyer dissolve 15 g of sodium dichromate dihydrate in 50 ml of acetic acid by swirling and heating on a hot plate. Adjust the temperature of the dichromate solution to 80°, transfer the thermometer and adjust the fluorene-acetic acid solution to 80°, and then, **under the hood**, pour in the dichromate solution. Note the time, the temperature of the solution, and heat on the steam bath for 30 min. Observe the maximum and final temperature, and then cool the solution and add 150 ml of water. Swirl the mixture for a full two minutes to coagulate the product and so promote rapid filtration, and collect the yellow solid in an 8.5-cm Büchner funnel (in case filtration is slow, empty the funnel and flask into a beaker and stir vigorously for a few minutes). Wash the filter cake well with water and then suck the filter cake as dry as possible. Either let the product dry overnight, or dry it quickly as follows: Put the moist solid into a 50-ml Erlenmeyer, add ether (20 ml) and swirl to dissolve, and add anhydrous sodium sulfate (10 g) to scavenge the water. Decant the ethereal solution through a cone of anhydrous sodium sulfate into a 125-ml Erlenmeyer, and rinse the flask and funnel with ether. Evaporate on the steam bath under an aspirator, heat until the ether is all removed, and pour the hot oil into a 50-ml beaker to cool and solidify. Scrape out the yellow solid; yield 4.0 g. Save for experiments in Section 12 of this chapter and Section 3 of Chapter 16.

12. Girard Separation of a Fluorene-Fluorenone Mixture

▼
Girard reagent:
$Cl^-[CH_3)_3\overset{+}{N}CH_2CONHNH_2]$
MW 167.64

Girard's reagent, trimethylaminoacetohydrazide chloride, of structure similar to that of semicarbazide, condenses with carbonyl compounds in ethanol containing a little acetic acid as catalyst to give derivatives of the type $Cl^-[(CH_3)_3\overset{+}{N}CH_2CONHN{=}CR_2]$, which are soluble in water because of the presence of the dipolar ionic grouping. A Girard derivative, once formed and extracted from a water-ether mixture into the aqueous phase, is hydrolyzed easily with excess water under catalysis by mineral acid, with regeneration of the carbonyl compound.

In a 25 × 150-mm test tube, place 0.5 g of the fluorene-fluorenone mixture, 0.5 g of Girard reagent, 0.5 ml of acetic acid, and 5 ml of 95% ethanol. Reflux the mixture for 30 min. using a 10-ml round-bottomed flask and condenser or, better, a cold finger condenser, made from a 20 × 150-mm test tube with side arm and conveniently supported at any height desired by a No. 3 neoprene adapter (Fig. 14.1). Then cool the solution, pour it into a separatory funnel, and rinse the tube with 25 ml of ether and then with 10 ml of water. Add 25 ml of saturated sodium chloride solution (to avoid an emulsion), shake well and let the layers separate. Run the lower aqueous layer into a second separatory funnel, wash the ethereal layer with a little water (10 ml), add the wash water to the main water extract, and extract this once with ether. Then run the aqueous layer into a 125-ml Erlenmeyer, add 1 ml of concentrated hydrochloric acid, and heat on the steam bath for 10 min to hydrolyze the Girard derivative and drive off dissolved ether.

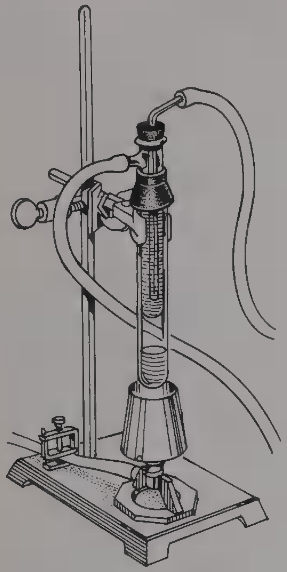


FIGURE 14.1 Cold finger reflux condenser.

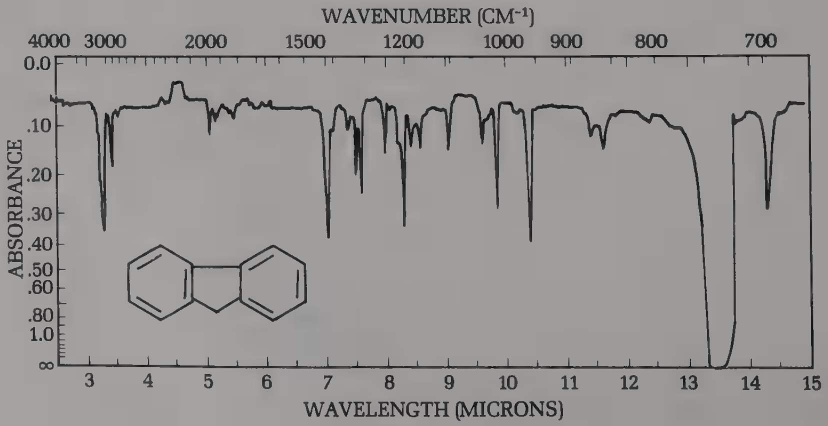
During the heating period, wash the ethereal solution in turn with 5% sodium bicarbonate solution and saturated sodium chloride solution and filter it through anhydrous sodium sulfate. This ethereal solution of nonketonic material may be colored from impurities; if so, it should be shaken briefly with decolorizing charcoal, filtered, and evaporated to dryness under an aspirator tube. Dissolve the residue in the least amount of methanol required (about 5 ml), let crystallization proceed at room temperature, and then at 0°; collect and wash the product with chilled methanol. The resulting fluorene should be colorless and the melting point close to 114°; yield 140 mg.

Hydrolysis of the Girard derivative gives a yellow oil, which solidifies on cooling in ice to a bright yellow solid. Collect the solid (crude fluorenone) and let it dry (170 mg, mp 75–78°); crystallize it from a very small volume of 66–75° ligroin. The fluorenone forms beautiful, large, bright, yellow spars, mp 82–83°.

QUESTIONS

1. What is the purpose of making derivatives of unknowns?
2. Why are 2,4-dinitrophenylhydrazones better derivatives than phenylhydrazones?
3. Using chemical tests how would you distinguish among 2-pentanone, 3-pentanone, and pentanal?

FIGURE 14.2 Infrared spectrum of fluorene in CS_2.



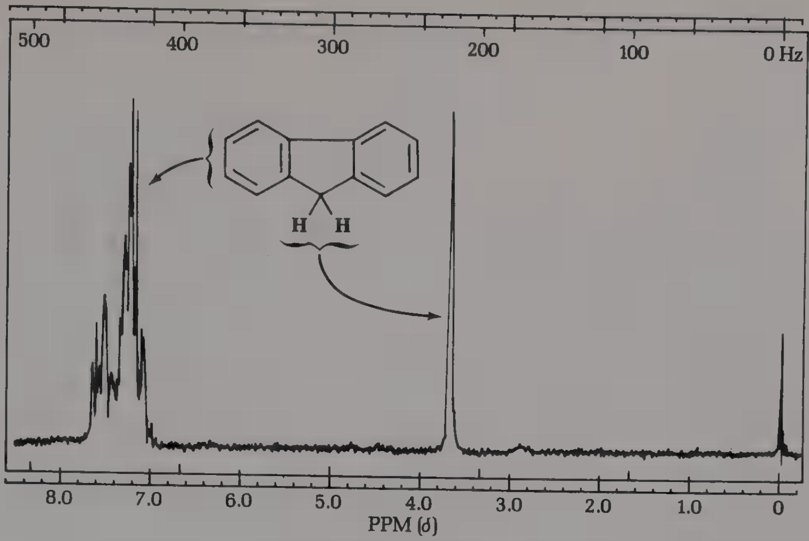


FIGURE 14.3 Nmr spectrum of fluorene.

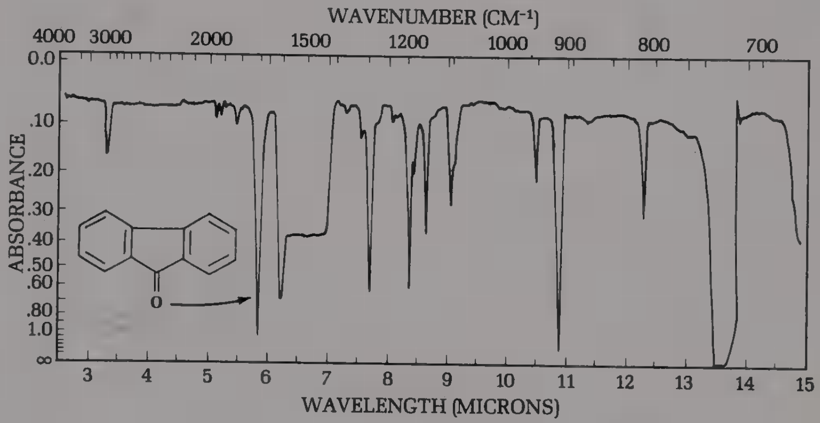


FIGURE 14.4 Infrared spectrum of fluorenone.

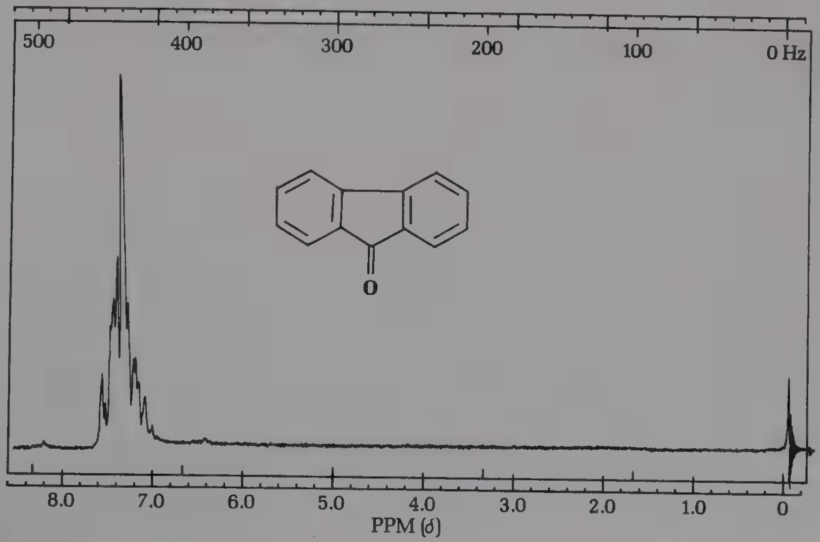


FIGURE 14.5 Nmr spectrum of fluorenone.

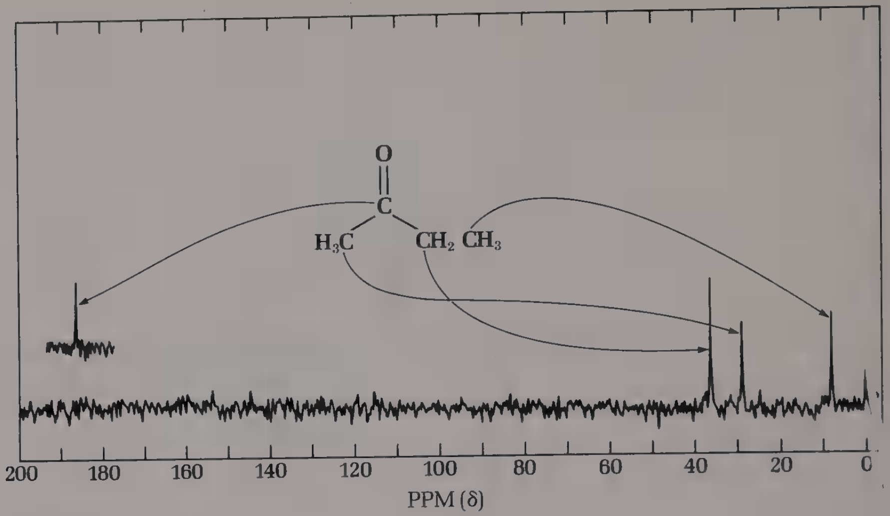


FIGURE 14.6 ^{13}C nmr spectrum of 2-butanone.

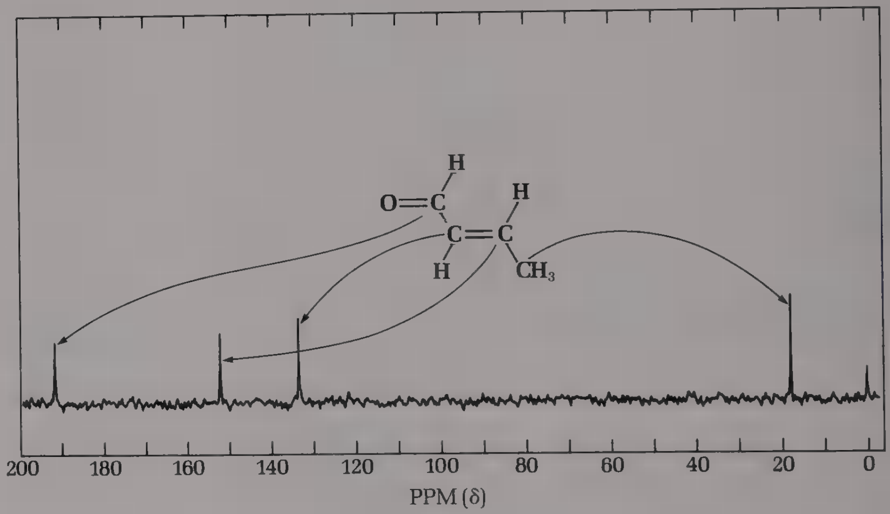


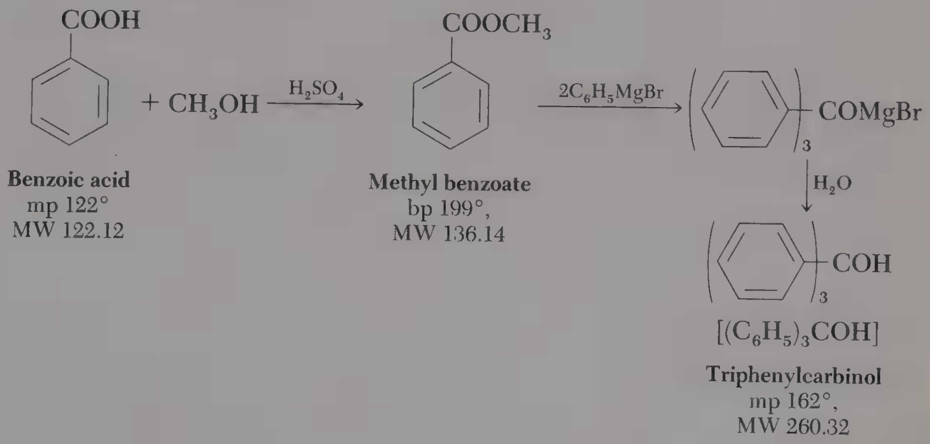
FIGURE 14.7 ^{13}C nmr spectrum of crotonaldehyde.

15

Grignard Synthesis of Triphenylcarbinol

KEYWORDS

Grignard synthesis
Fischer esterification
Lewis acid catalyst, $BF_3 \cdot Et_2O$
Methyl benzoate
Phenylmagnesium bromide
Biphenyl, by-product
Calcium chloride drying tube
Absolute ether
Starting the Grignard reaction
Triphenylmethyl
Beilstein test, copper wire



Benzoic acid
mp 122°
MW 122.12

Methyl benzoate
bp 199°,
MW 136.14

$[(C_6H_5)_3COH]$
Triphenylcarbinol
mp 162°,
MW 260.32

Benzoic acid is esterified by the Fischer method, and the methyl benzoate produced is used for the Grignard synthesis of triphenylcarbinol by interaction with two equivalents of phenylmagnesium bromide.

▼
Fischer esterification

In Fischer esterification, methanol is used in excess both to shift the equilibrium in favor of the product and to serve as solvent for the solid acid component. Of the acid and Lewis acid catalysts commonly employed, sulfuric acid is preferable to hydrogen chloride because of greater convenience and to boron fluoride etherate because it is more effective. In case either the alcohol or the acid involved in an esterification contains reactive double bonds or is sensitive to dehydration, boron trifluoride etherate becomes the catalyst of choice.

In this two-step synthesis, directions are given for the Grignard reaction of 5.0 g (0.037 mole) of pure methyl benzoate; the quantities of reagents are to be adjusted according to the amount of this intermediate available. Satisfactory yield and quality of the final product require care in the preparation and purification of the intermediate ester, as well as in the second step. The chief impurity in the Grignard reaction mixture is the hydrocarbon biphenyl, formed by the coupling reaction:

$$C_6H_5MgBr + C_6H_5Br \longrightarrow C_6H_5{-}C_6H_5 + MgBr_2$$

This hydrocarbon by-product is easily eliminated, however, since it is much more soluble in hydrocarbon solvents than triphenylcarbinol.

EXPERIMENTS

1. Methyl Benzoate

▼
One-hour reflux period; this can be done in advance and the mixture let stand

Place 10.0 g of benzoic acid and 25 ml of methanol in a 125-ml round-bottomed flask, and pour 3 ml of conc'd sulfuric acid *slowly and carefully down the walls of the flask*, and then swirl to mix the components. Attach a reflux condenser, add a boiling chip, and reflux the mixture gently with a microburner for 1 hr. Cool the solution, decant it into a separatory funnel containing 50 ml of water, and rinse the flask with a part of 35 ml of ether that is to be used for extraction of the product. Shake the separatory funnel and drain off the water, which contains the sulfuric acid and the bulk of the methanol. Wash the remaining solution with a second portion of water (25 ml) and then with 25 ml of 5% sodium bicarbonate to remove unreacted benzoic acid. Again shake, with frequent release of pressure, until no further reaction is apparent, and then drain off and acidify the extract. Any benzoic acid that precipitates is collected and the amount recovered should be allowed for in calculation of the percentage yield. Repeat the washing with bicarbonate until no precipitate forms on acidification of the aqueous layer. Wash with saturated sodium chloride solution and filter the solution through anhydrous sodium sulfate into an Erlenmeyer flask.

▼
Tared: previously weighed

Remove the ether by evaporation on the steam bath under an aspirator tube. See Fig. 6.4 or use a rotary evaporator (Chapter 16). When evaporation ceases, add 2–3 g of anhydrous sodium sulfate to the residual oil and heat for about 5 min longer. Then decant the methyl benzoate into a 50-ml round-bottomed flask, attach a stillhead, and distil downward through an empty fractionation column which functions as an air condenser; the boiling point of the ester is so high (199°) that a water-cooled condenser is liable to crack. Use a tared 25-ml Erlenmeyer as the receiver and collect material boiling above 190°. Yield 8 g.

Add a drop of the ester to a few drops of concentrated sulfuric acid and see if the ester dissolves, as an oxygen-containing compound should, and if the solution is colorless; a yellow color indicates lack of purity. Keep the flask of methyl benzoate stoppered until the ester is to be used.

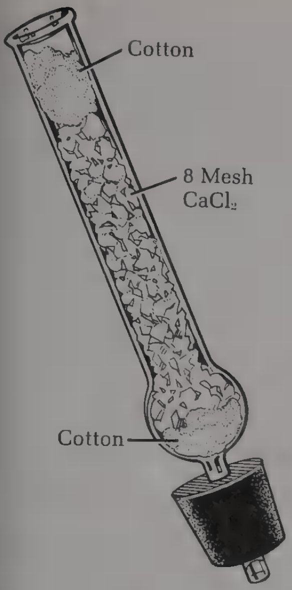


FIGURE 15.1 Calcium chloride drying tube fitted with a rubber stopper. Store for future use with cork in top, pipette bulb on bottom.

2. Phenylmagnesium Bromide (Phenyl Grignard Reagent)

The Grignard reagent is prepared in a dry 125-ml round-bottomed flask fitted with a long reflux condenser. A calcium chloride drying tube inserted in a cork that will fit either the flask or the top of the condenser is also made ready (Fig. 15.1). The flask and condenser should be as dry as possible to begin with, and then, as a further precaution to eliminate a possible film of moisture, the magnesium to be used (2 g = 0.082 mole of magnesium turnings) is placed in the flask, the calcium chloride tube is attached directly, and the flask is heated gently but thoroughly on the steam bath or with a cool luminous flame. The flask on cooling pulls dry air through the calcium chloride. Cool to room temperature before proceeding! **Extinguish all flames!**

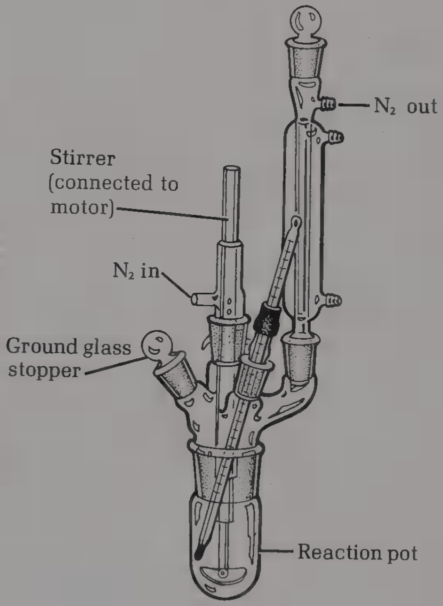


FIGURE 15.2 Semimicro-scale, research-type apparatus for Grignard reaction, with provision for a motor-driven stirrer and an inlet and outlet for dry nitrogen.

▼
Specially dried ether is required

Make an ice bath ready in case control of the reaction becomes necessary, remove the drying tube and fit it to the top of the condenser. Then pour into the flask 15 ml of *absolute* ether (absolutely dry, anhydrous) and 9 ml (13.5 g = 0.086 mole) of bromobenzene. (More ether is to be added as soon as the reaction starts, but at the outset the concentration of bromobenzene is kept high to promote easy starting.) If there is no immediate sign of reaction, insert a *dry* stirring rod with a flattened end and crush a piece of magnesium firmly against the bottom of the flask under the surface of the liquid, giving a twisting motion to the rod. When this is done properly the liquid becomes slightly cloudy, and ebullition commences at the surface of the compressed metal.

Attach the condenser at once, swirl the flask to provide fresh surfaces for contact, and, as soon as you are sure that the reaction has started, add ~~25~~ 15ml ml more absolute ether through the top of the condenser before spontaneous boiling becomes too vigorous (replace the drying tube). Note the volume of ether in the flask. Cool in ice if necessary to slow the reaction. If difficulty is experienced in initiating the reaction, try in succession the following expedients.

▼
Starting the Grignard Reaction

a) Warm on the steam bath with swirling. Then see if boiling continues when the flask (condenser attached) is removed from the heating bath.
b) Try further mashing of the metal with a stirring rod.
c) Add a tiny crystal of iodine as a starter (in this case the ethereal solution of the final reaction product should be washed with sodium bisulfite solution in order to remove the yellow color).
d) Add a few drops of a solution of phenylmagnesium bromide or of methylmagnesium iodide (which can be made in a test tube).
e) Add a drop of ethylene dibromide. This reactive halide will sometimes react where less reactive halides will not.
f) Start afresh, taking greater care with respect to the dryness of apparatus and reagents and sublime a crystal or two of iodine on the surface of the magnesium to generate Gattermann's "activated magnesium" before beginning the reaction again.

▼
Use minimum steam to avoid condensation on condenser

Once the reaction begins, spontaneous boiling in the diluted mixture may be slow or become slow. If so, mount the flask and condenser on the steam bath (one clamp supporting the condenser suffices) and reflux gently until the magnesium has disintegrated and the solution has acquired a cloudy or brownish appearance. The reaction is complete when only a few small remnants of metal (or metal contaminants) remain. Check to see that the volume of ether has not decreased. If it has, add more. Since the solution of Grignard reagent deteriorates on standing, the next step should be started at once.

3. Triphenylcarbinol

▼
This is a suitable stopping point

Mix 5 g (0.037 mole) of methyl benzoate and 15 ml of absolute ether in a separatory funnel, cool the flask containing phenylmagnesim bromide solution briefly in an ice bath, remove the drying tube, and insert the stem of the separatory funnel into the top of the condenser. Run in the methyl benzoate solution *slowly* with only such cooling as is required to control the mildly exothermic reaction, which affords an intermediate addition compound which separates as a white solid. Replace the calcium chloride tube, swirl the flask until it is at room temperature and the reaction has subsided. The reaction is then completed by either refluxing the mixture for one-half hour, or stoppering the flask with the calcium chloride tube and letting the mixture stand overnight (subsequent refluxing is then unnecessary).[1]

---

[1] A rule of thumb for organic reactions: a 10° rise in temperature will double the rate of the reaction.

Pour the reaction mixture into a 250-ml Erlenmeyer flask containing 50 ml of 10% sulfuric acid and about 25 g of ice and use both ordinary ether and 10% sulfuric acid to rinse the flask. Swirl well to promote hydrolysis of the addition compound; basic magnesium salts are converted into water-soluble neutral salts and triphenylcarbinol is distributed into the ether layer. An additional amount of ether (ordinary) may be required. Pour the mixture into a separatory funnel (rinse the flask with ether), shake, and draw off the aqueous layer. Shake the ether solution with 10% sulfuric acid to further remove magnesium salts, wash with saturated sodium chloride solution to remove water that has dissolved in the ether. The amounts of liquid used in these washing operations is not critical. In general, an amount of wash liquid equal to one third of the ether volume is adequate.

▼
Saturated aqueous sodium chloride solution removes water from ether

To effect final drying of the ether solution pour the ether layer out of the neck of the separatory funnel into an Erlenmeyer flask, add about 5 g of granular anhydrous sodium sulfate, swirl the flask from time to time, and after 5 min remove the drying agent by gravity filtration through a filter paper held in a funnel into a tared Erlenmeyer flask. Add a boiling chip and remove the ether by boiling on a steam bath, removing ether vapors with an aspirator tube (See Fig. 6.4). Remove the last traces of solvent by warming the flask under vacuum (See Fig. 4.8). The residue will be found to be a solid (9.5 g) of low and wide melting range (110–115°), since it contains considerable biphenyl. The by-product can be removed by crystallization from ligroin, but instead of removing the ether and dissolving the residue in ligroin it is simpler to add the ligroin to the ethereal solution, distil, and so displace the ether by the less volatile crystallization solvent. Use 25 ml of 66–77° ligroin and concentrate the ether-ligroin solution (steam bath) in an Erlenmeyer flask under an aspirator tube. Evaporate slowly until crystals of triphenylcarbinol just begin to separate and then let crystallization proceed, first at room temperature and then at 0°. The product should be colorless and should melt not lower than 160°. Concentration of the mother liquor may yield a second crop of crystals. Typical student yield 5.0 g. Evaporate the mother liquors to dryness and save the residue for later isolation of the components by chromatography.

4. Reactions of Triphenylcarbinol

Prepare a stock solution of 4.0 g of triphenylcarbinol in 80 ml of warm (steam bath) glacial acetic acid for use in the first four reactions (a-d) which follow.

a) To 10 ml of the stock solution add 2 ml of 47% hydrobromic acid, heat for 5 min on the steam bath, cool in ice, collect the product (0.8 g), and wash it with ligroin.

b) To 10 ml of the stock solution add 2 ml of an acetic acid solution containing 5% of chloroacetic acid and 1% of sulfuric acid, heat 5 min, add water (5–6 ml) to produce a saturated solution, and let the mixture stand for crystallization. Collect the product by suction filtration and wash the crystals with 1:1 methanol-water; yield 0.8 g.

c) To 10 ml of the stock solution add 2 ml of 47% hydriodic acid, heat for 1 hr, cool, add 1 g of sodium bisulfite in 20 ml of water, collect the precipitate, wash well with water, and crystallize the moist solid from methanol (15–20 ml).

d) To 10 ml of the stock solution add a hot solution of 2 g of stannous chloride in 5 ml of conc'd hydrochloric acid, heat for 1 hr, cool, collect, and wash well with methanol; yield 0.8 g.

e) Dissolve 1 g of triphenylcarbinol in 25 ml of methanol, add 1 ml of 47% hydrobromic acid, heat gently for 15 min, and let cool. Crystallization can be hastened by scratching. Wash the product (1.0 g) with methanol.

f) Weigh 1 g of triphenylcarbinol and 2 g of malonic acid (crush any lumps) into a 25-ml Erlenmeyer flask, and heat the mixture for 7 min on the hot plate (about 150°) under an aspirator tube for removal of fumes. Cool, dissolve in 2 ml of toluene, add 10 ml of ligroin (60–90°) and set the solution aside. Wash the product with ligroin; yield 0.8 g.

	Mp
$(C_6H_5)_3CCl$	113°
$(C_6H_5)_3CBr$[2]	
$(C_6H_5)_3CI$	
$CH_3COOC(C_6H_5)_3$	97°
$CH_2ClCOOC(C_6H_5)_3$	144°
$(C_6H_5)_3CH$	94°
$(C_6H_5)_3COOC(C_6H_5)_3$	186°
9-Phenylfluorene	148°
p-$(C_6H_5)_3CHC_6H_4C(C_6H_5)_3$	224°
$(C_6H_5)_3COCH_3$	97°
$(C_6H_5)_3COC(C_6H_5)_3$	237°
$(C_6H_5)_3CCH_2CO_2H$	176°

---

[2] Put a small spatulaful of this compound in a 13 × 100-mm test tube, dissolve in benzene and cool, add a few mg of zinc dust, and stir with a rod to produce a yellow solution containing triphenylmethyl radical. Decant into a clean tube and shake with air; the cloudy precipitate is the peroxide, $(C_6H_5)_3COOC(C_6H_5)_3$.

5. Identification

▼ *Beilstein test for halogen*

Identify each product in reactions (a)–(f), and present some reason for your conclusion in addition to correspondence in mp. Some of the possible products are listed in the table in the margin. Halogens can be detected by the **Beilstein test:** heat a 2–3 in. section of copper wire to redness in the oxidizing flame of a burner (nonluminous) until the flame is no longer colored, let the wire cool and touch it to a solid (or dip it in a liquid) and reheat; a volatile copper halide imparts a green color to the flame.

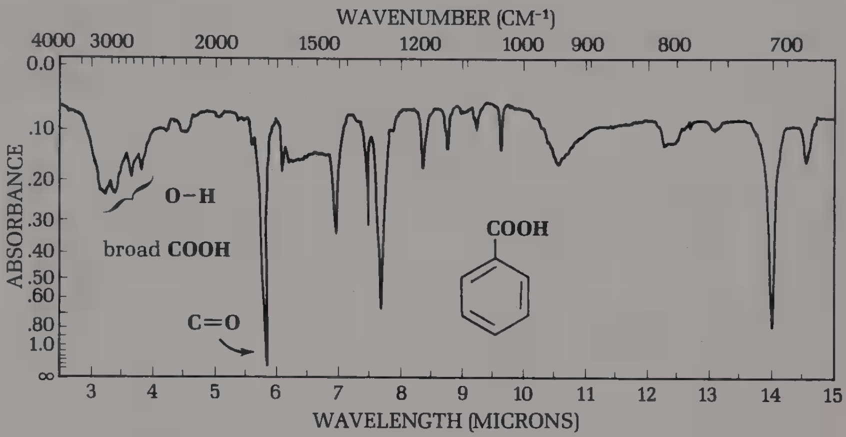


FIGURE 15.3 Infrared spectrum of benzoic acid in CS_2.

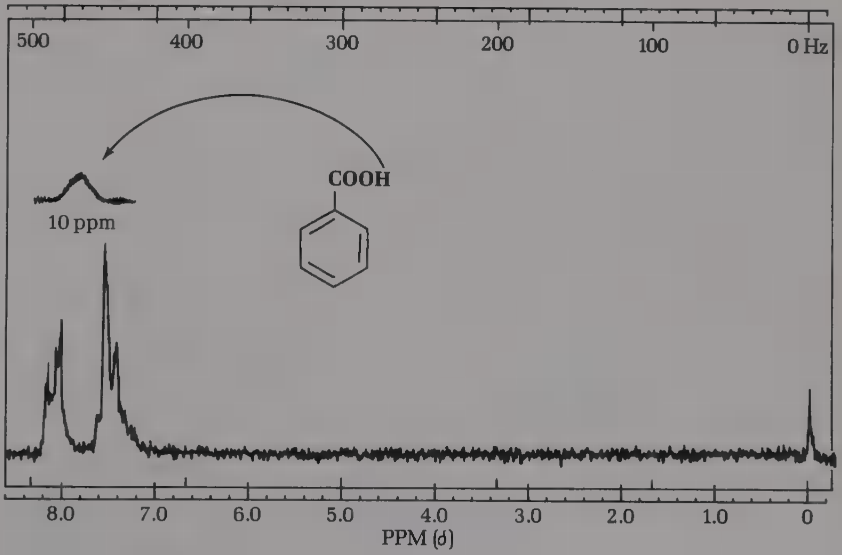


FIGURE 15.4 ^1H nmr spectrum of benzoic acid.

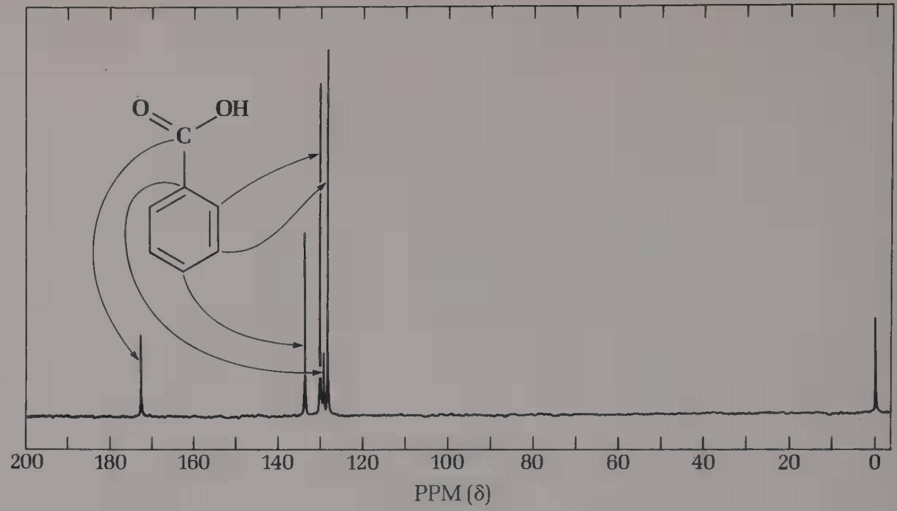


FIGURE 15.5 ^{13}C nmr spectrum of benzoic acid.

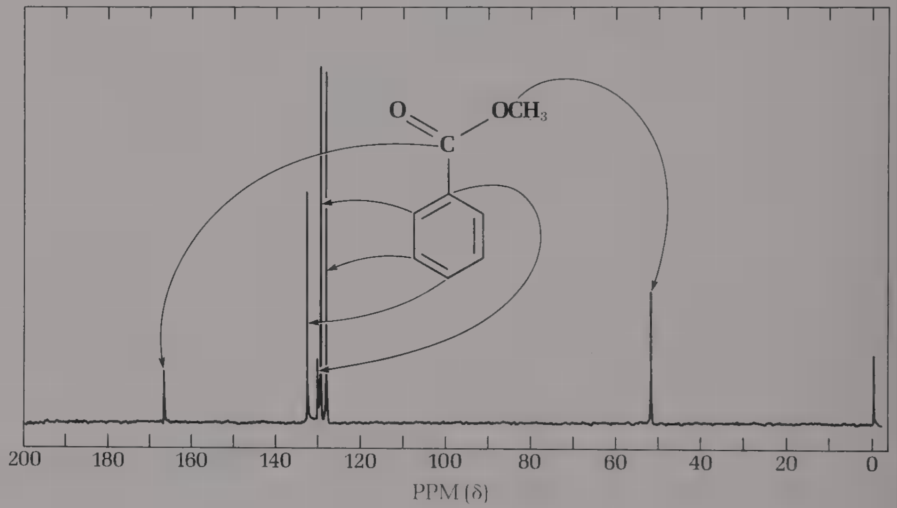


FIGURE 15.6 ^{13}C nmr spectrum of methyl benzoate.

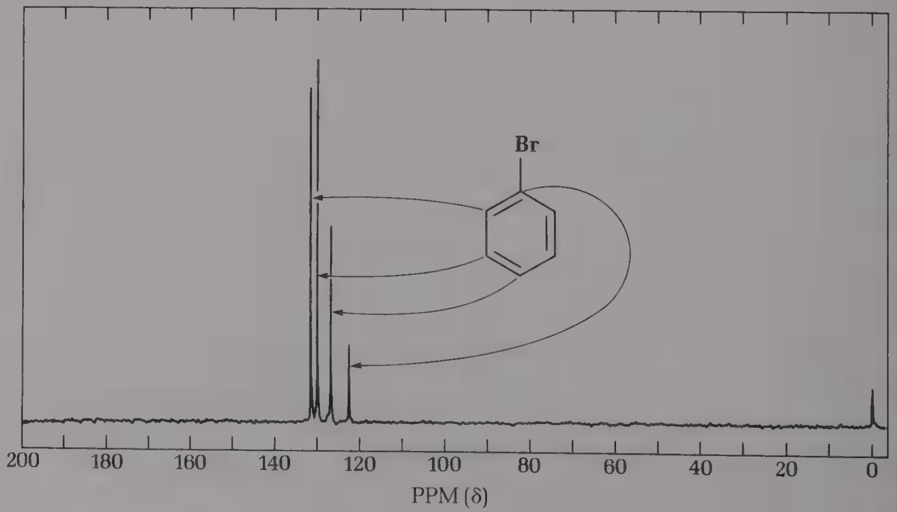


FIGURE 15.7 ^{13}C nmr spectrum of bromobenzene.

16

Column and Thin Layer Chromatography

KEYWORDS

Adsorption
Alumina column
Eluant, elute
Packing the column
Solvent bubbler
Acetylation
Mother liquor
Fractions
Trituration

Part 1. Column Chromatography

Mixtures of organic substances often can be separated by adsorbing the substances from a small volume of solvent onto a column of alumina and then leaching the column with a sequence of solvents of increasing ability to dislodge the adsorbed substances from the adsorbate (Fig. 16.1). The eluant liquid is collected in numbered fractions, each of which is evaporated for examination of the residue. If the components of the mixture are of different chemical types, some will be eluted at an early stage of the process and others in intermediate or late fractions. The usual order of elution is:

Alkanes (*first*)
Alkenes
Dienes
Aromatic hydrocarbons
Ethers
Esters, acetates
Ketones
Alcohols
Diols
Acids (*last*)

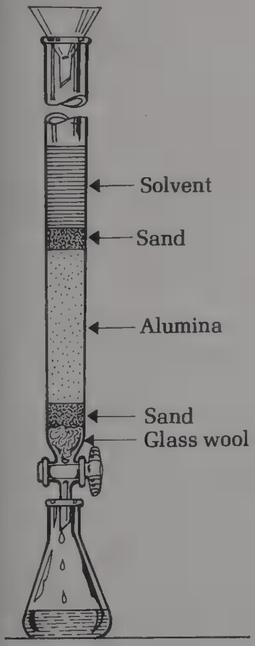


FIGURE 16.1 Chromatographic column.

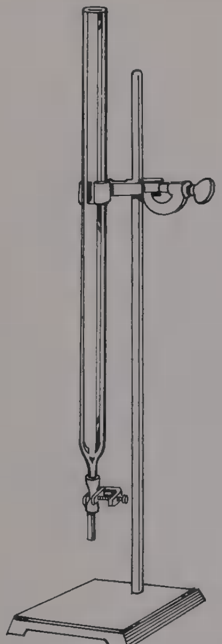


FIGURE 16.2 Chromatograph tube on ring stand.

Solvents employed for elution, listed in the order of increasing eluant power, are: ligroin, toluene, ether, methanol. In an investigation of an unknown mixture, elute first with petroleum ether, then with ligroin–diethyl ether mixtures, varying from 1% to 2%, 5%, 10%, 25%, and 50% ether in ligroin, pure ether, and then ether-methanol mixtures.

The directions which follow are for the preparation of a column and for three experiments involving applications of the technique. In the first experiment an alcohol is partially acetylated and the extent of reaction determined by separation of the easily eluted acetate from the strongly adsorbed alcohol; you should conduct the acetylation while you are preparing the column. The second experiment is separation of yellow fluorenone from the colorless hydrocarbon from which it is obtained by oxidation, and the third is an investigation of the triphenylcarbinol mother liquor of Chapter 16.3.

Ordinarily, 25 g of alumina is needed per gram of substance to be chromatographed, and this is the ratio specified in the first two experiments. In the third experiment, however, the difference in adsorbability is sufficient to permit a decrease in the proportion of alumina. In all three experiments the transition from solvents of low to high solvent power is done more rapidly than would be done if you were working with an unknown.

EXPERIMENTS

1. Preparation of the Chromatograph Column[1]

The column can be prepared using a burette, such as the one shown in Fig. 16.1, or using the less expensive and equally satisfactory chromatograph tube shown in Fig. 16.2, in which the flow of solvent is controlled by a screw pinchclamp. Weigh out the required amount of alumina (12.5 g in the first experiment), close the pinchclamp on the tube, and fill about half full with low-boiling ligroin. With a wooden dowel push a small plug of glass wool through the liquid to the bottom of the tube, dust in through a funnel enough sand to form a 1-cm layer over the glass wool, and level the surface by taping the tube. Unclamp the tube and, with the right hand, grasp both the top of the tube and the funnel so that the whole assembly can be shaken to dislodge alumina that may stick to the walls, and with the left hand pour in the alumina slowly while tapping the column with a rubber stopper fitted on the end of a pencil (Fig. 16.3). When the alumina has settled, add a little sand to provide a protective layer at the top. Open the pinchclamp, let the solvent level fall until it is just a little above the upper layer of sand, and then stop the flow. Alternatively the alumina can be added to the column (half filled with ligroin) by slurrying the alumina with ligroin in a beaker. The powder is stirred to suspend it in the solvent and *immediately* poured through a wide mouth funnel into the chromatograph tube. Rap the column with a rubber stopper to cause the alumina to settle. Add a protective layer of sand to the top. The column is now ready

FIGURE 16.3 Technique useful when filling a chromatographic tube with alumina.

---

[1] Aluminum oxide Merck (acid-washed) is suitable. Purified sand is supplied by J. T. Baker Chemical Co. A dowel ($\frac{3}{16}$″ or $\frac{1}{4}$″) is preferred to a fragile glass rod.

▼
Do not allow column to become dry during chromatography

for use. Prepare 50-ml Erlenmeyer flasks as receivers by taring (weighing) each one carefully and marking them with numbers on the etched circle.

After use, the tube is conveniently emptied by pointing the open end into a beaker, opening the pinchclamp, and applying gentle air pressure to the tip. If the plug of glass wool remains in the tube after the alumina leaves, wet it with acetone and reapply air pressure.

2. Acetylation of Cholesterol

Cover 0.5 g of cholesterol with 5 ml of acetic acid in a small Erlenmeyer flask, swirl, and note that the initially thin slurry soon sets to a stiff paste of the molecular compound $C_{27}H_{45}OH \cdot CH_3CO_2H$. Add 1 ml of acetic anhydride and heat the mixture on the steam bath for any convenient period of time from 15 min to 1 hr; record the actual heating period. Cool, add water, and extract with ether. Wash the ethereal extract twice with water and once with 10% sodium hydroxide, dry by shaking the ether extracts with saturated sodium chloride solution, then dry the ether over anhydrous sodium sulfate in an Erlenmeyer flask, filter, and evaporate the ether. Dissolve the residue in 3–4 ml of ether, transfer the solution with a capillary dropping tube onto a column of 12.5 g of alumina and rinse the flask with another small portion of ether.[2] Open the pinchclamp, run the eluant solution into a 50-ml Erlenmeyer flask, and as soon as the solvent in the column has fallen to the level of the upper layer of sand fill the column with a part of a measured 50 ml of ligroin. When about 25 ml of eluate has collected in the flask (fraction 1), change to a fresh flask; add a boiling stone to the first flask and evaporate the solution to dryness on the steam bath under an aspirator tube (Fig. 6.4). Evacuation using the aspirator helps to remove last traces of ligroin (see Fig. 4.8). If this fraction 1 is negative (no residue), use the flask for collecting further fractions. Continue adding ligroin until the 50-ml portion is exhausted and then use 50 ml of a 90:10 ligroin–ether mixture, followed by 75–100 ml of a 1:1 mixture of ligroin and ether. A convenient bubbler (Fig. 16.4) made from a 125-ml Erlenmeyer flask, a short piece of 10-mm dia glass tubing, and a cork will automatically add solvent. Collect and evaporate successive 25-ml fractions of eluate. The ideal method for removal of solvents involves the use of a rotary evaporator (Fig. 16.5).

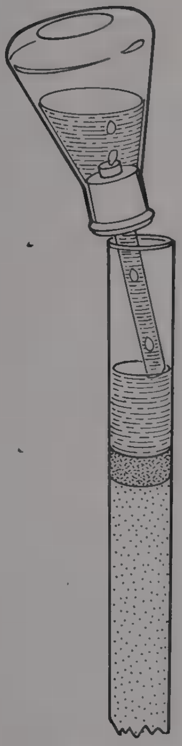


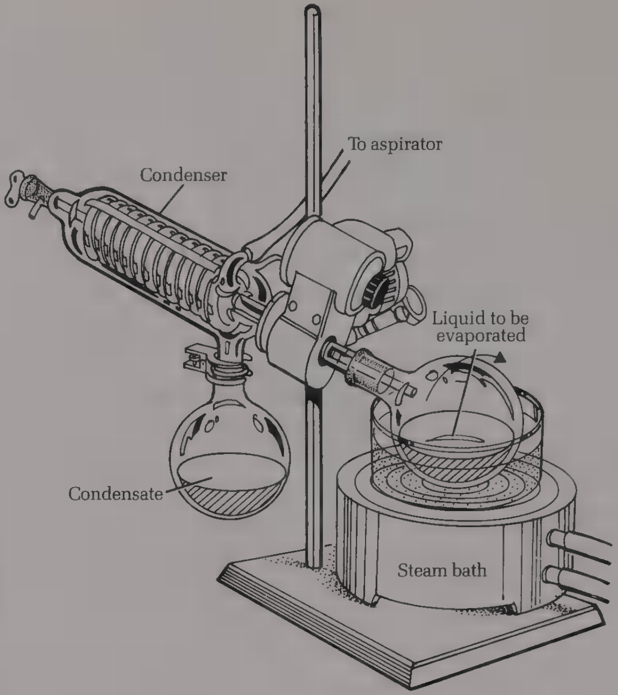
FIGURE 16.4 Bubbler for adding solvent automatically.

Cholesteryl acetate (mp 115°) and cholesterol (mp 149°) should appear, respectively, in early and late fractions with one or two negative fractions (no residue) in between. If so, combine consecutive fractions of early and of late material and determine the weights and melting points. Calculate the percentage of the acetylated material to the total recovered and compare your result with those of others in your class employing different reaction periods. The total recovery is about 80%.

---

[2] Ideally, the material to be adsorbed is dissolved in ligroin, the solvent of least eluant power. The present mixture is not soluble enough in ligroin and so ether is used, but the volume is kept to a minimum.

FIGURE 16.5 Rotary evaporator. The rate of evaporation with this apparatus is very fast due to the thin film of liquid over the entire inner surface of the rotating flask that is heated under a vacuum. Foaming and bumping are also greatly reduced.



3. Separation of Fluorene and Fluorenone

Prepare a column of 12.5 g of alumina, run out excess solvent, and pour onto the column a solution of 0.5 g of the fluorene-fluorenone mixture previously separated with Girard reagent (Chapter 14, Section 11). See if you can better the yields by chromatography. Elute at first with ligroin and use tared 50-ml flasks as receivers. The yellow color of fluorenone provides one index of the course of the fractionation, and the appearance of solid around the delivery tip provides another. Wash the solid frequently into the receiver with ether. When you think that one component has been eluted completely, change to another receiver until you judge that the second component is beginning to appear. Then, when you are sure the second component is being eluted, change to a 1:1 ligroin–ether mixture and continue until the column is exhausted. It is possible to collect practically all the two components in the two receiving flasks, with only a negligible intermediate fraction. After evaporation of solvent, evacuate each flask under vacuum (Fig. 4.8) and determine the weight and melting point of the products. Compare the outcome of the separation with that obtained with Girard reagent.

4. Examination of a Mother Liquor

Dissolve the residues from the purification of triphenylcarbinol in a little ether, pour the solution into a tared flask, evaporate and evacuate at the aspirator

(Fig. 4.8), and weigh. Digest 2 g of the oil or semi-solid with 15–20 ml of ligroin and prepare a column of 12.5 g of alumina. If solid has separated from the solution of sample, filter the mixture through a paper into the column and save the crystals for possible combination with more of the same material which might be isolated by chromatography. Since the early eluates are liable to contain mixtures, the first five fractions should be limited to 5 ml each and collected in small Erlenmeyer flasks (10- or 25-ml). When the fifth fraction has been collected, close the pinchclamp and defer further chromatography until the early eluates have been evaporated, pumped out, and examined (appearance and odor, particularly when warm). Let these fractions stand to see if, in time, crystals appear and continue with the chromatogram. If fraction 5 afforded only a trace of oil, elute with 20 ml of ligroin and collect the liquid in a 50-ml flask, as fraction 6. Then collect three fractions eluted by 20-ml portions of a 90:10 ligroin–ether mixture, stop the chromatogram, and evaluate the results.

Late fractions consisting of oils usually crystallize readily when scratched. If so, add a little ligroin, digest briefly on the steam bath, rub the material off the walls with a stirring rod, cool, collect, and take the melting point. If one or more of the early fractions crystallize, the crystals will probably be contaminated with oil, which can be separated as follows: Make ready a Hirsch funnel and paper, chill a mixture of 2 ml of water and 8 ml of methanol in ice and cool the flask as well, introduce solvent and stir to loosen the crystals (this process is known as trituration), and then collect the product by suction filtration and wash it with chilled solvent.

Identify all crystalline products encountered and account for their presence.

Part 2. Thin Layer Chromatography

▼
TLC

This microanalytical technique employs alumina as adsorbent, as in column chromatography, and silica gel as well but in the form of a thin layer on a glass plate. In this experiment the plate is a 3 × 1-inch microscope slide. A 1% solution of the substance to be examined is spotted onto the plate about 1 cm from the bottom end and the plate is inserted into a 4-oz wide-mouth bottle containing 4 ml of an organic solvent. The glass stopper is put in place and the time noted. The solvent travels rapidly up the thin layer by capillary action, and if the substance is a pure colored compound one soon sees a spot either traveling along with the solvent front or, more usually, at some distance behind the solvent front. One can remove the slide, quickly mark the front before the solvent evaporates, and calculate the Rf value. The Rf value is the ratio of the distance the spot travels from the point of origin to the distance the solvent travels.

▼
Colored compounds

If two colored compounds are present and an appropriate solvent is selected, two spots will appear.

▼
And colorless ones

Fortunately the method is not limited to colored substances. Any organic compound capable of being eluted from alumina will form a spot, which soon becomes visible when the solvent is let evaporate and the plate let stand in a stoppered 4-oz bottle containing a few crystals of iodine. Iodine vapor is adsorbed by the organic compound to form a brown spot. A spot should be outlined at once with a pencil, since it will soon disappear as the iodine sublimes away; brief return to the iodine chamber will regenerate the spot. The order of elution and the elution-power for solvents are the same as for column chromatography.

The adsorbent recommended for making TLC plates is a preparation of finely divided alumina containing plaster of paris as binder (Fluka[1]). A slurry of this material in water can be applied to microscope slides by simple techniques of dipping (A) or coating (B); for a small class, or for occasional preparation of a few slides by an individual, the more economical coating method is recommended. Either of two techniques of spraying (C) is applicable to the mass production of plates but requires special equipment.

FIGURE 16.6 **Preparation of TLC plates by dipping. The two microscope slides are grasped at one end, dipped into the silica gel slurry, removed and separated, and dried and activated by heating.**

(A) By Dipping. Place 15 g of Fluka, or similar alumina formulated for TLC, in a 40 × 80 mm weighing bottle[2] and stir with a glass rod while gradually pouring in 75 ml of distilled water. Stir until lumps are eliminated and a completely homogeneous slurry results. Grasp a pair of clean slides[3] at one end with the thumb and forefinger, dip them in the slurry of adsorbent (Fig. 16.6), withdraw with an even unhurried stroke, and touch a corner of the slides to the mouth of the weighing bottle to allow excess fluid to return to the container. Then dry the working surface by mounting the slides above a 70-watt hot plate, using as support two pairs of 1 × 7 cm strips of asbestos paper (or a pair of applicator sticks). Drying takes about a minute and a half and is evident by inspection. Remove the plate with a forceps as soon as it is dry, for cooling takes longer. If you dip and dry 8–10 slides in succession, finished plates will be ready for use when you are through. Alternatively, dry the slides in an oven at 110°. This heating activates the alumina. Coated slides dried at room temperature do not give as intense spots as those that have been heat-dried.

Keep the storage bottle or adsorbent closed when not in use. Note that this is not a stable emulsion but that the solid settles rapidly on standing. Stir thoroughly with a rod before each reuse.

Plates ready for use, as well as clean dry slides, are conveniently stored in a micro slide box (25 slides).

▼
Notes for the instructor

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[1] Aluminumoxid Fluka für Dünnschicht Chromatographie, Typ D5, Fluka AG, Buchs, S. G. Switzerland; Fluka Alumina is distributed by Tridom Chemical, Inc., 255 Oser Ave., Hauppauge, N.Y. 11787.

[2] Kimble Exax, 15146.

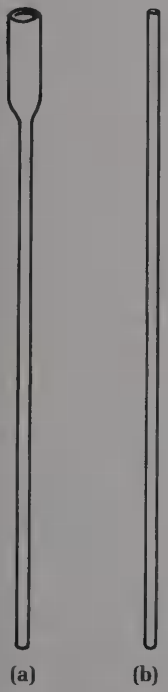
[3] The slides should be washed with water and a detergent, rinsed with tap water and then with distilled water, and placed on a clean towel to dry. Dry slides should be touched only on the sides, for a touch on the working surface may spoil a chromatogram.

(B) By Coating. Place 2 g of Fluka alumina and 10 ml of distilled water in a 25-ml Erlenmeyer, stopper the flask and shake to produce an even slurry. Keep the flask stoppered when not in use. Place a clean, dry slide on a block of wood or box with the slide projecting about 1 cm on the left-hand side (if you are right handed) so that it can be grasped easily on the two sides. Swirl the flask to mix the slurry and draw a portion into a medicine dropper. Hold the dropper vertically and, starting at the right end of the slide, apply emulsion until the entire upper face is covered; make further applications to repair pin holes and eliminate bubbles. Grasp the left end of the slide with a forceps and even the emulsion layer by tilting the slide to the left to cause a flow, and then to the right; tilt again to the front and to the rear. Dry the slide on a hot plate as you did when preparing slides by procedure A. The adsorbent should be about 0.25 mm thick.

(C) By Spraying. Two techniques which enable an operator to spray a hundred small slides in a few minutes are of particular use in research, where large plates are often required. A batch of slides for student use can be prepared by spraying and let air-dry at room temperature, but each plate should then be activated by heating.[4]

One technique utilizes the Sutter spray gun of metal.[5] Place 15 g of Fluka alumina and 75 ml of distilled water in a 125-ml Erlenmeyer, stopper the flask and shake to produce an even suspension, and pour this into the chamber of the spray gun. Connect the gun with a piece of suction tubing to a source of gas pressure, preferably a cylinder of nitrogen or oxygen set at a pressure of 15 lbs/sq in. Place a batch of slides side by side on a piece of cardboard or paper. Hold the gun vertically with the nozzle about 60 cm above the target, practice spraying a piece of paper, and then spray the slides evenly.

FIGURE 16.7 Micropipettes.



(D) Spotting Test Solutions. This is done with micropipettes of either style shown in Fig. 16.7, made by drawing out a 5-mm soft glass tube. Satisfactory micropipettes can also be made by drawing open-end mp capillaries in a microburner flame. The bore should be of such a size that, when the pipette is dipped deep into ligroin, the liquid flows in to form a tiny thread which, when the pipette is withdrawn, does not flow out to form a drop. Style (a) has the advantage that it can be cleaned by introducing solvent with a capillary dropping tube at the top and then shaking the solvent on through the tube. To spot a test solution, let a 2–3 cm column of solution flow into the pipette, hold this vertically over a coated plate (cold), aim it at a point on the right side of the plate and about 1 cm from the bottom, and lower the pipette until the tip just touches the adsorbent and liquid flows onto the plate; withdraw when the spot is about 2 mm in diameter. Make a second 2-mm spot on

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[4] Slides coated by dipping in a slurry of alumina in chloroform-methanol according to J. J. Peifer, *Michrochimica Acta,* 529 (1962), dry very rapidly but still have to be steamed and heat-activated for the best performance.

[5] Sprühgerät nach B. Sutter, Hormuth-Vetter, Wiesloch (Baden), Germany.

the left side of the plate, let it dry, and make two more applications of the same size (2-mm) at the same place. Determine whether the large or the small spot gives the better results.

EXPERIMENTS

1. Dyes

The first experiment utilizes a test solution[6] containing a green dye, a red dye, and a blue dye of the structures shown below. Or try mixtures of commercially available food colors. Chromatograms are to be run on the test solution, using the solvents listed:

(a) Ligroin (3.5 ml)–Ether (0.5 ml)
(b) Ligroin (2 ml)–Ether (2 ml)
(c) Cyclohexane (4 ml)
(d) Dichloromethane (4 ml)
(e) Cyclohexane-Ethyl acetate. (Use 4 ml of a mixture of 8.5 ml of cyclohexane and 1.5 ml of ethyl acetate.)

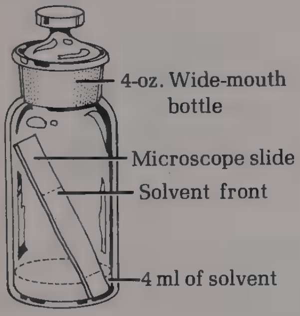
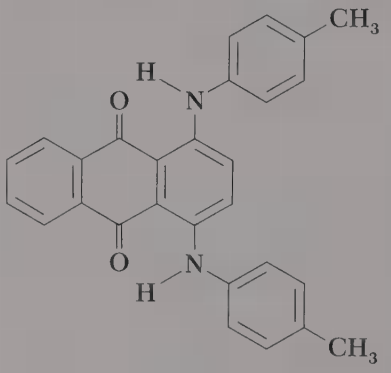
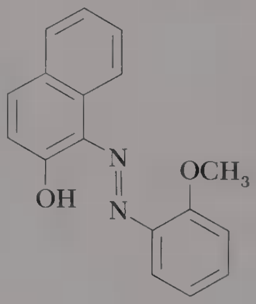


FIGURE 16.8 **A method of developing thin layer chromatographic plates.**

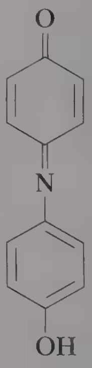
For each chromatogram, (a)-(e), make two 2-mm spots of the test solution on the right and left sides of the plate and about 1 cm from the end, one of the two spots of single strength and the other of triple strength. For chromatogram (a) prepare a running chamber by pouring 4 ml of ligroin into a 4-oz bottle, swirl to saturate the air with solvent, and put the stopper in place. With a forceps, grasp a plate that has been spotted and lower it into the bottle and rest it in a slanting position against the wall (Fig. 16.8). Replace the stopper, observe the plate carefully, and see if the dyes separate. Let chromatogram (a) continue for a time while starting others in the series. In each case, after a clear-cut pattern of separation or nonseparation has been achieved make a drawing to record the position, shape, and color of each spot.



1,4-Di-*p*-toluidinoanthraquinone (D & C Green 6)



Sudan Red G



Indophenol (blue)

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Note to instructor

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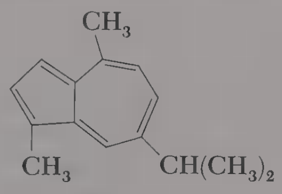
[6] Prepare an alcoholic solution containing 1% each of D & C Green 6, Sudan Red G, and Indophenol, available from Allied Chemical Corp., Specialty Chemicals Division, 40 Rector St., New York, N. Y., 10006.

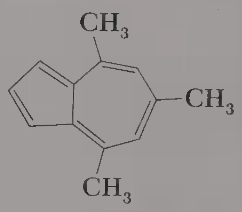
▼
Questions

1. You probably have found that the dyes are separated effectively with ligroin–ether mixtures and that the relative rate of travel indicates the same order of elution in each case. Examine the structures of the three dyes. Can you account for the order of travel? Think particularly about hydrogen bonding.
2. If you noted a reversal in the order of elution from that in (1) above, can you account for the reversal? Again consider hydrogen bonding and remember that a hydrogen bond to oxygen is stronger than one to nitrogen. A solvent capable of hydrogen bonding might interfere with internal hydrogen bonding.
3. Have you thought of an explanation for the results with chromatograms (c) and (d)? If not, consider the fact that a liquid of poor solvent power may not move a substance spotted on a plate at all and that a liquid of high solvent power may move all the dyes along with the solvent front.
4. Suppose the results of the thin layer experiments were to be used as a guide to running a column chromatographic separation of these pigments on alumina, what solvent or solvents would you select and how would you proceed?

2. Hydrocarbon Pigments



Guaiazulene



4,6,8-Trimethylazulene

▼
Solubility in hexane at 0°, mg/ml: lycopene, 7.7 mg; β-carotene, 109 mg

Commercial β-carotene is expensive and lycopene more so, but isolation of the two carotenoids from natural resources (Chapter 49) provides adequate material for thin layer chromatography. Add about 0.5 ml dichloromethane to the small tube containing the two carotenoids and shake to dissolve. The carotenoids may not all dissolve because of partial air oxidation to insoluble material, but there should be enough colored supernatant liquid for numerous chromatographic experiments. Keep the solutions out of the light, and when no more is required evaporate the solvent for safe storage.

If guaiazulene from guaiene is not available for testing, 4,6,8-trimethylazulene[7] is recommended (1% solution in toluene, protected from light).

▼
Autoxidation

The highly unsaturated hydrocarbons are subject to rapid photochemical autoxidation, but during a chromatogram they are protected by solvent vapor.

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[7] Aldrich Chemical Co. Aldrich stocks azulene itself, but at a higher price than that of 4,6,8-trimethylazulene.

However, on removal of a plate from the chamber a spot may disappear rapidly and so should be outlined in pencil immediately. Save a plate from which outlined spots of the three hydrocarbons have disappeared and develop it in an iodine chamber as described in Experiment 3.

With care to make small spots, you should be able to run a chromatogram of the three hydrocarbons on one plate; put the blue hydrocarbon in the center lane. Spot two plates with the three hydrocarbons and run one in toluene and the other in cyclohexane.

▼
Questions

Does either solvent effect a separation? If one of them fails, what is the explanation? The tomato is reported to contain one part of β-carotene to 10 parts of lycopene; do your chromatograms provide evidence for the presence or absence of β-carotene in the lycopene sample? Suggest a procedure for the separation of a mixture of 1 g of lycopene and 1 g of β-carotene, using a combination of crystallization and column chromatography. How would you control the separation? Which of the three hydrocarbons is the most sensitive to air oxidation?

3. Colorless Compounds

You are now to apply the thin layer technique to the more general case in which the chromatograms are developed in a 4-oz bottle containing crystals of iodine. During development, spots appear rapidly, but remember that they also disappear rapidly. Therefore, outline each spot with a pencil immediately on withdrawal of the plate from the iodine chamber. Solvents suggested are as follows:

Cyclohexane
Toluene
Toluene (3 ml)—Chloroform (1 ml)
9:1 Toluene—Methanol (use 4 ml)

Compounds for trial are to be selected from the following list (all 1% solutions in toluene):

▼
Make your own selections

1. Anthracene* (Fluorescent under uv light.)
2. Cholesterol
3. 2,7-Dimethyl-3,5-octadiyne-2,7-diol
4. Diphenylacetylene
5. *trans,trans*-1,4-Diphenyl-1,3-butadiene*
6. *p*-Di-*t*-butylbenzene
7. 1,4-Di-*t*-butyl-2,5-dimethoxybenzene
8. *trans*-Stilbene
9. 1,2,3,4-Tetraphenylnaphthalene*
10. Tetraphenylthiophene
11. *p*-Terphenyl*
12. Triphenylcarbinol
13. Triptycene

It is up to you to make selections and to plan your own experiments. Do as many as time permits. One plan would be to select a pair of compounds estimated to be separable and which have *Rf* values determinable with the same solvent. One can assume that a hydroxyl compound will travel less rapidly with a hydrocarbon solvent than a hydroxyl-free compound, and so you will know what to expect if the solvent contains a hydroxylic component. An aliphatic solvent should carry along an aromatic compound with aliphatic substituents better than one without such groups. However, instead of relying on assumptions, you can do brief preliminary experiments on used plates on which previous spots are visible or outlined. If you spot a pair of compounds on such a plate and let the solvent rise about 3 cm from the starting line before development, you may be able to tell if a certain solvent is appropriate for a given sample. If so, run a complete chromatogram on the two compounds on a fresh plate. If separation of the two seems feasible, put two spots of one compound on a plate, let the solvent evaporate, and put spots of the second compound over the first ones. Run a chromatogram and see if you can detect two spots in either lane (with colorless compounds, it is advisable not to attempt a three-lane chromatogram until you have acquired considerable practice and skill).

▼
Preliminary trials on used plates

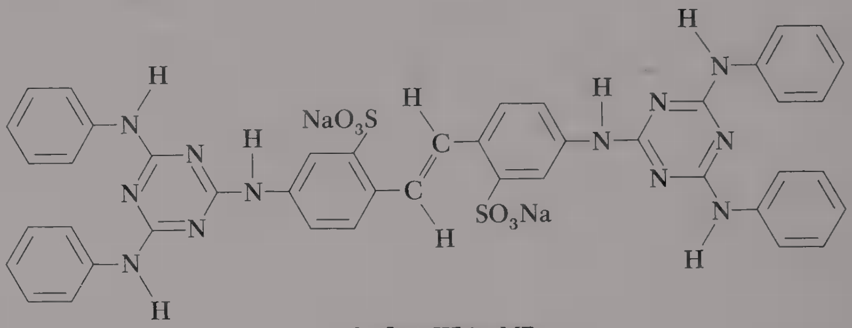
4. Discussion

In case you have investigated hydroxylated compounds, you doubtless have found that it is reasonably easy to separate a hydroxylated from a nonhydroxylated compound, or a diol from a mono-ol. How, by a simple reaction followed by a thin layer chromatogram, could you separate cholesterol from triphenylcarbinol? Heating a sample of each with acetic anhydride-pyridine for 5 min on the steam bath, followed by chromatography, should do it. Study of the rate of acetylation of cholesterol by acetic acid (Chapter 16, Section 2) could be done more simply by the thin layer technique than by column chromatography and with less material. On first trial of a new reaction, one wonders if anything has happened and how much, if any, starting material is present. A comparative chromatogram of reaction mixture with starting material may tell the story. How crude is a crude reaction product? How many components are present? The thin layer technique may give the answers to these questions and suggest how best to process the product. A preparative column chromatogram may afford a large number of fractions of eluent (say 1 to 30). Some fractions probably contain nothing and should be discarded, while others should be combined for evaporation and workup. How can you identify the good and the useless fractions? Take a few used plates and put numbered circles on clean places of each, spot samples of each of the fractions, and, without any chromatography, develop the plates with iodine. Negative fractions for discard will be obvious and the pattern alone of positive fractions may allow you to infer which fractions can be combined. Thin layer chromatograms of the first and last fractions of each suspected group would then show whether or not your inferences are correct.

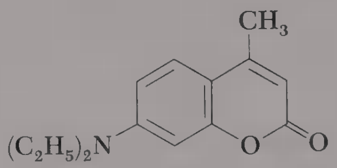
5. Fluorescence

▼
Do not look into a uv lamp

Four of the compounds listed in Section 3 are fluorescent under ultraviolet light and such compounds give colorless spots which can be picked up on a chromatogram by fluorescence (after removal from the uv-absorbing glass bottle). If a uv-light source is available, spot the four compounds on a used plate and observe the fluorescence. Take this opportunity to examine a white shirt or handkerchief under uv light to see if it contains a brightener, that is, a fluorescent white dye or optical bleach. Brighteners of the type of Calcofluor White MR, a sulfonated *trans*-stilbene derivative, are commonly used in detergent formulations for cotton, and the substituted coumarin derivative formulated is typical of brighteners used for nylon, acetate, and wool. Detergents normally contain 0.1–0.2% of optical bleach. The amount of dye on a freshly laundered shirt is approximately 0.01% of the weight of the fabric.



Calcofluor White MR



7-Diethylamino-4-methylcoumarin

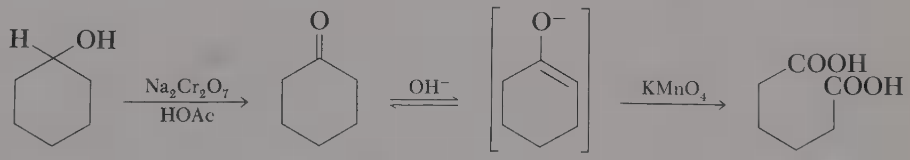
A technique of some use in the chromatography of nonfluorescent compounds is to incorporate an inorganic fluorescent indicator in the adsorbent. The whole plate is then fluorescent under a uv lamp and a dark spot appears where a migrating compound has quenched the fluorescence.

17

Adipic Acid by Chromic Acid Oxidation

KEYWORDS

Oxidation
Acidic dichromate
Alkaline permanganate
Exothermic reaction
Cyclohexanol, cyclohexanone
Manganese dioxide



Cyclohexanol
bp 161.5°, den 0.96,
MW 100.16

Cyclohexanone
bp 157°, den 0.95,
MW 98.14,
solubility 1.5 g/100 ml $H_2O^{10°}$

Adipic acid
mp 153°, MW 146.14
solubility 1.4g/100 g $H_2O^{15°}$

Cyclohexanol can be oxidized directly to adipic acid by nitric acid in about 50% yield, but a stirring motor is required and toxic fumes have to be disposed of. Direct oxidation with permanganate is free from these limitations but takes several days. Since the first step in the permanganate reaction is the slow one, the overall reaction time is greatly reduced by oxidation of cyclohexanol to cyclohexanone with dichromate in acetic acid followed by permanganate oxidation of cyclohexanone catalyzed by alkali to promote formation of the intermediate enolate.

Before beginning the experiment write complete, *balanced* equations for both reactions in your laboratory notebook.

EXPERIMENTS

1. Cyclohexanone

In a 125-ml Erlenmeyer flask dissolve 15 g of sodium dichromate dihydrate in 25 ml of acetic acid by swirling the mixture on the hot plate and then cool the solution with ice to 15°. In a second Erlenmeyer flask chill a mixture of 15.0 g of cyclohexanol and 10 ml of acetic acid in ice. After the first solution is cooled to 15°, transfer the thermometer and adjust the temperature in the second flask to 15°. Wipe the flask containing the dichromate solution, pour the solution into the cyclohexanol-acetic acid mixture, rinse the flask with a little solvent (acetic acid), note the time, and take the initially light orange solution from the ice bath, but keep the ice bath ready for use when required. The exothermic reaction that is soon evident can get out of hand unless controlled. When the temperature rises to 60° cool in ice just enough to prevent a further rise and then, by intermittent brief cooling, keep the temperature close to 60° for 15 min. No further cooling is needed, but the flask should be swirled occasionally and the temperature watched. The usual maximal temperature is 65° (25–30 min). When the temperature begins to drop and the solution becomes pure green, the reaction is over. Allow 5–10 min more reaction time and then pour the green solution into a 250-ml round-bottomed flask, rinse the Erlenmeyer flask with 100 ml of water, and add the solution to the flask for steam distillation of the product (apparatus, Fig. 5.2). Distil as long as any oil passes over with the water and, since cyclohexanone is appreciably soluble in water, continue somewhat beyond this point (about 80 ml will be collected).

▼
Reaction time 45 min

Alternatively, instead of setting up the apparatus for steam distillation, simply add a boiling chip to the 250-ml flask and distil 40 ml of liquid, cool the flask slightly, add 40 ml of water to it and distil 40 ml more. Note the temperature during the distillation. This is a steam distillation in which steam is generated *in situ* rather than from an outside source.

Cyclohexanone is fairly soluble in water. Dissolving inorganic salts such as potassium carbonate or sodium chloride in the aqueous layer will decrease the solubility of cyclohexanone such that it can be completely extracted with ether. This process is known as "salting out."

To salt out the cyclohexanone add to the distillate 0.2 g of sodium chloride per ml of water present and swirl to dissolve the salt. Then pour the mixture into a separatory funnel, rinse the flask with ether, add more ether to a total volume of 25–30 ml, shake, and draw off the water layer. Then wash the ether layer with 25 ml of 10% sodium hydroxide solution to remove acetic acid, test a drop of the wash liquor to make sure it contains excess alkali, and draw off the aqueous layer.

To dry the ether, since it contains dissolved water, shake the ether layer with an equal volume of saturated aqueous sodium chloride solution. Draw off the aqueous layer, pour the ether out of the neck of the separatory funnel into an Erlenmeyer flask, add about 5 g of anhydrous sodium sulfate, and complete final drying of the ether solution by occasional swirling of the solution over a 5-min period. Remove the drying agent by gravity filtration into a tared

Erlenmeyer flask and rinse the flask that contained the drying agent, the sodium sulfate, and the funnel with ether. Add a boiling chip to the ether solution and evaporate the ether on the steam bath under an aspirator tube (Fig. 6.4). Cool the contents of the flask to room temperature, evacuate the crude cyclohexanone under aspirator vacuum to remove final traces of ether (Fig. 4.8), and weigh the product. Yield 11–12.5 g.[1]

The crude cyclohexanone can be purified by simple distillation or used directly in the following experiment.

2. Adipic Acid

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Choice of controlling the temperature for 30 min or running an unattended overnight reaction

The reaction to prepare adipic acid is conducted with 10.0 g of cyclohexanone, 30.5 g of potassium permanganate, and amounts of water and alkali that can be adjusted to provide an attended reaction period of one-half hour, procedure (a), or an unattended overnight reaction, procedure (b).

a) For the short-term reaction, mix the cyclohexanone and permanganate with 250 ml of water in a 500-ml Erlenmeyer flask, adjust the temperature to 30°, note that there is no spontaneous temperature rise, and then add 2 ml of 10% sodium hydroxide solution. A temperature rise is soon registered by the thermometer. It may be of interest to determine the temperature at which you can just detect warmth, by holding the flask in the palm of the hand and by touching the flask to your cheek. When the temperature reaches 45° (15 min) slow the oxidation process by brief ice-cooling and keep the temperature at 45° for 20 min. Wait for a slight further rise (47°) and an eventual drop in temperature (25 min), and then heat the mixture by swirling it over a flame to complete the oxidation and to coagulate the precipitated manganese dioxide. Make a spot test by withdrawing reaction mixture on the tip of a stirring rod and touching it to a filter paper; permanganate, if present, will appear in a ring around the spot of manganese dioxide. If permanganate is still present, add small amounts of sodium bisulfite until the spot test is negative. Then filter the mixture by suction on an 11-cm Büchner funnel, wash the brown precipitate well with water, add a boiling chip, and evaporate the filtrate over a flame from a large beaker to a volume of 70 ml. If the solution is not clear and colorless, clarify it with decolorizing charcoal and evaporate again to 70 ml. Acidify the hot solution with concentrated hydrochloric acid to pH 1–2, add 10 ml acid in excess, and let the solution stand to crystallize. Collect the crystals

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[1] When the acetic acid solutions of cyclohexanol and dichromate were mixed at 25° rather than at 15° the yield of crude cyclohexanone was only 6.9 g. A clue to the evident importance of the initial temperature is suggested by an experiment in which the cyclohexanol was dissolved in 12.5 ml of benzene instead of in 10 ml of acetic acid and the two solutions were mixed at 15°. Within a few minutes orange-yellow crystals separated and soon filled the flask; the substance probably is the chromate ester, $(C_6H_{11}O)_2CrO_2$. When the crystal magma was let stand at room temperature the crystals soon dissolved, exothermic oxidation proceeded, and cyclohexanone was formed in high yield. Perhaps a low initial temperature insures complete conversion of the alcohol into the chromate ester before side reactions set in.

on a small Büchner funnel, wash them with a very small quantity of *cold* water, press the crystals between sheets of filter paper to remove excess water, and set them aside to dry. A typical yield of adipic acid, mp 152–153°, is 6.9 g.

b) In the alternative procedure the weights of cyclohexanone and permanganate are the same but the amount of water is doubled (500 ml) to moderate the reaction and make temperature control unnecessary. All the permanganate must be dissolved before the reaction is begun. Heat the flask and swirl the contents vigorously. Test for undissolved permanganate with a glass rod. After adjusting the temperature of the solution to 30°, 10 ml of 10% sodium hydroxide is added and the mixture is swirled briefly and let stand overnight (maximum temperature 45–46°). The work-up is the same as in procedure (a) and a typical yield of adipic acid is 8.3 g.

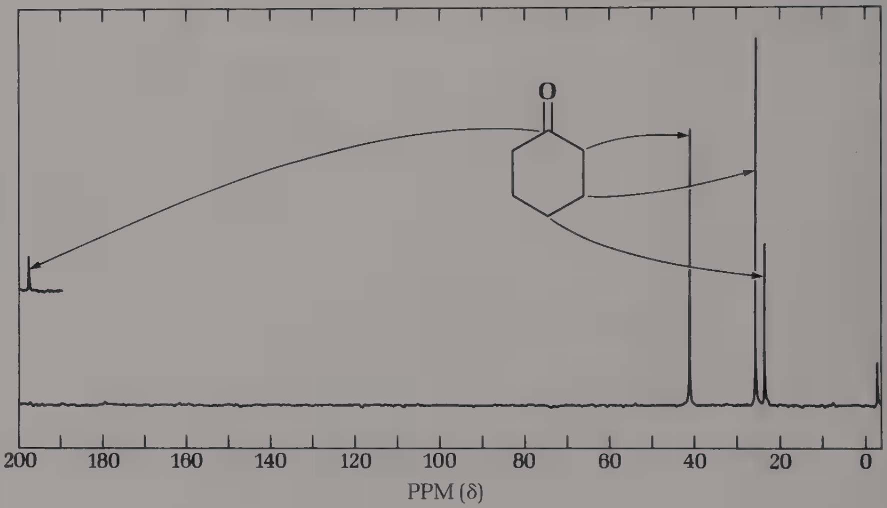


FIGURE 17.1 ^{13}C nmr spectrum of cyclohexanone.

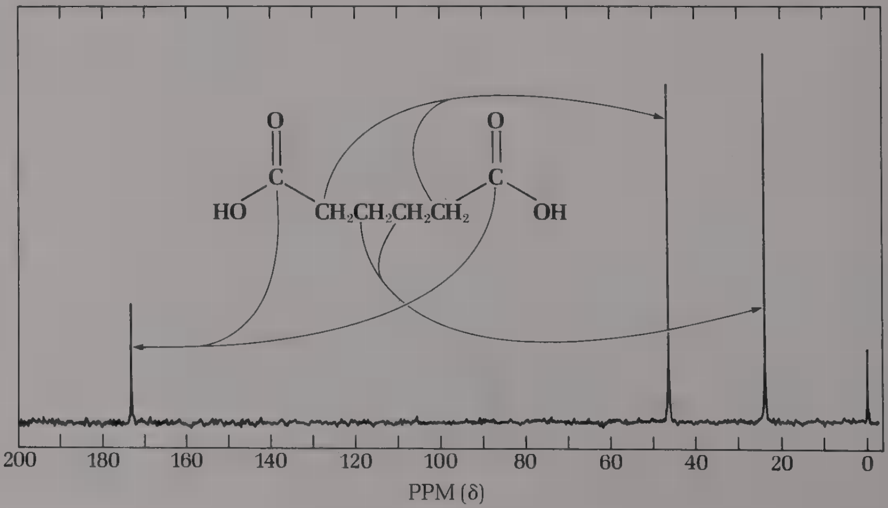


FIGURE 17.2 ^{13}C nmr spectrum of adipic acid.

18

Cholesterol from Gallstones

KEYWORDS

Gallstones
Bilirubin
Zinc dust debromination
Jones, Collins reagents
4- and 5-Cholesten-3-one

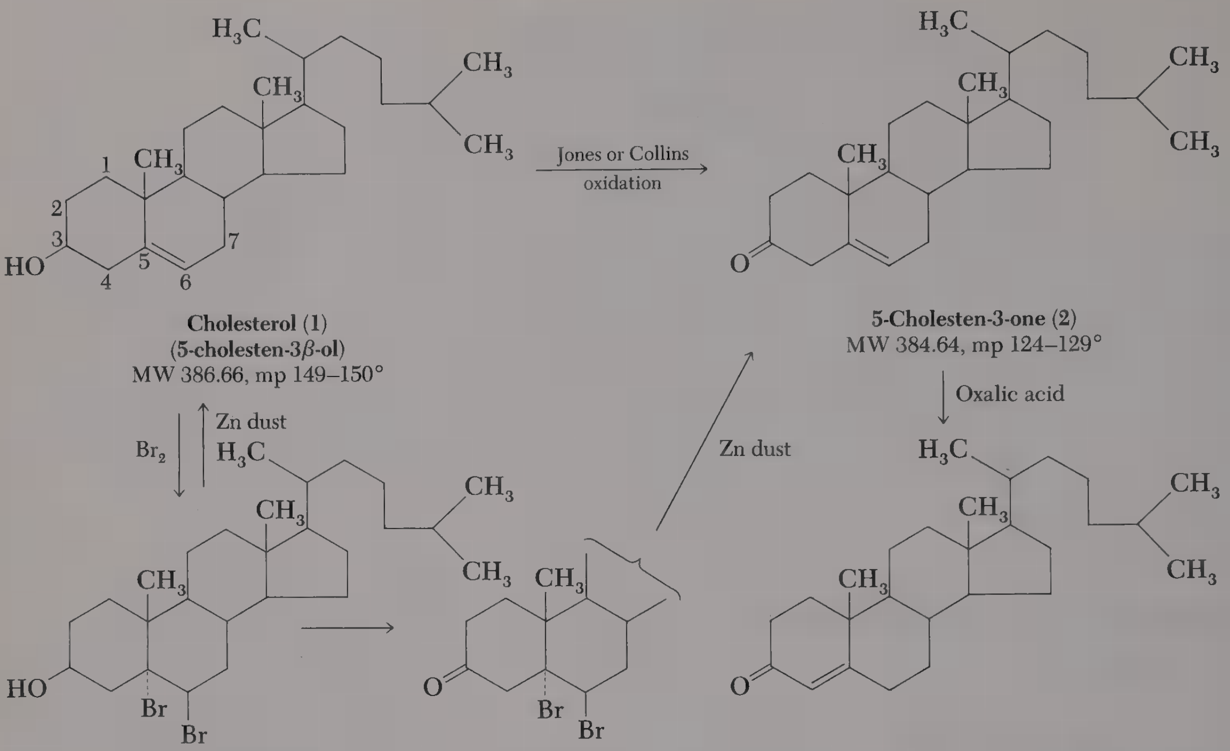
Bromine
Cholesterol dibromide
Isomerization
Liebermann-Burchard test

Acetic anhydride + sulfuric acid
Selenium dioxide test
Tetranitromethane test, unsaturation

▼
Role in atherosclerosis

Human gallstones are composed primarily of the crystalline unsaturated alcohol, cholesterol, along with the bile pigment bilirubin, a metabolite of hemoglobin. Cholesterol is also the substance which thickens arterial walls leading to decreased blood flow in the disease atherosclerosis. The average adult human male contains about 200 g of cholesterol located principally in the brain and nerve tissue; the commercial substance is isolated from the spinal cords of cattle.

In this experiment cholesterol is isolated from human gallstones and freed from various steroidal impurities by conversion to the dibromide, followed by zinc dust debromination. The pure cholesterol, a secondary alcohol, can be oxidized to the corresponding ketone by very versatile procedures: oxidation with Jones reagent or Collins reagent. This ketone, 5-cholesten-3-one, is labile (unstable) and can be isomerized, via the enol, to the conjugated ketone 4-cholesten-3-one. These conversions can be followed spectroscopically. (See the following page.)

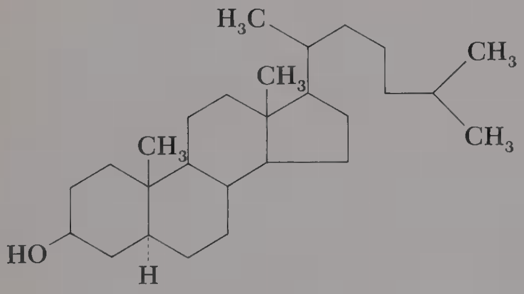


Cholesterol (1)
(5-cholesten-3β-ol)
MW 386.66, mp 149–150°

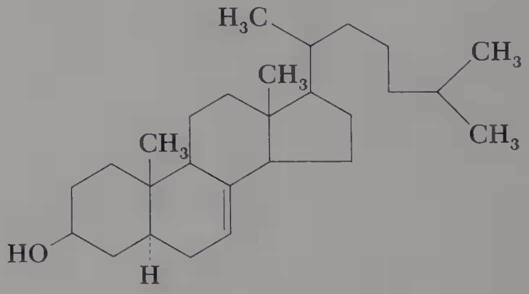
5-Cholesten-3-one (2)
MW 384.64, mp 124–129°

5α,6β-Dibromocholestan-3β-ol (3)

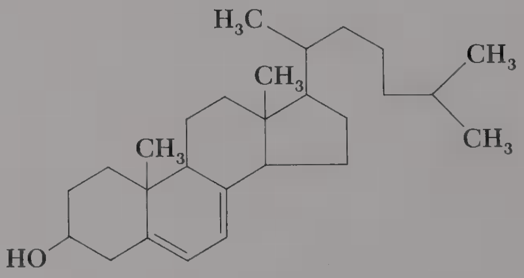
4-Cholesten-3-one (4)
mp 81–82°, $\lambda_{\max}^{\mathrm{EtOH}}$ 241 nm



3β-Cholestanol



7-Cholesten-3β-ol



5,7-Cholestadien-3β-ol

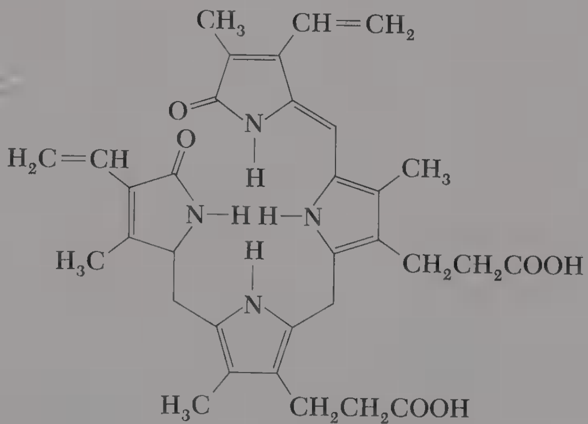
Cholesterol isolated from natural sources contains small amounts (0.1–3%) of 3β-cholestanol, 7-cholesten-3β-ol, and 5,7-cholestadien-3β-ol.[1] These are so very similar to cholesterol in solubility that their removal by crystallization is not feasible. However, complete purification can be accomplished through the sparingly soluble dibromo derivative 5α,6β-dibromocholestan-3β-ol. 3β-Cholestanol being saturated does not react with bromine and remains in the mother liquor. 7-Cholesten-3β-ol and 5,7-cholestadien-3β-ol are dehydrogenated by bromine to dienes and trienes, respectively, that likewise remain in the mother liquor and are eliminated along with colored by-products.

The cholesterol dibromide that crystallizes from the reaction solution is collected, washed free of the impurities or their dehydrogenation products, and debrominated with zinc dust, with regeneration of cholesterol in pure form. Specific color tests can differentiate between pure cholesterol and tissue cholesterol purified by ordinary methods.

EXPERIMENTS

1. Cholesterol from Gallstones[2]

Swirl 2 g of crushed gallstones in a 25-ml Erlenmeyer flask with 15 ml of 2-butanone on a hot plate for a few minutes until the solid has disintegrated and the cholesterol has dissolved. Filter the solution while hot, taking precautions to warm the stemless funnel prior to the filtration. Its dirty-yellow appearance is from a brown residue of the bile pigment bilirubin, a metabolite of hemoglobin.



Bilirubin,
$\lambda_{max}^{CHCl_3}$ 450 nm

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[1] A fourth companion, cerebrosterol, or 24-hydroxycholesterol, is easily eliminated by crystallization from alcohol.

[2] Obtainable from the department of surgery of a hospital. Wrap the stones in a towel and crush by light pounding with a hammer. (See also *Instructor's Manual for Organic Experiments*, 4th ed. for a procedure for making "artificial gallstones.")

▼
Crystallization from mixed solvents. Chapter 4.

If the filtrate is highly colored, add decolorizing charcoal and refilter the solution in the usual way (see Chapter 4). This will ordinarily not be necessary. Dilute the filtrate with 10 ml of methanol, reheat the filtrate to the boiling point, add a little water gradually until the solution is saturated with cholesterol at the boiling point, cool the solution in ice, and let the solution stand for crystallization. Collect the crystals, wash, dry, and take the melting point. Typical result: 1.5 g of large colorless plates or needles, mp 146–147.5°. Use 1 g for purification and save the rest for color tests.

In a 25-ml Erlenmeyer flask dissolve 1 g of gallstone cholesterol or of commercial cholesterol (content of 7-cholesten-3β-ol about 0.6%) in 7 ml of ether by gentle warming and, with a pipette fitted with a pipetter (Fig. 18.1), or with a plastic syringe (Fig. 18.2), or from a burette in the hood, add 5 ml of a solution of bromine and sodium acetate in acetic acid.[3] Cholesterol dibromide begins to crystallize in a minute or two. Cool in an ice bath and stir the crystalline paste with a stirring rod for about 10 minutes to ensure complete crystallization, and at the same time cool a mixture of 3 ml of ether and 7 ml of acetic acid in ice. Then collect the crystals on a small suction funnel and wash with the iced ether-acetic acid solution to remove the yellow mother liquor. Finally wash with a little methanol, continuing to apply suction, and transfer the white solid without drying it (dry weight 1.2 g) to a 50-ml Erlenmeyer flask. Add 20 ml of ether, 5 ml of acetic acid, and 0.2 g of zinc dust[6] and swirl. In about 3 min the dibromide dissolves; after 5–10 min swirling, zinc acetate

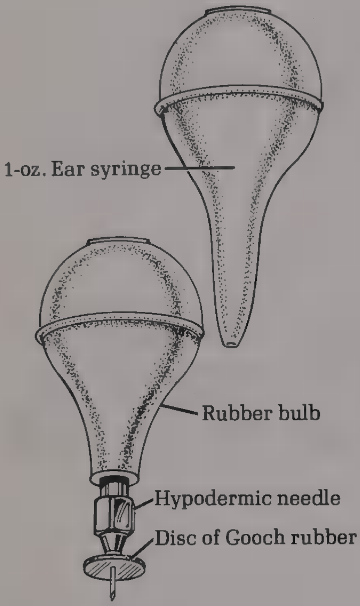

FIGURE 18.1 Pipetters.[4]

FIGURE 18.2 Plastic syringes.[5]

Notes for the instructor

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[3] Weigh a 125-ml Erlenmeyer flask on a balance placed in the hood, add 4.5 g of bromine by a capillary dropping tube (avoid breathing the vapor), and add 50 ml of acetic acid and 0.4 g of sodium acetate (anhydrous).

[4] The pipetter at the top is a 1-oz ear syringe (#526, Davol, Inc., Providence, R.I.) available at most drug stores. The pipetter below is made from a rubber bulb, a hypodermic needle, and a disc of Gooch rubber. A commercial version of the latter pipetter is available from Wilkens-Anderson Co.

[5] Becton, Dickinson Co. (Rutherford, N.J.) disposable 2.5 ml plastic syringe "Plastipak."

[6] If the reaction is slow add more zinc dust. The amount specified is adequate if material is taken from a freshly opened bottle, but zinc dust deteriorates on exposure to air.

usually separates to form a white precipitate (the dilution sometimes is such that no separation occurs). Stir for 5 min more and then add water by drops (no more than 0.5 ml) until any solid present (zinc acetate) dissolves to make a clear solution. Decant the solution from the zinc into a separatory funnel, wash the ethereal solution twice with water and then with 10% sodium hydroxide (to remove traces of acetic acid). Then shake the ether solution with an equal volume of saturated sodium chloride solution to reduce the water content, dry the ether with anhydrous sodium sulfate, remove the drying agent, add 10 ml of methanol (and a boiling stone), and evaporate the solution on the steam bath to the point where most of the ether is removed and the purified cholesterol begins to crystallize. Remove the solution from the steam bath, let crystallization proceed at room temperature and then in an ice bath, collect the crystals, and wash them with cold methanol; you should obtain 0.6–0.7 g, mp 149–150°. Save about 0.1 g of the pure cholesterol for tests.

▼
Aspirator tube

2. Oxidation of Cholesterol with Jones Reagent

▼
Jones Reagent:
$CrO_3–H_2SO_4–H_2O$,
acetone solvent

Oxidation of the secondary hydroxyl group of cholesterol to a ketone (*e.g.*, Oppenauer oxidation) leads to the α,β-unsaturated ketone 4-cholesten-3-one (**4**); the intermediate 5-cholesten-3-one (**2**) is labile and easily isomerized under the reaction conditions to the thermodynamically more stable α,β-unsaturated isomer. One way to avoid this isomerization is to brominate the double bond, oxidize the alcohol, and then debrominate with zinc dust.[7] In this experiment cholesterol is oxidized with either Jones reagent or Collins reagent. The initially formed 5-isomer does not isomerize under the reaction conditions, even though Jones reagent contains sulfuric acid and Collins reagent contains pyridine, both of which will catalyze the isomerization. In a separate experiment the 5-compound is isomerized to the 4-isomer using oxalic acid as the catalyst. These reactions are tests of laboratory skill because both 5- and 4-cholesten-3-one are difficult to crystallize.

Procedure

5-Cholesten-3-one. Dissolve 0.5 g of purified cholesterol in 65 ml of acetone by warming on the steam bath. Cool to 15° and add Jones reagent[8] dropwise with swirling until the orange color persists (about 0.56 ml of the reagent is required). After 2 to 5 min the reaction mixture is poured into 300 ml of 5% sodium bicarbonate solution. The aqueous solution is extracted three times with 30-ml portions of ether, the combined ether extracts are washed with an equal volume of water, and then shaken with 50 ml of saturated sodium

▼
Notes for the instructor

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[7] L. F. Fieser, *Organic Syntheses*, Collect. Vol. 4, John Wiley & Sons, Inc., New York, N.Y., 1963, p. 195.

[8] Jones reagent is prepared by dissolving 26.72 g of chromium trioxide in 23 ml of conc'd sulfuric acid and diluting carefully with water to a total volume of 100 ml.

chloride solution. The ether solution is dried for a few minutes over anhydrous sodium sulfate and evaporated to dryness. The residual oil is often difficult to crystallize; reserve about 60 mg for spectroscopic examination. Dissolve the oil in 5 ml of hot methanol, cool slowly to room temperature and then to 0–4°. The large colorless prisms are collected by suction filtration; yield 0.3 g, mp 124–129° (camphorlike odor).

3. Oxidation of Cholesterol with Collins Reagent

Collins reagent, a chromium trioxide-pyridine complex, is another very versatile oxidizing agent, which usually gives high yields of pure products. It can be used to oxidize primary allylic and benzylic alcohols to aldehydes in 50–80% yield, and it is a superior reagent for the oxidation of secondary alcohols to ketones. In this experiment it is employed for the oxidation of cholesterol to the corresponding ketone without isomerization of the double bond.

The reagent is prepared *in situ*[9] in methylene chloride, as a dark red solution. A one-sixth molar equivalent of the alcohol to be oxidized (in dichloromethane) is added at or near room temperature. After a few minutes a dark sticky precipitate of chromium salts separates; the product is isolated from the methylene chloride solution by the usual process of extracting, washing, drying, and evaporating the solvent.

Procedure

5-Cholesten-3-one. Add 1.2 g (12 mmoles) of anhydrous chromium trioxide with swirling to a solution of 1.9 g (24 mmoles) of pyridine in 30 ml of dry dichloromethane in a 125-ml Erlenmeyer flask. Continue swirling until all the chromium trioxide has dissolved (10 min), then cool the dark red solution to 10° and add 0.77g (2 mmoles) of cholesterol dissolved in 10 ml dichloromethane. Maintain the temperature of the mixture at 10° for 20 min, then decant the dichloromethane solution from the tarry black residue into a separatory funnel. Rinse the reaction flask with 20 ml of ether and add these washings to the separatory funnel. Wash the ether–dichloromethane solution with three 20-ml portions of 5% aqueous sodium hydroxide solution, two 20-ml portions of 3% hydrochloric acid, one 20-ml portion of 5% sodium bicarbonate solution, and 20 ml of saturated sodium chloride solution. Dry the organic layer over anhydrous sodium sulfate and evaporate the solvent (preferably under reduced pressure). Dissolve the residual oil in 8 ml of hot methanol and cool the solution slowly to room temperature and finally to 0–4°. The yield of colorless prisms, which are collected by suction filtration, is usually about 0.5 g, mp 124–129°.

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[9] J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Lett.*, 3363 (1968); and R. Ratcliffe and R. Rhodehorst, *J. Org. Chem.*, 35 4000 (1970). See also Louis F. Fieser and Mary Fieser, *Reagents for Organic Synthesis, Vol. 1,* John Wiley and Sons, Inc., New York, N.Y., 1967, pp. 142–147.

4. Isomerization of 5-Cholesten-3-one

The ketone 5-cholesten-3-one, resulting from the oxidation of cholesterol, is easily isomerized to the conjugated ketone, 4-cholesten-3-one, with oxalic acid.

Procedure

4-Cholesten-3-one. A mixture of 0.25 g of 5-cholesten-3-one (either crystalline material or the oil from the preceding experiment), 25 mg of anhydrous oxalic acid, and 2 ml of 95% ethanol is heated on the steam bath until all the solid has dissolved and for 10 min longer, and then allowed to stand at

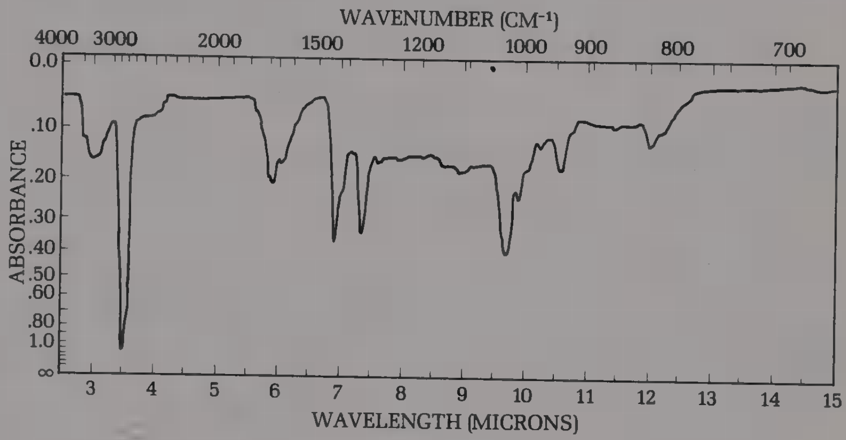

FIGURE 18.3 Infrared spectrum of cholesterol.

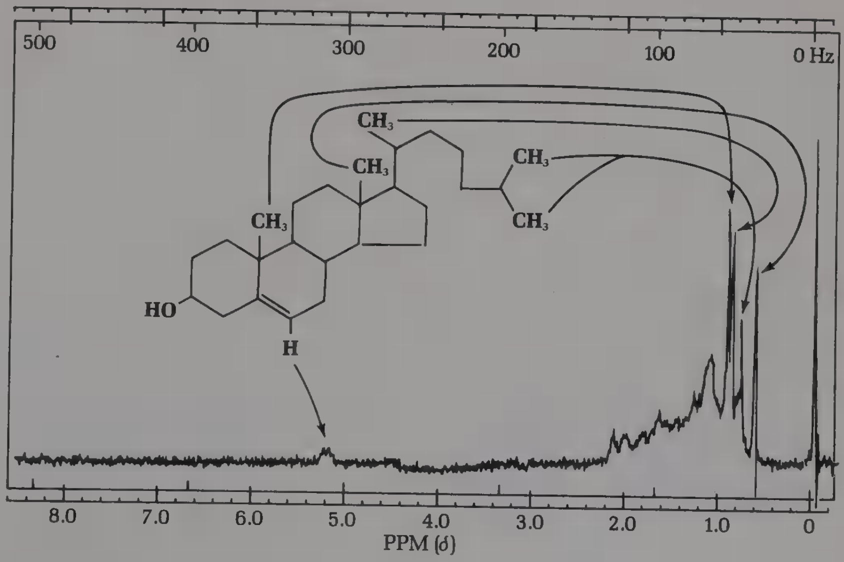

FIGURE 18.4 ^1H nmr spectrum of cholesterol.

room temperature. If crystallization does not start after 30 min the solution is seeded or scratched. After crystallization has proceeded at room temperature and then at 0–4°, the large colorless prismatic needles that separate are collected by suction filtration; yield about 0.2 g, mp 81–82°. This compound is also difficult to crystallize; noncrystalline material can be used for spectroscopic examination.

From an examination of the ir (Chapter 7), uv (Chapter 9) and nmr spectra (Chapter 8) of your cholesterol and the two isomeric ketones, assess your success in carrying out these transformations.

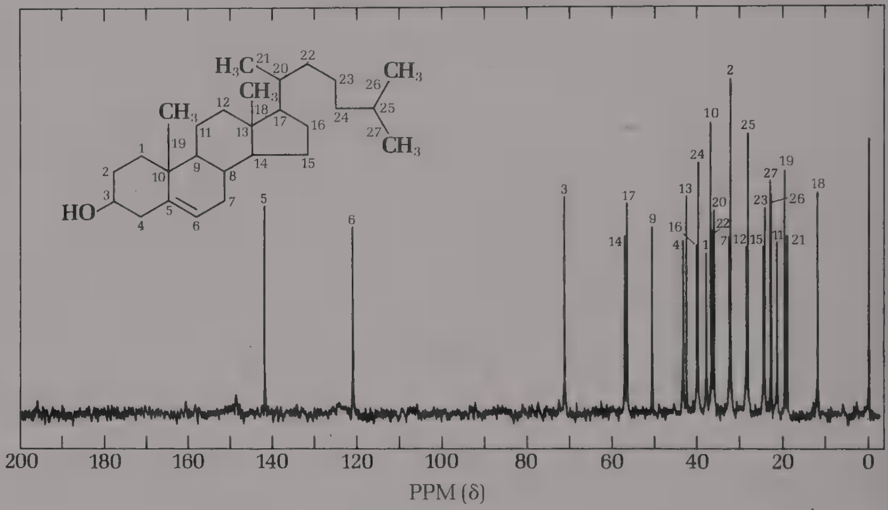

FIGURE 18.5 ^{13}C nmr spectrum of cholesterol.

19

Blood Cholesterol

KEYWORDS

Liebermann-Burchard test
Blood plasma
Heparin
7-Cholesten-3β-ol
Visible spectrometer
Absorbance

Cholesterol reacts with acetic anhydride and sulfuric acid to give a transient purple color changing to blue and then green. This color reaction is the basis for the clinical determination of cholesterol in blood; serum is extracted with ether, the extract mixed with acetic anhydride and sulfuric acid, and the absorbance of the mixture at 620 nm read on a visible spectrometer after a predetermined time. The results are compared with a set of standards; normal human blood contains 160–200 mg of cholesterol per 100 ml. Of this about 25% is free, the remainder esterified.

In the present experiments qualitative Liebermann-Burchard tests are used with pure, commercial, and gallstone cholesterol. A variety of colors are produced in varying lengths of time due to the various cholestenols present. Repeated experiments have demonstrated, however, that cholesterol gives a reproducible color with a maximum absorption at 620 nm, which reaches its maximum intensity in 30 minutes. Even though the exact chemical structure of the colored species (presumably a carbonium ion) is not known, this color reaction is still the method generally used for measuring the cholesterol level in blood.

The cholesterol content of whole human blood can be measured on outdated blood obtained from a blood bank or you may wish to employ your own blood. This should be drawn by competent medical personnel, preferably into a heparinized Vacu-tainer. This serum-capped tube is evacuated and coated on the interior with a layer of heparin, which prevents the blood from clotting. It can be placed directly in the centrifuge to separate cells from plasma.

As in ultraviolet spectroscopy (Chapter 9), the absorbance of visible light at a given wavelength is a function of the path length of the light-absorbing nature of the substance being measured. In the present experiment, the absorbance of known concentrations of cholesterol is measured at the beginning and end of the laboratory period and then is compared with the absorbance of the unknowns to determine quantitatively the amount of cholesterol in the blood. The customary method of reporting blood cholesterol is in milligrams per 100 milliliters of blood.

EXPERIMENTS

1. Qualitative Liebermann-Burchard Tests

Introduce a very small quantity of your purified cholesterol from the previous experiment, commercial cholesterol, and unpurified gallstone cholesterol into separate melting point capillaries (to give a layer no more than 0.5 mm thick) and add chloroform (from a micro pipette made by drawing out another melting point capillary) to give a column 10 mm high. Shake the chloroform down into the capillaries the same way you would "whip" down the mercury column in a clinical thermometer. With a second micro pipette introduce a 3-mm column of the test reagent into the melting point tubes without allowing it to mix with the cholesterol solution. The test reagent is made by chilling 1 ml of acetic anhydride in an ice bath and adding one drop of concentrated sulfuric acid (the solution is stable for 3–4 hr if kept on ice). Grasp all three tubes at the open end and mix the two solutions by a quick whip. Note the time to the nearest second and place the tubes on a white background.

Gallstone cholesterol contains 2–3% of 7-cholesten-3β-ol, which reacts very rapidly with the test reagent to give an initial blue color changing to green; pure cholesterol reacts more slowly and the initial transient color is purplish.

The test is still more sensitive when conducted at 0° because at this temperature 7-cholesten-3β-ol reacts to the point of maximum color density in 5 min, whereas cholesterol is negative indefinitely. See if, by this modified test, you can differentiate between pure cholesterol and commercial cholesterol, which contains about 0.6% 7-cholesten-3β-ol. The only change necessary is to cool the chloroform solution in an ice bath and keep the tube in ice while introducing the acid reagent into the open end; whip the tube and return it to the ice bath.

A 3-mm column of cholesterol in a melting point capillary weighs approximately 1 mg. Estimate the weight of 7-cholesten-3β-ol that you are just able to detect.

2. Blood Cholesterol[1]

To 0.5 ml of blood plasma (obtained by centrifugation of whole blood for 10 min) in a 25-ml glass-stoppered flask add 5 ml of alcoholic potassium hydroxide solution[2]. Stopper, shake well, and incubate in a water bath at 37° for 55 min. Cool to room temperature, add 10 ml of either hexane or ligroin (bp 68°) and 5 ml of water, and shake vigorously for one minute. Transfer a 4-ml aliquot of the hexane layer to a dry test tube and evaporate the solvent by heating the tube to about 60° while pulling air into the tube with an aspirator tube (Fig. 6.4). Should the hexane not separate in the separatory funnel, centrifuge the emulsion at slow speed for a minute or two until it breaks and two clear layers are formed.

Standard Cholesterol Solutions. A 5-ml sample of a standard solution[3] containing 400 mg of cholesterol per liter of ethanol is mixed with 0.3 ml of 33% potassium hydroxide solution in a 25-ml glass-stoppered flask and treated in exactly the same manner as described above for blood plasma. After the layers separate (by centrifugation if necessary) 1-, 2-, and 3-ml aliquots of the hexane layer are measured into three dry test tubes and evaporated to dryness. These three dried samples are to be used as standards and contain the equivalent of 200, 400, and 600 mg of cholesterol per 100 ml of blood.

Color Development.[4] Arrange the test tubes containing the dried samples in the following order: first an empty tube (used as blank), the three standards, the unknowns, and finally a second set of three standards. Place all test tubes in a water bath (or room) held at 25° and add 6 ml of modified Liebermann-Burchard reagent[5] to each of the test tubes sequentially at regular time intervals (one minute). Stir the contents of each test tube vigorously with a clean stirring rod and return the tubes to the constant temperature bath. Thirty minutes after the addition of the reagent to the first test tube and at one-minute intervals thereafter, determine the absorbance of each sample at 620 nm, setting the visible spectrometer to read zero with the blank (the first test tube, which contains only the reagent).

Knowing the absorbances and concentrations of the six standard solutions, calculate the concentration of cholesterol in your unknown. Report the value as mg per 100 ml of blood.

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[1] This experiment is best conducted by a large group so that the labor of preparing the six standard solutions and the reagents can be shared. This procedure is based on that of Abell, Levy, Brodie, and Kendall, *J. Biol. Chem.*, **195**, 357(1952).

[2] Just before use, prepare the alcoholic hydroxide solution by adding 1 ml of 33% potassium hydroxide solution to 16 ml of absolute (100%) ethanol.

[3] Dissolve 400 mg of purified cholesterol in 100 ml of hot ethanol, cool to room temperature, and make up the solution to 1 liter in a volumetric flask.

[4] The absorbance at 620 nm can be measured, using 1-cm cells, on any spectrometer or colormeter set to operate at this wavelength. The Bausch and Lomb Spectronic 20 is satisfactory.

[5] The Liebermann-Burchard reagent is prepared by adding 1 volume of concentrated sulfuric acid to 20 volumes of chilled acetic anhydride (10°) in a glass-stoppered container. The mixture is shaken well and kept cold for 9 min. Then 10 volumes of acetic acid are added and the mixture warmed to room temp. The reagent should be used within one hour of preparation.

20

Nitration of Methyl Benzoate

KEYWORDS

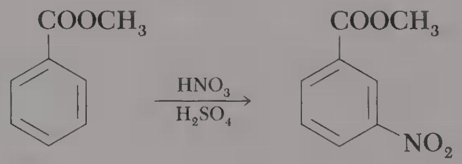
Nitration

Nitronium ion

Electrophilic substitution

meta-Directing, deactivating group

$$HNO_3 + 2\,H_2SO_4 \rightleftharpoons \underset{\text{Nitronium ion}}{NO_2^+} + 2\,HSO_4^- + H_3O^+$$



Methyl benzoate
MW 136.16
bp 199.6°, den 1.09

Methyl *m*-nitrobenzoate
MW 181.15
mp 78°

In the presence of concentrated sulfuric acid, nitric acid ionizes to generate the electrophilic nitronium ion, the bisulfate ion, and the hydronium ion. In this experiment sulfuric acid also serves as solvent for methyl benzoate. Nitration under these conditions proceeds with relative ease despite the fact that the ester substituent on the benzene ring deactivates the ring to electrophilic substitution. The nitration of nitrobenzene to give *m*-dinitrobenzene requires fuming nitric acid and a temperature of 100° because the nitro group is a more strongly deactivating substituent than the methyl ester group.

In the present experiment the *meta*-nitro substituted product can be isolated in greater than 80% yield. Washing the crude crystalline product with cold methanol removes an oil consisting of the *ortho*-nitro product, some of the desired *meta* product, and a dinitro ester. Unchanged methyl benzoate has not been detected.[1]

EXPERIMENT

In a 125-ml Erlenmeyer flask cool 12 ml of concentrated sulfuric acid to 0° and then add 6.1 g of methyl benzoate. Again cool the mixture to 0–10°. Now add dropwise, using a Pasteur pipette, a cooled mixture of 4 ml of concentrated sulfuric acid and 4 ml of concentrated nitric acid. During the addition of the acids, swirl the mixture frequently and maintain the temperature of the reaction mixture in the range of 5–15°.

When all the nitric acid has been added, warm the mixture to room temperature and after 15 min pour it on 50 g of cracked ice in a 250-ml beaker. Isolate the solid product by suction filtration using a small Büchner funnel and wash well with water, then with two 10-ml portions of ice-cold methanol. A small sample is saved for a melting point determination. The remainder is weighed and crystallized from an equal weight of methanol. The crude product should be obtained in about 80% yield and with a mp of 74–76°. The recrystallized product should have a mp of 78°.

QUESTION

1. Why does methyl benzoate dissolve in concentrated sulfuric acid?

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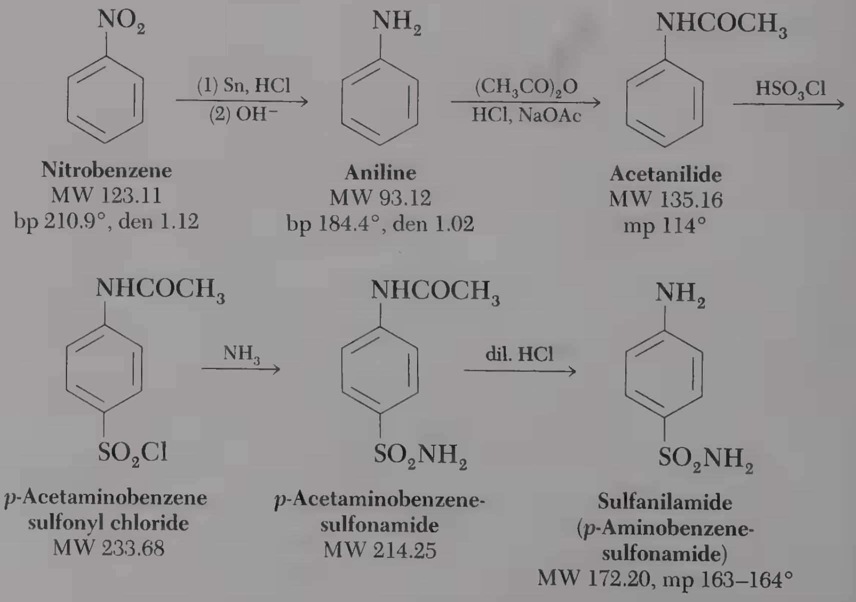
[1] O. Kamm and J. B. Segar, *Org. Syn., Coll. Vol., 1*, 372 (1941).

21

Sulfanilamide from Nitrobenzene

KEYWORDS

- Sulfa drug, sulfanilamide
- Antibacterial agent
- Nitrobenzene (toxic)
- Reduction, tin and hydrochloric acid
- Aniline double salt
- Steam distillation
- Acetylation in aqueous solution, acetic anhydride
- Acetanilide, *p*-acetaminobenzenesulfonyl chloride
- Chlorosulfonic acid
- Ammonolysis
- Speed, high yield, pure product



Nitrobenzene
MW 123.11
bp 210.9°, den 1.12

Aniline
MW 93.12
bp 184.4°, den 1.02

Acetanilide
MW 135.16
mp 114°

***p*-Acetaminobenzene sulfonyl chloride**
MW 233.68

***p*-Acetaminobenzenesulfonamide**
MW 214.25

Sulfanilamide (*p*-Aminobenzenesulfonamide)
MW 172.20, mp 163–164°

This experiment is a five-step synthesis of the first known sulfa drug, sulfanilamide. A number of N-substituted sulfanilamides are still important antibacterial agents. These drugs compete with *p*-aminobenzoic acid in bacterial enzyme systems and thus inhibit bacterial growth. Higher organisms, such as man, do not require *p*-aminobenzoic acid in their enzyme systems and are therefore not affected by the sulfanilamides.

Nitrobenzene is reduced to aniline by tin and hydrochloric acid. A double salt with tin having the formula $(C_6H_5NH_3)_2SnCl_4$ separates partially during the reaction, and at the end it is decomposed by addition of excess alkali, which converts the tin into water-soluble stannite or stannate (Na_2SnO_2 or Na_2SnO_3). The aniline liberated is separated from inorganic salts and the insoluble impurities derived from the tin by steam distillation and is then dried, distilled, and acetylated, either with acetic anhydride in aqueous solution or by refluxing with acetic acid. Treatment of the resulting acetanilide with excess chlorosulfonic acid effects substitution of the chlorosulfonyl group and affords *p*-acetaminobenzenesulfonyl chloride. The alternative route to this intermediate via sulfanilic acid is unsatisfactory, because sulfanilic acid being dipolar is difficult to acetylate. In both processes the amino group must be protected by acetylation to permit formation of the acid chloride group. The next step in the synthesis is ammonolysis of the sulfonyl chloride and the terminal step is removal of the protective acetyl group.

Use the total product obtained at each step as starting material for the next step and adjust the amounts of reagents accordingly. You are to choose between alternative procedures for the acetylation of aniline and decide whether to purify an intermediate or use it as such to avoid purification losses. Aim for a high overall yield of pure final product in the shortest possible time. Keep a record of your actual working time. Study the procedures carefully before coming to the laboratory so that your work will be efficient. A combination of consecutive steps that avoids a needless isolation saves time and increases the yield.

EXPERIMENT

1. Aniline

▼
Measure nitrobenzene in the hood. Do not breathe the vapors

The reduction of the nitrobenzene is carried out in a 500-ml round-bottomed flask suitable for steam distillation of the reaction product. Put 25 g of granulated tin and 12.0 g of nitrobenzene in the flask, make an ice-water bath ready, add 55 ml of concentrated hydrochloric acid, insert a thermometer, and swirl well to promote reaction in the three-phase system. Let the mixture react until the temperature reaches 60° and then cool briefly in ice just enough to prevent a rise above 60°, so the reaction will not get out of hand. Continue to swirl, cool as required, and maintain the temperature in the range 55–60° for 15 min. Remove the thermometer and rinse it with water, fit the flask with a reflux condenser, and heat on the steam bath with frequent swirling until droplets of nitrobenzene are absent from the condenser, and the color due to an intermediate reduction product is gone (about 15 min). During this period dissolve 40 g of sodium hydroxide in 100 ml of water and cool to room temperature.

▼
Reaction time: 0.5 hr

▼
Suitable point of interruption

At the end of the reduction reaction, cool the acid solution in ice (to prevent volatilization of aniline) during gradual addition of the solution of alkali. This alkali neutralizes the aniline hydrochloride releasing aniline, which will now be volatile in steam. Attach a stillhead with steam-inlet tube, condenser, adapter, and receiving Erlenmeyer (Fig. 5.2); heat the flask with a microburner to prevent the flask from filling with water from condensed steam and proceed to steam distil. Since aniline is fairly soluble in water (3.6 g/100 $g^{18°}$) distillation should be continued somewhat beyond the point where the distillate has lost its original turbidity (50–60 ml more). Make an accurate estimate of the volume of distillate by filling a second flask with water to the level of liquid in the receiver and measuring the volume of water.

▼
Alternative choices

▼
Salting out aniline

At this point consider the conversion of aniline into acetanilide and make a choice between alternative procedures. You could reduce the solubility of aniline by dissolving in the steam distillate 0.2 g of sodium chloride per ml, extract the aniline with 2–3 portions of dichloromethane, dry the extract, distil the dichloromethane (bp 41°), and then distil the aniline (bp 184°). You can then select either of the two following procedures for the acetylation of aniline. The second (b) requires pure, dry aniline, as you would obtain it by extraction and distillation, both processes which might be attended with losses; furthermore a 4-hr reflux period is required. Procedure (a) also calls for pure aniline, but note that the first step is to dissolve the aniline in water and hydrochloric acid. Your steam distillate is a mixture of aniline and water, both of which have been distilled. Are they not both water-white and presumably pure? Hence, an attractive procedure would be to assume that the steam distillate contains the theoretical amount of aniline and to add to it, in turn, appropriate amounts of hydrochloric acid, acetic anhydride, and sodium acetate, calculated from the quantities given in 2 (a).

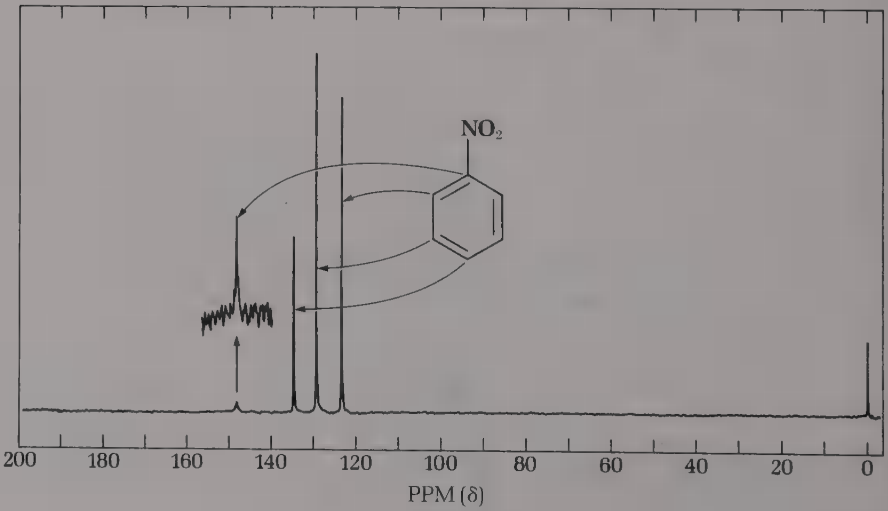


FIGURE 21.1 ^{13}C nmr spectrum of nitrobenzene.

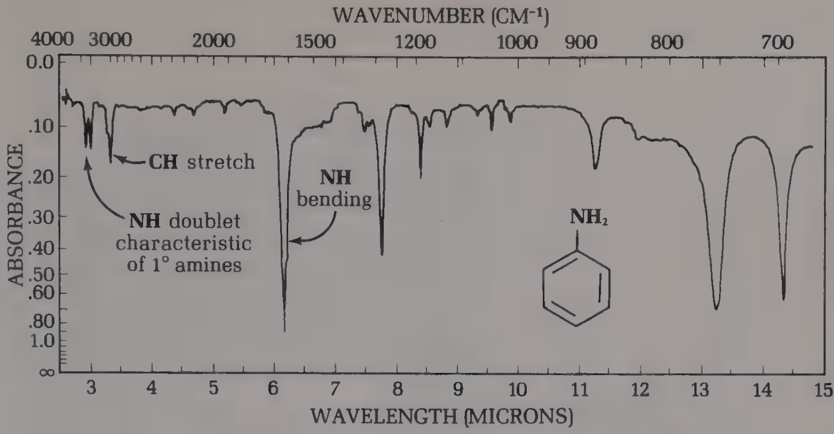


FIGURE 21.2 Infrared spectrum of aniline.

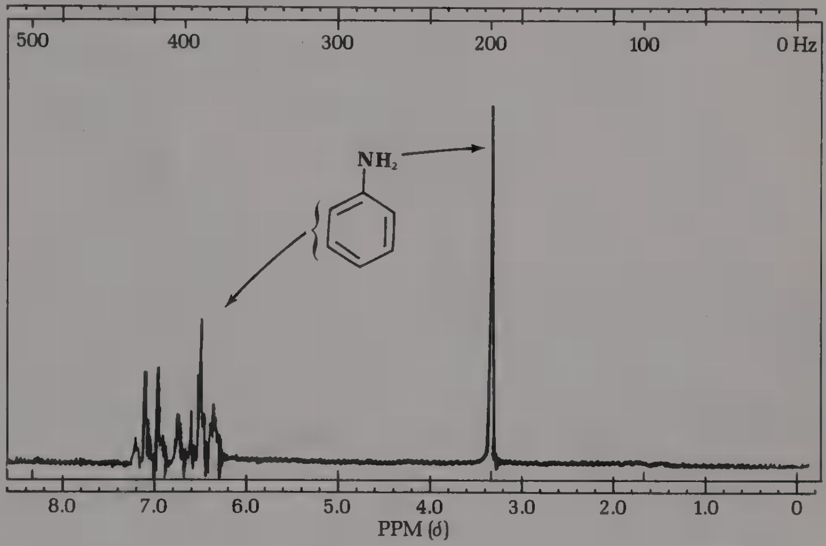


FIGURE 21.3 ^1H nmr spectrum of aniline.

2. Acetanilide

Choice of procedures (a) and (b)

(a) Acetylation in Aqueous Solution

Dissolve 5.0 g (0.054 mole) of aniline in 135 ml of water and 4.5 ml (0.054 mole) of concentrated hydrochloric acid, and, if the solution is colored, filter it by suction through a pad of decolorizing charcoal. Measure out 6.2 ml (0.065 mole) of acetic anhydride, and also prepare a solution of 5.3 g (0.065 mole) of anhydrous sodium acetate in 30 ml of water. Add the acetic anhydride to the solution of aniline hydrochloride with stirring and at once add the sodium acetate solution. Stir, cool in ice, and collect the product. It should be colorless and the mp close to 114°. Since the acetanilide *must be completely dry* for use in the next step, it is advisable to put the material in a tared 125-ml Erlenmeyer flask and to heat this on the steam bath under evacuation until the weight is constant. (See Fig. 4.8.)

(b) Refluxing with Acetic Acid

In a 125-ml flask equipped with an air condenser set for reflux, place 5.0 g of aniline and 20 ml of acetic acid; in case the aniline is discolored add a small pinch of zinc dust which will reduce colored oxidation products to aniline. Adjust the flame so that the ring of condensate rises to about 15 cm from the top of the condenser. Reflux for at least 4 hr and pour the hot reaction mixture in a thin stream into 200 ml of cold water. Collect the product and wash it with water. Pure acetanilide is colorless and melts at 114°.

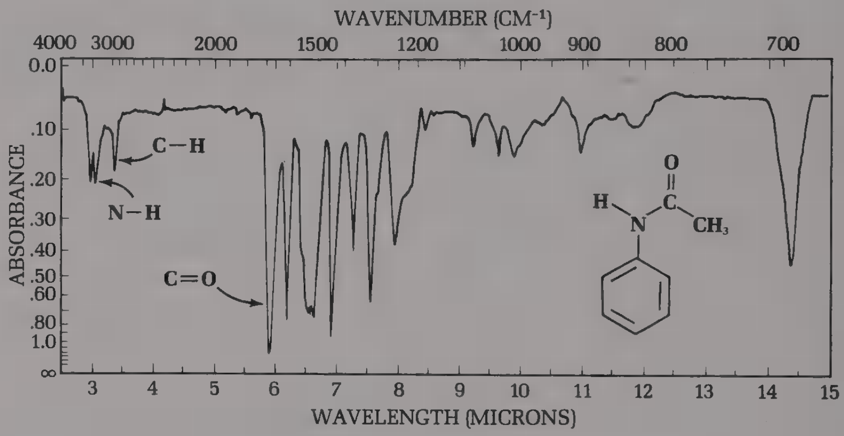


FIGURE 21.4 Infrared spectrum of acetanilide in $CHCl_3$.

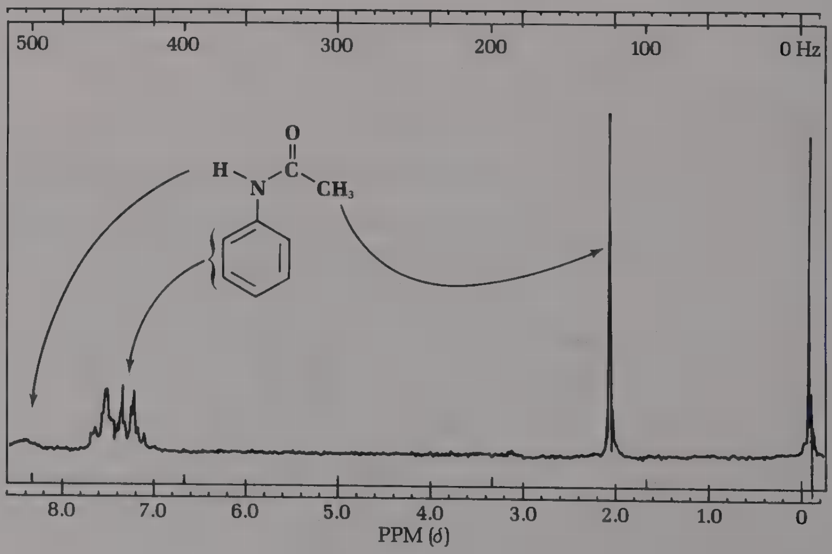


FIGURE 21.5 ^{1}H nmr spectrum of acetanilide. The amide proton shows a characteristically broad peak.

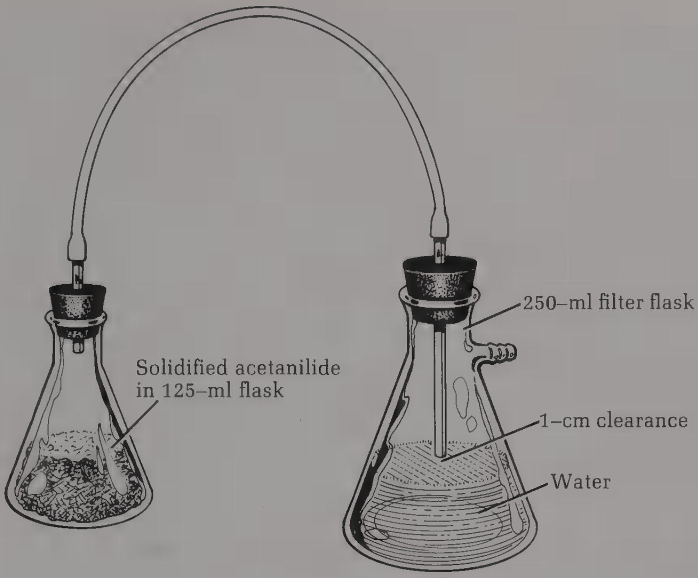


FIGURE 21.6 Chlorosulfonation apparatus fitted with HCl gas trap.

3. Sulfanilamide

▼
Caution! Corrosive chemical, reacts violently with water. Withdraw with pipette and pipetter (See Figs. 1.24 and 1.25)

The chlorosulfonation of acetanilide in the preparation of sulfanilamide is conducted without solvent in the 125-ml Erlenmeyer flask used for drying the precipitated acetanilide from procedure 2(a). Since the reaction is most easily controlled when the acetanilide is in the form of a hard cake, the dried solid is melted by heating the flask over a free flame; as the melt cools, the flask is swirled to distribute the material as it solidifies over the lower walls of the flask. Let the flask cool while making provision for trapping the hydrogen chloride evolved in the chlorosulfonation. Fit the Erlenmeyer with a stopper connected by a section of rubber tubing to a glass tube fitted with a stopper into the neck of a 250-ml filter flask half-filled with water. The tube should be about 1 cm above the surface of the water and *must not dip into the water.* See Fig. 21.6. Cool the flask containing the acetanilide thoroughly in an ice-water bath, and for 5.0 g of acetanilide measure 12.5 ml of chlorosulfonic acid in a graduate (supplied with the reagent and kept away from water). Add the reagent in 1–2 ml portions with a capillary dropping tube, and connect the flask to the gas trap. The flask is now removed from the ice bath and swirled until a part of the solid has dissolved and the evolution of hydrogen chloride is proceeding rapidly. Occasional cooling in ice may be required to prevent too brisk a reaction. In 5–10 min the reaction subsides and only a few lumps of acetanilide remain undissolved. When this point has been reached, heat the mixture on the steam bath for 10 min to complete the reaction, cool the flask under the tap, and deliver the oil by drops with a capillary dropper while stirring it into 75 ml of ice water contained in a beaker cooled in an ice bath (hood). Rinse the flask with cold water and stir the precipitated *p*-acetaminobenzenesulfonyl chloride for a few minutes until an even suspension of granular white solid is obtained.

▼
Do not let the mixture stand before addition of ammonia

Collect and wash the solid on a Büchner funnel. After pressing and draining the filter cake, transfer the solid to the rinsed reaction flask, add (for 5 g of aniline) 15 ml of concentrated aqueous ammonia solution and 15 ml of water, and heat the mixture over a flame with occasional swirling (hood). Maintain the temperature of the mixture just below the boiling point for 5 min. During this treatment a change can be noted as the sulfonyl chloride undergoes transformation to a more pasty suspension of the amide. Cool the suspension well in an ice bath, collect the *p*-acetaminobenzenesulfonamide by suction filtration, press the cake on the funnel, and allow it to drain thoroughly. Any excess water will unduly dilute the acid used in the next step.

Transfer the still moist amide to the well-drained reaction flask, add 5 ml of concentrated hydrochloric acid and 10 ml of water (for 5 g of aniline), boil the mixture gently until the solid has all dissolved (5–10 min), and then continue the heating at the boiling point for 10 min longer (do not evaporate to dryness). The solution when cooled to room temperature should deposit no solid amide, but, if it is deposited, heating should be continued for a further period. The cooled solution of sulfanilamide hydrochloride is shaken with decolorizing charcoal and filtered by suction. Place the solution in a beaker and cautiously add an aqueous solution of 5 g of sodium bicarbonate with stirring to neutralize the hydrochloride. After the foam has subsided, test the suspension with litmus, and, if it is still acidic, add more bicarbonate until the neutral point is reached. Cool thoroughly in ice and collect the granular, white precipitate of sulfanilamide. The crude product (mp 161–163°) on crystallization from alcohol or water affords pure sulfanilamide, mp 163–164°, with about 90% recovery.

QUESTIONS

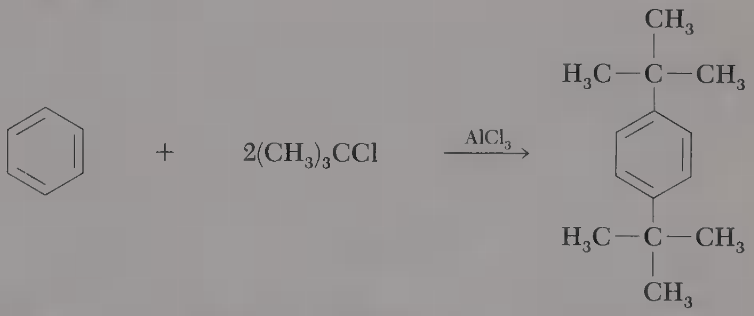
1. Why must the central glass tube in the HCl gas trap (Fig. 21.6) be *above* the water level?
2. Why is an acetyl group added to aniline (making acetanilide) and then removed to regenerate the amine group in sulfanilamide?
3. What happens when chlorosulfonic acid comes in contact with water?
4. Acetic anhydride, like any anhydride, reacts with water to form a carboxylic acid. How then is it possible to carry out an acetylation in aqueous solution? What is the purpose of the hydrochloric acid and the sodium acetate in this reaction?

22

Friedel-Crafts Alkylation of Benzene and Dimethoxybenzene

KEYWORDS

Friedel-Crafts alkylation
Lewis acid catalyst, $AlCl_3$
t-Butyl chloride, benzene
Thiourea inclusion complex
Lewis acid catalyst, fuming H_2SO_4
t-Butyl alcohol, 1,4-dimethoxybenzene
Trimethylcarbonium ion
Strange crystallization behavior



Benzene
MW 78.11, den 0.88,
bp 80°

2-Chloro-2-methylpropane
(*t*-Butyl chloride)
MW 92.57, den 0.85,
bp 51°

***p*-Di-*t*-butylbenzene**
MW 190.32, mp 77–79°,
bp 167°

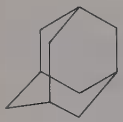
The classical illustration of Friedel-Crafts alkylation, the reaction of benzyl chloride with benzene to form diphenylmethane, has the disadvantage that separation of the liquid reaction product from a complex mixture is not satisfactory with equipment ordinarily available. In this experiment *t*-butyl chloride is used instead of benzyl chloride. *t*-Butyl chloride reacts rapidly with benzene at 0° under the catalytic influence of aluminum chloride via the *t*-butyl carbonium ion to give first *t*-butylbenzene, a liquid, and then *p*-di-*t*-butylbenzene, a beautifully crystalline solid (symmetrical structure). The crystalline reaction product is then isolable with ease in reasonable yield.

▼
By-products

The chief factor limiting the yield appears to be the lability of the *t*-butyl group of the product. The reaction of *p*-di-*t*-butylbenzene with *t*-butyl chloride and aluminum chloride (1.3 moles) at 0–5° gives *m*-di-*t*-butylbenzene, 1,3,5-tri-*t*-butylbenzene, and unchanged starting material. Thus, the mother liquor from crystallization of *p*-di-*t*-butylbenzene probably contains *t*-butylbenzene, the desired *p*-di product, the *m*-di isomer, and 1,3,5-tri-*t*-butylbenzene.

▼
Thiourea inclusion complex

Although the mother liquor probably contains a mixture of several components, the *p*-di-*t*-butylbenzene present can be isolated easily as an inclusion complex (see Chapter 48, Oleic Acid). If you construct a molecular model of *p*-di-*t*-butylbenzene and try fitting it into cellulose acetate cylinders representing urea channels (dia 14.3 cm and 16.2 cm) and a thiourea channel (dia 26.4 cm), you will find that it fits as nicely in the thiourea channel as does adamantane. The adamantane complex is made up of 3.4 molecules of thiourea per molecule of hydrocarbon. Compare the length of the *p*-di-*t*-butylbenzene molecule with the length of *n*-alkanes (consult Table 2 of Chapter 48) and predict the host/guest ratio. You can then check your prediction experimentally.



Adamantane

EXPERIMENTS

1. *p*-Di-*t*-butylbenzene

▼
Caution! Benzene is a mild carcinogen. Handle in the hood, do not breathe vapors or allow liquid to come in contact with the skin.

▼
Reaction time about 15 min

Measure in the hood 20 ml of 2-chloro-2-methylpropane (*t*-butyl chloride) and 10 ml of benzene in a 125-ml filter flask equipped with a one-holed rubber stopper fitted with a thermometer. Place the flask in an ice-water bath to cool. Weigh 1 g of fresh aluminum chloride onto a creased paper, and scrape it with a small spatula into a 10 × 75-mm test tube; close the tube at once with a cork.[1] Connect the side arm of the flask to the aspirator (preferably one made of plastic) and operate it at a rate sufficient to carry away hydrogen chloride formed in the reaction. Cool the liquid to 0–3°, add about one quarter of the aluminum chloride, replace the thermometer and swirl the flask vigorously in the ice bath. After an induction period of about 2 min a vigorous reaction sets in, with bubbling and liberation of hydrogen chloride. Add the remainder of the catalyst in three portions at intervals of about 2 min. Toward the end, the reaction product begins to separate as a white solid. When this occurs, remove the flask from the bath and let stand at room temperature for 5 min. Add ice and water to the reaction mixture and then ether for extraction of the product, stirring with a rod or spatula to help bring the solid into solution. Transfer the solution to a separatory funnel and shake; draw off the lower layer and wash the upper ether layer with water and then with a saturated sodium

---

[1] Alternative scheme: Put a wax pencil mark on the test tube 37 mm from the bottom and fill the tube with aluminum chloride to this mark.

chloride solution. Dry the ether solution over anhydrous sodium sulfate for 5 min, filter the solution to remove the drying agent, remove the ether by evaporation on the steam bath, and evacuate the flask using the aspirator to remove traces of solvent until the weight is constant (Fig. 4.8); yield of crude product, 15 g.

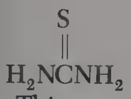
▼
Aspirator tube to remove ether vapor

The oily product should solidify on cooling. For crystallization, dissolve the product in 20 ml of methanol and let the solution come to room temperature without disturbance. If you are in a hurry, lift the flask without swirling; place it in an ice-water bath and observe the result. After thorough cooling at 0°, collect the product and rinse the flask and product with a little ice-cold methanol. The yield of *p*-di-*t*-butylbenzene from the first crop is 8.2–8.6 g of satisfactory material. Save the product for the next step as well as the mother liquor, in case you later wish to work it up for a second crop.

▼
Spontaneous crystallization gives beautiful needles or plates

In a 125-ml Erlenmeyer flask dissolve 5 g of thiourea (Caution! See marginal note.) and 3 g of *p*-di-*t*-butylbenzene in 50 ml of methanol (break up lumps with a flattened stirring rod) and let the solution stand for crystallization of the complex, which occurs with ice cooling. Collect the crystals and rinse with a little methanol and dry to constant weight; yield 5.8 g. Bottle a small sample, determine carefully the weight of the remaining complex, and place the material in a separatory funnel along with about 25 ml each of water and ether. Shake until the crystals disappear, draw off the aqueous layer containing thiourea, wash the ether layer with saturated sodium chloride, and dry the ether layer over anhydrous sodium sulfate. Remove the drying agent by filtration; collect the filtrate in a tared 125-ml Erlenmeyer. Evaporate and evacuate as before, making sure the weight of hydrocarbon is constant before you record it. Calculate the number of molecules of thiourea per molecule of hydrocarbon (probably *not* an integral number).

▼
Inclusion complex starts to crystallize in 10 min



$H_2N\overset{\overset{S}{\|}}{C}NH_2$

Thiourea
MW 76.12

▼
Caution! Thiourea is a mild carcinogen. Handle the solid in a hood. Do not breathe dust.

To work up the mother liquor from the crystallization from methanol, first evaporate the solvent. Note that the residual oil does not solidify on ice cooling. Next, dissolve the oil, together with 5 g of thiourea (*Caution!*), in 50 ml of methanol, collect the inclusion complex that crystallizes (3.2 g), and recover *p*-di-*t*-butylbenzene from the complex as before (0.8 g before crystallization).

▼
Work-up of mother liquor

The infrared spectrum of *p*-di-*t*-butylbenzene is presented in Fig. 22.1 and

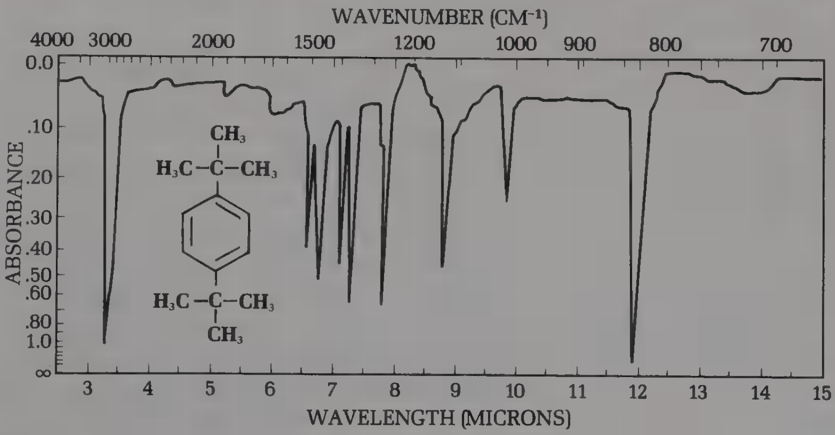
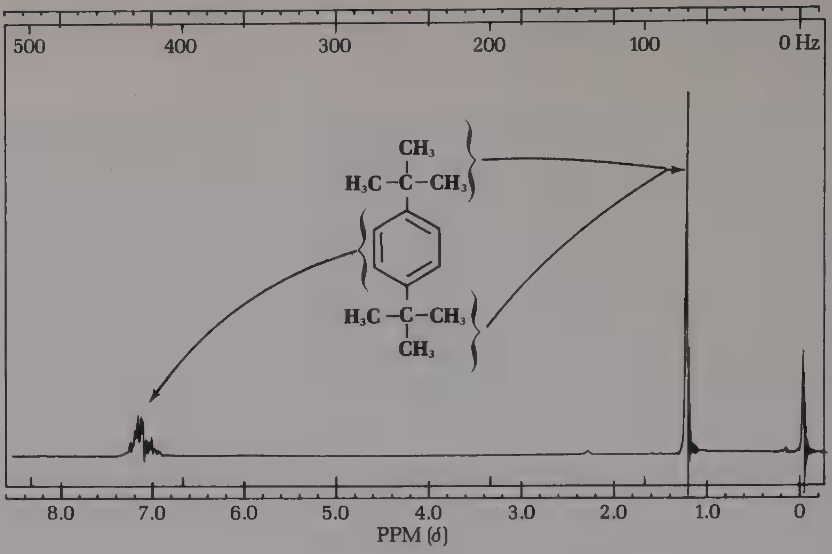


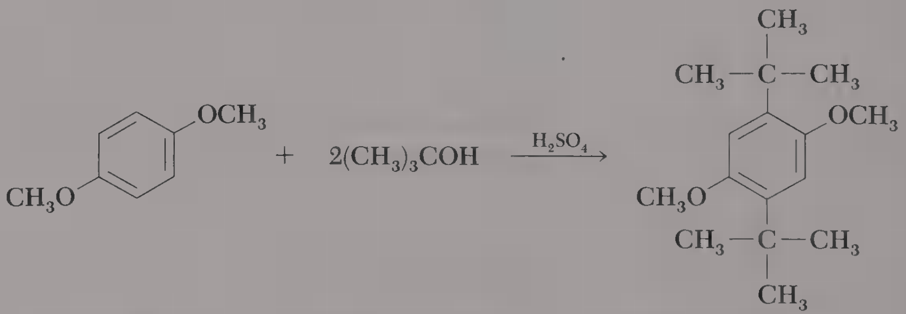
FIGURE 22.1 Infrared spectrum of *p*-di-*t*-butylbenzene.

FIGURE 22.2 ^1H nmr spectrum of *p*-di-*t*-butylbenzene.



the nmr spectrum in Fig. 22.2. Note the simplicity of the nmr spectrum, a result of symmetry in the molecule.

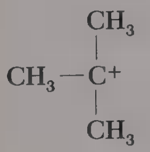
2. 1,4-Di-*t*-butyl-2,5-dimethoxybenzene



Hydroquinone dimethyl ether
MW 138.16, mp 57°

2-Methyl-2-propanol (*t*-Butyl alcohol)
MW 74.12, den 0.79, mp 25.5°, bp 82.8°

1,4-Di-*t*-butyl-2,5-dimethoxybenzene
MW 250.37, mp 104–105°


$$\mathrm{CH_3-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C^+}}}$$

Trimethylcarbonium ion

This experiment illustrates the Friedel-Crafts alkylation of an activated benzene molecule with a tertiary alcohol in the presence of sulfuric acid as the Lewis acid catalyst. As in the reaction of benzene and *t*-butyl chloride the substitution involves attack by the electrophilic trimethylcarbonium ion.

$H_2SO_4 + SO_3$
Fuming sulfuric acid
Caution! Highly corrosive. Reacts violently with water

Place 6 g of hydroquinone dimethyl ether in a 125-ml Erlenmeyer flask, add 10 ml of *t*-butyl alcohol and 20 ml of acetic acid, and put the flask in an ice-water bath to cool. Measure 10 ml of concentrated sulfuric acid into a 50-ml Erlenmeyer, add 10 ml of 30% fuming sulfuric acid, and put the flask, properly supported, in the ice bath to cool. Put a thermometer in the larger flask and swirl in the ice bath until the temperature is in the range 0–3°, and remove the thermometer (solid, if present, will dissolve later). Clamp a small separa-

▼
Reaction time about 12 min

tory funnel in a position to deliver into the 125-ml Erlenmeyer so that the flask can remain in the ice-water bath, wipe the smaller flask dry, and pour the chilled sulfuric acid solution into the funnel. While constantly swirling the 125-ml flask in the ice bath, run in the chilled sulfuric acid by rapid drops during the course of 4–7 min. By this time considerable solid reaction product should have separated, and insertion of a thermometer should show that the temperature is in the range 20–25°. Swirl the mixture while maintaining the temperature at about 25° for 5 minutes more and then cool in ice. Add ice to the mixture to dilute the sulfuric acid, then add water to nearly fill the flask, cool, and collect the product on a Büchner funnel with suction. Apply only very gentle suction at first to avoid breaking the filter paper, which is weakened by the strong sulfuric acid solution. Wash liberally with water and then turn on the suction to full force. Press down the filter cake with a spatula and let drain well. Meanwhile, cool a 30-ml portion of methanol for washing to remove a little oil and a yellow impurity. Release the suction, cover the filter cake with a third of the chilled methanol, and then apply suction. Repeat the washing a second and a third time.

Since air-drying of the crude reaction product takes time, the following short procedure is suggested: Place the moist material in a 125-ml Erlenmeyer, add a little dichloromethane to dissolve the organic material, and note the appearance of nonlipid droplets. Add enough calcium chloride to bind the nonlipid material and filter the supernatant into another 125-ml Erlenmeyer; notice that this process eliminates considerable extraneous purple pigment. As the last of the solution is passing through the paper, add 30 ml of methanol (bp 65°) to the filtrate and start evaporation (on the steam bath) to eliminate the dichloromethane (bp 41°). When the volume is estimated to be about 30 ml, let the solution stand for crystallization. When crystallization is complete cool in ice and collect. The yield of large plates of pure 1,4-di-*t*-butyl-2,5-dimethoxybenzene is 6–7 g.

Figure 22.3 and Fig. 22.4 present the infrared and nmr spectra of the starting hydroquinone dimethyl ether. Can you predict the appearance of the nmr spectrum of the product?

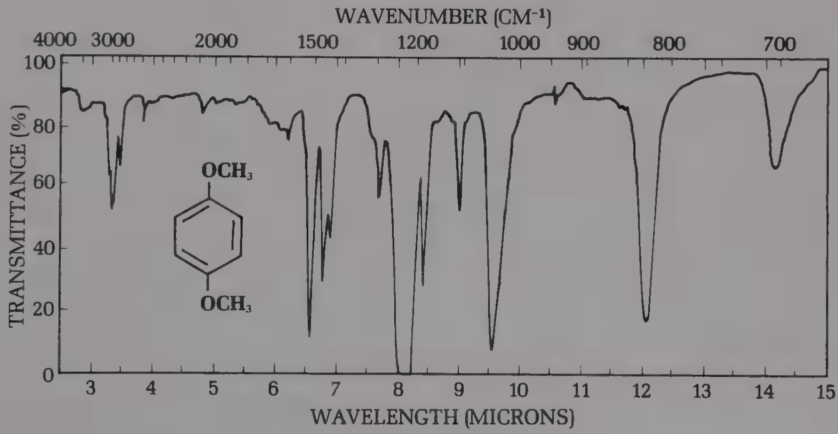


FIGURE 22.3 Infrared spectrum of *p*-dimethoxybenzene.

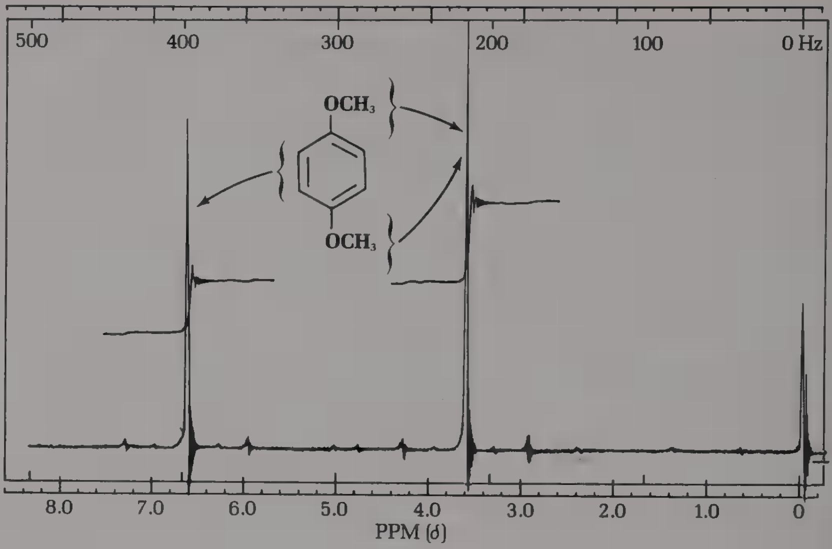


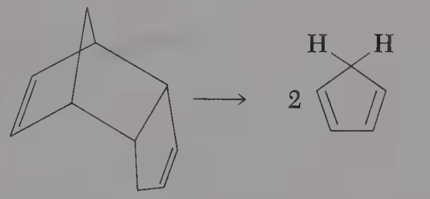
FIGURE 22.4 ^{1}H nmr spectrum of *p*-dimethoxybenzene.

23

Ferrocene [Bis(cyclopentadienyl)iron]

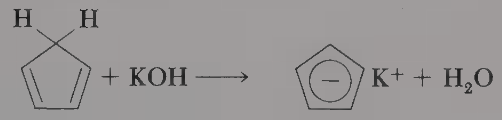
KEYWORDS

Allylic protons
Cyclopentadienyl anion
Metallocene
Reverse Diels-Alder reaction

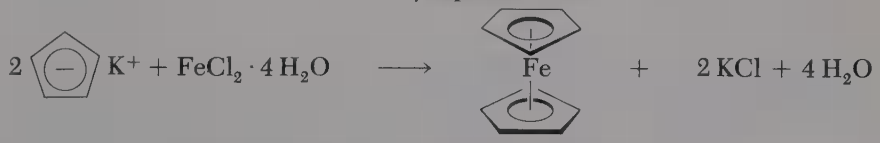


Dicyclopentadiene
den. 0.98
MW 132.20

Cyclopentadiene
bp 41°, den. 0.80
MW 66.10



Potassium cyclopentadienide



Iron(II) chloride
MW 198.81

Ferrocene
Bis(cyclopentadienyl)iron
MW 186.04, mp 172–174°

Although organometallic compounds have been known for over a century, the accidental discovery, by P. L. Pauson in 1951, of the organometallic compound ferrocene began a period of very active research into the chemistry of compounds of carbon and the transition metals. Subsequently, it has been found that most transition metals form ferrocene-like compounds, which differ in stability to oxidation and to heat.

In the present experiment ferrocene is prepared by reaction of the anion of cyclopentadiene with iron(II) chloride. Abstraction of one of the acidic allylic protons of cyclopentadiene with base gives the aromatic cyclopentadienyl anion. It is considered aromatic because it conforms to the Hückel rule in having $4n + 2$ π electrons (where n is 1). Two molecules of this anion will react with iron(II) to give ferrocene, the most common member of the class of metal-organic compounds referred to as metallocenes. In this centrosymmetric sandwich-type π complex, all carbon atoms are equidistant from the iron atom, and the two cyclopentadienyl rings rotate more or less freely with respect to each other. The extraordinary stability of ferrocene (stable to 500°) can be attributed to the sharing of the 12 π electrons of the two cyclopentadienyl rings with the six outer shell electrons of iron(II) to give the iron a stable 18-electron inert gas configuration. Ferrocene is soluble in organic solvents, can be dissolved in concentrated sulfuric acid and recovered unchanged, and is resistant to other acids and bases as well (in the absence of oxygen). This behavior is consistent with that of an aromatic compound; ferrocene is found to undergo electrophilic aromatic substitution reactions with ease.

Cyclopentadiene readily dimerizes at room temperature by a Diels-Alder reaction to give dicyclopentadiene. This dimer can be "cracked" by heating (an example of the reversibility of the Diels-Alder reaction) to give low-boiling cyclopentadiene. In most syntheses of ferrocene the anion of cyclopentadiene is prepared by reaction of the diene with metallic sodium. Subsequently, this anion is allowed to react with anhydrous iron(II) chloride. In the present experiment the anion is generated using powdered potassium hydroxide, which functions as both a base and a dehydrating agent.

The anion of cyclopentadiene rapidly decomposes in air, and iron(II) chloride, although reasonably stable in the solid state, is readily oxidized to the iron(III) (ferric) state in solution. Consequently this reaction must be carried out in the absence of oxygen, accomplished by bubbling nitrogen gas through the solutions to displace dissolved oxygen and to flush air from the apparatus. In research laboratories rather elaborate apparatus is used to carry out an experiment in the absence of oxygen. In the present experiment, because no gases are evolved, no heating is necessary, and the reaction is only mildly exothermic, very simple apparatus is used.

EXPERIMENT

Measure 20 ml of technical grade dicyclopentadiene (85% pure) into a 50-ml flask and arrange for fractional distillation into an ice-cooled receiver as in Fig. 11.1 Heat the dimer with a microburner until it refluxes briskly. In about 5 min the monomeric diene will begin to distil slowly and will have a boiling

▼
Potassium hydroxide is extremely corrosive and hygroscopic. Immediately wash any spilled powder or solutions from the skin and wipe up all spills. Keep containers tightly closed.

▼
Dimethyl sulfoxide is rapidly absorbed through the skin. Wash off spills with water.

point in the range of 40–42°. While this distillation is taking place, rapidly weigh 25 g of finely powdered potassium hydroxide[1] into a 125-ml Erlenmeyer flask, add 60 ml of dimethoxyethane ($CH_3OCH_2CH_2OCH_3$) and immediately cool the mixture in an ice bath. Swirl the mixture in the ice bath for a minute or two, then bubble nitrogen through the solution for about 2 min. Quickly cork the flask and shake the mixture to dislodge the cake of potassium hydroxide from the bottom of the flask and to dissolve as much of the base as possible (much will remain undissolved).

Grind 7 g of iron(II) chloride tetrahydrate to a fine powder and then add 6.5 g of the green salt to 25 ml of dimethyl sulfoxide (DMSO) in a 50-ml Erlenmeyer flask. Pass nitrogen through the DMSO mixture for about 2 min, cork the flask, and shake it vigorously to dissolve all the iron(II) chloride. Gentle warming of the flask on a steam bath may be necessary to dissolve the last traces of iron(II) chloride. Transfer the solution rapidly to a 60-ml separatory funnel equipped with a cork to fit the 125-ml Erlenmeyer flask, flush air from the funnel with a stream of nitrogen, and stopper it.

Transfer 5.5 ml of the freshly distilled cyclopentadiene to the slurry of potassium hydroxide in dimethoxyethane. Shake the flask vigorously and note the color change as the potassium cyclopentadienide is formed. After waiting about 5 min for the anion to form, replace the cork on the Erlenmeyer flask with the separatory funnel quickly (to avoid admission of air to the flask). See

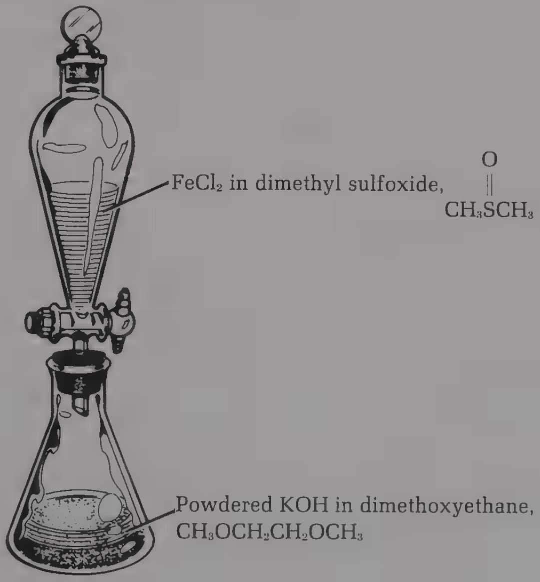


FIGURE 23.1 Apparatus for ferrocene synthesis.

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[1] Potassium hydroxide is easily ground to a fine powder in 75-g batches in one minute employing an ordinary food blender (e.g. Waring, Osterizer). The finely powdered base is transferred in a hood to a bottle with a tightly fitting cap. If a blender is unobtainable, crush and then grind 27 g of potassium hydroxide pellets in a large mortar and quickly weigh 25 g of the resulting powder into the 125-ml Erlenmeyer flask.

Fig. 23.1. Add the iron(II) chloride solution to the base dropwise over a period of 20 min with vigorous swirling and shaking. Dislodge the potassium hydroxide should it cake on the bottom of the flask. The shaking will allow nitrogen to pass from the Erlenmeyer flask into the separatory funnel as the solution leaves the funnel. Continue to shake and swirl the solution for 10 min after all the iron(II) chloride is added, then pour the dark slurry onto a mixture of 90 ml of 6 *M* hydrochloric acid and 100 g of ice in a 500-ml beaker. Stir the contents of the beaker thoroughly to dissolve and neutralize all the potassium hydroxide. Collect the crystalline orange ferrocene on a Büchner funnel (save the filtrate), wash the crystals with water, press out excess water, and allow the product to dry on a watchglass overnight. Add a solution of 1 g of tin(II) chloride (stannous chloride) in 10 ml of water to the filtrate. How do you account for the color changes?

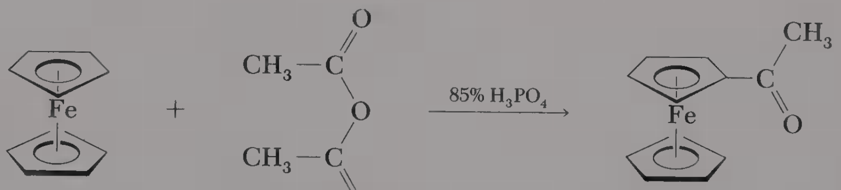
Recrystallize the ferrocene from methanol or, better, from ligroin. It is also very easily sublimed. In a hood place about 0.5 g of crude ferrocene on a watchglass on a hot plate set to about 150°. Invert a glass funnel over the watchglass. Ferrocene will sublime in about one hour, leaving nonvolatile impurities behind. Pure ferrocene melts at 172–174°. Compare the melting points of your sublimed and recrystallized materials.

24

Friedel-Crafts Acylation of Ferrocene: Acetylferrocene

KEYWORDS

Friedel-Crafts acylation
"Superaromatic"
Phosphoric acid catalyst
Deactivating substituent



Ferrocene
Bis(cyclopentadienyl)iron
MW 186.04

Acetic Anhydride
MW 102.09
bp 139.5°, den 1.08

Acetylferrocene
(Acetylcyclopentadienyl)cyclopentadienyliron
MW 228.08, mp 85–86°

The Friedel-Crafts acylation of benzene requires aluminum chloride as the catalyst, but ferrocene, which has been referred to as a "superaromatic" compound, can be acylated under much milder conditions with phosphoric acid as catalyst. Since the acetyl group is a deactivating substituent the addition of a second acetyl group, which requires more vigorous conditions, will occur in the nonacetylated cyclopentadienyl ring to give 1,1′-diacetylferrocene.

EXPERIMENT

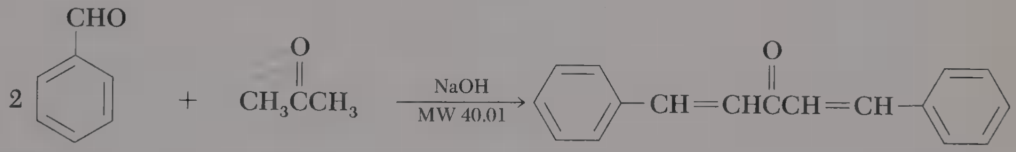
In a 25-ml round-bottomed flask place 3.0 g of ferrocene, 10.0 ml of acetic anhydride, and 2.0 ml of 85% phosphoric acid. Equip the flask with a reflux condenser and a calcium chloride drying tube. Warm the flask gently on the steam bath with swirling to dissolve the ferrocene, then heat strongly for 10 min more. Pour the reaction mixture onto 50 g of crushed ice in a 400-ml beaker and rinse the flask with 10 ml of ice water. Stir the mixture for a few minutes with a glass rod, add 75 ml of 10% sodium hydroxide solution (the solution should still be acidic), then add solid sodium bicarbonate (be careful of foaming) until the remaining acid has been neutralized. Stir and crush all lumps, allow the mixture to stand for 20 min, then collect the product by suction filtration. Press the crude material as dry as possible between sheets of filter paper, transfer it to an Erlenmeyer flask, and add 40 ml of hexane or ligroine to the flask. Boil the solvent for a few minutes, then decant the dark orange solution into another Erlenmeyer flask, leaving a gummy residue of polymeric material. Treat the solution with decolorizing charcoal and filter it, through a fluted filter paper placed in a warm stemless funnel, into an appropriately sized Erlenmeyer flask. Evaporate the solvent (use an aspirator tube, Fig. 6.4) until the volume is about 20 ml. Set the flask aside to cool slowly to room temperature. Beautiful rosettes of dark orange-red needles of acetylferrocene will form. After the product has been cooled in ice, collect it on a Büchner funnel and wash the crystals with a small quantity of cold solvent. Pure acetylferrocene has mp 84–85°. Your yield should be about 1.8 g.

25

Dibenzalacetone by the Aldol Condensation

KEYWORDS

Aldol condensation
Claisen-Schmidt reaction
Benzalacetone
Geometric isomers



Benzaldehyde
MW 106.13, bp 178°,
den 1.04

Acetone
(2-Propanone)
MW 58.08, bp 56°,
den 0.790

Dibenzalacetone
(1,5-Diphenyl-1,4-pentadien-3-one)
MW 234.30, mp 110–111°

The reaction of an aldehyde with a ketone employing sodium hydroxide as the base is an example of a mixed aldol condensation reaction known as the Claisen-Schmidt reaction. Dibenzalacetone is readily prepared by condensation of acetone with two equivalents of benzaldehyde. The aldehyde carbonyl is more reactive than that of the ketone and therefore reacts rapidly with the anion of the ketone to give a β-hydroxyketone, which easily undergoes base-catalyzed dehydration. Depending on the relative quantities of the reactants, the reaction can give either mono- or dibenzalacetone.

In the present experiment sufficient ethanol is present as solvent to readily dissolve the starting material, benzaldehyde, and also the intermediate, benzalacetone. The benzalacetone, once formed, can then easily react with another mole of benzaldehyde to give the product, dibenzalacetone.

EXPERIMENT

Mix 0.05 mole of benzaldehyde with the theoretical quantity of acetone, add one-half the mixture to a solution of 5 g of sodium hydroxide dissolved in 50 ml of water and 40 ml of ethanol at room temperature (<25°). After 15 min add the remainder of the aldehyde–ketone mixture and rinse the container with a little ethanol to complete the transfer. After one half hour, during which time the mixture is swirled frequently, collect the product by suction filtration on a Büchner funnel. Break the suction and carefully pour 100 ml of water on the product. Reapply the vacuum. Repeat this process three times in order to remove all traces of sodium hydroxide. Finally, press the product as dry as possible on the filter using a cork, then press it between sheets of filter paper to remove as much water as possible. Save a small sample for melting point determination and then recrystallize the product from ethanol using about 10 ml of ethanol for each 4 g of dibenzalacetone. Pure dibenzalacetone melts at 110–111°, and the yield after recrystallization should be about 4 g.

QUESTIONS

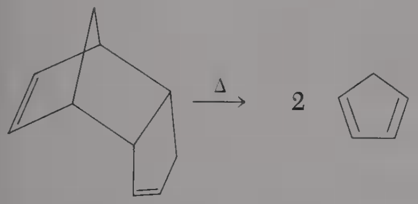
1. Write a mechanism for the aldol condensation of one mole of benzaldehyde with one mole of acetone.
2. Write a mechanism for the base-catalyzed dehydration of the product from the reaction in Question 1 to give benzalacetone.
3. Why is it important to maintain equivalent proportions of reagents in this reaction?
4. What side products do you expect in this reaction? How are they removed?
5. What do the melting points of the crude and recrystallized products tell you about purity?
6. Write formulas to show the possible geometric isomers of dibenzalacetone. Which isomer would you expect to be most stable? Why?
7. What evidence do you have that your product consists of a single geometric isomer or a mixture of isomers? Does the melting point give such information?
8. From the ^1H nmr spectrum of dibenzalacetone can you deduce what geometric isomer(s) is (are) formed?
9. How would you change the above procedure if you wished to synthesize benzalacetone, $C_6H_5CH{=}CHCOCH_3$? Benzalacetophenone, $C_6H_5CH{=}CHCOC_6H_5$?

26

Diels-Alder Reaction

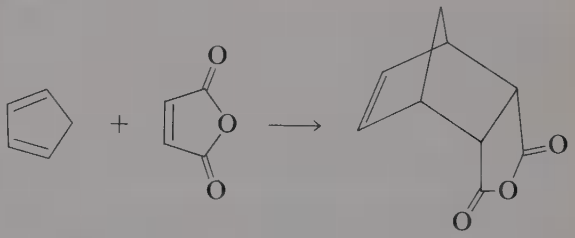
KEYWORDS

Diels-Alder reaction
Dicyclopentadiene, dimer
Reverse Diels-Alder reaction
Cyclopentadiene, monomer
Maleic anhydride
Endo-, exo-
Compound X



Dicyclopentadiene
den 0.98,
MW 132.20

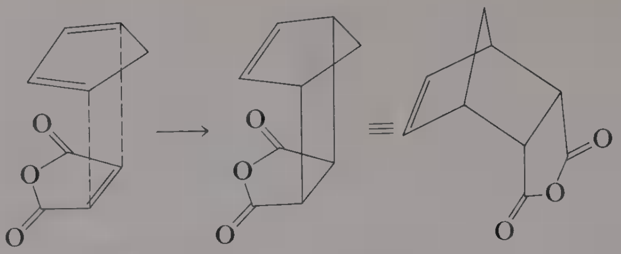
Cyclopentadiene
Bp 41°, den 0.80,
MW 66.10



Maleic anhydride
mp 53°, MW 98.06

***cis*-Norbornene-5,6-*endo*-dicarboxylic anhydride**
mp 165°, MW 164.16

Cyclopentadiene is obtained from the light oil from coal tar distillation but exists as the stable dimer, dicyclopentadiene, which is the Diels-Alder adduct from two molecules of the diene. Thus, generation of cyclopentadiene by pyrolysis of the dimer represents a reverse Diels-Alder reaction. In the Diels-Alder addition of cyclopentadiene and maleic anhydride the two molecules approach each other in the orientation shown in the drawing (see p. 178), as this orientation provides maximal overlap of π-bonds of the two reactants and favors formation of an initial π-complex and then the final *endo*-product. Dicyclopentadiene also has the *endo*-configuration.



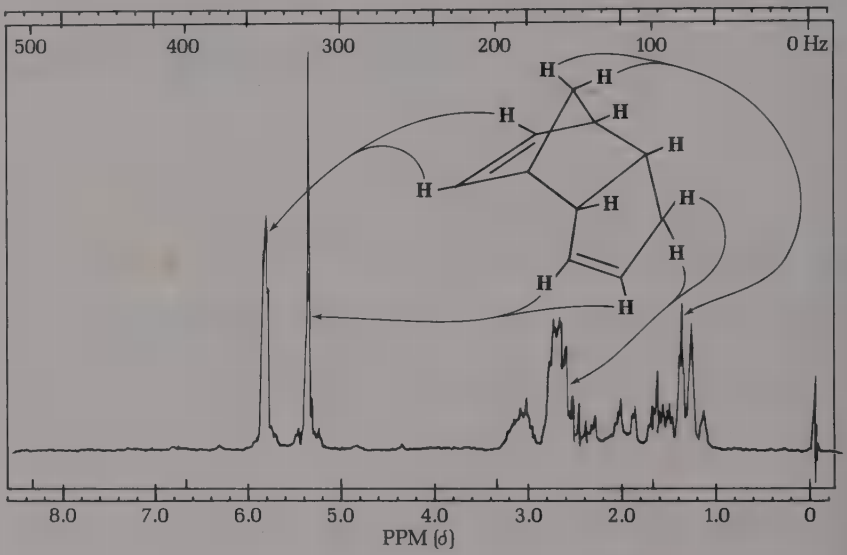


FIGURE 26.1 ^{1}H nmr spectrum of dicyclopentadiene.

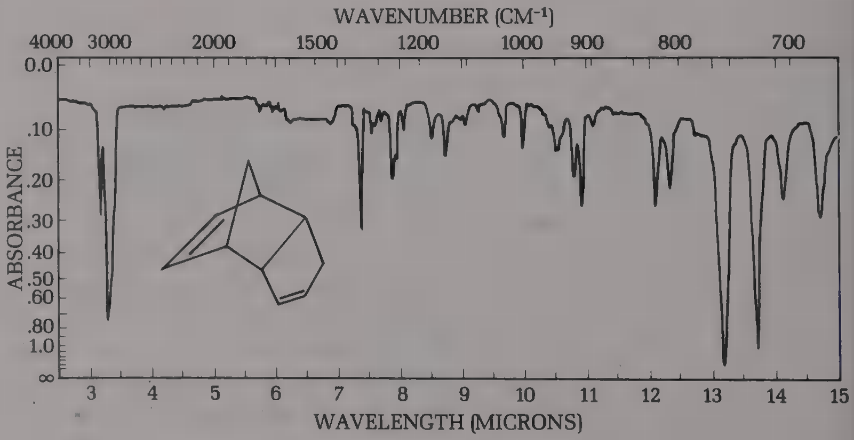


FIGURE 26.2 Infrared spectrum of dicyclopentadiene.

EXPERIMENTS

1. *cis*-Norbornene-5,6-*endo*-dicarboxylic Anhydride

▼
Reverse Diels-Alder

Measure 30 ml of technical dicyclopentadiene (85% pure) into a 125-ml flask and arrange for fractional distillation into an ice-cooled receiver as in Fig. 11.1. Heat the dimer with a microburner until it refluxes briskly and at such a rate that the monomeric diene begins to distil in about 5 min and soon reaches a steady boiling point in the range 40–42°. Apply heat continuously to promote rapid distillation without exceeding the boiling point of 42°. Distillation for 45 min should provide the 12 ml of cyclopentadiene required for two preparations of the adduct; continued distillation for another half hour gives a total of about 20 ml of monomer.

While the distillation is in progress, place 6 g of maleic anhydride[1] in a 125-ml Erlenmeyer flask and dissolve the anhydride in 20 ml of ethyl acetate by heating on a hot plate or steam bath. Add 20 ml of ligroin, bp 60–90°, cool the solution thoroughly in an ice-water bath, and leave it in the bath (some anhydride may crystallize).

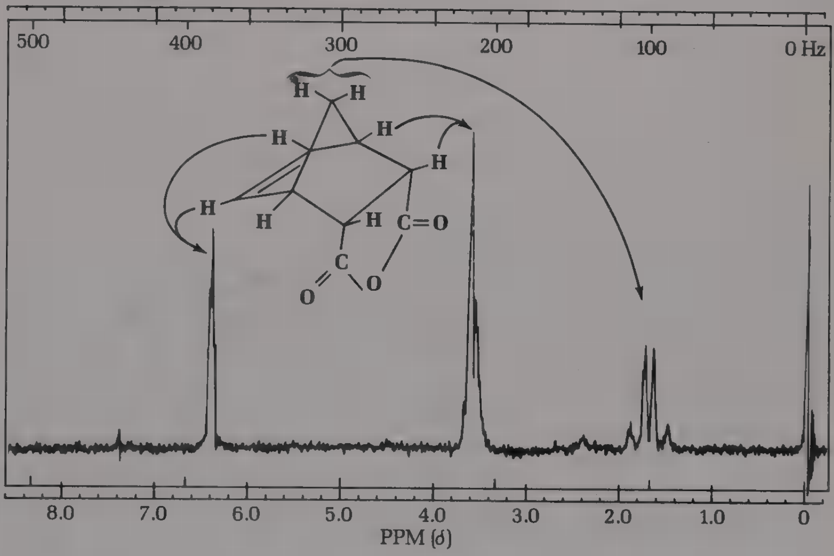


FIGURE 26.3 ^{1}H nmr spectrum of *cis*-norbornene-5,6-*endo*-dicarboxylic anhydride.

▼
Rapid addition at 0°

The distilled cyclopentadiene may be slightly cloudy, because of the condensation of moisture in the cooled receiver and water in the starting material. Add about 1 g of calcium chloride to remove the moisture. Measure 6 ml of dry cyclopentadiene, and add it to the ice-cold solution of maleic anhydride. Swirl the solution in the ice bath for a few minutes until the exothermic reaction is over and the adduct separates as a white solid. Then heat the mixture

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[1] Material available in the form of cast ⅞-in rods should be crushed in a mortar and preferably crystallized from benzene-ligroin.

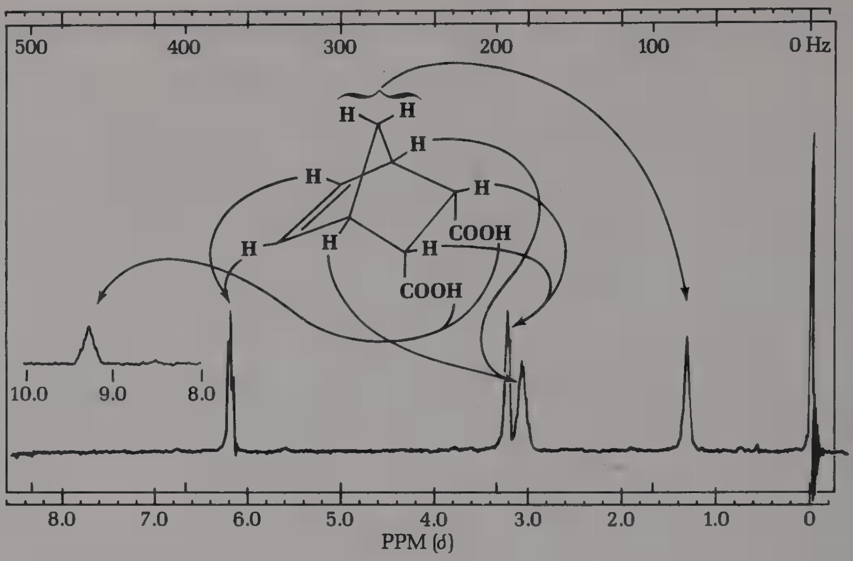
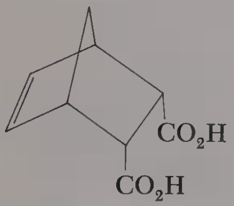


FIGURE 26.4 ^{1}H nmr spectrum of *cis*-norbornene-5,6-*endo*-dicarboxylic acid.

on a hot plate or steam bath until the solid is all dissolved.[2] If you let the solution stand undisturbed, you will be rewarded with a beautiful display of crystal formation. The anhydride crystallizes in long spars, mp 164–165°; yield, 8.2 g.[3]



endo,-cis-Diacid

endo,cis-Diacid

For preparation of the *endo,cis*-diacid, place 4.0 g of bicyclic anhydride and 50 ml of distilled water in a 125-ml Erlenmeyer flask, grasp this with a clamp, swirl the flask over a free flame, and bring the contents to the boiling point, at which point the solid partly dissolves and partly melts. Continue to heat until the oil is all dissolved and let the solution stand undisturbed. Since the diacid has a strong tendency to remain in supersaturated solution, allow half an hour or more for the solution to cool to room temperature and then drop in a carborundum boiling stone. Observe the stone and its surroundings carefully, waiting several minutes before applying the more effective method of making one scratch with a stirring rod on the inner wall of the flask at the air-liquid interface. Let crystallization proceed spontaneously to give large needles,

---

[2] In case moisture has gotten into the system, a little of the corresponding diacid may remain undissolved at this point and should be removed by filtration of the hot solution.

[3] The student need not work up the mother liquor but may be interested in learning the result. Concentration of the solution to a small volume is not satisfactory because of the presence of dicyclopentadiene, formed by dimerization of excess monomer; the dimer has high solvent power. Hence the bulk of the solvent is evaporated on the steam bath, the flask is connected to the water pump with a rubber stopper and glass tube and heated under vacuum on the steam bath until dicyclopentadiene is removed and the residue solidifies. Crystallization from 1:1 ethyl acetate-ligroin affords 1.3 g of adduct, mp 156–158°; total yield, 95%.

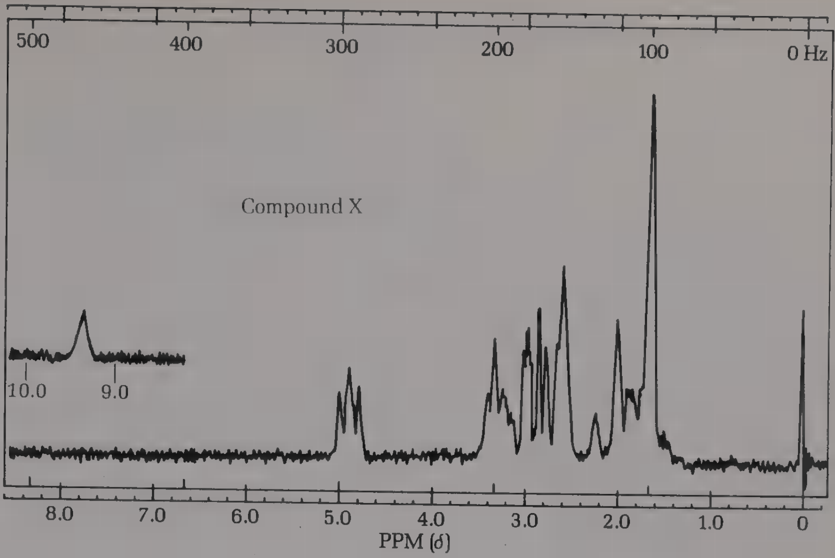


FIGURE 26.5 ^{1}H nmr spectrum of Compound X, prepared from *endo,cis*-diacid.

The temperature of decomposition is variable

which will dry quickly, then cool in ice and collect. Yield, 4.0 g; mp 180–185°, dec (anhydride formation).[4]

Problem:[5] Compound X

For preparation of X, place 1 g of the *endo,cis*-diacid and 5 ml of concentrated sulfuric acid in a 50-ml Erlenmeyer and heat gently on the hot plate for a minute or two until the crystals are all dissolved. Then cool in an ice bath, add a small piece of ice, swirl to dissolve, and add further ice until the volume is about 20 ml. Heat to the boiling point and let the solution simmer on the hot plate for 5 minutes. Cool well in ice, scratch the flask (see Chapter 4) to induce crystallization, and allow for some delay in complete separation. Collect, wash with water, and crystallize from water. Compound X (about 0.7 g) forms large prisms, mp 203°.

What is X?

To determine the formula for X try to answer the following questions: What intermediate is formed when the diacid dissolves in concentrated sulfuric acid? Why is the nmr spectrum of X so much more complex than the diacid and anhydride spectra? What functional group is missing from X that is seen in Figs. 19.3 and 19.4? Write formulas for possible structures of X and devise tests to distinguish among them.

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[4] The *endo,cis*-diacid is stable to alkali but can be isomerized to the *trans*-diacid (mp 192°) by conversion to the dimethyl ester (3 g of acid, 10 ml methanol, 0.5 ml concentrated H_2SO_4; reflux 1 hr). This ester is equilibrated with sodium ethoxide in refluxing ethanol for 3 days and saponified. For an account of a related epimerization and discussion of the mechanism, see J. Meinwald and P. G. Gassman, *J. Am. Chem. Soc.*, **82**, 5445 (1960). See also K. L. Williamson, Y.-F. Li, R. Lacko and C. H. Youn, *J. Am. Chem. Soc.*, **91**, 6129 (1969) and K. L. Williamson and Y.-F. Li, *J. Am. Chem. Soc.*, **92**, 7654 (1970).

[5] Introduced by James A. Deyrup.

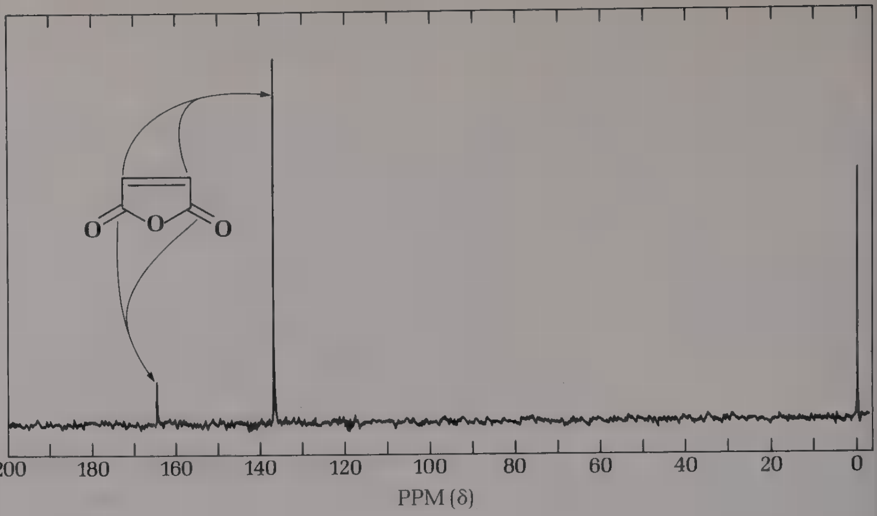


FIGURE 26.6 ^{13}C nmr spectrum of maleic anhydride.

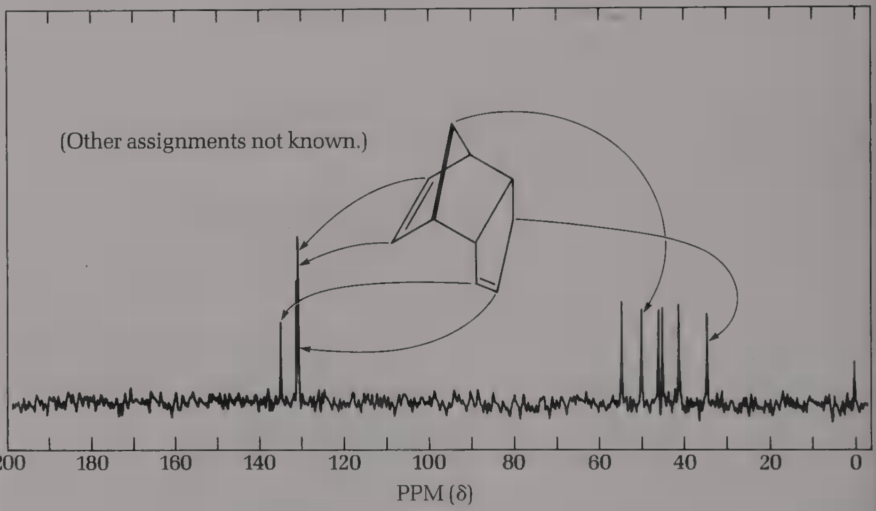


FIGURE 26.7 ^{13}C nmr spectrum of dicyclopentadiene.

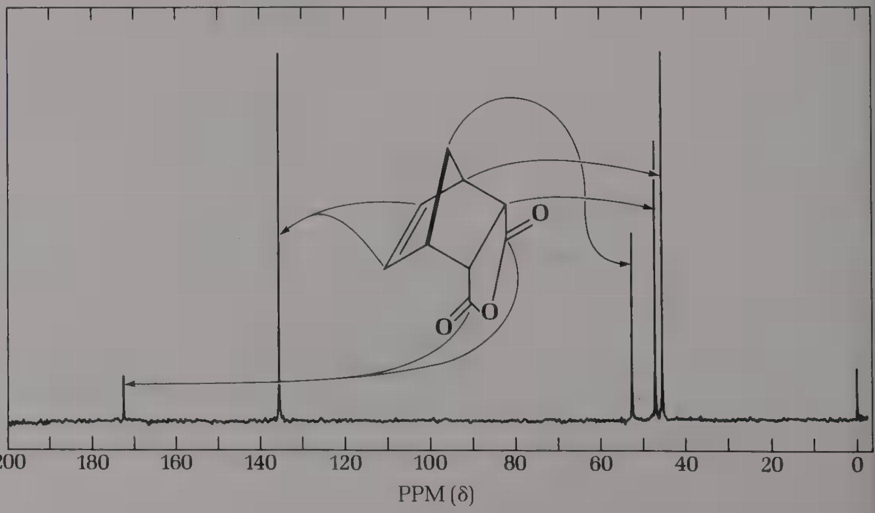


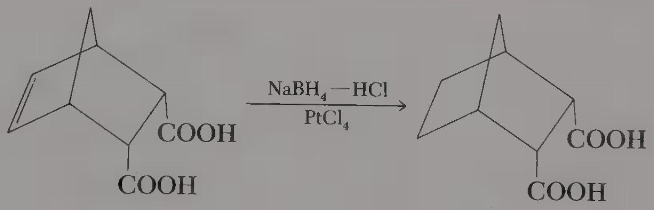
FIGURE 26.8 ^{13}C nmr spectrum of *cis*-norbornene-5,6-*endo*-dicarboxylic anhydride.

27

Catalytic Hydrogenation

KEYWORDS

Platinum catalyst
Sodium borohydride
Brown procedure
Pressure balloon



cis-Norbornene-5,6-_endo_-dicarboxylic acid
mp 180–190°, dec;
MW 182.17

cis-Norbornane-5,6-_endo_-dicarboxylic acid
mp 170–175°, dec;
MW 184.19

▼
Hydrogen generated in situ

Conventional procedures for hydrogenation in the presence of a platinum catalyst employ hydrogen drawn from a cylinder of compressed gas and require elaborate equipment. H. C. Brown and C. A. Brown[1] introduced the simple procedure of generating hydrogen *in situ* from sodium borohydride and hydrochloric acid and prepared a highly active supported catalyst by reduction of platinum chloride with sodium borohydride in the presence of decolorizing carbon. The special apparatus described by these authors is here dispensed with in favor of a balloon technique also employed for catalytic oxygenation (Chapter 47).

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[1] H. C. Brown and C. A. Brown, *J. Am. Chem. Soc.*, 84, 1495 (1962).

EXPERIMENT

The reaction vessel is a 125-ml filter flask with a white rubber pipette bulb wired onto the side arm. Introduce 10 ml of water, 1 ml of platinum (IV) chloride solution,[2] and 0.5 g of decolorizing charcoal and swirl during addition of 3 ml of stabilized 1*M* sodium borohydride solution.[3] While allowing 5 minutes for formation of the catalyst, dissolve 1 g of *cis*-norbornene-5,6-*endo*-dicarboxylic acid in 10 ml of hot water. Pour 4 ml of concentrated hydrochloric acid into the reaction flask, followed by the hot solution of the unsaturated acid. Cap the flask with a large serum stopper and wire it on. Draw 1.5 ml of the stabilized sodium borohydride solution into the barrel of a plastic syringe, thrust the needle through the center of the stopper, and add the solution dropwise with swirling. The initial uptake of hydrogen is so rapid that the balloon may not inflate until you start injecting a second 1.5 ml of borohydride solution through the stopper. When the addition is complete and the reaction appears to be reaching an end point (about 5 min), heat the flask on the steam bath with swirling and try to estimate the time at which the balloon is deflated to a constant size (about 5 min). When balloon size is constant, heat and swirl for 5 min more and then release the pressure by injecting the needle of an open syringe through the stopper.

▼
Reaction time about 15 min

Filter the hot solution by suction and place the catalyst in a jar marked "Catalyst Recovery."[4] Cool the filtrate and extract it with three 15-ml portions of ether. The combined extracts are to be washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the ether gives about 0.8–0.9 g of white solid.

▼
Common ion effect

The only solvent of promise for crystallization of the saturated *cis*-diacid is water, and the diacid is very soluble in water and crystallizes extremely slowly and with poor recovery. However, the situation is materially improved by addition of a little hydrochloric acid to decrease the solubility of the diacid.

Scrape out the bulk of the solid product and transfer it to a 25-ml Erlenmeyer flask. Add 1–2 ml of water to the 125-ml flask, heat to boiling to dissolve residual solid, and pour the solution into the 25-ml flask. Bring the material into solution at the boiling point with a total of not more than 3 ml of water (as a guide, measure 3 ml of water into a second 25-ml Erlenmeyer). With a capillary dropping tube add 3 drops of concentrated hydrochloric acid and let the solution stand for crystallization. Clusters of heavy prismatic needles soon separate; the recovery is about 90%. The product should give a negative test for unsaturation with acidified permanganate solution.

Observe what happens when a sample of the product is heated in a melting point capillary to about 170°. Account for the result. You may be able to confirm your inference by letting the oil bath cool until the sample solidifies and then noting the mp temperature and behavior on remelting.

---

[2] A solution of 1 g of $PtCl_4$ in 20 ml of water.

[3] Dissolve 1.6 g of sodium borohydride and 0.3 g of sodium hydroxide (stabilizer) in 40 ml of water. When not in use, the solution should be stored in a refrigerator. If left for some time at room temperature in a tightly stoppered container, gas pressure may develop sufficient to break the vessel.

[4] Used catalyst can be sent for recovery of the $PtCl_4$ to Engelhard Industries, 865 Ramsey St., Hillside, N. J., 07205.

28

Amines

KEYWORDS

Organic bases
Primary, secondary, tertiary
Solubility in acid
Hinsberg test
Benzenesulfonyl chloride
Sulfonamides
Acidic hydrogen
Acetyl and benzoyl derivatives

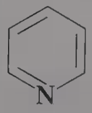
Amines, whether primary, secondary, or tertiary, differ from other classes of organic compounds, including other nitrogen-containing substances, in being basic. Some amines are not soluble enough in water to give a basic response to test paper, but nevertheless they combine with mineral acids to form salts. Hence their basic character can be recognized by a simple test with acid that distinguishes amines from neutral substances, such as amides ($RCONH_2$), N-acylamines ($RNHCOCH_3$), and nitriles (RCN). An amine salt can be recognized by its reaction with base. Procedures for the tests for basicity are given in Section 1. Section 2 presents a test for distinguishing between primary, secondary, and tertiary amines, Section 3 gives procedures for preparation of solid derivatives for melting point characterizations, and Section 4 gives spectral characteristics. After applying the procedures to known substances you are to identify a series of unknowns.

EXPERIMENTS

1. Basicity

Substances to be tested:

Aniline, $C_6H_5NH_2$, bp 184°
p-Toluidine, $CH_3C_6H_4NH_2$, mp 43°
Pyridine, C_5H_5N, bp 115° (a tertiary amine)
Methylamine hydrochloride, dec. (salt of CH_3NH_2, bp −6.7°)
Aniline hydrochloride, dec. (salt of $C_6H_5NH_2$)
Aniline sulfate, dec.


Pyridine

First, see if the substance has a fishy, ammonia-like odor, for if so it probably is an amine of low molecular weight. Then test the solubility in water by putting 2 drops if a liquid or an estimated 20 mg if a solid into a 10 × 75-mm test tube, adding 0.2 ml of water (a 5-mm column) and first seeing if the substance dissolves in the cold. If the substance is a solid, rub it well with a stirring rod and break up any lumps before drawing a conclusion.

If the substance is *readily soluble in cold water* and if the odor is suggestive of an amine, test the solution with pH paper and further determine if the odor disappears on addition of a few drops of 10% hydrochloric acid. If the properties are more like those of a salt, add a few drops of 10% sodium hydroxide solution. If the solution remains clear, addition of a little sodium chloride may cause separation of a liquid or solid amine.

If the substance is not soluble in cold water, see if it will dissolve on heating; be careful not to mistake the melting of a substance for dissolving. If it *dissolves in hot water,* add a few drops of 10% alkali and see if an amine precipitates. (If you are in doubt as to whether a salt has dissolved partially or not at all, pour off supernatant liquid and make it basic.)

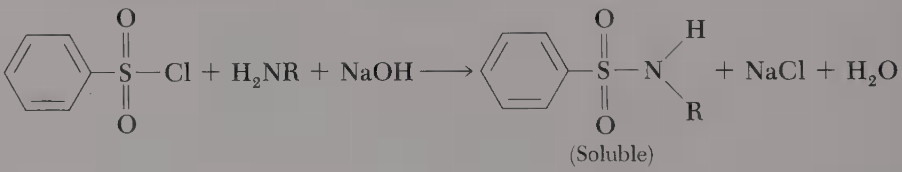
If the substance is *insoluble in hot water,* add 10% hydrochloric acid, heat if necessary, and see if it dissolves. If so, make the solution basic and see if an amine precipitates.

2. Hinsberg Test

The procedure for distinguishing amines with benzenesulfonyl chloride is to be run in parallel on the following substances:

Aniline, $C_6H_5NH_2$ (bp 184°)
N-Methylaniline, $C_6H_5NHCH_3$ (bp 194°)
Triethylamine, $(CH_3CH_2)_3N$ (bp 90°)

Primary and secondary amines react in the presence of alkali with benzenesulfonyl chloride, $C_6H_5SO_2Cl$, to give sulfonamides.



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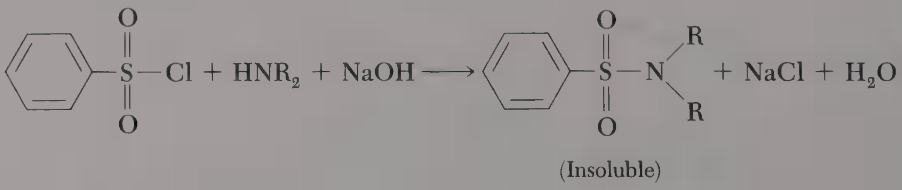
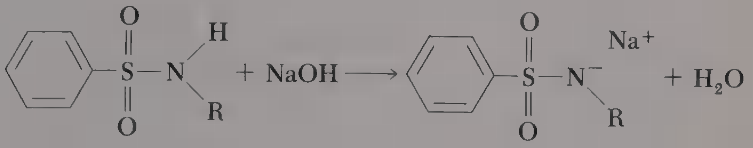


(2)

The sulfonamides are distinguishable because the derivative from a primary amine has an acidic hydrogen which renders the product soluble in alkali (reaction 1), whereas the sulfonamide from a secondary amine is insoluble (reaction 2). Tertiary amines lack the necessary acidic hydrogen for formation of benzenesulfonyl derivatives.



▼ *Distinguishing among primary (1°), secondary (2°) and tertiary (3°) amines*

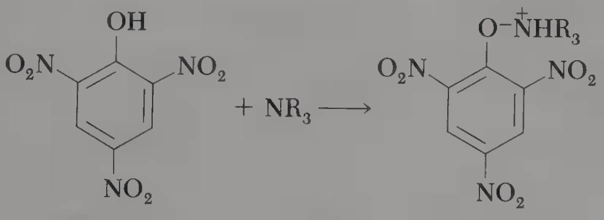
To about 4 ml of water in a 13 × 100-mm test tube (4-cm column) add either 4 drops of a liquid amine or an estimated 100 mg of an amine salt, 1 ml of 10% sodium hydroxide solution, and 7 drops of benzenesulfonyl chloride. Stopper the tube, shake it *vigorously* for 5 min, note if there is any heat effect and if a solid separates. Warm the tube slightly, shake for a few minutes longer *until the odor of benzenesulfonyl chloride is no longer apparent,* and cool the mixture. If an oil separates at this point cool the tube in an ice bath, rub the oil against the walls with a stirring rod, and see if this is a product which will solidify or if it is unreacted amine. Make sure that the solution is still alkaline, and if there is a precipitate at this point it should be collected by suction filtration and a portion tested with dilute alkali to prove that it is actually insoluble in alkali. (An alkali-soluble product may precipitate at this point as the sodium salt if too much alkali is used.) If there is no precipitate the alkaline solution may contain an alkali-soluble sulfonamide. Acidify with concentrated hydrochloric acid and if a precipitate forms collect it and confirm the solubility in alkali.

3. Solid Derivatives

Acetyl derivatives of primary and secondary amines are usually solids suitable for melting point characterization and are readily prepared by reaction with acetic anhydride, even in the presence of water. Benzoyl and benzenesulfonyl derivatives are made by reaction of the amine with the appropriate acid chloride in the presence of alkali, as in Section 2 (the benzenesulfonamides of aniline and of *N*-methylaniline melt at 110° and 79°, respectively).

Solid derivatives suitable for characterization of tertiary amines are the methiodides and picrates:

$$R_3N + CH_3I \longrightarrow R_3\overset{+}{N}CH_3\ I^-$$



2,4,6-Trinitrophenol
(Picric Acid)

Amine picrate

Typical derivatives are to be prepared, and although determination of melting points is not necessary since the values are given, the products should be saved for possible identification of unknowns.

a) Measure 4–5 drops (about 1 millimole) of aniline into a 13 × 100-mm test tube and add 5 drops of acetic anhydride. Note the heat effect, allow 2–3 min for completion of the reaction, and then cool and add water. The oily precipitate soon solidifies, mp 114°. Test its solubility in dilute HCl.
b) Dissolve about 100 mg of aniline hydrochloride in 1 ml of water, add 5 drops of acetic anhydride, and then (at once) about 100 mg of sodium acetate.
c) Dissolve about 100 mg of picric acid in 1 ml of methanol and to the warm solution add 4 drops of triethylamine and let the solution stand. The picrate melts at 171°.

Write balanced equations for the above reactions.

4. NMR and IR Spectra of Amines

The proton bound to nitrogen can appear between 0.6 and 7.0 ppm on the nmr spectrum, the position depending upon solvent, concentration, and structure of the amine. The peak is sometimes extremely broad owing to slow exchange and interaction of the proton with the electric quadrupole of the nitrogen. If addition of a drop of D_2O to the sample causes the peak to disappear, this is evidence for an amine hydrogen, but alcohols, phenols, and enols will also exhibit this exchange behavior. See Fig. 28.1 for the nmr spectrum of aniline, in which the amine hydrogens appear as a sharp peak at 3.3 ppm. Infrared spectroscopy can also be very useful for identification purposes. Primary amines, both aromatic and aliphatic, show a weak doublet between 3300 and

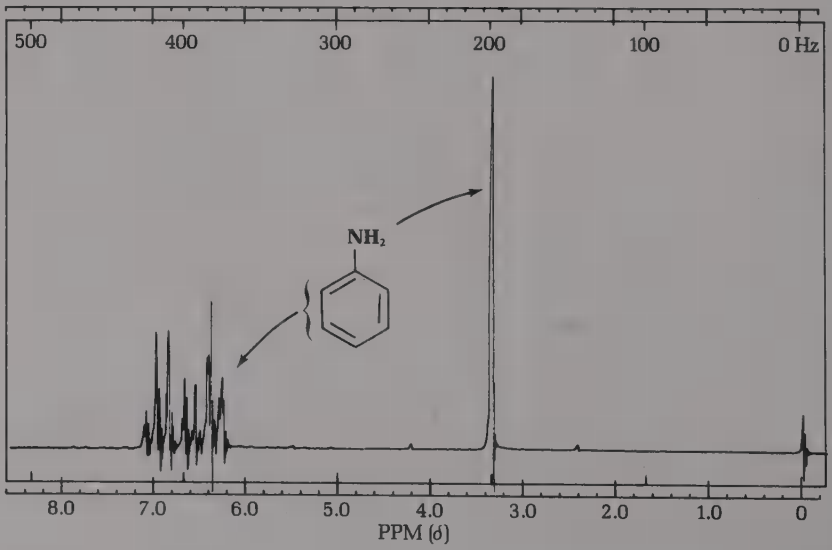


FIGURE 28.1 Nmr spectrum of aniline.

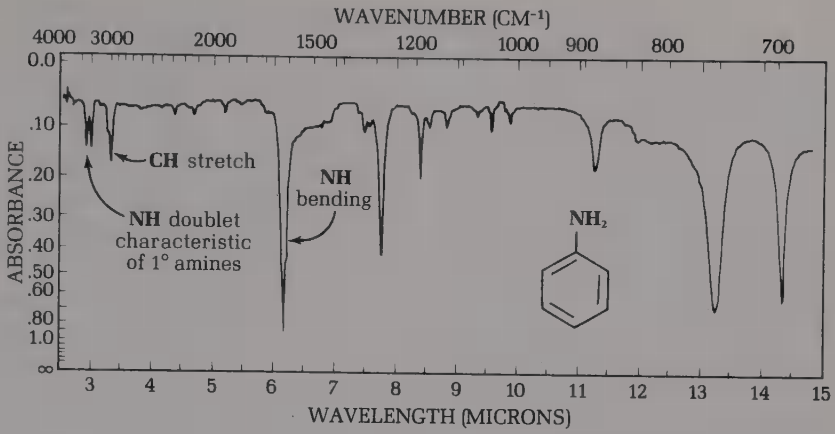


FIGURE 28.2 Infrared spectrum of aniline in CS_2.

3500 cm^{-1} and a strong absorption between 1560 and 1640 cm^{-1} due to NH bending (Fig. 28.2). Secondary amines show a single peak between 3310 and 3450 cm^{-1}. Tertiary amines have no useful infrared absorptions.

In Chapter 9 the characteristic ultraviolent absorption shifts of aromatic amines in the presence and absence of acids were discussed.

5. Unknowns (see Tables 58.5 and 58.6)

Determine first if the unknown is an amine or an amine salt and then determine whether the amine is primary, secondary, or tertiary. Complete identification of your unknown may be required.

QUESTIONS

1. How could you most easily distinguish between samples of 2-aminonaphthalene and of acetanilide?
2. Would you expect the reaction product from benzenesulfonyl chloride and ammonia to be soluble or insoluble in alkali?
3. Is it safe to conclude that a substance is a tertiary amine because it forms a picrate?
4. Why is it usually true that amines that are insoluble in water are odorless?
5. Technical dimethylaniline contains traces of aniline and of methylaniline. Suggest a method for elimination of these impurities.
6. How would you prepare aniline from aniline hydrochloride?

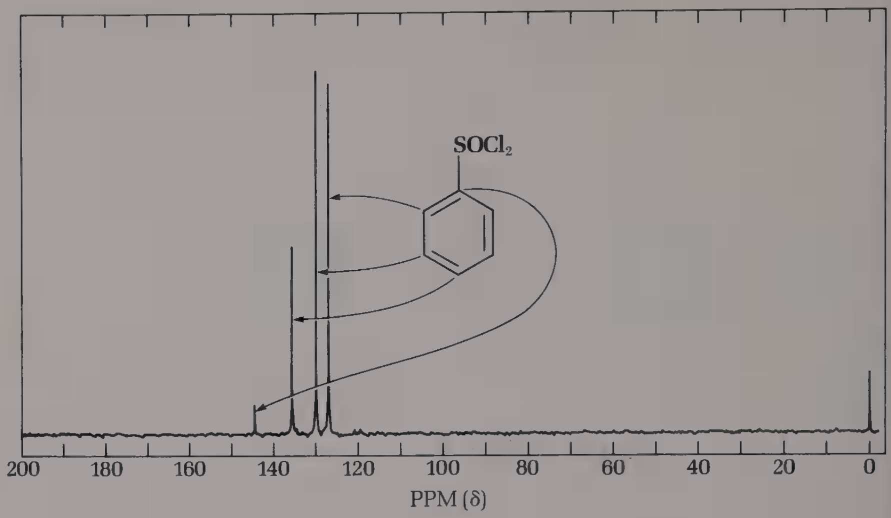


FIGURE 28.3 ^{13}C nmr spectrum of benzenesulfonyl chloride.

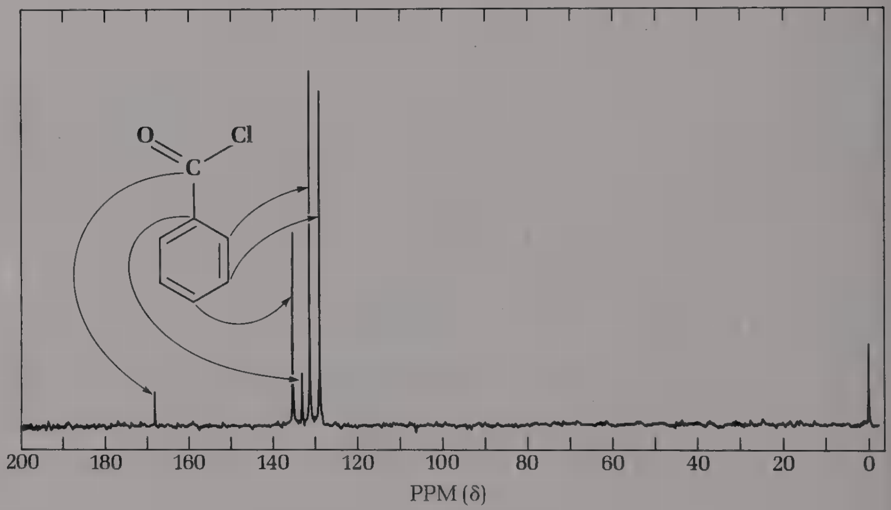


FIGURE 28.4 ^{13}C nmr spectrum of benzoyl chloride.

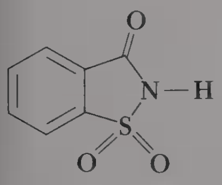
29

Sugars

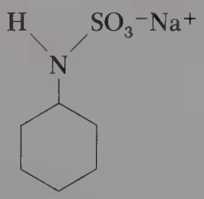
KEYWORDS

Mono-, di-, and oligosaccharides
Polysaccharides
Saccharin
Sodium cyclamate
Osazones
Phenylhydrazine
Fehling solution
Copper(II) ion
Tollens reagent
Red tetrazolium
Disaccharide hydrolysis
Trityl, triphenylmethyl

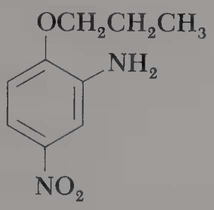
The term sugar applies to mono-, di-, and oligosaccharides, which are all soluble in water and thereby distinguished from polysaccharides. Many natural sugars are sweet, but data of Table 29.1 show that sweetness varies greatly with stereochemical configuration and is exhibited by compounds of widely differing structural type.



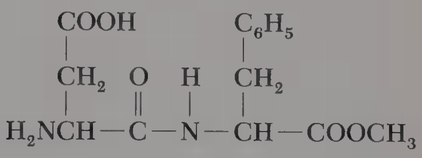
Saccharin



Sodium cyclamate



2-Amino-4-nitro-1-*n*-propoxybenzene



$$\begin{array}{ccccccccc} \text{COOH} & & & & & & \text{C}_6\text{H}_5 & & \\ | & & & & & & | & & \\ \text{CH}_2 & & \text{O} & & \text{H} & & \text{CH}_2 & & \\ | & & \| & & | & & | & & \\ \text{H}_2\text{NCH} & - & \text{C} & - & \text{N} & - & \text{CH} & - & \text{COOCH}_3 \end{array}$$

Aspartame
L-Aspartyl-L-phenylalanine methyl ester

Sugars are neutral and combustible and these properties distinguish them from other water-soluble compounds. Some polycarboxylic acids and some lower amines are soluble in water, but the solutions are acidic or basic. Water-soluble amine salts react with alkali with liberation of the amine, and sodium salts of acids are noncombustible.

Table 29.1 Relative Sweetness of Sugars and Sugar Substitutes

Compound	Sweetness	
	To man	*To bees*
Monosaccharides		
D-Fructose	1.5	+
D-Glucose	0.55	+
D-Mannose	Sweet, then bitter	−
D-Galactose	0.55	−
D-Arabinose	0.70	−
Disaccharides		
Sucrose (glucose, fructose)	1	+
Maltose (2 glucose)	0.3	+
α-Lactose (glucose, galactose)	0.2	−
Cellobiose (2 glucose)	Indifferent	−
Gentiobiose (2 glucose)	Bitter	−
Synthetic sugar substitutes		
Aspartame	180	
Saccharin	550	
2-Amino-4-nitro-1-*n*-propoxybenzene	4000	

▼ *Osazones*

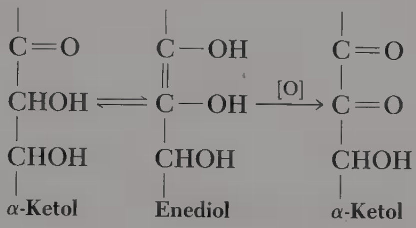
One gram of sucrose dissolves in 0.5 ml of water at 25° and in 0.2 ml at the boiling point, but the substance has marked, atypical crystallizing properties. In spite of the high solubility it can be obtained in beautiful, large crystals (rock candy). More typical sugars are obtainable in crystalline form only with difficulty, particularly in the presence of a trace of impurity, and even then give small and not well-formed crystals. Alcohol is often added to a water solution to decrease solubility and thus to induce crystallization. The amounts of 95% ethanol required to dissolve 1-g samples at 25° are: sucrose, 170 ml; glucose, 60 ml; fructose, 15 ml. Some sugars have never been obtained in crystalline condition and are known only as viscous sirups. With phenylhydrazine many sugars form beautiful crystalline derivatives called osazones. Osazones are much less soluble in water than the parent sugars, since the molecular weight is increased by 178 units and the number of hydroxyl groups reduced by one. It is easier to isolate an osazone than to isolate the sugar, and sugars that are sirups often give crystalline osazones. Osazones of the more highly hydroxylic disaccharides are notably more soluble than those of monosaccharides.

Some disaccharides do not form osazones, but a test for formation or nonformation of the osazone is ambiguous, because the glycosidic linkage may suffer hydrolysis in a boiling solution of phenylhydrazine and acetic acid, with formation of an osazone derived from a component sugar and not from the disaccharide. If a sugar has reducing properties it is also capable of osazone formation; hence an unknown sugar is tested for reducing properties before preparation of an osazone is attempted. Three tests for differentiation between reducing and nonreducing sugars are described below; two are classical and the third modern.

EXPERIMENTS

1. Fehling Solution[1]

The reagent is made just prior to use by mixing equal volumes of Fehling solution I, containing copper(II) sulfate, with solution II, containing tartaric acid and alkali. The copper, present as a deep blue complex anion, if reduced by a sugar from the copper(II) to the copper(I) state, precipitates as red copper(I) oxide. If the initial step in the reaction involved oxidation of the aldehydic group of the aldose to a carboxyl group, a ketose should not reduce Fehling solution, or at least should react less rapidly than an aldose, but the comparative experiment that follows will show that this supposition is not the case. Hence, attack by an alkaline oxidizing agent must attack the α-ketol grouping common to aldoses and hexoses, and perhaps proceeds through an enediol, the formation of which is favored by alkali. A new α-ketol grouping is produced, and thus oxidation proceeds down the carbon chain.



One milliliter of mixed solution will react with 5 mg of glucose; the empirically determined ratio is the basis for quantitative determination of the sugar. The Fehling test is not specific to reducing sugars, since ordinary aldehydes reduce the reagent although by a different mechanism and at a different rate.

The following sugars are to be tested: 0.1 *M* solutions of glucose, fructose, lactose, maltose, sucrose (cane sugar).[2]

Introduce 10 drops of the 0.1 *M* solutions to be tested into each of five 13 × 100-mm test tubes carrying some form of serial numbers resistant to heat and water (rubber bands), and prepare a beaker of hot water in which all the tubes can be heated at once. Measure 5 ml of Fehling solution I into a small flask

▼
Note for the instructor

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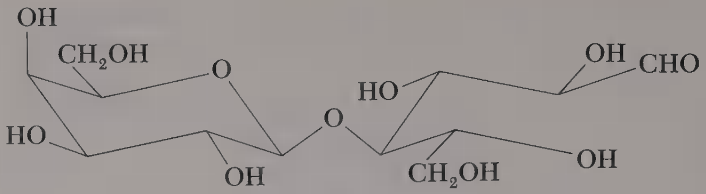
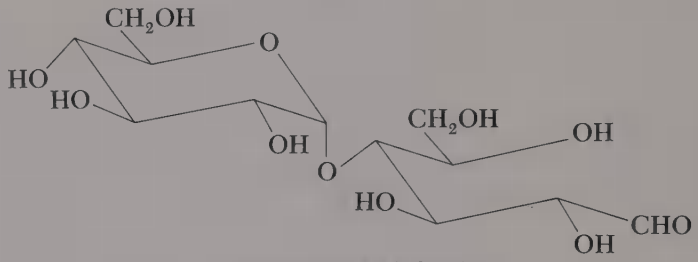
[1] *Solution I:* 34.64 g of $CuSO_4 \cdot 5H_2O$ dissolved in water and diluted to 500 ml. *Solution II:* 173 g of sodium potassium tartrate (Rochelle salt) and 65 g of sodium hydroxide dissolved in water and diluted to 500 ml.

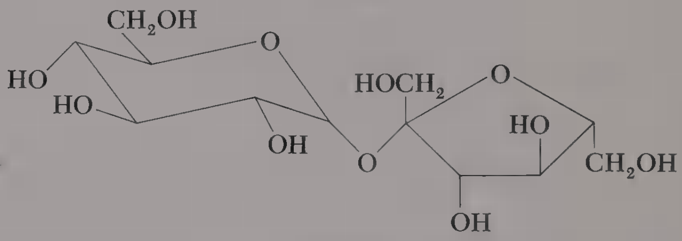
[2] To prepare 0.1 *M* test solutions dissolve the following amounts of substance in 100 ml of water each: D-glucose monohydrate, 1.98 g; fructose, 1.80 g; α-lactose monohydrate, 3.60 g; maltose monohyrate, 3.60 g; sucrose, 3.42 g; *n*-butanol, 0.72 g.



Lactose (aldehyde form)



Maltose (aldehyde form)



Sucrose

and wash the graduate before using it to measure 5 ml of solution II into the same flask. Mix until all precipitate dissolves, measure 2 ml of mixed solution into each of the five test tubes, shake, put the tubes in the heating bath, and observe the results. Empty, and wash the tubes with water and then with dilute acid (leave the markers in place and continue heating the beaker of water on a hot plate).

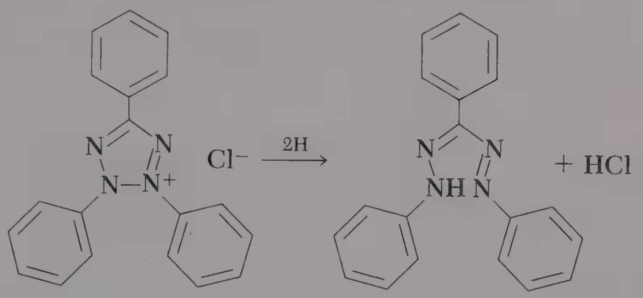
2. Tollens Reagent

Tollens reagent is a solution of a silver ammonium hydroxide complex that is reduced by aldoses and ketoses as well as by simple aldehydes. See Chapter 14.4 for the preparation of this reagent and the procedure for carrying out the test. The test is more sensitive than the Fehling test and better able to reveal small differences in reactivity, but it is less reliable in distinguishing between reducing and nonreducing sugars.

Prepare the five test tubes according to Chapter 14.4. Into each tube put one drop of 0.1 *M* solution of glucose, fructose, lactose, maltose, and *n*-butanol.[2] Add 1 ml of Tollens reagent to each tube and let the reaction proceed at room temperature. Watch closely and try to define the order of reactivity as measured not by the color of solution but by the time of the first appearance of metal.

3. Red Tetrazolium[3]

The reagent (RT) is a nearly colorless, water-soluble substance that oxidizes aldoses and ketoses, as well as other α-ketols, and is thereby reduced. The reduced form is a water-insoluble, intensely colored pigment, a diformazan.



Red tetrazolium RT-Diformazan

Red tetrazolium affords a highly sensitive test for reducing sugars and distinguishes between α-ketols and simple aldehydes more sharply than Fehling and Tollens tests.

Put one drop of each of the five 0.1 M test solutions of Section 1 in the cleaned, marked test tubes, and to each tube add 1 ml of a 0.5% aqueous solution of red tetrazolium and one drop of 10% sodium hydroxide solution. Put the tubes in the beaker of hot water and note the order of development of color.

For estimation of the sensitivity of the test, use the substance that you regard as the most reactive of the five studied. Dilute 1 ml of the 0.1 M solution with water to a volume of 100 ml, and run a test with RT on 0.2 ml of the diluted solution.

4. Phenylosazones

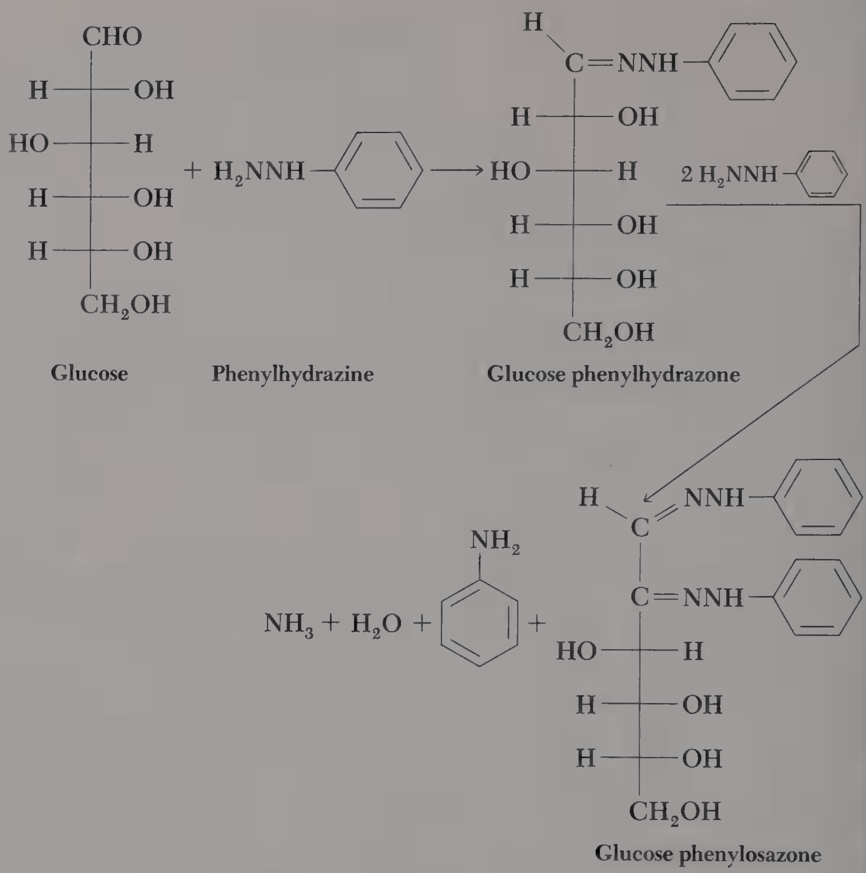
Prepare 10 ml of stock phenylhydrazine reagent as in Chapter 14, Section 1, and put 1-ml (1-millimole) portions of it into four of the cleaned, numbered test tubes. Add 3.3-ml (0.3-millimole) portions of 0.1 M solutions of glucose, fructose, lactose, and maltose and heat the tubes in the beaker of hot water for 20 min. Shake the tubes occasionally to relieve supersaturation and note the times at which osazones separate. If after 20 min no product has separated, cool and scratch the test tube to induce crystallization. (Save unused phenylhydrazine reagent.)

Collect and save the products for possible later use in identification of unknowns. Since osazones melt with decomposition, the bath in a mp determination should be heated at a standard rate (0.5° per sec).

▼
Note for the instructor

---

[3] 2,3,5-Triphenyl-2H-tetrazolium chloride. Available from Aldrich, Dajac, Eastman, Fischer, MCB. Freshly prepared aqueous solutions should be used in tests. Any unused solution should be acidified and discarded.

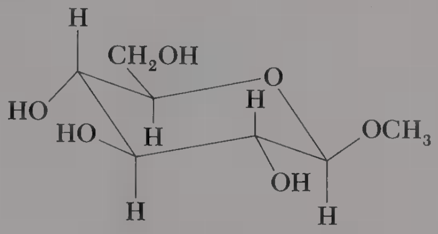


5. Hydrolysis of a Disaccharide

▼
Sucrose is heated with dil HCl

The object of this experiment is to determine conditions suitable for hydrolysis of a typical disaccharide. Put 1-ml portions of a 0.1 *M* solution of sucrose in each of five numbered test tubes and add 5 drops of concentrated hydrochloric acid to tubes 2–5. Let tube 2 stand at room temperature and heat the other four tubes in the hot water bath for the following periods of time: tube 3, 2.5 min; tube 4, 5 min; tubes 1 and 5, 15 min. As each tube is removed from the bath, it is cooled to room temperature, and if it contains acid, adjusted to approximate neutrality by addition of 15 drops of 10% sodium hydroxide. Measure one drop of each neutral solution into a new numbered test tube, add 1 ml of red tetrazolium solution and a drop of 10% sodium hydroxide, and heat the five tubes together for 2 min and watch them closely.

In which of the tubes was hydrolysis negligible, incomplete, and extensive? Does the comparison indicate the minimum heating period required for complete hydrolysis? If not, return the stored solutions to the numbered test tubes, treat each with 1 ml of stock phenylhydrazine reagent and heat the tubes together for 5 min. On the basis of your results, decide upon a hydrolysis procedure to use in studying unknowns; the same method is applicable to the hydrolysis of methyl glycosides.



Methyl β-D-glucoside

6. Evaporation Test

Few solid derivatives suitable for identification of sugars are available. Osazones are not suitable since the same osazone can form from more than one sugar. Acetylation, in the case of a reducing sugar, is complicated by the possibility of formation of the α- or the β-anomeric form, or a mixture of both. The unknown (next section) is supplied in 0.1 *M* aqueous solution, and if this is evaporated and the residue acetylated the ratio of α- to β-acetates may not be the same as that reported for a crystalline starting material. In the case of glucose, acetylation after evaporation and crystallization of the product from water gives β-glucose pentaacetate (two polymorphic forms, mp 111° and 135°), but the yield is only 36% (α-pentaacetate, mp 114°). The other sugars give less favorable results.

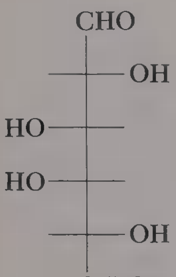
Triphenylmethyl (trityl) ethers can be made by reaction of an anhydrous sugar in pyridine solution with trityl chloride, $(C_6H_5)_3CCl$. Primary alcoholic groups are attacked in preference to secondary, and hence the amount of reagent required varies: glucose requires one mole; fructose, lactose, and maltose require two; and sucrose three. 6-Tritylglucose forms solvated crystals of indefinite melting point. Acetylation of this product gives tetraacetyl trityl-α-glucose, mp 130°, whereas if glucose is tritylated in pyridine, and the product then acetylated in the same solution without being isolated, the substance formed is tetraacetyl trityl-β-glucose, mp 163°.

Although preparation of the above derivatives is not recommended, the first step in the preparation, evaporation of the aqueous solution of the sugar, provides useful guidance in identification. On thorough evaporation of all the water from a solution of glucose, fructose, mannose, or galactose, the sugar is left as a sirup that appears as a glassy film on the walls of the container. Evaporation of solutions of lactose or maltose gives white solid products, which are distinguishable because the temperature ranges at which they decompose differ by about 100°.

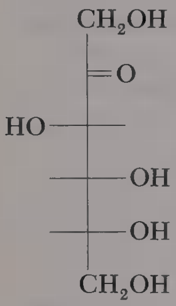
▼ *Test for lactose, maltose*

Measure 2 ml of a 0.1 *M* solution of either lactose or maltose into a 25 × 150-mm test tube, and add an equal volume of cyclohexane (to hasten the evaporation). Connect the test tube through a filter trap to the suction pump with a rubber stopper that fits the test tube snugly. Make sure that the pressure-gauge of the filter trap is adjusted correctly so that it will show whether or not all connections are tight and if the aspirator is operating efficiently. Then turn the water running through the aspirator on at full force and rest the tube horizontally in the steam bath with all but the largest ring removed, so that the whole tube will be heated strongly. If evaporation does not occur rapidly, check the connections and trap to see what is wrong. If a water layer persists for a long time, disconnect and add 1–2 ml of cyclohexane to hasten evaporation. When evaporation appears to be complete, disconnect, rinse the walls of the tube with 1–2 ml of methanol, and evaporate again, when a solid should separate on the walls. Rinse this down with methanol and evaporate again to produce a thoroughly anhydrous product. Then scrape out the solid and determine the melting point, or actually the temperature range of decomposition.

Anhydrous α-maltose decomposes at about 100–120°, and anhydrous α-lactose at 200–220°. Note that in the case of an unknown a temperature of decomposition in one range or the other is valid as an index of identity only if the substance has been characterized as a reducing sugar. Before applying the test to an unknown, perform a comparable evaporation of a 0.1 *M* solution of glucose, fructose, galactose, or mannose.



D-Galactose



D-Fructose

7. Unknowns

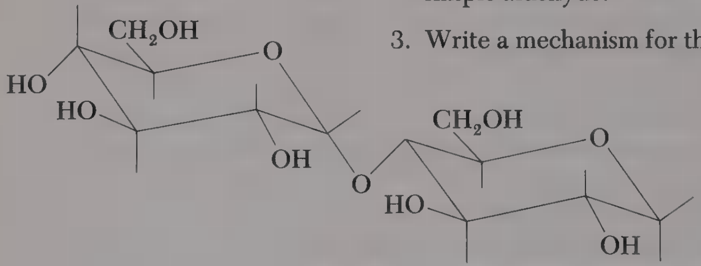
The unknown, supplied as a 0.1 *M* solution, may be any one of the following substances:

D-Glucose
D-Fructose
D-Galactose
Lactose
Maltose
Sucrose
Methyl β-D-glucoside

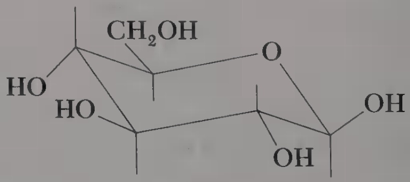
You are to devise your own procedure of identification.

QUESTIONS

1. What, do you conclude, is the order of relative reactivity in the RT test of the compounds studied?
2. Which test do you regard as the most reliable for distinguishing reducing from nonreducing sugars, and which for differentiating an α-ketol from a simple aldehyde?
3. Write a mechanism for the acid-catalyzed hydrolysis of a disaccharide.



Maltose



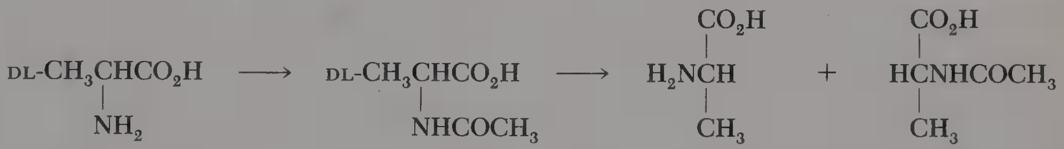
β-D-Glucose

30

Enzymic Resolution of DL-Alanine

KEYWORDS

Resolution
Enzyme
Acylase, N-acyl derivatives
N-acetyl-DL-alanine
L-alanine
Levorotatory
Azlactone



$$\text{DL-}CH_3\underset{\underset{\displaystyle NH_2}{|}}{C}HCO_2H \longrightarrow \text{DL-}CH_3\underset{\underset{\displaystyle NHCOCH_3}{|}}{C}HCO_2H \longrightarrow H_2N\underset{\underset{\displaystyle CH_3}{|}}{\overset{\overset{\displaystyle CO_2H}{|}}{C}}H + H\underset{\underset{\displaystyle CH_3}{|}}{\overset{\overset{\displaystyle CO_2H}{|}}{C}}NHCOCH_3$$

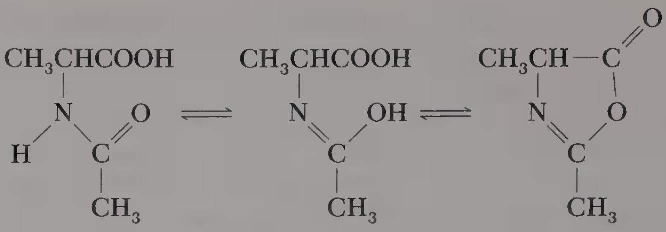
DL-Alanine (**1**)
mp 295°,
MW 89.10

N-Acetyl-DL-alanine (**2**)
mp 137°,
MW 131.13

L-Alanine (**3**)
mp 297°,
α_D + 14.4°

N-Acetyl-D-alanine (**4**)
mp 125°,
α_D + 66.5°

Resolution of DL-alanine (**1**) is accomplished by heating the N-acetyl derivative (**2**) in weakly alkaline solution with acylase, a proteinoid preparation from porcine kidney containing an enzyme that promotes rapid hydrolysis of N-acyl derivatives of natural L-amino acids but acts only immeasurably slowly on the unnatural D-isomers. N-Acetyl-DL-alanine (**2**) can thus be converted into a mixture of L-(+)-alanine (**3**) and N-acetyl-D-alanine (**4**). The mixture is easily separable into the components, since the free amino acid (**3**) is insoluble in ethanol and the N-acetyl derivative (**4**) is readily soluble in this solvent. Note that, in contrast to the weakly levorotatory D-(−)-alanine (− 14.4°), its acetyl derivative is strongly dextrorotatory.



(4) (5) (6)

The acetylation of an α-amino acid presents the difficulty that, if the conditions are too drastic, the N-acetyl derivative (4) is converted in part through the enol (5) to the azlactone (6).[1] However, under critically controlled conditions of concentration, temperature, and reaction time, N-acetyl-DL-alanine can be prepared easily in high yield.

Place 2 g of DL-alanine and 5 ml of acetic acid in a 25 × 150-mm test tube, insert a thermometer, and clamp the tube in a vertical position. Measure 3 ml of acetic anhydride, which is to be added when the alanine/acetic acid mixture is at exactly 100°. Heat the test tube with a small flame, with stirring, until the temperature of the suspension has risen a little above 100°. Stir the suspension, let the temperature gradually fall, and when it reaches 100° add the 3-ml portion of acetic anhydride and note the time. In the course of 1 min the temperature falls (91–95°, cooled by added reagent), rises (100–103°, the acetylation is exothermic), and begins to fall with the solid largely dissolving. Stir to facilitate reaction of a few remaining particles of solid, let the temperature drop to 80°, pour the solution into a tared 125-ml round-bottomed flask, and rinse the thermometer and test tube with a little acetone. Add 10 ml of water to react with excess anhydride, connect the flask to the aspirator operating at full force, put the flask *inside* the rings of the steam bath and wrap the flask with a towel. Evacuation and heating for about 5–10 minutes should remove most of the acetic acid and water and leave an oil or thick sirup. Add 10 ml of cyclohexane and evacuate and heat as before for 5–10 min. Traces of water in the sirup are removed as a cyclohexane/water azeotrope. If the product has not yet separated as a white solid or semisolid, determine the weight of the product, add 10 ml more cyclohexane, and repeat the process. When the weight becomes constant, the yield of acetyl DL-alanine should be close to the theoretical amount. The product has a pronounced tendency to remain in supersaturated solution and hence does not crystallize readily.

▼
Check the pressure gauge

Add 10 ml of distilled water[2] to the reaction flask, grasp this with a clamp, swirl the mixture over a free flame to dissolve all the product, and cool under the tap. Remove a drop of the solution on a stirring rod, add it to 0.5 ml of a 0.3% solution of ninhydrin in water, and heat to boiling. If any unacetylated DL-alanine is present a purple color will develop. Pour the solution into a 20 ×

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[1] The azlactone of DL-alanine is known only as a partially purified liquid.

[2] Tap water may contain sufficient heavy metal ion to deactivate the enzyme.

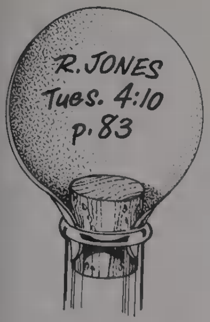

FIGURE 30.1 Filter paper identification marker.

150-mm test tube and rinse the flask with a little water. Add 1.5 ml of concentrated ammonia solution, stir to mix, check the pH with Hydrion paper, and if necessary adjust to pH 8 by addition of more ammonia with a capillary dropping tube. Add 10 mg of commercial acylase powder, or 2 ml of fresh acylase solution,[3] mix with a stirring rod, rinse the rod with distilled water and make up the volume until the tube is about half full. Then stopper the tube, mark it for identification (Fig. 30.1), and let the mixture stand at room temperature overnight, or at 37°[4] for 4 hrs.

▼
Work-up time $\frac{1}{2}$–$\frac{3}{4}$ hr

At the end of the incubation period add 3 ml of acetic acid to denature the enzyme and if the solution is not as acidic as pH 5 add more acid. Rinse the cloudy solution into a 125-ml Erlenmeyer flask, add 100 mg of decolorizing carbon (0.5-cm column in a 13 × 100-mm test tube), heat and swirl over a free flame for a few moments to coagulate the protein, and filter the solution by suction. Transfer the solution to a 125-ml round-bottomed flask and evaporate on a rotary evaporator under vacuum, or add 20 ml of cyclohexane (to prevent frothing) and a boiling stone and evaporate on the steam bath under vacuum to remove water and acetic acid as completely as possible. Remove the last traces of water and acid by adding 15 ml of cyclohexane and evaporating again to remove water and acetic acid as azeotropes. The mixture of L-alanine and acetyl D-alanine separates as a white scum on the walls. Add 15 ml of 95% ethanol, digest on the steam bath, and dislodge some of the solid with a spatula. Cool well in ice for a few minutes, and then scrape as much of the crude L-alanine as possible onto a suction funnel, and wash it with ethanol. Save the ethanol mother liquor.[5] To recover the L-alanine retained by the flask, add 2 ml of water and warm on the steam bath until the solid is all dissolved, then transfer the solution to a 25-ml Erlenmeyer flask by means of a capillary dropping

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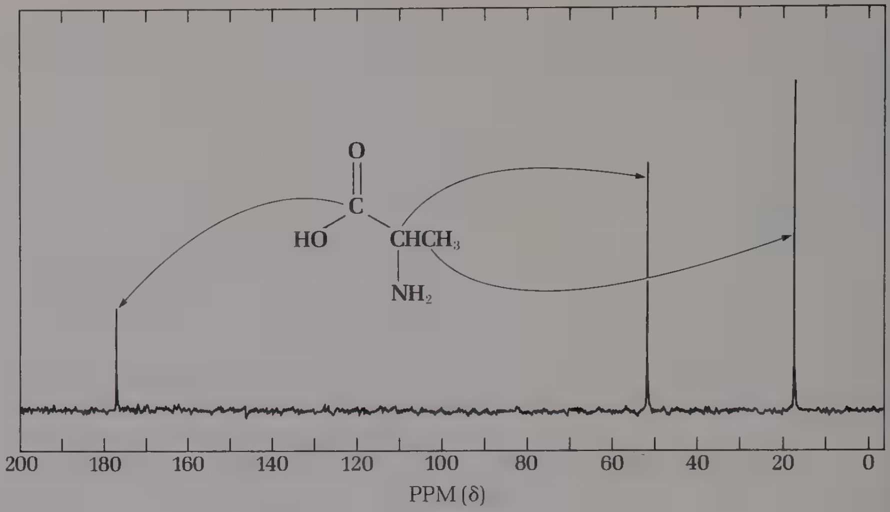
[3] The preparation of the acylase solution should be started on the day of the week that fresh pork kidneys are available at a slaughter house. The fat is sliced off two kidneys (about 150 g) and the kidneys cut into small pieces and either (a) ground in a mortar with sand and suspended in 300 ml of distilled water or (b) placed in a Waring blender with 300 ml of water and ground for 2 min. The homogenate prepared by either method is centrifuged in the cold until the supernatant liquid is clear (3000 × g for 30 min, or 2000 × g fro 3 hr). A 46-cm length of cellulose sausage casing (2.5 cm in diameter) is wetted so that it can be opened and tied off at one end. The enzyme solution is carefully decanted into the sack, the other end is tied off, and the sack is let soak overnight in a pan of running tap water (dialysis removes soluble kidney components that would interfere with isolation of the amino acid; colored impurities are removed in the course of the isolation and do not interfere). The enzyme solution is centrifuged again to remove debris and stored at 5° until required; the volume is about 150 ml.

Commercial porcine kidney acylase is available from Schwarz/Mann, Division of Becton, Dickinson and Co., Orangeburg, N.Y. 10962.

[4] A reasonably constant heating device that will hold 15 tubes is made by filling a 1-liter beaker with water, adjusting to 37°, and maintaining this temperature by the heat of a 250-watt infrared drying lamp shining horizontally on the beaker from a distance of about 40 cm. The capacity can be tripled by placing other beakers on each side of the first one and a few cm closer to the lamp.

[5] In case the yield of L-alanine is low, evaporation of this mother liquor may reveal the reason. If the residue solidifies readily and crystallizes from acetone to give acetyl-DL-alanine, mp 130° or higher, the acylase preparation is recognized as inadequate in activity or amount. Acetyl-D-alanine is much more soluble and slow to crystallize.

FIGURE 30.2 ^{13}C nmr spectrum of alanine.



tube, rinse the flask with 2 ml more water, and transfer in the same way. Add the filtered L-alanine, dissolve by warming, and filter the solution by gravity into a 50-ml Erlenmeyer flask (use the dropping tube to effect the transfer of solution to filter). Rinse the flask and funnel with 1 ml of water and then with 5 ml of warm 95% ethanol. Then heat the filtrate on the steam bath and add more 95% ethanol (10–15 ml) in portions until crystals of L-alanine begin to separate from the hot solution. Let crystallization proceed. Collect the crystals and wash with ethanol. The yield of colorless needles of L-alanine, α_D + 13.7 to + 14.4°[6] (in 1 *N* hydrochloric acid) varies from 0.40 to 0.56 g, depending on the activity of the enzyme.

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[6] Determination of optical activity can be made in the student laboratory with a Zeiss Pocket Polarimeter, which requires no monochromatic light source and no light shield. For construction of a very inexpensive polarimeter, see W. H. R. Shaw, *J. Chem. Ed.*, 32, 10 (1955).

31

Paper Chromatography of Amino Acids

KEYWORDS

- Partition chromatography
- Stationary phase, adsorbed water
- Moving phase, phenol
- Lipophilic, hydrophilic
- *Rf* values of amino acids
- Molecular flow
- Protein hydrolyzate
- Filter paper strip
- Applicator stick
- Solvent front
- Capillary action
- Chromatogram
- Ninhydrin and permanganate spot tests
- Detection of proline

Filter paper under ordinary atmospheric conditions consists of cellulose containing 22% of adsorbed water (hydrogen-bonded; about two molecules per $C_6H_{10}O_5$ unit). When a folded strip of filter paper is inserted into a slightly slanting test tube (Fig 31.1) with the lower end of the paper dipping into phenol, an organic solvent which is only partially miscible with water, the phenol ascends the strip by capillary flow without material disturbance of the adsorbed water. In paper partition chromatography, the adsorbed water is the stationary phase and the organic solvent the moving phase, just as silicone oil is the stationary phase and helium the moving phase in gas chromatography.

Before the folded strip is inserted, the paper is impregnated with minute amounts of an amino acid solution at the starting line (Fig. 31.2) that will be a little above the level of the phenol. As phenol rises toward the top of the strip the amino acids are subjected to innumerable partitions between the moving lipid phase and the stationary water phase. Highly lipophilic amino

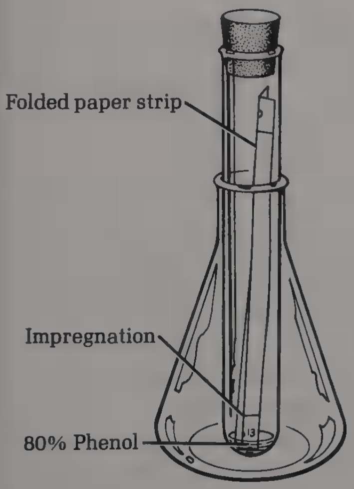


FIGURE 31.1 Apparatus for partition chromatography.

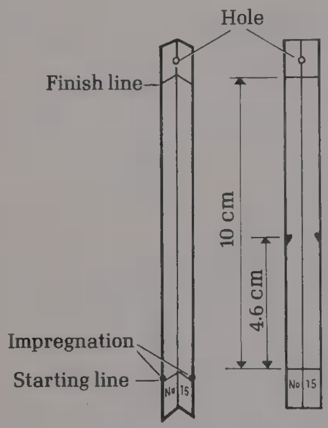
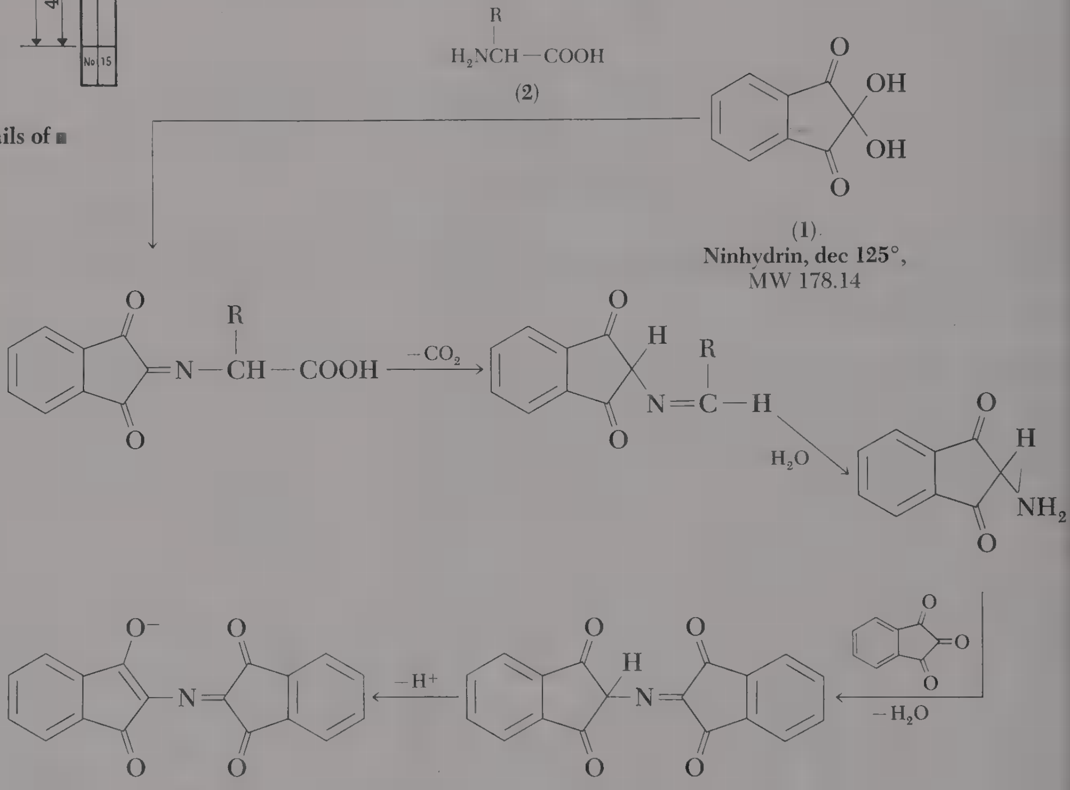


FIGURE 31.2 Details of a chromatogram.

acids travel almost as fast as the organic solvent, whereas very hydrophilic ones are largely retained by the adsorbed water and make little progress. When the easily discernible solvent front has reached the finish line, 10 cm from the starting line (Fig. 31.2b), the chromatogram is terminated and the strip removed and hung on a hook. Amino acids are all colorless and the strip bears no indication of their distribution until it is sprayed with a solution of ninhydrin. The oxidation-reduction reaction between ninhydrin (1) and an amino acid (2) produces a pigment (3) that contains only the nitrogen atom from the original amino acid:



(2)

(1)
Ninhydrin, dec 125°,
MW 178.14

(3)

The pigment appears as a small purple spot (b). The position of the front (top) of the spot is noted and its distance from the starting line measured with a ruler. The rate of flow, or *Rf* value, of a particular amino acid is the ratio of the distance travelled by the acid to the distance travelled by the solvent. In the example of Fig. 31.2b, $Rf = (4.6/10) = 0.46$. The acids having primary α-amino groups give pink to purple spots when sprayed with ninhydrin; the secondary amines proline and hydroxyproline give yellow spots.

Rf values vary with the solvent system and type of filter paper used; those reported in Table 31.1 were determined by the procedure you will follow in this experiment. In the series glycine, alanine, valine, and leucine, *Rf* increases

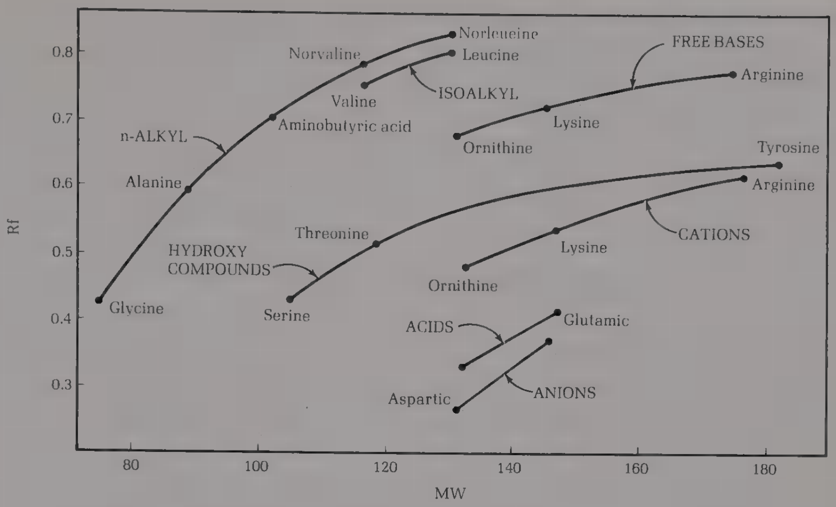


FIGURE 31.3 *Rf* values of amino acids as a function of MW. Lines connect similar amino acids.

with increasing molecular weight; the larger the alkyl group the more the acid tends to move along with the organic solvent. Figure 31.3 includes *Rf* values for three rarer, straight-chain acids that are the higher *n*-alkyl homologs of alanine; that the five points for the *n*-alkyl series fall on a smooth curve demonstrates a regular relationship, if not a strict proportionality, between *Rf* and molecular weight. The structure of the alkyl group is of minor but noticeable influence, since the isoalkyl compounds valine and leucine do not travel quite as fast as, and hence are slightly more hydrophilic than, the *n*-isomers.

The balance between the hydrophilic and lipophilic character of a given acid is expressed by a quantity defined as the molecular flow (*Mf*), calculated thus:

$$Mf = \frac{\text{MW}}{100\,Rf}$$

Inspection of the fifth column of the table shows that structurally related compounds have *Mf* values in a range characteristic of the acid type. For the seven *n*- and *i*-alkyl acids plotted in Fig. 31.3, the average *Mf* is 1.6. That the *Mf* values for methionine (1.9) and phenylalanine (2.0) are slightly higher means that the sulfur atoms and the phenyl group render the substances a little more hydrophilic, weight for weight, than the alkyl acids. Proline is distinctive in being a cyclic secondary amine; it has the lowest *Mf* value (1.4) of all the acids. Cystine is anomalous in both *Mf* and solubility. The high molecular weight would seem to be offset by the presence of two hydrophilic dipolar ion groupings, but the *Rf* is very low and the solubility in water (25°) is only 0.112 g/l, as compared to 24.3 g/l for leucine. The anomaly may be associated with the existence of the substance in a chelated endocyclic structure.

Introduction of polar groups into the side chains of the alkyl acids (av. $Mf =$ 1.6) produces successive hydrophilic shifts as follows: basic group, $Mf = 2.1$; hydroxyl group, $Mf = 2.5$; carboxyl group, $Mf = 4.0$. Figure 31.3 shows that

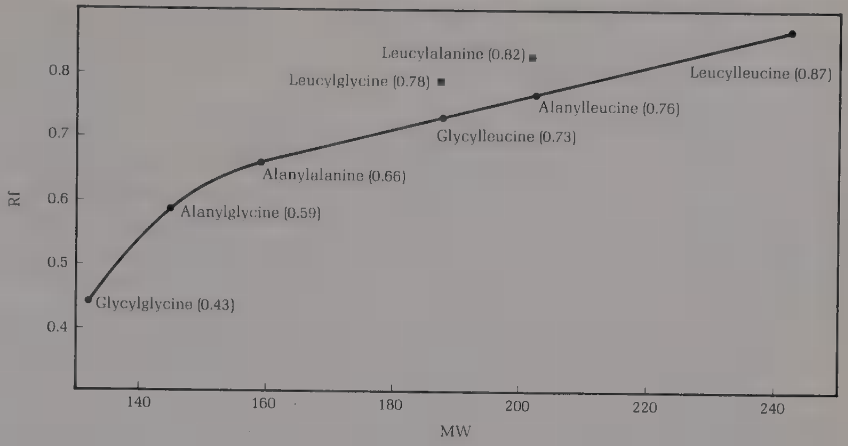
Table 31.1 Rf Values of Amino Acids

Acid	Formula	Rf	MW	$Mf = \frac{MW}{100\,Rf}$	Cost[a]	Yield: mg/100 mg of		
						Hair	*Silk*	*Gelatin*[b]
Cystine (CyS-SCy)	$HO_2CCH(NH_2)CH_2SSCH_2CH(NH_2)CO_2H$	.16	240.30	15	low	18.0		
Glycine (Gly)	$CH_2(NH_2)CO_2H$	.42	75.07	1.8	v. low	4.1	42.3	25.5
Alanine (Ala)	$CH_3CH(NH_2)CO_2H$	.59	89.09	1.5	v. low	2.8	24.5	11.4
Valine (Val)	$(CH_3)_2CHCH(NH_2)CO_2H$	.75	117.15	1.6	v. low	5.5	3.2	2.0
Leucine (Leu)	$(CH_3)_2CHCH_2CH(NH_2)CO_2H$	.79	131.18	1.7	v. low	11.2	0.8	2.5
Isoleucine (Ile)	$CH_3CH(CH_2CH_3)CH(NH_2)COOH$	—	131.18	—	high	—	—	2.5
Methionine (Met)	$CH_3SCH_2CH_2CH(NH_2CO_2H$	.77	149.21	1.9	v. low	0.7	—	2.0
Phenylalanine (Phe)	$C_6H_5CH_2CH(NH_2)CO_2H$	.82	165.19	2.0	low	2.4	—	2.5
Proline (Pro)	$\underline{HNCH_2CH_2CH_2CH}CO_2H$	.85	115.13	1.4	high	4.3	1.5	14.1
Ornithine (Orn)	$H_2NCH_2CH_2CH_2CH(NH_2)CO_2H$	.67	132.16	2.0	high			
cation	$^+H_3NCH_2CH_2CH_2CH(NH_2)CO_2H$	.47	133.17	2.8	—			
Lysine (Lys)	$H_2NCH_2CH_2CH_2CH_2CH(NH_2)CO_2H$	.71	146.19	2.1	high	1.9	0.4	3.6
cation	$^+H_3NCH_2CH_2CH_2CH_2CH(NH_2)CO_2H$	.53	147.20	2.8	—			
Arginine (Arg)	$HN{=}C(NH_2)NHCH_2CH_2CH_2CH(NH_2)CO_2H$	.76	174.20	2.3	low	8.9	1.1	9.0
cation	$^+H_2N{=}C(NH_2)NHCH_2CH_2CH_2CH(NH_2)CO_2H$	.60	175.21	2.9	—			
Histidine (His)	$\underline{HNCH{=}NCH{=}C}-CH_2CH(NH_2)COOH$	—	155.16	—	high			
Tryptophan (Try)	$\underline{HN-C_6H_4-CH{=}C}-CH_2CH(NH_2)COOH$	—	204.23	—	high			
Serine (Ser)	$HOCH_2CH(NH_2)CO_2H$	.43	105.09	2.4	low	10.6	12.6	4.4
Threonine (Thr)	$HOCH(CH_3)CH(NH_2)CO_2H$	.51	119.21	2.3	v. high	8.5	1.5	2.9
Tyrosine (Tyr)	$HOC_6H_4CH_2CH(NH_2)CO_2H$	.62	181.19	2.9	high	2.2	10.6	1.7
Aspartic acid (Asp)	$HO_2CCH_2CH(NH_2)CO_2H$	.32	133.10	4.2	v. low	3.9	—	6.1
anion	$HO_2CCH_2CH(NH_2)CO_2^-$	.25	132.09	5.3	—			
Glutamic acid (Glu)	$HO_2CCH_2CH_2CH(NH_2)CO_2H$	.40	147.13	3.7	v. low	13.1	—	12.1
anion	$HO_2CCH_2CH_2CH(NH_2)CO_2^-$	.35	146.12	4.0				

[a] Per 100 g of L- or DL-form: v. low < \$5.00, low < \$10.00, high < \$15.00, v. high < \$25.00 (1977 prices—Sigma).

[b] Also 9.7 mg of hydroxyproline, unique to gelatin.

FIGURE 31.4 *Rf* values of dipeptides as a function of MW. Average *Mf* = 2.6.



the curves are of similar types but displaced to lower *Rf* values. The average *Mf* value of 2.6 found for dipeptides (Fig. 31.4) indicates that the lipophilic effect of the peptide link is intermediate between those of the hydroxyl and carboxyl groups. If ornithine, lysine, or glutamic acid is chromatographed not as the free base but as the monohydrochloride, the ionic group provides better retention by the stationary water phase and in each case *Rf* is about 0.2 unit lower than found for the free base. Since lysine perchlorate gives the same *Rf* as the hydrochloride, the rate of flow is determined by the lysine cation alone. A smaller hydrophilic displacement results when aspartic and glutamic acid are chromatographed as anions. The displacements are sometimes useful aids in the identification of unknowns, which is the major objective of the present experiment.

▼
Scheduling of experiments

Sections 1–3 of the following experiments describe general procedures, and Section 4 lists five chromatograms that are to be run on known acids and mixtures; these should be started at the very beginning of the period so that they can be carried to a stopping point by the end of the day. As soon as the chromatograms are under way the two protein hydrolysis experiments should be started; during the 1-hr reflux period the characterizing tests of Sections 5–8 can be carried out. The protein hydrolyzate should be worked up on the first day and an overnight chromatogram started.

EXPERIMENTS

1. Procedure of Chromatography[1]

A strip of paper 13 cm long and 1.3 cm wide is to be cut from a roll of half inch, Eaton-Dikeman No. 613 filter paper,[2] marked, punched, folded, and impregnated as shown in Fig. 31.5 without being touched at the critical part with the

▼
Notes for the instructor

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[1] Wilkins-Anderson supplies the filter paper recommended, the strip dispenser (Fig. 31.6), the amino acid dispenser, and stock amino acid solutions.

[2] Supplied in rolls $7\frac{1}{4}$ and $9\frac{5}{8}$ in. in diameter. Half-inch Whatman No. 1 paper ($8\frac{3}{8}$ in. roll, 600 ft) can be used but gives slightly lower *Rf* values.

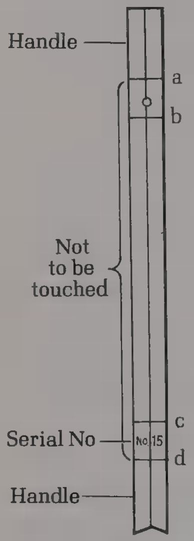


FIGURE 31.5 Details of chromatograms for the partition of proteins.

fingers, since contact with skin protein can give rise to false spots. This can be done by initially cutting a strip about 4 cm longer than required and using the 2-cm section at each end as a handle that is to be cut off after all other operations have been completed. Figure 31.5 shows such a strip marked with starting and finish lines (*c* and *b*, 10 cm apart) and boundary lines (*a* and *d*); it is also punched and marked with a serial number. A strip dispenser (Fig. 31.6) protects the paper and expedites the preparation of strips. Grasp the end of the paper tape and pull out enough to give a 10-cm (or a 25-cm) strip, mark pencil lines at *a, b, c,* and *d* (or, for a 25-cm strip at *a, b, e,* and *f*), punch a hole centered between *a* and *b* with the point of a sharp pencil, and then cut off the strip cleanly at a right angle at a point about 2 cm above *a*. Record the serial number of the experiment in the space between *c* and *d,* and then fold the strip down the center. A convenient technique of folding (without contact of the fingers in the vital area) is to place the strip with the markings down on a clean, smooth surface, such as the back of a notebook or writing pad, place a transparent 30–60° triangle so that it covers exactly one half of the paper (Fig. 31.7), press it down firmly, insert under the paper the tip of a second triangle, fold the paper up against and over the first one (*b*), and then withdraw the first triangle while flattening the strip on the crease (*c*). Note that the triangle for drawing formulas (Fig. 31.8) serves also for folding paper strips.

Now grasp the folded strip by the lower handle and apply a tiny spot of amino acid solution to each edge of the strip at the ends of the starting line (*d*). The wetted area should be no more than 1–2 mm in diameter and may be visible only from the under side. The application is conveniently made with the

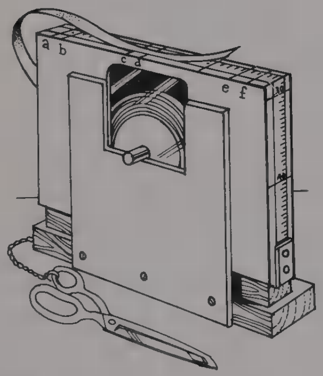


FIGURE 31.6 Strip dispenser. This dispenser, which will hold any of the rolls mentioned in Footnote 2, can be made from a 3 cm × 23 cm × 30 cm cardboard box. For accommodation of the 24 cm roll, the box is mounted on blocks as shown and its middle section cut away. The slot is 8 cm from the top; marking-line (*a*) is 1.3 cm from the edge, and the other lines are at the following distances from (*a*): (*b*), 1.5 cm; (*c*), 11.5 cm; (*d*), 13 cm; (*e*), 26.5 cm; (*f*), 28 cm. A small hole, centered between (*a*) and (*b*), is punched with an awl through both layers of cardboard.

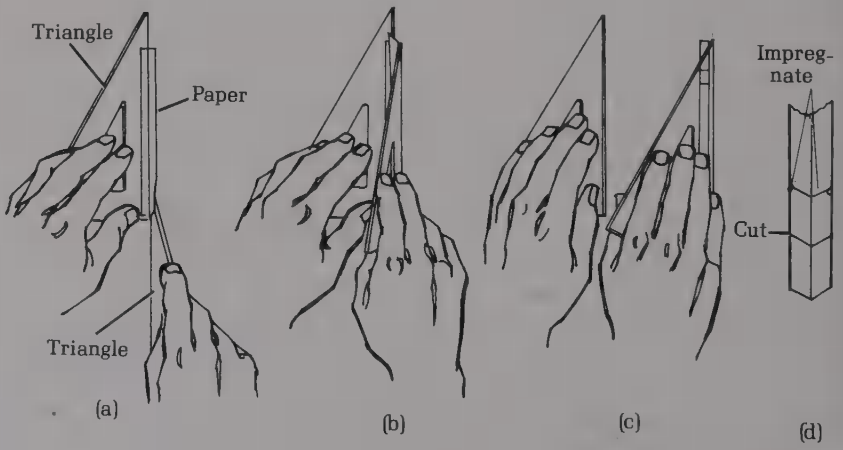
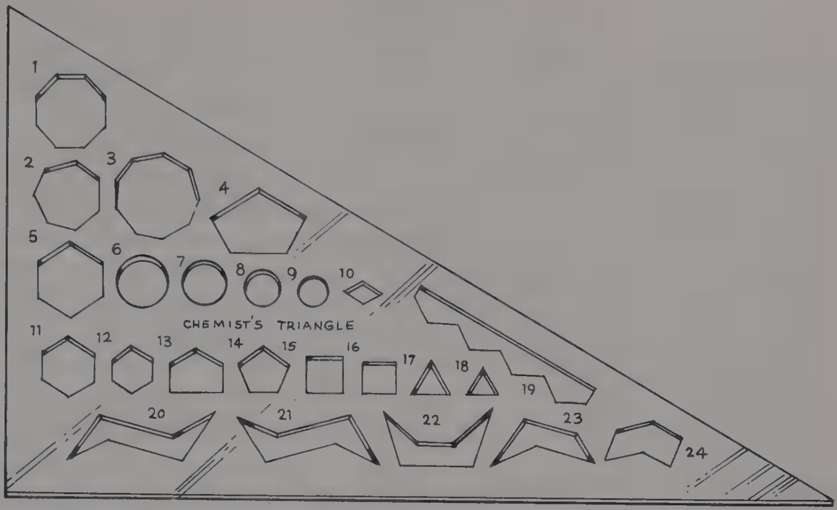


FIGURE 31.7 A technique for folding a chromatogram.

FIGURE 31.8 Chemist's triangle



smaller end of a toothpick that has been soaked in the solution until the wood is saturated.[3] To remove excess solution, touch the end first to the neck of the bottle and then to the strip handle; make practice spots on the handle and then make the actual applications and let the spots dry (1–2 min). About 0.2 ml of 80% phenol is to be withdrawn from a bottle in which the solution is overlayered with 90–120° ligroin to prevent oxidation[4] and introduced to the bottom of a 20 × 150-mm test tube without getting it on the walls of the tube. This can be done by inserting the capillary tip of a Pasteur pipette through the ligroin layer while expelling air from the pipette, drawing up phenol solution, inserting the dropper carefully into the test tube until the tip is at the bottom, and running in a 2-cm column of liquid. Mount the tube in a stand, an Erlenmeyer flask, or beaker in a slightly slanting position, cut off both handles of the impregnated strip, and with a forceps, lower the strip carefully into the test tube so that it touches only the top and bottom, as in Fig. 31.1. Make sure that the hole is up and the amino acid spots down,[5] cork the tube, and note the time. It takes 2–3 hours for the solvent front to complete the travel of 10 cm and reach the finish line. When this point is reached (note the time) grasp the strip with a

▼

Caution: Phenol is toxic and caustic. If it gets on the skin wash the area well with soap and water and then with dilute alcohol

▼

Notes for the instructor

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[3] An amino acid dispenser can be made by inserting a toothpick through a hole drilled in the plastic cap of a specimen vial containing each amino acid solution. Matching colors on a base board, the vial, and the cap for each amino acid help to ensure return to the proper place.

[4] Either (a) warm a mixture of 80 g of analytical grade phenol with 20 g of distilled water until dissolved and cool, or (b) pour 114 ml of boiling water into a 1-lb bottle of phenol and shake for a few minutes; the solid soon dissolves (and the temperature drops to about 13°). The solution is at once covered with a generous layer of 90–120° ligroin and shaken to effect saturation (very little ligroin dissolves). If the solution is not protected from air it soon turns pink and then brown and eventually acquires capacity for destroying amino acids by oxidation.

[5] If you notice soon enough that the strip is upside down you may be able to withdraw it with a forceps, cut off the wetted section, and insert it in the correct way.

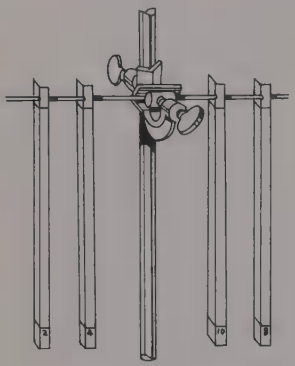


FIGURE 31.9 Chromatograph strips hung on an applicator stick for drying. The ends of the stick can be pointed in a pencil sharpener.

forceps and withdraw it while rinsing it with a stream of acetone to remove phenol and promote rapid drying. Hang the strip on either an applicator stick (Fig. 31.9), a hook, or a pin. When it is dry, grasp the lower end with a forceps and spray the strip uniformly but lightly with ninhydrin solution[6] (the paper should become moist but not dripping).

Spots usually begin to appear within 5–10 min; with aspartic acid there may be an induction period of several hours. Each application of a test solution should give rise to a separate spot, not more than 3–4 mm in diameter, on each side of the strip, but one may appear better defined and more reliable than the other. Outline the boundary of each spot in pencil, measure the distance of spot front from the starting line, and calculate the *Rf*.

It is not imperative that the chromatogram be extended to the point where the solvent has exactly reached the finish line. If you wish to stop it either somewhat short of or somewhat beyond this point, withdraw the strip and *mark the position of the solvent front,* and then rinse it with acetone and proceed as above.

2. Overnight Chromatograms

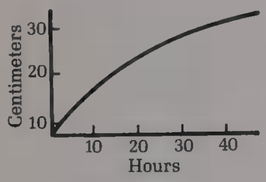


FIGURE 31.10 Rate of solvent travel.

Figure 31.10 shows that the rate of solvent flow decreases markedly with time.[7] Hence, use of a longer strip gives much greater flexibility in the time of terminating the chromatogram. With a 25-cm strip, the chromatograms can be run for 3–5 hrs, 15 hrs, or any time up to 24 hrs. Such a strip can be measured on the same dispenser (Fig. 31.7), marked with lines at *a, b, e,* and *f,* and folded as before. The amino acid sample is doubled by making one pair of applications, drying, and repeating the process. The amount of phenol is increased to 1 ml (4-cm column in the Pasteur pipette); the phenol is introduced as before into a 20 × 150-mm test tube, and this is slid into a 250-ml graduate and adjusted to a slanting position. The strip is lowered into place and the graduate stoppered.

3. Basification

For conversion of either lysine or arginine hydrochloride to the free base or conversion of either aspartic or glutamic acid to the anion, the test solution is spotted on a strip and let dry and then 10% sodium hydroxide is applied at the same spots (use a fresh toothpick and discard it) and let dry.

▼
Notes for the instructor

---

[6] Dissolve 400 mg of ninhydrin and 1.5 ml of *s*-collidine in 100 ml of 95% ethanol. A basic solution is required since the test solutions are acidic; pyridine can be substituted for collidine but gives less range in the color of the spots. Ethanol is superior to the frequently used butanol because it evaporates more rapidly and promotes quick development of spots at room temperature. A perfume atomizer works well as a sprayer.

[7] The curve conforms to the equation:

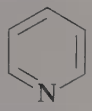
$$\log T\,(\text{min}) = \sqrt{0.42 \times \text{distance (cm)}}$$

4. Knowns

Prepare five 10-cm strips; impregnate them with 0.1 M solutions[8] of each of the following acids or pairs. In the case of a pair, apply one acid in two spots, dry, and then apply the other acid over the first in the same two spots.

Arginine hydrochloride	Phenylalanine and glycine
Arginine (see Section 3)	Alanine and aspartic acid
Leucine and glutamic acid	

See if you can effect good separation of spots, and compare your *Rf* values with those of Table 31.1. You may note a general displacement of values, which will be of guidance in studying unknowns.



Pyridine

5. Detection of Basic Amino Acids

Put a tiny drop of arginine hydrochloride solution on the bottom of an inverted beaker. With a fresh toothpick (that is to be discarded after the test) add a tiny drop of 2% phosphotungstic acid solution and see if a white precipitate forms. Repeat with lysine hydrochloride and, for comparison, with some one of the nonbasic acids.

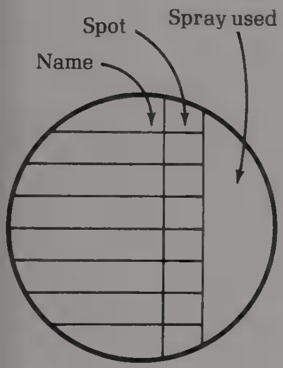


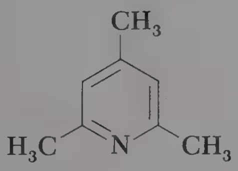
FIGURE 31.11 Details of filter paper used in ninhydrin spot test.

6. Ninhydrin Spot Test

Mark a piece of filter paper as in Fig. 31.11 and enter the names: alanine, proline (make two applications), methionine, aspartic acid, glutamic acid, arginine. In the box opposite each name make an appropriate spot of any size desired, let the spots dry, spray with ninhydrin-collidine, and let dry. Repeat, using ninhydrin-pyridine spray. The papers can be entered in the notebook, but the initial colors should be recorded; application of label lacquer helps to retard fading.

7. Permanganate Spot Test

Spot a paper as in Section 6 with alanine, methionine, cystine, tyrosine, and proline (two applications); dry, and spray lightly with an aqueous solution containing 1% potassium permanganate and 2% sodium carbonate. Put a check mark beside any spots that at once appear yellow on a pink background, and without delay bleach the paper by holding it briefly under a watch glass covering a beaker (hood) in which sulfur dioxide is generated by occasional addition of sodium bisulfite and hydrochloric acid. Let the excess dioxide evaporate, spray with ninhydrin-collidine, and let dry.



s-Collidine

▼
Note for the instructor

---

[8] The solutions are made by dissolving 0.01 mole of a hydrochloride in 100 ml of water, or dissolving 0.01 mole of a free amino acid and 1 ml of concentrated hydrochloric acid in 100 ml of water (cystine and tyrosine require 4 ml of acid). In the case of the compounds of higher molecular weight the acid is required to effect solution; in other cases it is required to prevent growth of microorganisms.

Permanganate oxidizes the sulfur atom of methionine and cystine and the phenolic group of tyrosine, but the recovery test shows that original acid is still present. The nature of the yellow pigment is not known.[9]

8. Differentiation of Tyrosine from Sulfur Amino Acids

This test is based upon the fact that the phenolic tyrosine couples with diazotized amines, whereas methionine and cystine do not.

Dip a 10-cm strip of filter paper halfway into a 1% solution of 2,5-dichlorobenzenediazonium chloride[10] and hang it up to dry. Mark one-half of the wetted area T and the other M, and put two spots each of tyrosine and methionine in the appropriate areas. Then dip the strip into 1% aqueous sodium carbonate solution and hang the strip up and again let dry; record the initial result (note that both solutions contain hydrochloric acid) and the final appearance (5–10 min).

9. Protein Hydrolyzate

▼
One-hour reflux; 20-min workup; overnight chromatogram

In a 25 × 150-mm test tube clamped so that it can be heated over a free flame, place 100 mg of either hair, silk, or gelatin (cooking gelatin serves well), 3 ml of constant boiling (20%) hydrochloric acid,[11] and a boiling stone. Insert a cold finger condenser and let its side-tube rest on the rim of the test tube,[12] and reflux for 1 hr. Alternatively, reflux the solution in a $^{14}\!/_{20}$ 10-ml round-bottomed flask equipped with a $^{14}\!/_{20}$ condenser. Remove the condenser, introduce an aspirator tube, and boil the solution down to a volume of about 1 ml. Then rinse down the walls with 3 ml of acetone, add 3 ml of cyclohexane, and heat the tube in an open steam bath while evacuating at the full force of the aspirator to remove the hydrochloric acid and solvents (azeotropic distillation) and leave a film of amino acid hydrochlorides. Add 1 ml of water, warm briefly, and then cool.

Try to identify the major component acids of the mixture (see Table 31.1) by applying such of the tests (Sections 5–8) as seem appropriate and by running an overnight chromatogram (Section 2). In some instances it may be possible to identify a group of components of comparable Rf values but not to differentiate between the members of the group. Treatment of a small sample with phosphotungstic acid and chromatography of the filtrate may be helpful.

---

[9] Proline gives a weak positive response in the permanganate test if the spray is left on for ½–1 min but not if it is bleached at once as specified; proline is adequately identified by the yellow ninhydrin spot test.

[10] Du Pont Naphthanil Diazo Scarlet GG.

[11] Mix 2.5 ml of concentrated hydrochloric acid with 2 ml of water.

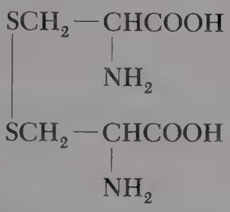
[12] The cooling tube close to the boiling liquid breaks up an otherwise troublesome foam.

10. Unknowns

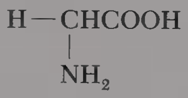
Test solutions (containing hydrochloric acid) of the following acids (0.1 M) serve as satisfactory unknowns:

Cystine
Glycine
Alanine
Leucine
Methionine
Proline
Lysine
Arginine
Tyrosine
Aspartic acid
Glutamic acid

A 0.1-ml sample (2 drops delivered by a medicine dropper into a specimen vial) should suffice for identification by a combination of spot tests (Sections 5–8) and chromatography, either on the material as supplied or after basification (Section 3). Estimate the weight of sample that you actually used for a successful identification.

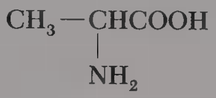


Cystine CyS
CyS



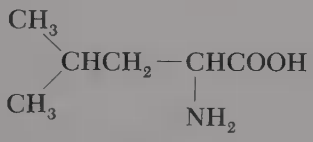
$\mathrm{H{-}\underset{\substack{|\\ NH_2}}{C}HCOOH}$

Glycine
Gly

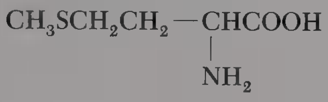


$\mathrm{CH_3{-}\underset{\substack{|\\ NH_2}}{C}HCOOH}$

Alanine
Ala

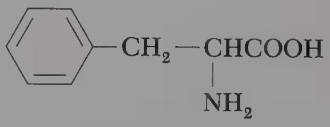


Leucine
Leu

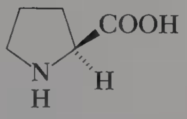


$\mathrm{CH_3SCH_2CH_2{-}\underset{\substack{|\\ NH_2}}{C}HCOOH}$

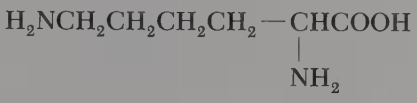
Methionine
Met



Phenylalanine
Phe

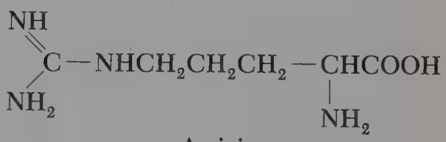


Proline
Pro

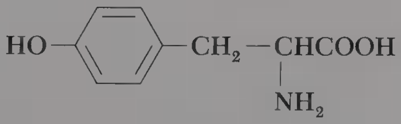


$\mathrm{H_2NCH_2CH_2CH_2CH_2{-}\underset{\substack{|\\ NH_2}}{C}HCOOH}$

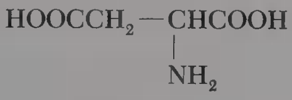
Lysine
Lys



Arginine
Arg

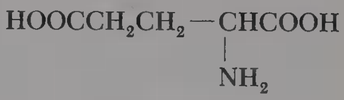


Tyrosine
Tyr



$\mathrm{HOOCCH_2{-}\underset{\substack{|\\ NH_2}}{C}HCOOH}$

Aspartic acid
Asp



$\mathrm{HOOCCH_2CH_2{-}\underset{\substack{|\\ NH_2}}{C}HCOOH}$

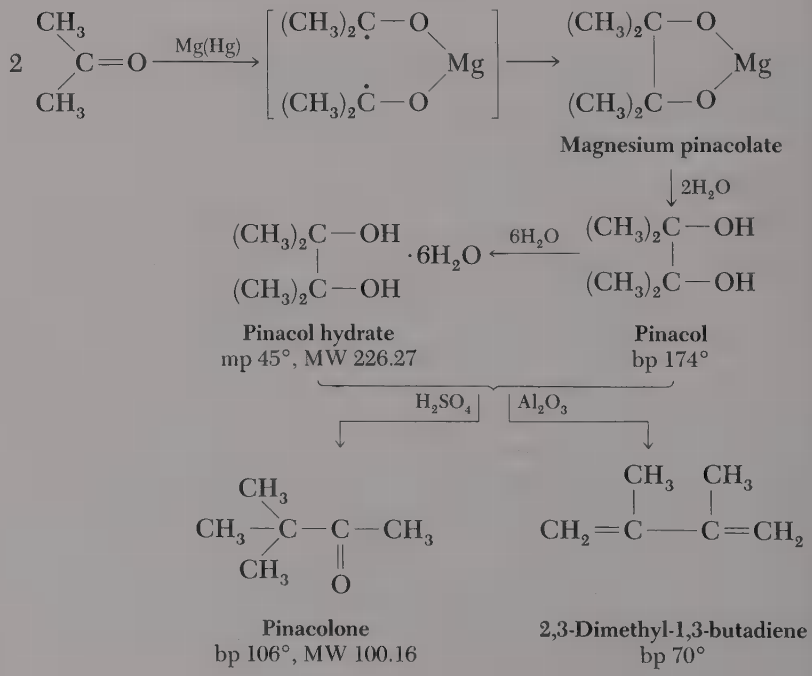
Glutamic acid
Glu

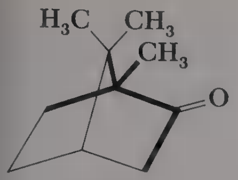
32

Pinacol and Pinacolone

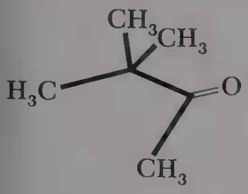
KEYWORDS

Bimolecular reduction
Diradical
Pinacol-pinacolone rearrangement
Oxime





Camphor



Pinacolone

Acetone on treatment with metallic reducing agents is converted mainly into the product of bimolecular reduction, pinacol. The diol is a liquid but can be isolated as the crystalline hexahydrate. In the following procedure dry acetone is treated under Grignard conditions (reagents and apparatus strictly anhydrous) with magnesium that has been amalgamated with 0.02 mole of mercury by reaction with mercury(II) chloride in the presence of a portion of the acetone: $HgCl_2 + Mg \longrightarrow Hg + MgCl_2$. Bimolecular reduction probably proceeds through a diradical and affords magnesium pinacolate as a voluminous precipitate, which on treatment with water affords pinacol and then pinacol hydrate. This crystalline solid is easily separated from unreacted acetone and isopropyl alcohol, the product of normal, unimolecular reduction.

Pinacol on catalytic dehydration over alumina undergoes normal dehydration to 2,3-dimethyl-1,3-butadiene, but on dehydration with sulfuric acid the ditertiary glycol largely undergoes rearrangement and affords pinacolone in 70% yield. Fully purified pinacolone, as well as its oxime, has a fine camphor-like odor, and a similarity in structure is evident from the formulas.

You are to prepare and purify pinacol as the hydrate, and convert it into pinacolone, which is to be characterized as the crystalline oxime. As a special experiment, pinacolone can be further converted by the haloform reaction into trimethylacetic acid.

EXPERIMENTS

1. Pinacol Hydrate

▼

Caution! Benzene is toxic and a mild carcinogen. Perform this experiment in a hood. Avoid contact with benzene liquid or vapor.

In a 500-ml round-bottomed flask equipped with a long reflux condenser, place 8 g of magnesium turnings and 100 ml of dry benzene. The apparatus should be thoroughly dry, as in a Grignard reaction. A solution of 9 g of mercury(II) chloride in 75 ml of dry acetone is placed in a small dropping funnel fitted into the top of the condenser by means of a grooved cork. Add about one fourth of this solution, and if the reaction does not start in a few minutes (vigorous ebullition) warm the mixture gently on the steam bath, but be prepared to plunge the flask into cold water if moderation of the reaction becomes necessary. Once started, the reaction will proceed vigorously at this stage without further heating. Run in the remainder of the acetone solution at such a rate as to keep the reaction in progress. The boiling should be vigorous, but some cooling may be necessary in order to prevent escape of uncondensed acetone. When the reaction slows down (5–10 min), mount the flask on the steam bath with a single clamp placed as high as possible on a ring stand to keep the condenser upright. If the jaws of the clamp fit only loosely on the condenser, the flask can be grasped and swirled vigorously to dislodge the magnesium pinacolate that separates. Keep the mixture boiling briskly and swirl frequently for one hour.

▼

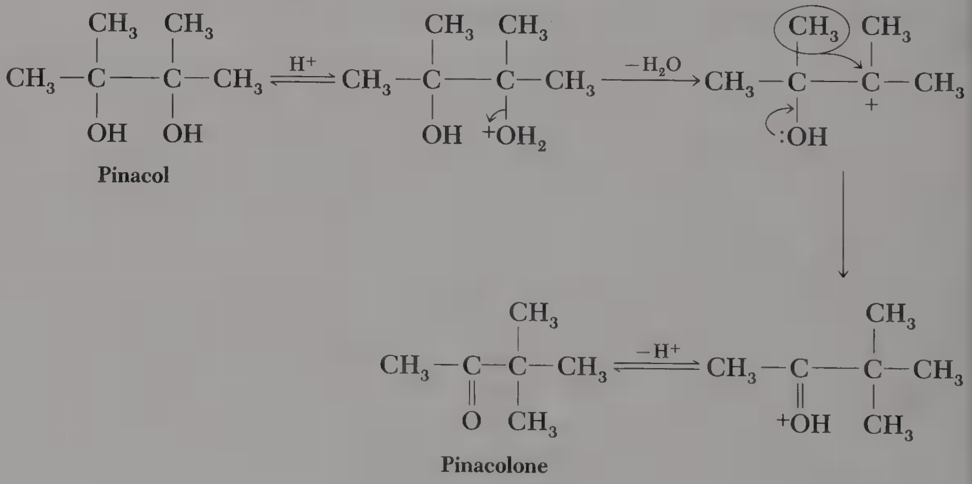
Extinguish flames

Then add 20 ml of water through the condenser and boil the mixture for 0.5 hr with frequent shaking. Water converts the pinacolate into pinacol (soluble in benzene) and a precipitate of magnesium hydroxide. Filter the hot solution by suction, return the solid magnesium hydroxide to the flask and reflux it with 50 ml of ordinary benzene for 5–10 min, and filter this solution as before. Pour the combined filtrates into an Erlenmeyer flask and evaporate the solution on a steam bath under an aspirator tube to one third the original volume. Then add 15 ml of water, cool well in an ice bath, and scratch the walls of the flask with a stirring rod. Pinacol hydrate separates as an oil which soon solidifies; it should be collected only after thorough cooling and stirring for maximum crystallization. Scrape the crude material onto a suction funnel, wash it with a little cold benzene, press it well with a spatula, and let it drain for about 5 min in order to remove as much benzene as possible.

The crude material is purified by crystallization from water, in which it is very soluble. Dissolve the product in 25 ml of water in a small Erlenmeyer flask, and boil the solution gently for about 5 min in order to remove traces of benzene. If the solution is appreciably colored it should be clarified with decolorizing charcoal. Filter the hot solution through a rather large funnel, which has been warmed on the steam bath, into a second flask and set it aside to crystallize. Cool in ice before collecting the large crystals of pinacol hydrate. The purified product should be dried in a cool place since it sublimes easily. The yield is 18–20 g; mp 46–47°. Calculate the theoretical yield from the amount of magnesium employed, for acetone is taken in considerable excess.

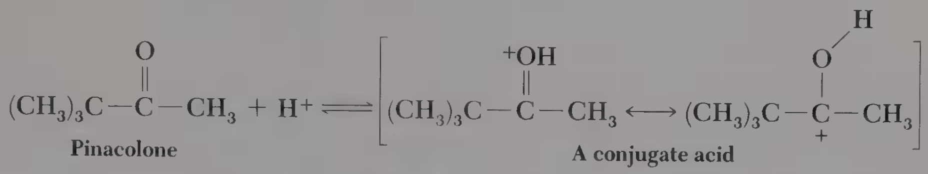
▼
Pinacol rearrangement

2. Pinacolone



Into a 250-ml round-bottomed flask pour 80 ml of water, then 20 ml of concentrated sulfuric acid, and dissolve 20 g of pinacol hydrate in a warm solution. Attach a reflux condenser, boil the mixture for 15 min, observe, and note carefully the changes that take place. Cool until the boiling ceases, attach a stillhead with a steam-inlet tube, and steam distil (Chapter 5) until no more pinacolone comes over. Separate the upper layer of crude pinacolone, dry it with calcium chloride, and purify it by fractional distillation. Use a fractionating column and tetrachloroethane as chaser and see if you can detect and remove a small forerun containing dimethylbutadiene.

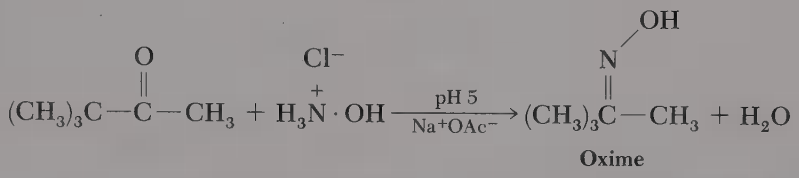
3. Pinacolone Oxime



$$(CH_3)_3C-\overset{\overset{O}{\|}}{C}-CH_3 + H^+ \rightleftharpoons \left[(CH_3)_3C-\overset{\overset{+OH}{\|}}{C}-CH_3 \longleftrightarrow (CH_3)_3C-\underset{+}{\overset{\overset{O-H}{|}}{C}}-CH_3\right] \quad (1)$$

$$H_2N-OH + H^+ \rightleftharpoons H_3\overset{+}{N}-OH \quad (2)$$

Hydroxylamine A conjugate acid



$$(CH_3)_3C-\overset{\overset{O}{\|}}{C}-CH_3 + \overset{Cl^-}{H_3\overset{+}{N}}\cdot OH \xrightarrow[Na^+OAc^-]{pH\ 5} (CH_3)_3\overset{\overset{\overset{OH}{N}}{\|}}{C}-CH_3 + H_2O \quad (3)$$

Pinacolone (eq. 1) becomes *more* electrophilic on protonation and on protonation hydroxylamine (eq. 2) becomes *less* nucleophilic, as conjugate acids. A compromise is struck in the formation of the oxime (eq. 3) by adding sodium acetate to buffer the solution at pH 5 where pinacolone is converted to the electrophilic conjugate acid and yet there is a sufficient concentration of the nonprotonated nucleophile hydroxylamine, to allow the reaction to proceed.

Measure into a 25-ml test tube provided with a cold finger condenser 1 ml of pinacolone and 3 ml each of 5 *M* hydroxylamine hydrochloride solution and 5 *M* sodium acetate solution, and add 5 ml of 95% ethanol in order to bring the oil completely into solution. Add a boiling chip and reflux the solution gently on the steam bath for 2 hr, at which time there should be sufficient oxime for identification. The oxime usually separates as an oily layer; on very thorough cooling and by rubbing the walls of the test tube with a stirring rod the material can be caused to solidify. Collect the product on a suction funnel, dry a portion on a filter paper, and determine the melting point. Pure pinacolone oxime melts at 77–78°. It is soluble in cold dilute hydrochloric acid, and the pleasant odor becomes particularly apparent on boiling the solution. The oxime evaporates (sublimes) rapidly, even at room temperature, and should not be left exposed to the air for more than a few hours.

QUESTION

1. Oximes can exhibit geometric isomerism; e.g., in pinacolone oxime the hydroxyl groups can be *cis* or *trans* to the *t*-butyl group. How can you demonstrate that your product is one isomer and not a mixture?

33

Succinic Anhydride

KEYWORDS

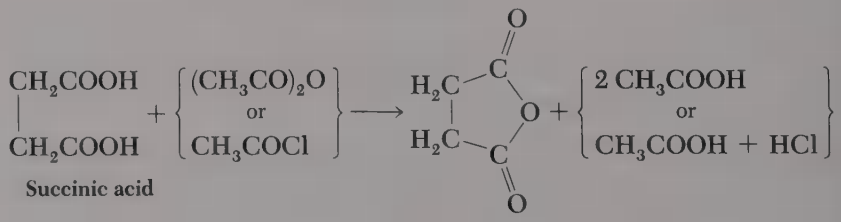
Dehydration of a diacid
Alcoholysis
Ammonolysis
γ-Lactone
Succinanilic acid
Succinanil

Acetic anhydride can be prepared by the interaction of sodium acetate and acetyl chloride or by the addition of acetic acid to ketene:

$$CH_3COONa + CH_3COCl \longrightarrow (CH_3CO)_2O + NaCl$$

$$CH_2{=}C{=}O + CH_3COOH \longrightarrow (CH_3CO)_2O$$

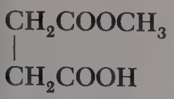
The anhydride of an acid also can be prepared by treatment of the acid with a dehydrating agent, and in the case of a dibasic acid of the type of succinic acid this direct method is the only one applicable. This particular dehydration can be accomplished by use of either acetic anhydride or acetyl chloride:



Succinic acid

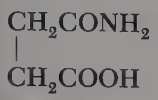
Succinic anhydride
mp 120°
MW 100.07

The cyclic anhydride formed is not contaminated with inorganic reagents and the acetic acid produced serves as a solvent for crystallization. The procedure may be used for preparation of the anhydrides of glutaric, maleic, and phthalic acid.



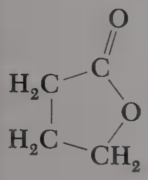
$$\begin{array}{l} CH_2COOCH_3 \\ | \\ CH_2COOH \end{array}$$

(1)



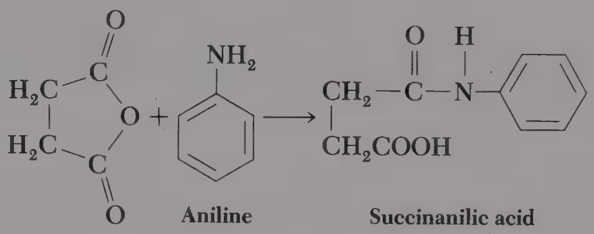
$$\begin{array}{l} CH_2CONH_2 \\ | \\ CH_2COOH \end{array}$$

(2)



(3)

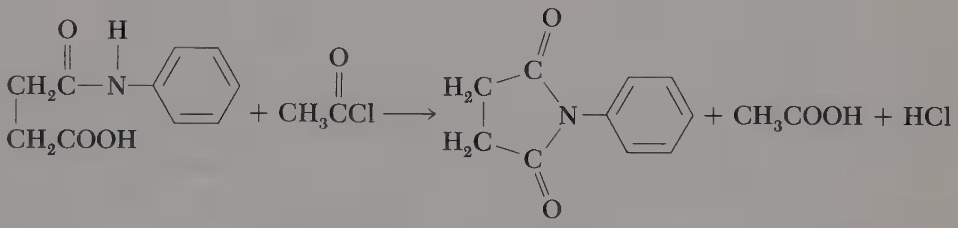
Succinic anhydride is useful as a precursor to derivatives not available directly from the acid. On alcoholysis succinic anhydride yields the monomethyl ester (**1**); the acid amide (**2**) is formed on ammonolysis of the anhydride; reduction gives butyrolactone (3), a typical γ-lactone. The reaction of the anhydride with aniline can be used for the identification of this primary amine,



Aniline

Succinanilic acid
Mp 150°

since the product is a crystalline substance of sharp melting point. On treatment with acetyl chloride, succinanilic acid is cyclized to succinanil.



Acetyl chloride

Succinanil
Mp 154°

EXPERIMENTS

1. Succinic Anhydride

▾
Reaction time 15 min

In a 125-ml round-bottomed flask fitted with a reflux condenser closed with a calcium chloride tube, place 15 g of succinic acid and 20 ml of acetic anhydride. Heat to the boiling point, noting that the crystals soon dissolve, and reflux gently for 15 min. Let the solution cool for a time undisturbed and observe the crystallization. Finally, cool in ice, collect the crystals on a dry suction funnel, and use several small portions of ether to rinse the reaction flask and wash the crystalline anhydride. The yield is 10–11 g; mp 119–120°. Test the product with cold sodium bicarbonate solution for the presence of unchanged acid.

2. Succinanilic Acid

Dissolve 1 g of succinic anhydride in 30 ml of toluene on the steam bath and to the boiling solution add all at once a solution of 0.9 ml of aniline in about 5 ml of toluene. The separation of the reaction product occurs in a striking manner. Cool the mixture, collect the crystalline product, and wash it with toluene. Remove the last traces of solvent under vacuum. Determine the yield and melting point and note the action on a few mgs of the substance of cold sodium bicarbonate solution. Pure succinanilic acid melts at 150°.

The reaction of succinic anhydride with a primary amine can be carried out rapidly and with very small amounts of material. Dissolve a few small crystals of succinic anhydride in 1 ml of hot toluene in a 10 × 75-mm test tube, add one small crystal of *p*-toluidine (another primary amine), and observe the result.

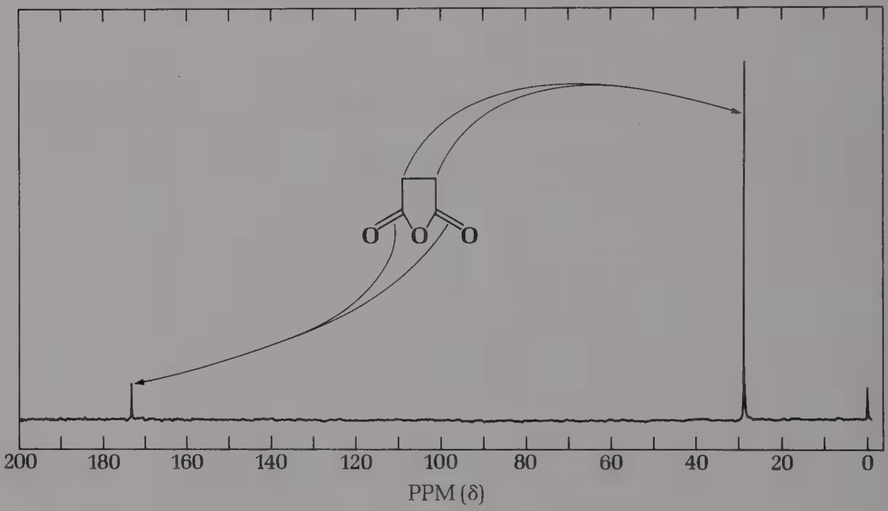


FIGURE 33.1 ^{13}C nmr spectrum of succinic anhydride.

3. Succinanil

Convert the remainder of the succinanilic acid to the cyclized form, succinanil (apparatus as in Section 1). Cover the succinanilic acid (about 1.8 g) with 5 ml of acetyl chloride and reflux until the reaction is complete (5 min). Allow crystallization to take place, collect the product, and wash it with ether as before. Test the solubility in bicarbonate solution. The pure material melts at 154°.

QUESTIONS

1. How could succinic acid be converted into β-aminopropionic acid?
2. Devise a method for the resolution of a *dl*-alcohol, based on the assumption that an optically active derivative of aniline is available, and noting that the succinanilic acid, as other acids, can be esterified without difficulty.

34

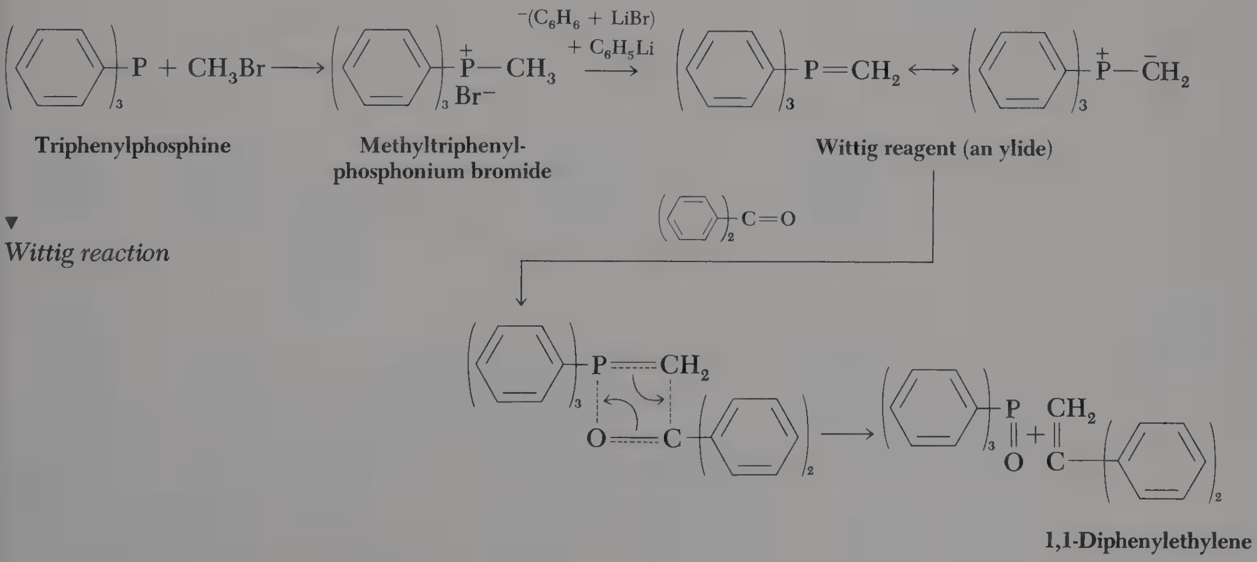
Wittig-Horner Reaction

KEYWORDS

Olefin synthesis
Wittig reaction
Wittig-Horner reaction
Ylide
Phosphonium salt
Phosphonate
Dimethylformamide, DMF

The Wittig reaction affords an invaluable method for the conversion of a carbonyl compound to an olefin, for example the conversion of benzophenone to 1,1-diphenylethylene:

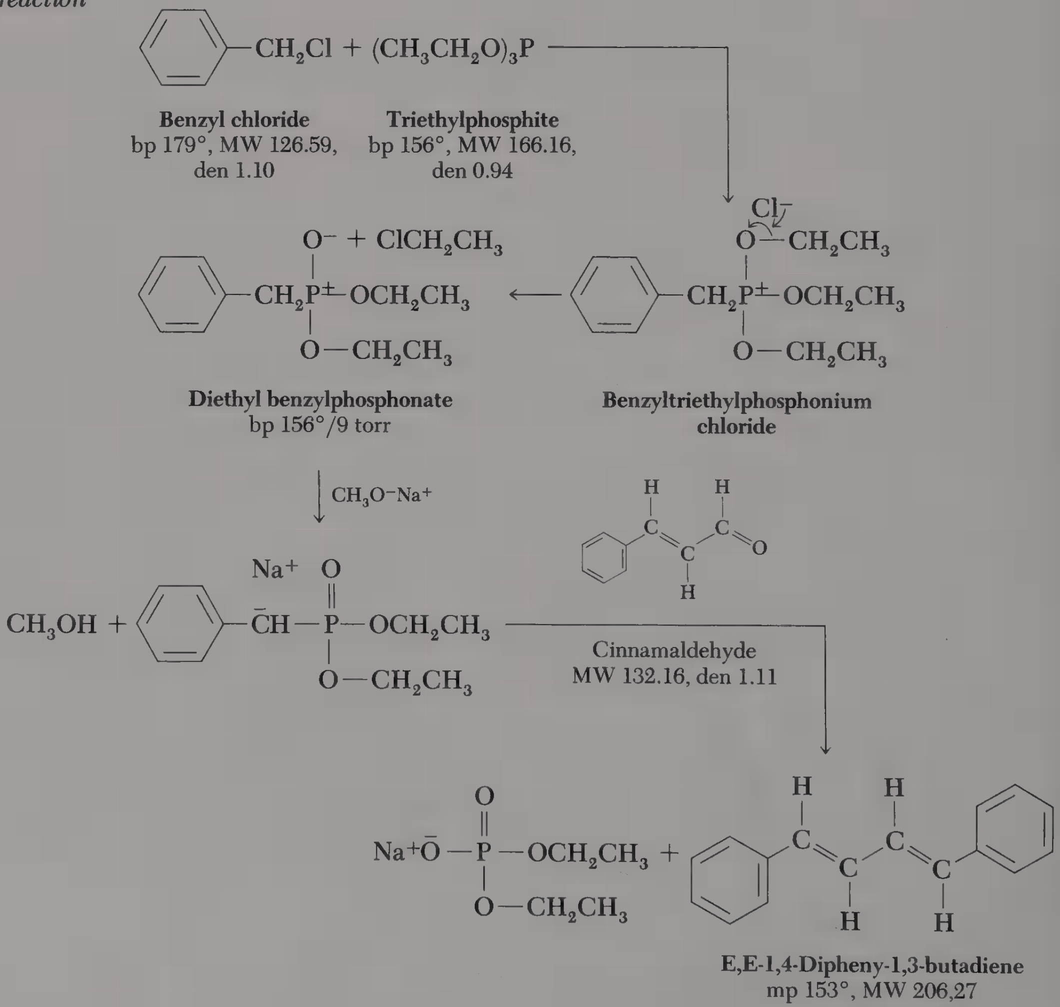
▼
Wittig reaction



Since the active reagent, an ylide, is unstable, it is generated in the presence of the carbonyl compound to be used by dehydrohalogenation of an alkyltriphenylphosphonium bromide with phenyllithium in dry ether in a nitrogen atmosphere.

When the halogen compound employed in the first step has an activated halogen atom ($RCH{=}CHCH_2X$, $C_6H_5CH_2X$, $XCH_2CO_2C_2H_5$) a simpler procedure known as the Horner phosphonate modification of the Wittig reaction is applicable. When benzyl chloride is heated with triethyl phosphite, ethyl chloride is eliminated from the initially formed phosphonium chloride with the production of diethyl benzylphosphonate. This phosphonate is stable, but in the presence of a strong base it condenses with a carbonyl component in the same way that a Wittig ylide condenses.

▼
Simplified Wittig reaction



Caution: Take care to keep organophosphorus compounds off the skin.

Thus, it reacts with benzaldehyde to give *E*-stilbene and with cinnamaldehyde to give *E*,*E*-1,4-diphenyl-1,3-butadiene.

EXPERIMENT

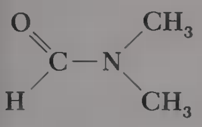
▼

Time for first step: 1 hr

With the aid of pipettes and a pipetter, measure into a 25 × 150 mm test tube 5 ml of benzyl chloride (α-chlorotoluene) and 7.7 ml of triethyl phosphite. Add a boiling stone, insert a cold finger condenser, and with a small flame reflux the liquid gently for 1 hr. Alternatively, carry out the reaction in a 25-ml round-bottomed flask equipped with a reflux condenser. (Elimination of ethyl chloride starts at about 130°, and in the time period specified the temperature of the liquid rises to 190–200°.) Let the phosphonate ester cool to room temperature, pour it into a 125-ml Erlenmeyer flask containing 2.4 g of sodium methoxide, and add 40 ml of dimethylformamide, using a part of this solvent to rinse the test tube. Swirl the flask vigorously in a water-ice bath to thoroughly chill the contents and continue swirling while running in 5 ml of cinnamaldehyde by pipette. The mixture soon turns deep red and then crystalline hydrocarbon starts to separate. When there is no further change (about 2 min) remove the flask from the cooling bath and let it stand at room temperature for about 10 min. Then add 20 ml of water and 10 ml of methanol, swirl vigorously to dislodge crystals, and finally collect the product on a suction funnel using the red mother liquor to wash the flask. Wash the product with water until the red color of the product is all replaced by yellow. Then wash with methanol to remove the yellow impurity, and continue until the wash liquor is colorless. The yield of the crude faintly yellow hydrocarbon, mp 150–151°, is 5.7 g. This material is satisfactory for use in the next experiment (1.5 g required). A good solvent for crystallization of the remainder of the product is methylcyclohexane (bp 101°, 10 ml per g; use more if the solution requires filtration). Pure *E*,*E*-1,4-diphenyl-1,3-butadiene melts at 153°.

▼

Use freshly opened sodium methoxide



Dimethylformamide
MW 73.10, bp 153°

A highly polar solvent capable of dissolving ionic compounds such as sodium methoxide yet miscible with water.

35

p-Terphenyl by the Diels-Alder Reaction

KEYWORDS

Diels-Alder reaction
Isomerization
Hydrolysis
Decarboxylation
Dimethyl acetylenedicarboxylate
Lachrymator, vesicant

Diels-Alder reaction

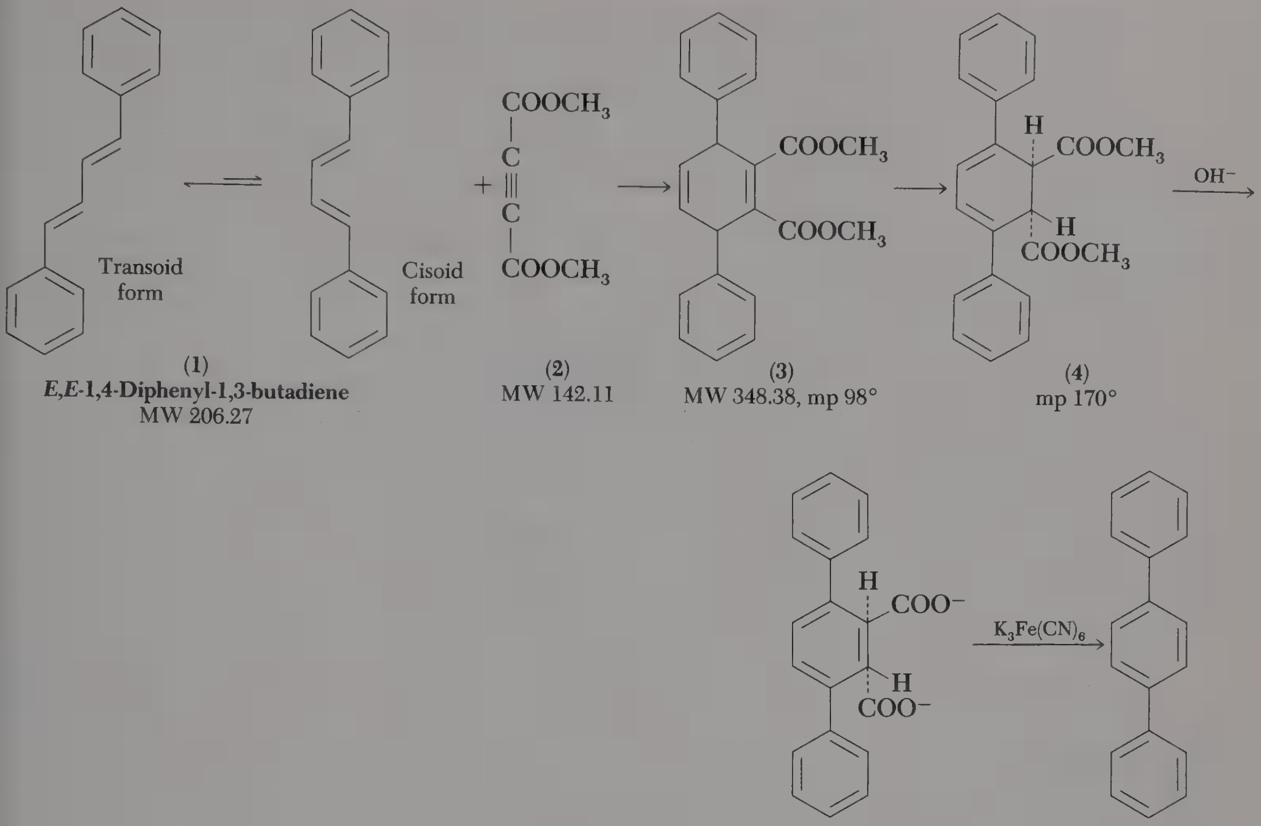
E,E-1,4-Diphenyl-1,3-butadiene (**1**) is most stable in the transoid form, but at a suitably elevated temperature the cisoid form present in the equilibrium adds to dimethyl acetylenedicarboxylate (**2**) to give dimethyl 1,4-diphenyl-1,4-dihydrophthalate (**3**). This low-melting ester is obtained as an oil and when warmed briefly with methanolic potassium hydroxide is isomerized to the high-melting *E*-ester (**4**). The free *E*-acid can be obtained in 86% yield by refluxing the suspension of (**3**) in methanol for 4 hr, but in the recommended procedure the isomerized ester is collected, washed to remove dark mother liquor, and hydrolyzed by brief heating with potassium hydroxide in a high-boiling solvent. The final step, an oxidative decarboxylation, is rapid and nearly quantitative. It probably involves reaction of the oxidant with the dianion (**5**) with removal of two electrons and formation of a diradical, which loses carbon dioxide with formation of *p*-terphenyl (**6**).

EXPERIMENT

Place 1.5 g of *E,E*-1,4-diphenyl-1,3-butadiene and 1.0 ml (1.1 g) of dimethyl acetylenedicarboxylate (*caution, skin irritant*[1]) in a 25 × 150 mm test tube

---

[1] This ester is a powerful lachrymator (tear producer) and vesicant (blistering agent) and should be dispensed from a bottle provided with a pipet and a pipetter. Even a trace of ester on the skin should be washed off with methanol, followed by soap and water.



(1)
E,*E*-1,4-Diphenyl-1,3-butadiene
MW 206.27

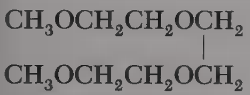
(2)
MW 142.11

(3)
MW 348.38, mp 98°

(4)
mp 170°

(5)

(6)
p-Terphenyl
MW 230.29, mp 211°



$$\begin{array}{l}CH_3OCH_2CH_2OCH_2\\ \qquad\qquad\qquad\quad |\\ CH_3OCH_2CH_2OCH_2\end{array}$$

Triethylene glycol dimethyl ether (triglyme). Miscible with water. bp 222°

▼
Reaction time: 30 min

and rinse down the walls with 5 ml of triethylene glycol dimethyl ether (triglyme) (bp 222°). Clamp the test tube in a vertical position, introduce a cold finger condenser, and reflux the mixture gently for 30 min. Alternatively, carry out the experiment in a 25-ml round-bottomed flask equipped with a reflux condenser. Cool the yellowish solution under the tap, pour into a separatory funnel, and rinse out the reaction vessel with a total of about 50 ml of ether. Extract twice with water (50–75 ml portions) to remove the high-boiling solvent, shake the ethereal solution with saturated sodium chloride solution, and dry the ether layer over anhydrous sodium sulfate. Filter or decant the ether solution into a tared 125-ml Erlenmeyer flask and evaporate the ether on the steam bath, eventually with evacuation at the aspirator, until the weight of yellow oil is constant; yield 2.5–2.8 g.

While evaporation is in progress, dissolve 0.5 g of potassium hydroxide (about 5 pellets) in 10 ml of methanol by heating and swirling; the process is greatly hastened by crushing the lumps with a stirring rod with a flattened head. Crystallization of the yellow oil containing (3) can be initiated by cooling and scratching; this provides assurance that the reaction has proceeded properly. Pour in the methanolic potassium hydroxide and heat with swirling on the hot plate for about 1 min until a stiff paste of crystals of the isomerized ester (4) appears. Cool, thin the mixture with methanol, collect the product and wash it free of dark mother liquor, and spread it thinly on a paper for rapid drying. The yield of pure, white ester (4) is 1.7–1.8 g. Solutions in methanol are strongly fluorescent.

▼
Rapid hydrolysis of the hindered ester (4)

Place the ester (4) in a 25 × 150 mm test tube, add 0.7 g of potassium hydroxide (7–8 pellets), and pour in 5 ml of triethylene glycol. Stir the mixture with a thermometer and heat, raising the temperature to 140° in the course of about 5 min. By intermittent heating, keep the temperature close to 140° for 5 min longer and then cool the mixture under the tap. Pour into a 125-ml Erlenmeyer flask and rinse the tube with about 50 ml of water. Heat to boiling and, in case there is a small precipitate or the solution is cloudy, add a little decolorizing charcoal, swirl, and filter the alkaline solution by gravity. Then add 3.4 g of potassium ferricyanide and heat on the hot plate with swirling for about 5 min to dissolve the oxidant and to coagulate the white precipitate which soon separates. The product can be air-dried overnight or dried to constant weight by heating in an evacuated Erlenmeyer flask on the steam bath. The yield of colorless *p*-terphenyl, mp 209–210°, is 0.7–0.8 g. The *p*-terphenyl crystallizes well from dioxane; however, dioxane is a carcinogen and must be handled with great care.

QUESTIONS

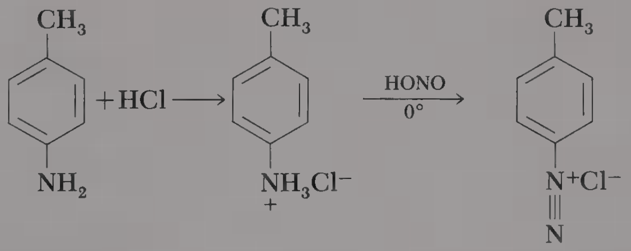
1. What is the driving force for the isomerization of 3 to 4?
2. Why is the *trans* and not the *cis* diester 4 formed in the isomerization of 3 to 4?
3. Why does hydrolysis of 4 in methanol require 4 hours whereas hydrolysis in triethylene glycol requires only 10 minutes?

36

p-Chlorotoluene by the Sandmeyer Reaction

KEYWORDS

Sandmeyer reaction
Cuprous chloride
Nitrous acid
Diazonium salt
Toluidine



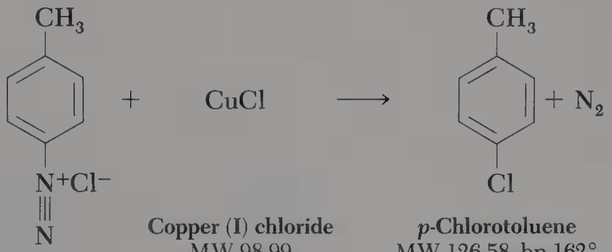
p-Toluidine MW 107.15, mp 45°

p-Toluidine hydrochloride

p-Methylbenzenediazonium chloride

$$NaNO_2 + HCl \xrightarrow{0°} HONO + NaCl$$

Sodium nitrite MW 69.01



Copper (I) chloride MW 98.99

p-Chlorotoluene MW 126.58, bp 162°

The Sandmeyer reaction is a versatile means of replacing an aromatic amine group with a chlorine or bromine atom, through reaction of the copper(I) halide with a diazonium salt.

p-Toluidine is dissolved in the required amount of hydrochloric acid, two more equivalents of acid are added, and the mixture cooled in ice to produce a paste of the crystalline amine hydrochloride. When this salt is treated at 0–5° with one equivalent of sodium nitrite, nitrous acid is liberated and reacts to produce the diazonium salt. The excess hydrochloric acid beyond the two equivalents required to form the amine hydrochloride and react with sodium nitrite maintains acidity sufficient to prevent formation of the diazoamino compound and rearrangement of the diazonium salt.

Copper(I) chloride is made by reduction of copper(II) sulfate with sodium sulfite (which is produced as required from the cheaper sodium bisulfite). The white solid is left covered with the reducing solution for protection against air oxidation until it is to be used and then dissolved in hydrochloric acid. On addition of the diazonium salt solution a complex forms and rapidly decomposes to give *p*-chlorotoluene and nitrogen. The mixture is very discolored, but steam distillation leaves most of the impurities and all salts behind and gives material substantially pure except for the presence of a trace of yellow pigment which can be eliminated by distillation of the dried oil.

EXPERIMENTS

1. Copper(I) Chloride Solution

$$2\ CuSO_4 \cdot 5H_2O + 4\ NaCl + NaHSO_3 + NaOH \longrightarrow 2CuCl + 3\ Na_2SO_4 + 2\ HCl + 10\ H_2O$$

MW 249.71 MW 58.45 MW 104.97 MW 40.01 MW 99.02

▼
$NaHSO_3$, *not* $Na_2S_2O_2$

▼
One may stop here

In a 500-ml round-bottomed flask (to be used later for steam distillation) dissolve 30 g of copper(II) sulfate crystals ($CuSO_4 \cdot 5H_2O$) in 100 ml of water by boiling and then add 10 g of sodium chloride, which may give a small precipitate of basic copper(II) chloride. Prepare a solution of sodium sulfite from 7 g of sodium bisulfite, 4.5 g of sodium hydroxide, and 50 ml of water and add this, not too rapidly, to the hot copper(II) sulfate solution (rinse flask and neck). Shake well and put the flask in a pan of cold water in a slanting position favorable for decantation and let the mixture stand to cool and settle during the diazotization. When you are ready to use the copper(I) chloride, decant the supernatant liquid, wash the white solid once with water by decantation, and dissolve the solid in 45 ml of concentrated hydrochloric acid. The solution is susceptible to air oxidation and should not stand for an appreciable time before use.

2. Diazotization

Put 11.0 g of *p*-toluidine and 15 ml of water in a 125-ml Erlenmeyer flask. Measure 25 ml of concentrated hydrochloric acid and add 10 ml of it to the flask. Heat over a free flame and swirl to dissolve the amine and hence ensure that it is all converted into the hydrochloride. Add the remaining acid and cool thoroughly in an ice bath and let the flask stand in the bath while preparing a solution of 7 g of sodium nitrite in 20 ml of water. To maintain a temperature of 0–5° during diazotization add a few pieces of ice to the amine hydrochloride suspension and add more later as the first ones melt. Pour in the nitrite solution in portions during 5 min with swirling in the ice bath. The solid should dissolve to a clear solution of the diazonium salt. After 3–4 min test for excess nitrous acid: dip a stirring rod in the solution, touch off the drop on the wall of the flask, put the rod in a small test tube, and add a few drops of water. Then insert a strip of starch-iodide paper; an instantaneous deep blue color due to a starch-iodine complex indicates the presence of nitrous acid. (The sample tested is diluted with water because strong hydrochloric acid alone produces the same color on starch-iodide paper, after a slight induction period.) Leave the solution in the ice bath.

3. Sandmeyer Reaction

Complete the preparation of copper(I) chloride solution, cool it in the ice bath, pour in the solution of diazonium chloride through a long-stemmed funnel, and rinse the flask. Swirl occasionally at room temperature for 10 min and observe initial separation of a complex of the two components and its decomposition with liberation of nitrogen and separation of an oil. Arrange for steam distillation (Fig. 5.2) or generate steam *in situ* by simply boiling the contents of the flask over a flame (add more water during the distillation). Do not start the distillation until bubbling in the mixture has practically ceased and an oily layer has separated. Then steam distil and note that *p*-chlorotoluene, although lighter than the solution of inorganic salts in which it was produced, is heavier (den 1.07) than water. Extract the distillate with a little ether, wash the extract with 10% sodium hydroxide solution to remove any *p*-cresol present, then wash with saturated sodium chloride solution; dry the ether solution over about 5 g of anhydrous sodium sulfate and filter or decant it into a tared flask, evaporate the ether, and determine the yield and percentage yield of product (your yield should be about 9 g). Pure *p*-chlorotoluene is obtained by simple distillation of this crude product.

▼
Caution! Use aspirator tube

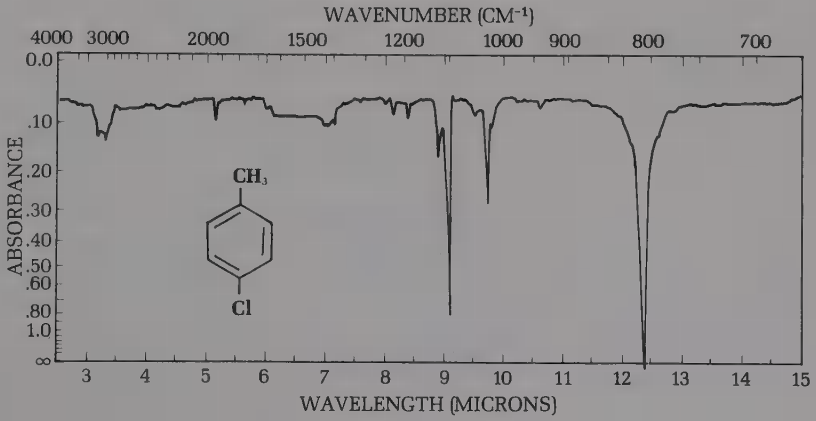


FIGURE 36.1 Infrared spectrum of *p*-chlorotoluene in CS_2.

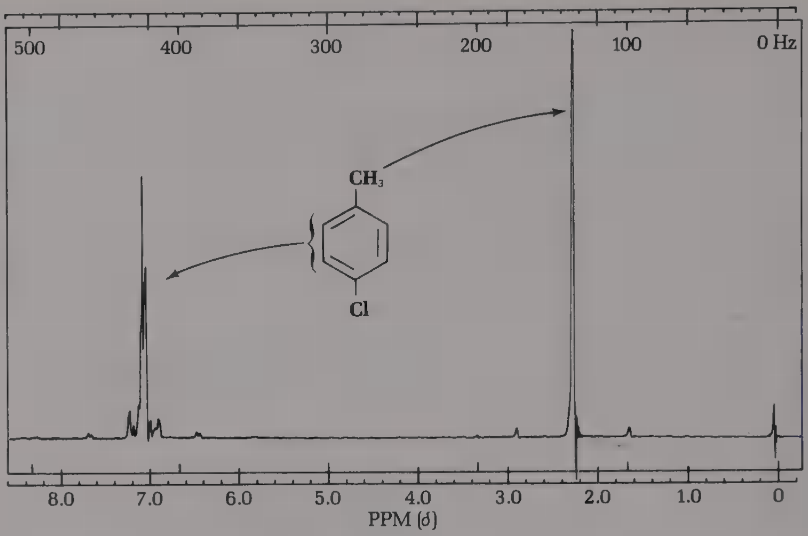


FIGURE 36.2 ^{1}H nmr spectrum of *p*-chlorotoluene.

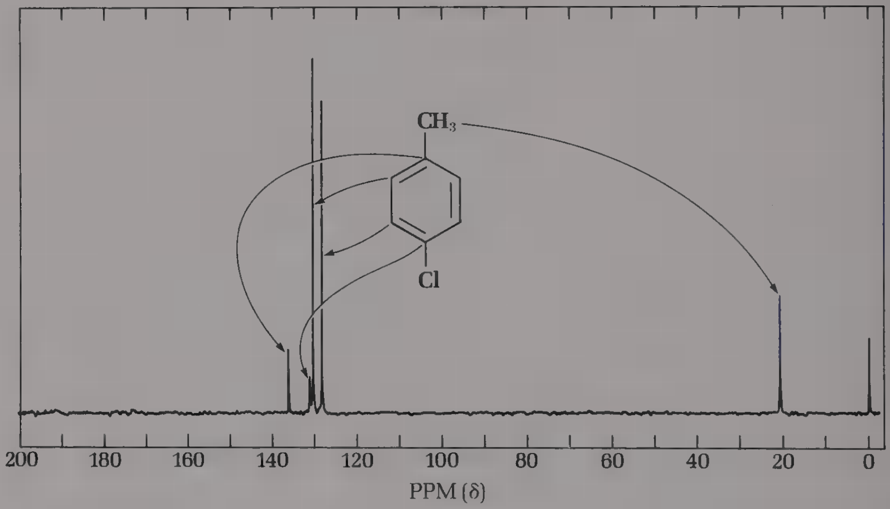


FIGURE 36.3 ^{13}C nmr spectrum of *p*-chlorotoluene.

QUESTIONS

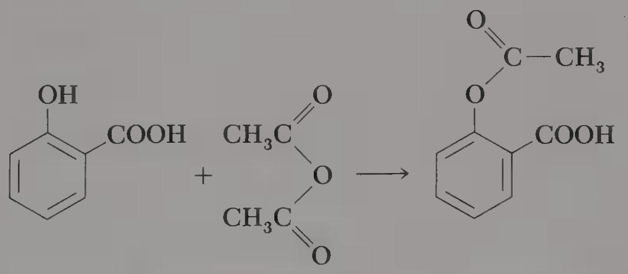
1. Nitric acid is generated by the action of sulfuric acid on sodium nitrate. Nitrous acid is prepared by the action of hydrochloric acid on sodium nitrite. Why is nitrous acid prepared *in situ*, rather than obtained from the reagent shelf?
2. What by-product would be obtained in high yield if the diazotization of *p*-toluidine were carried out at 30° instead of 0–5°?

37

Acetylsalicylic Acid (Aspirin)

KEYWORDS

Salicylic acid
Acetylation catalysts
Sodium acetate, pyridine, boron trifluoride etherate, H_2SO_4
Commercial aspirin



Salicyclic acid MW 138.12, mp 159° **Acetic anhydride** MW 102.09, bp 140° **Acetylsalicylic acid** MW 180.15, mp 128–137°

This experiment demonstrates the effect of four acetylation catalysts: two bases, sodium acetate and pyridine; a Lewis acid, boron trifluoride; and a mineral acid, sulfuric acid.

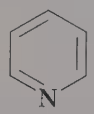
EXPERIMENT

Place 1 g of salicylic acid in each of four 13 × 100-mm test tubes and add to each tube 2 ml of acetic anhydride. To the first tube add 0.2 g of anhydrous sodium acetate, note the time, stir with a thermometer, and record the time required for a 4° rise in temperature and the estimated proportion of solid that has dissolved. Replace the thermometer and continue to stir occasionally

Acetylation Catalysts

(1) $CH_3COO^-Na^+$

(2)



(3) $BF_3 \cdot (C_2H_5)_2O$

(4) H_2SO_4

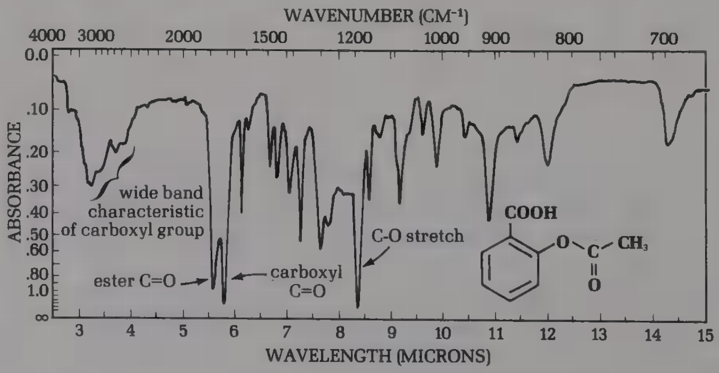
while starting the next acetylation. Obtain a clean thermometer, put it in the second tube, add 5 drops of pyridine, observe as before, and compare with the first results. To the third and fourth tubes add 5 drops of boron trifluoride etherate[1] and 5 drops of concentrated sulfuric acid, respectively. What is the order of activity of the four catalysts as judged by the rates of the reactions?

Put all tubes in hot water (beaker) for 5 min to dissolve solid material and complete the reactions, and then pour all the solutions into a 125-ml Erlenmeyer flask containing 50 ml of water and rinse the tubes with water. Swirl to aid hydrolysis of excess acetic anhydride and then cool thoroughly in ice, scratch the side of the flask with a stirring rod to induce crystallization, and collect the crystalline solid; yield 4 g.

Acetylsalicylic acid melts with decomposition at temperatures reported from 128 to 137°. It can be crystallized by dissolving it in ether, adding an equal volume of petroleum ether, and letting the solution stand undisturbed in an ice bath.

Test the solubility of your sample in toluene and in hot water and note the peculiar character of the aqueous solution when it is cooled and when it is then rubbed against the tube with a stirring rod. Note also that the substance dissolves in cold sodium bicarbonate solution and is precipitated by addition of an acid. Compare a tablet of commerical aspirin with your sample. Test the solubility of the tablet in water and in toluene and observe if it dissolves completely. Compare its behavior when heated in a melting point capillary with the behavior of your sample. If an impurity is found present, it is probably some substance used as binder for the tablets. Is it organic or inorganic? What harmless, edible type of substance do you suppose it is, judging from the various properties? Test with a drop of iodine-potassium iodide solution.

FIGURE 37.1 Infrared spectrum of acetylsalicylic acid (aspirin) in $CHCl_3$.



▼
Note for the instructor

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[1] Commercial reagent if dark should be redistilled (bp 126°, water-white).

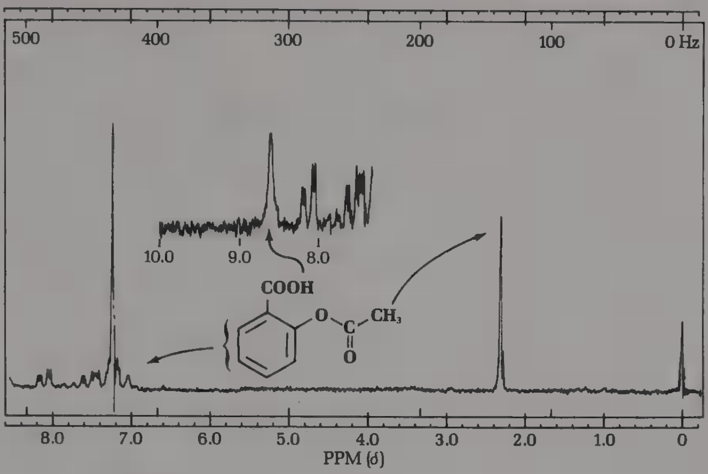


FIGURE 37.2 ^1H nmr spectrum of acetylsalicylic acid (aspirin).

38

Derivatives of 1,2-Diphenylethane — A Multistep Synthesis[1]

KEYWORDS

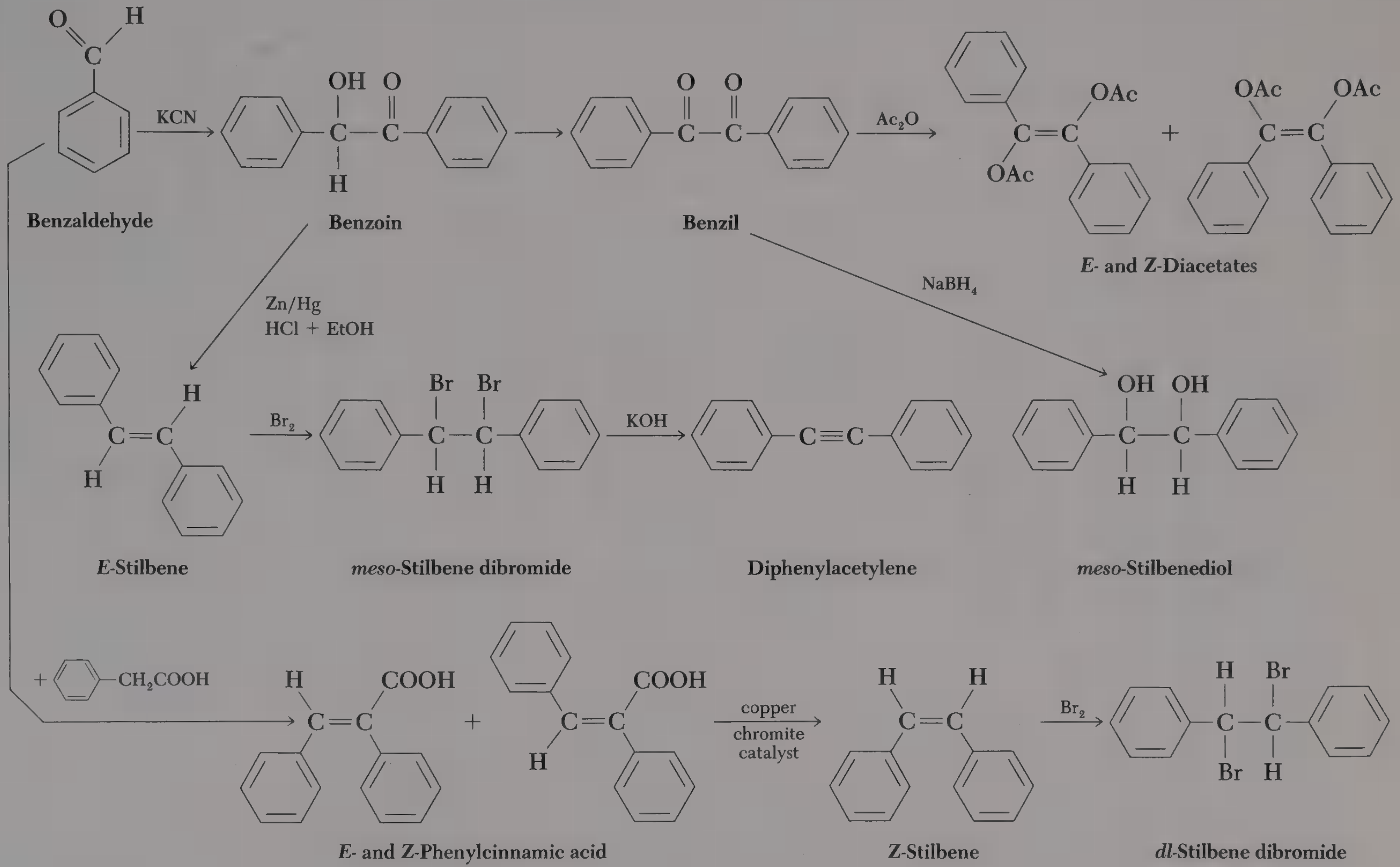
Benzoin condensation
Cyanide ion catalyst
Nitric acid oxidation
Quinoxaline
Sodium borohydride reduction
Reductive acetylation
Thionyl chloride
Dehydrohalogenation
Pyridinium hydrobromide perbromide
Triethylene glycol
Perkin condensation
Decarboxylation
Copper chromite catalyst
Quinoline
Steric inhibition of resonance

Procedures are given in this chapter for rapid preparation of small samples of twelve related compounds starting with benzaldehyde and phenylacetic acid. The quantities of reagents specified in the procedures are such as to provide somewhat more of each intermediate than is required for completion of all subsequent steps in the sequence of reactions. If the experiments are dovetailed, the entire series of preparations can be completed in very short working time. For example, one can start the preparation of benzoin (record the time of starting and do not rely on memory), and during the 30-min reflux period start the preparation of α-phenylcinnamic acid; this requires refluxing for 35 min, and while it is proceeding the benzoin preparation can be stopped when the time is up and the product let crystallize. The α-phenylcinnamic acid

▼
Note for the instructor

---

[1] If the work is well organized and proceeds without setbacks, the experiments can be completed in about four laboratory periods. The instructor may elect to name a certain number of periods in which the student is to make as many of the compounds as possible; the instructor may also decide to require submission only of the end products in each series.



Benzaldehyde
KCN
Benzoin
Benzil
Ac₂O
E- and Z-Diacetates
Zn/Hg
HCl + EtOH
NaBH₄
E-Stilbene
Br₂
meso-Stilbene dibromide
KOH
Diphenylacetylene
meso-Stilbenediol
+ CH₂COOH
+
E- and Z-Phenylcinnamic acid
copper
chromite
catalyst
Z-Stilbene
Br₂
dl-Stilbene dibromide

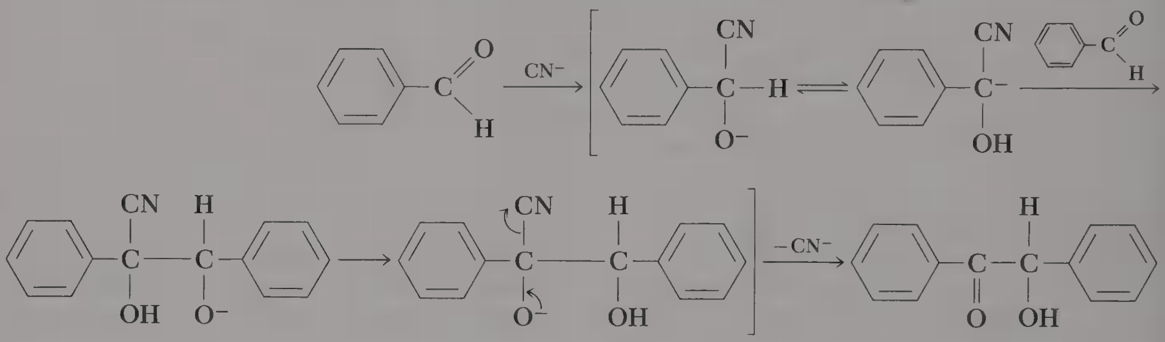
mixture can be let stand (and cool) until one is ready to work it up. Also, while a crystallization is proceeding one may want to observe the crystals occasionally but should utilize most of the time for other operations.

Points of interest concerning stereochemistry and reaction mechanisms are discussed in the introductions to the individual procedures. Since several of the compounds have characteristic ultraviolet or infrared absorption spectra, pertinent spectroscopic constants are recorded and brief interpretations of the data are presented (Figs. 38.1–38.11).

EXPERIMENTS

1. Benzoin

Condensation of two moles of benzaldehyde under the specific catalytic influence of cyanide ion affords *dl*-benzoin:



Benzoin
mp 135°, MW 212.24

Being an α-ketol (compare D-fructose), benzoin reduces Fehling solution (in alcoholic solution) and forms an osazone (mp 225°); the 2,4-dinitrophenylhydrazone forms orange-yellow plates from ethanol, mp 239°.

▼
Potassium cyanide
Poison!
Do not handle if you have open cuts on your hands.
Never acidify a cyanide solution (HCN gas is evolved).
Wash hands after handling cyanide.

▼
Reaction time: 30 min

Procedure

Place 1.5 g of potassium cyanide (**poison!**) in a 125-ml round-bottomed flask, dissolve it in 15 ml of water, add 30 ml of 95% ethanol and 15 ml of pure benzaldehyde,[2] introduce a boiling stone, attach a short condenser, and reflux the solution gently on the steam bath for 30 min. Remove the flask and, if no crystals appear within a few minutes, withdraw a drop on a stirring rod and rub it against the neck of the flask to induce crystallization. When crystallization is complete, collect the product and wash it free of yellow mother liquor with

▼
Note for the instructor

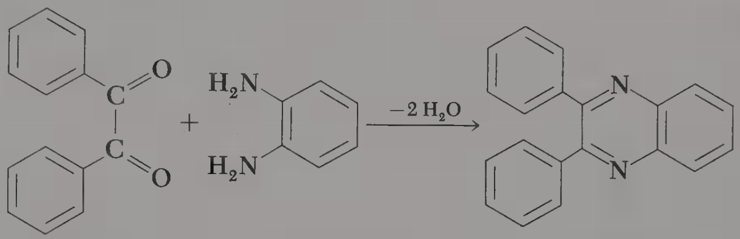
---

[2] Commercial benzaldehyde inhibited against autoxidation with 0.1% hydroquinone is usually satisfactory. If the material available is yellow or contains benzoic acid crystals it should be shaken with equal volumes of 5% sodium carbonate solution until carbon dioxide is no longer evolved and the upper layer dried over calcium chloride and distilled (bp 178–180°), with avoidance of exposure of the hot liquid to air.

a 1:1 mixture of 95% ethanol and water (wash the cyanide-containing mother liquor down the sink with plenty of water). Usually this first-crop material is colorless and of satisfactory melting point (134–135°); usual yield 10–12 g.[3]

2. Preparation of Benzil and its Quinoxaline Derivative

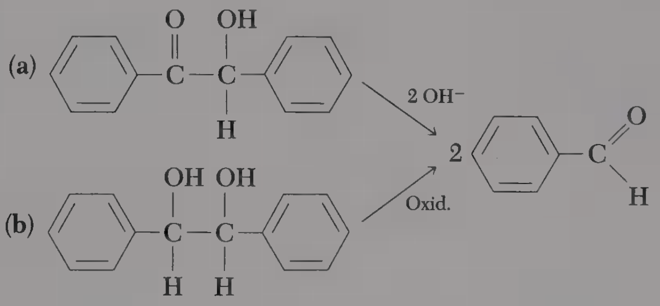
Benzoin can be oxidized to the α-diketone, benzil, very efficiently by nitric acid or by copper(II) sulfate in pyridine. On oxidation with sodium dichromate in acetic acid the yield is lower because some material is converted into benzaldehyde by cleavage of the bond between two oxidized carbon atoms and activated by both phenyl groups (a). Similarly, hydrobenzoin on oxidation with dichromate or permanganate yields chiefly benzaldehyde and only a trace of benzil (b).



Benzil
mp 96°, MW 210.22

o-**Phenylenediamine**
mp 103°,
MW 108.14

Quinoxaline derivative
mp 126°, MW 282.33



A reaction that characterizes benzil as an α-diketone is a condensation reaction with *o*-phenylenediamine to the quinoxaline derivative. The aromatic heterocyclic ring formed in the condensation is fused to a benzene ring to give a bicyclic system analogous to naphthalene.

▼
Note for the instructor

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[3] Concentration of the mother liquor to a volume of 20 ml gives a second crop (1.8 g, mp 133–134.5°); best total yield 13.7 g (87%). Recrystallization can be accomplished with either methanol (11 ml/g) or 95% ethanol (7 ml/g) with 90% recovery in the first crop.

Procedure (oxidation)

▼
Reaction time: 11 min

Heat a mixture of 4 g of benzoin and 14 ml of concentrated nitric acid on the steam bath for 11 min. Use an aspirator tube near the top of the flask to remove nitrogen oxides. Add 75 ml of water to the reaction mixture, cool to room temperature, and swirl for a minute or two to coagulate the precipitated product; collect and wash the yellow solid on a Hirsch funnel, pressing the solid well on the filter to squeeze out the water. The crude product (dry weight 3.7–3.9 g) need not be dried but can be crystallized at once from ethanol. Dissolve the product in 10 ml of hot ethanol, add water dropwise to the cloud point, and set aside to crystallize. Record the yield, crystalline form, color, and mp of the purified benzil.

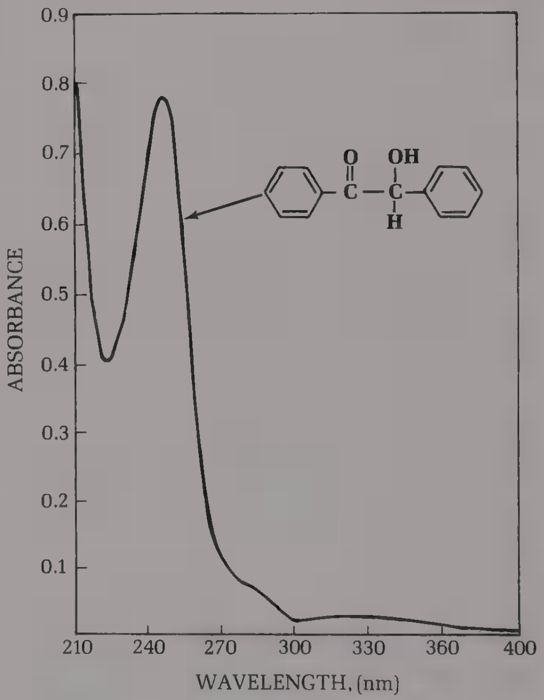
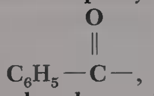


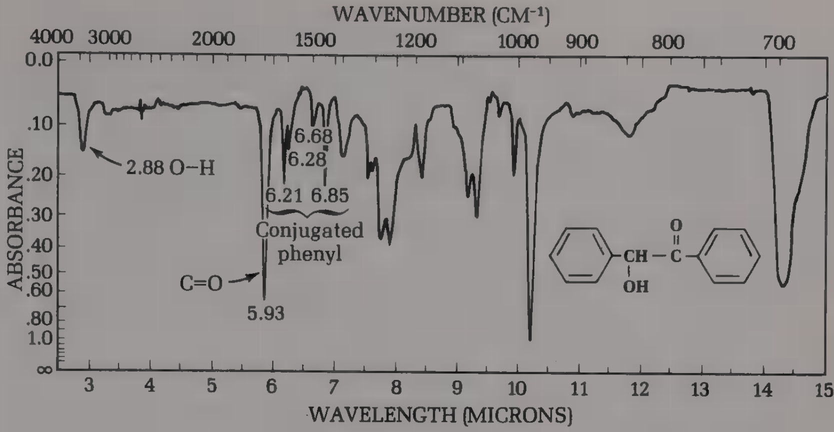
FIGURE 38.1 The ultraviolet spectrum of benzoin. λ_{max}^{EtOH} 247 nm (ϵ = 13,200). Concentration: 12.56 g/l = 5.92×10^{-5} mole/l. See Chapter 9 (Ultraviolet Spectroscopy) for the relationship between the extinction coefficient, ϵ, absorbance, A, and concentration, C. The absorption band at 247 nm is attributable to the presence of the phenyl ketone group,


$C_6H_5-\overset{\overset{\displaystyle O}{\|}}{C}-$,

in which the carbonyl group is conjugated with the benzene ring. Aliphatic α,β-unsaturated ketones, $R-CH{=}CH-C{=}O$, show selective absorption of ultraviolet light of comparable wavelength.

Test for the Presence of Unoxidized Benzoin. Dissolve about 0.5 mg of crude or purified benzil in 0.5 ml of 95% ethanol or methanol and add one drop of 10% sodium hydroxide. If benzoin is present the solution soon acquires a purplish color owing to a complex of benzil with a product of autoxidation of benzoin. If no color develops in 2–3 min, an indication that the sample is free from benzoin, add a small amount of benzoin, observe the color that develops, and note that if the test tube is stoppered and shaken vigorously the color momentarily disappears; when the solution is then let stand, the color reappears.

FIGURE 38.2 Infrared spectrum of benzoin in $CHCl_3$.



Benzil Quinoxaline Preparation

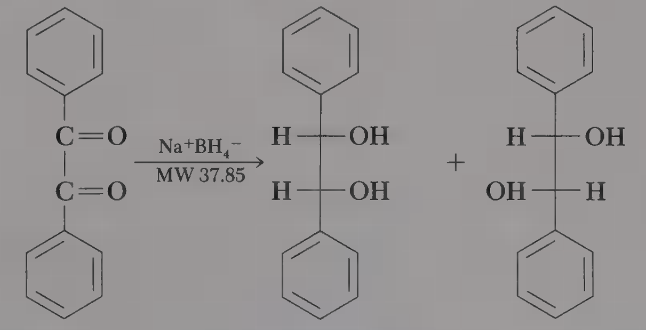
▼ *Handle o-phenylenediamine with care. Similar compounds (hair dyes) are mild carcinogens.*

Commercial *o*-phenylenediamine is usually badly discolored (air oxidation) and gives a poor result unless purified as follows. Place 200 mg of material in a 20 × 150-mm test tube, evacuate the tube at full aspirator suction, clamp it in a horizontal position, and heat the bottom of the tube with a flame to distil or sublime colorless *o*-phenylenediamine from the dark residue into the upper half of the tube. Let the tube cool in position until the melt has solidified, and scrape out the white solid.

▼ *Reaction time: 10 min*

Weigh 0.2 g of benzil (theory = 210 mg) and 0.1 g of your purified *o*-phenylenediamine (theory = 108 mg) into a 20 × 150-mm test tube and heat in a steam bath for 10 min, which changes the initially molten mixture to a light tan solid. Dissolve the solid in hot methanol (about 5 ml) and let the solution stand undisturbed. If crystallization does not occur within 10 min, reheat the solution and dilute it with a little water to the point of saturation. The crystals should be filtered as soon as formed, for brown oxidation products accumulate on standing. The quinoxaline forms colorless needles, mp 125–126°; yield 185 mg.

3. Sodium Borohydride Reduction of Benzil



Benzil
MW 210.22

meso-Hydrobenzoin
Mp 137°, MW 214.25

dl-Hydrobenzoin
Mp 120°

Addition of two atoms of hydrogen to benzoin or of four atoms of hydrogen to benzil gives a mixture of stereoisomeric diols, of which the predominant isomer is the nonresolvable *meso*-hydrobenzoin. Reduction is accomplished rapidly with sodium borohydride ($Na^+BH_4^-$) in ethanol. The high cost of the reagent is offset by its low molecular weight and the fact that one mole of hydride reduces four moles of a ketone.

$$4\,R_2C{=}O + Na^+BH_4^- \longrightarrow (R_2CHO)_4BNa$$

$$(R_2CHO)_4BNa + 2\,H_2O \longrightarrow 4\,R_2CHOH + NaBO_2$$

The procedure that follows specifies use of benzil rather than benzoin because the progress of the reduction can then be followed by the discharge of the yellow color. The amount of reagent required for reduction of 500 mg of benzil is calculated as follows:

$$500\text{ mg} \times \frac{37.85}{210.22} \times \frac{2}{4} = 45\text{ mg NaBH}_5$$

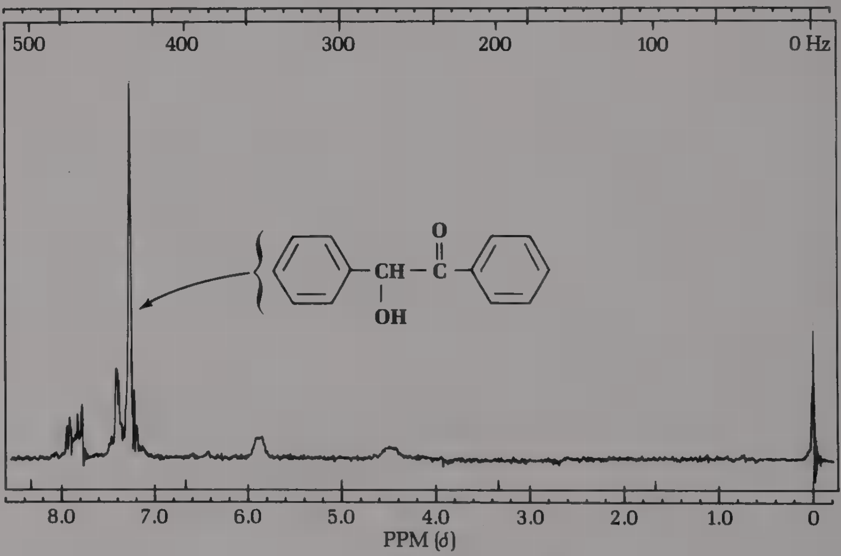


FIGURE 38.3 ^{1}H nmr spectrum of benzoin.

Procedure

▼
Reaction time: 10 min

In a 50-ml Erlenmeyer flask, dissolve 0.5 g of benzil in 5 ml of 95% ethanol and cool the solution under the tap to produce a fine suspension. Then add 0.1 g of sodium borohydride (large excess). The benzil dissolves, the mixture warms up, and the yellow color disappears in 2–3 min. After a total of 10 min, add 5 ml of water, heat to the boiling point, filter in case the solution is

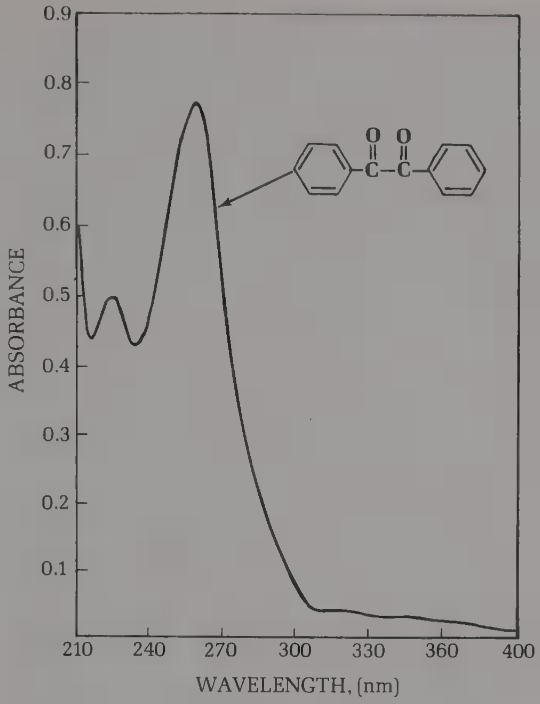


FIGURE 38.4 Ultraviolet spectrum of benzil. $\lambda_{\max}^{\mathrm{EtOH}}$ 260 nm ($\epsilon = 19{,}800$). One-cm cells and 95% ethanol have been employed for all the UV spectra in this chapter.

not clear, dilute to the point of saturation with more water (10 ml), and set the solution aside to crystallize. *meso*-Hydrobenzoin separates in lustrous thin plates, mp 136–137°; yield 0.35 g.

4. Reductive Acetylation of Benzil

In one of the first demonstrations of the phenomenon of 1,4-addition, Johannes Thiele (1899) established that reduction of benzil with zinc dust in a mixture of acetic anhydride-sulfuric acid involves 1,4-addition of hydrogen to the α-diketone grouping and acetylation of the resulting enediol before it can undergo ketonization to benzoin. The process of reductive acetylation results in a mixture of the *E*- and Z-isomers **1** and **2**. Thiele and subsequent investigators isolated the more soluble, lower-melting Z-stilbenediol diacetate (**2**) in only impure form, mp 110°. Separation of the two isomers by chromatography is not feasible because they are equally adsorbable on alumina. However, separation is possible by fractional crystallization (described in the following procedure) and both isomers can be isolated in pure condition. In the method prescribed here for the preparation of the isomer mixture, hydrochloric acid is substituted for sulfuric acid because the latter acid gives rise to colored impurities and is reduced to sulfur and to hydrogen sulfide.[4]

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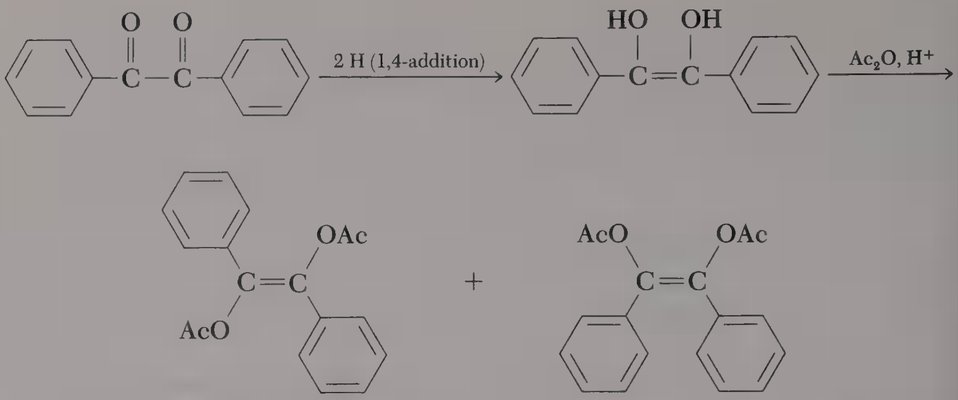
[4] If acetyl chloride (2 ml) is substituted for the hydrochloric acid-acetic anhydride mixture in the procedure, the Z-isomer is the sole product.



mp 155°, λ_{max}^{EtOH} 271nm (ϵ = 23,400),
MW 296.31
1

mp 119°, λ_{max}^{EtOH} 265nm (ϵ = 12,800),
MW 296.31
2

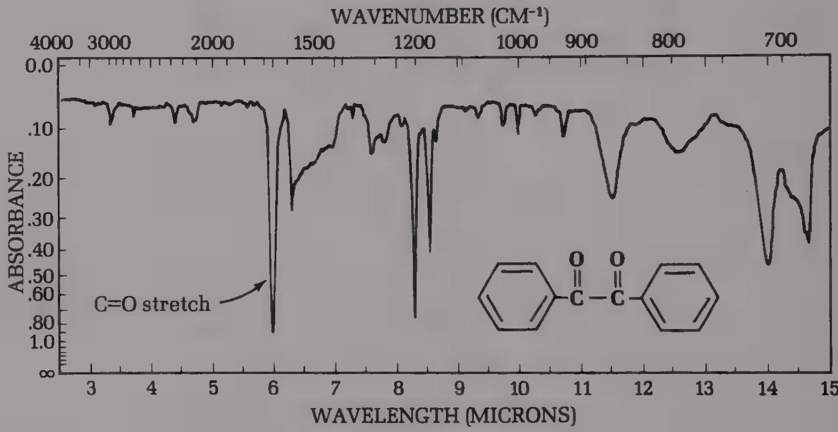


FIGURE 38.5 Infrared spectrum of benzil in $CHCl_3$.

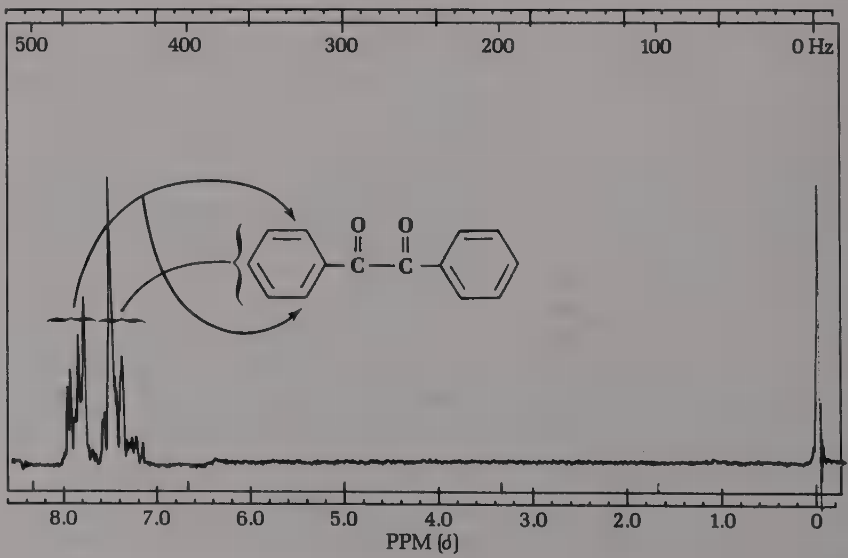


FIGURE 38.6 Nmr spectrum of benzil.

Assignment of configuration

The configurations of this pair of geometrical isomers remained unestablished for over 50 years, but the tentative inference that the higher-melting isomer has the more symmetrical *E* configuration eventually was found to be correct. Evidence of infrared spectroscopy is of no avail; the spectra are nearly identical in the interpretable region (2–8 μ) characterizing the acetoxyl groups, but differ in the fingerprint region (8–12 μ). However, the isomers differ markedly in ultraviolet absorption (Fig. 38.8) and, in analogy to *E*- and *Z*-stilbene (Section 5), the conclusion is justified that the higher-melting isomer, since it has an absorption band at longer wavelength and higher intensity than its isomer, does indeed have the configuration 1.

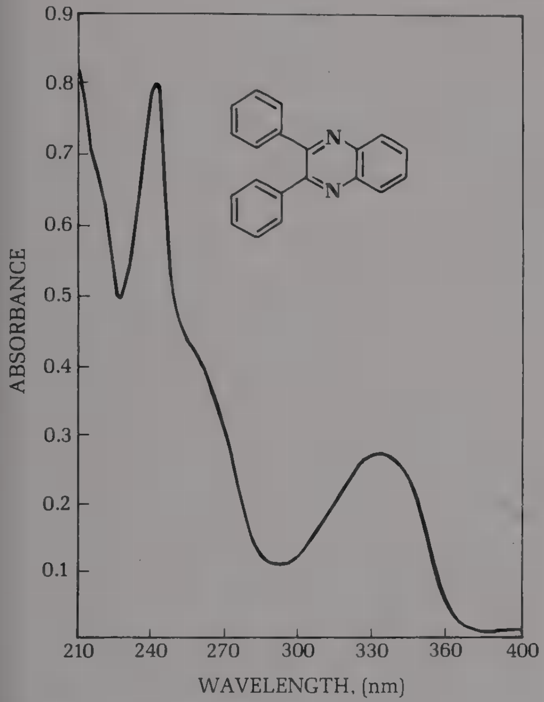


FIGURE 38.7 Ultraviolet spectrum of quinoxaline derivative. λ_{max}^{EtOH} 244 nm (ϵ = 37,400), 345 nm (ϵ = 12,700). Spectrum recorded on Cary Model 17 spectrometer.

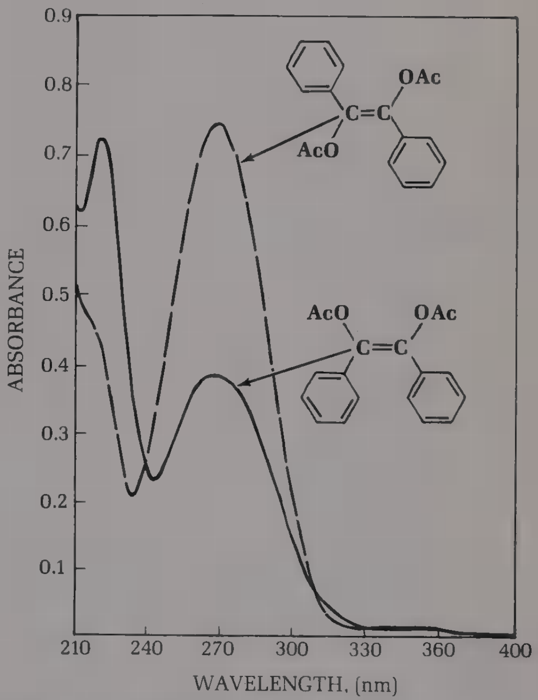


FIGURE 38.8 Ultraviolet spectra of *Z*- and *E*- stilbene diacetate. *Z*: λ_{max}^{EtOH} 223 nm (ϵ = 20,500), 269 nm (ϵ = 10,800); *E*: λ_{max}^{EtOH} 272 nm (ϵ = 20,800). In the *Z*-diacetate the two phenyl rings can not be coplanar which prevents overlap of the *p*-orbitals of the phenyl groups with those of the central double bond. This steric inhibition of resonance accounts for the diminished intensity of the *Z*-isomer relative to the *E*. Both spectra were run at the same concentration.

Procedure

▼
Reaction time: 10 min

Place one test tube (20 × 150-mm) containing 7 ml of acetic anhydride and another (13 × 100-mm) containing 1 ml of concentrated hydrochloric acid in an ice bath and, when both are thoroughly chilled, transfer the acid to the anhydride dropwise in not less than one minute by means of a capillary dropping tube. Wipe the test tube dry, pour the chilled solution into a 50-ml Erlenmeyer flask containing 1 g of pure benzil and 1 g of zinc dust, and swirl for 2–3 min in an ice bath. Remove the flask and hold it in the palm of the hand; if it begins to warm up, cool further in ice. When there is no further exothermic effect, let the mixture stand for 5 min and then add 25 ml of water. Swirl, break up any lumps of product, and allow a few minutes for hydrolysis of excess acetic anhydride. Then collect the mixture of product and zinc dust, wash with water, and press and apply suction to the cake until there is no further drip. Digest the solid (drying is not necessary) with 70 ml of ether to dissolve the organic material, add about 4 g of anhydrous sodium sulfate, and swirl briefly; filter the solution into a 125-ml Erlenmeyer flask, concentrate the filtrate (steam bath, boiling stone, water aspirator) to a volume of approximately 15 ml,[5] and let the flask stand, corked and undisturbed.

The *E*-diacetate (**1**) soon begins to separate in prismatic needles, and after 20–25 min crystallization appears to stop. Removal and evaporation of the mother liquor can be done with a rotary evaporator or as follows: Warm a 25-ml Erlenmeyer flask on the steam bath under an aspirator tube and, with use of a capillary dropping tube, suck up a portion of the mother liquor (ethereal solution) covering the crystals and transfer it, by drops, to the warm evaporation flask; if the ether is caused to evaporate at the same rate as the solution is added, the solvent can be eliminated rapidly without need for a boiling stone. When all the solution has been transferred, wash the remaining crystals with a little fresh ether and transfer the washings to the warm flask as previously. The crystals of *E*-diacetate (**1**) can then be scraped out of the larger flask and the weight and mp determined (e.g., 294 mg, mp 154–156°).

Dissolve the white solid left in the evaporation flask in 10 ml of methanol, let the solution stand undisturbed for about 10 min, and drop in one tiny crystal of the *E*-diacetate (**1**). This should give rise, in 20–30 min, to a second crop of the *E*-diacetate (e.g., 58 mg, mp 153–156°). Then concentrate the mother liquor and washings to a volume of 7–8 ml, let cool to room temperature as before, and again seed with a crystal of *E*-diacetate; this usually affords a third crop of the *E*-diacetate (e.g., 62 mg, mp 153–155°).

At this point the mother liquor should be rich enough in the more soluble Z-diacetate (**2**) for its isolation. Concentrate the methanol mother liquor and washings from the third crop of **1** to a volume of 4–5 ml, stopper the flask, and let the solution stand undisturbed overnight. The Z-diacetate (**2**) sometimes separates spontaneously in large rectangular prisms of great beauty. If the solution remains supersaturated, addition of a seed crystal of **2** causes

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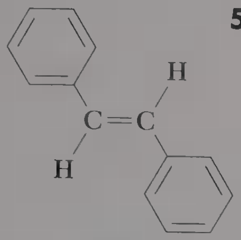
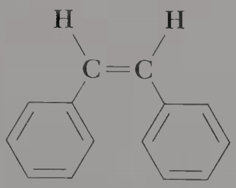
[5] Measure 15 ml of a solvent into a second flask of the same size and compare the levels in the two flasks.

prompt separation of the Z-diacetate in a paste of small crystals (e.g., 215 mg, mp 118–119°; then: 70 mg, mp 116–117°).

5. Stilbene

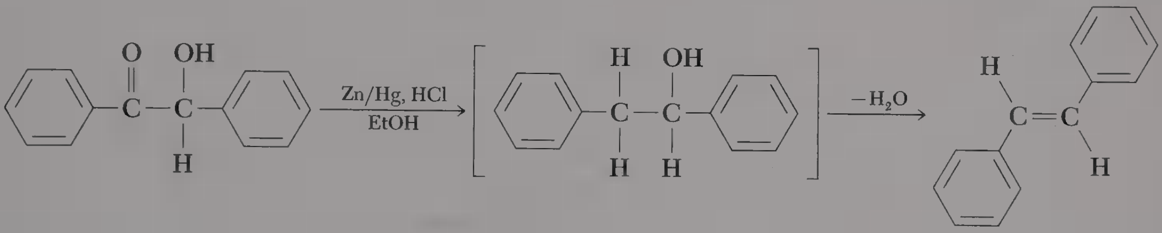


E-Stilbene
Mp 125°, MW 180.24
λ_{max}^{EtOH} 301nm ($\epsilon = 28{,}500$)
226nm ($\epsilon = 17{,}700$)
Heat of hydrogenation, −20.1 kcal/mole

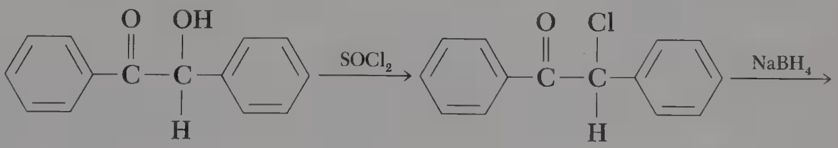


Z-Stilbene
Mp 6°, MW 180.24
λ_{max}^{EtOH} 280nm ($\epsilon = 13{,}500$)
223nm ($\epsilon = 23{,}000$)
Heat of hydrogenation, −25.8 kcal/mole

One method of preparing *E*-stilbene is reduction of benzoin with zinc amalgam in ethanol-hydrochloric acid, presumably through an intermediate:

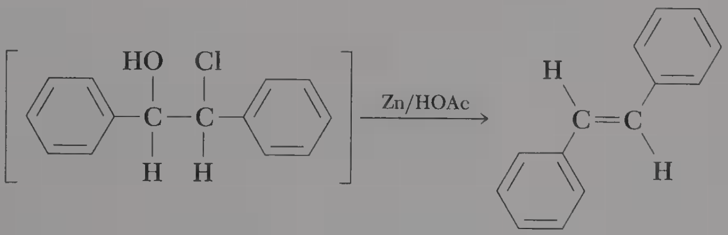


The procedure that follows is quick and affords very pure hydrocarbon. It involves three steps: (1) replacement of the hydroxyl group of benzoin by



Benzoin
MW 212.24

Desyl chloride
Mp 68°



Mixture of *d,l*- and *d′,l′*-isomers

E-Stilbene

chlorine to form desyl chloride (2), reduction of the keto group with sodium borohydride to give what appears to be a mixture of the two diastereoisomeric chlorohydrins, and (3) elimination of the elements of hypochlorous acid with zinc and acetic acid. The last step is analogous to the debromination of an olefin dibromide.

Procedure

Place 4 g of benzoin (crushed to a powder) in a 125-ml round-bottomed flask, cover it with 4 ml of thionyl chloride,[6] warm gently on the steam bath (hood) until the solid has all dissolved, and then more strongly for 5 min.

Caution: If the mixture of benzoin and thionyl chloride is let stand at room temperature for an appreciable time before being heated, an undesired reaction intervenes[7] and the synthesis of *E*-stilbene is spoiled.

To remove excess thionyl chloride (bp 77°), evacuate at the aspirator for a few minutes, add 10 ml of petroleum ether (bp 30–60°), boil it off, and evacuate again. Desyl chloride is thus obtained as a viscous, pale yellow oil (it will solidify if let stand). Dissolve this in 40 ml of 95% ethanol, cool under the tap, and add 360 mg of sodium borohydride (an excess is harmful). Stir, break up any lumps of the borohydride, and after 10 min add to the solution of chlorohydrins 2 g of zinc dust and 4 ml of acetic acid and reflux for 1 hr. Then cool under the tap. When white crystals separate add 50 ml of ether and decant the solution from the bulk of the zinc into a separatory funnel. Wash the solution twice with an equal volume of water containing 1–2 ml of concentrated hydrochloric acid (to dissolve basic zinc salts) and then, in turn, with 5% sodium carbonate solution and saturated sodium chloride solution. Dry the ether over anhydrous sodium sulfate (4 g), filter to remove the drying agent, evaporate the filtrate to dryness, dissolve the residue in the minimum amount of hot 95% ethanol (30–40 ml), and let the product crystallize. *E*-Stilbene separates in diamond-shaped iridescent plates, mp 124–125°; yield 1.8–2.2 g.

6. *meso*-Stilbene Dibromide

E-Stilbene reacts with bromine predominantly by the usual process of *trans*-addition and affords the optically inactive, nonresolvable *meso*-dibromide (see page 247); the much lower-melting *dl*-dibromide (Section 9) is a very minor product of the reaction.

Procedure

▼
Total time required: 10 min

In a 125-ml Erlenmeyer flask dissolve 2 g of *E*-stilbene in 40 ml of acetic acid, by heating on the steam bath, and then add 4 g of pyridinium hydrobromide

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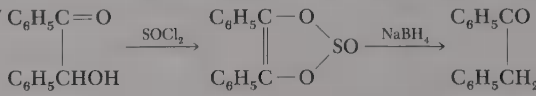
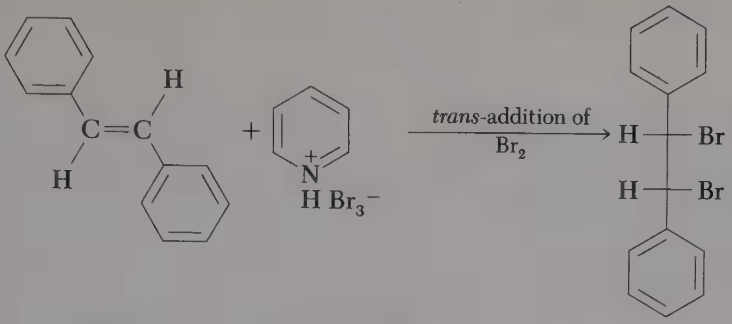
[6] The reagent can be dispensed from a burette or measured by pipette; in the latter case the liquid should be drawn into the pipette with a pipetter, **not by mouth.**

[7] 

Desoxybenzoin



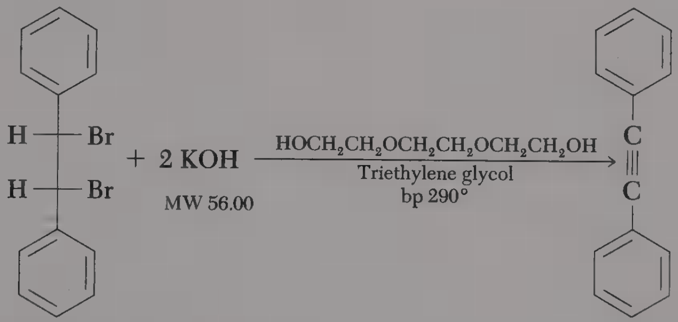
E-Stilbene
MW 180.24

Pyridinium hydrobromide perbromide
MW 319.86

meso-Dibromide
mp 238°, MW 340.07

perbromide. Mix by swirling, if necessary rinse crystals of reagent down the walls of the flask with a little acetic acid, and continue the heating for 1–2 min longer. The dibromide separates almost at once in small plates. Cool the mixture under the tap, collect the product, and wash it with methanol; yield of colorless crystals, mp 236–237°, 3.2 g. Use 0.5 g of this material for the preparation of diphenylacetylene and turn in the remainder.

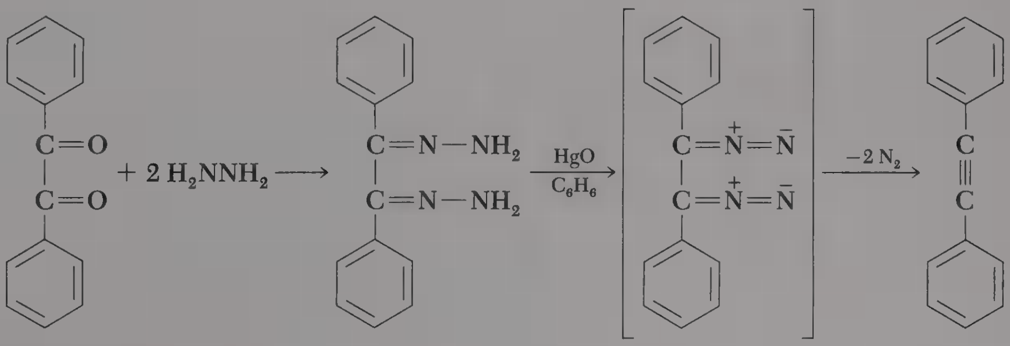
7. Diphenylacetylene



meso-Stilbene dibromide
MW 340.07

Diphenylacetylene
mp 61°, MW 178.22

One method for the preparation of diphenylacetylene involves oxidation of benzil dihydrazone with mercuric oxide; the intermediate diazo compound loses nitrogen as formed to give the hydrocarbon:



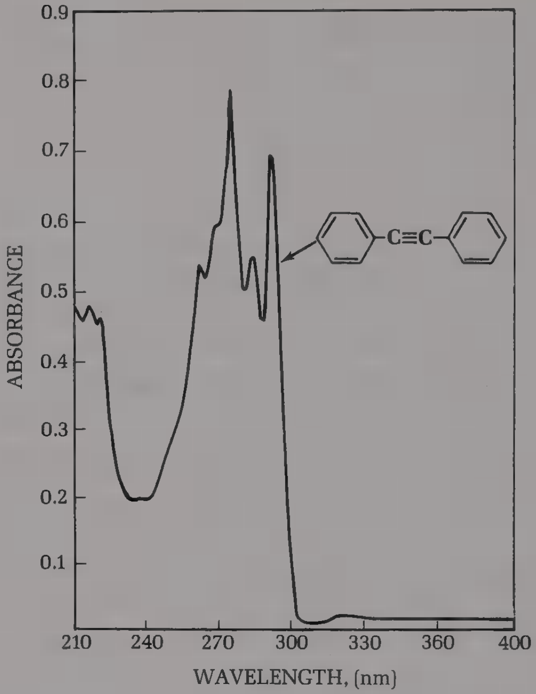


FIGURE 38.9 Ultraviolet spectrum of diphenylacetylene. λ_{max}^{EtOH} 279 nm (ϵ = 31,400). This spectrum is characterized by considerable fine structure (multiplicity of bands) and a high extinction coefficient.

The method used in this procedure involves dehydrohalogenation of *meso*-stilbene dibromide. An earlier procedure called for refluxing the dibromide with 43% ethanolic potassium hydroxide in an oil bath at 140° for 24 hours. In the following procedure the reaction time is reduced to a few minutes by use of the high-boiling triethylene glycol as solvent to permit operation at a higher reaction temperature.

Procedure

▼
Reaction time: 5 min

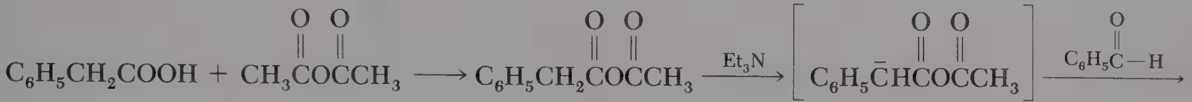
In a 20 × 150-mm test tube place 0.5 g of *meso*-stilbene dibromide, 3 pellets of potassium hydroxide[8] (250 mg), and 2 ml of triethylene glycol. Insert a thermometer into a 10 × 75-mm test tube containing enough triethylene glycol to cover the bulb, and slip this assembly into the larger tube. Clamp the tube in a vertical position about two inches above a microburner, and heat the mixture with a very small flame to a temperature of 160°, when potassium bromide begins to separate. By intermittent heating, keep the mixture at 160–170° for 5 min more, then cool to room temperature, remove the thermometer and small tube, and add 10 ml of water. The diphenylacetylene that separates as a nearly colorless, granular solid is collected by suction filtration. The crude

---

[8] Potassium hydroxide pellets are 85% KOH and 15% water.

product need not be dried but can be crystallized directly from 95% ethanol. Let the solution stand undisturbed in order to observe the formation of beautiful, very large spars of colorless crystals. After a first crop has been collected, the mother liquor on concentration affords a second crop of pure product; total yield, 0.23 g; mp 60–61°.

8. *α*-Phenylcinnamic Acid (*E* and *Z*)[9]



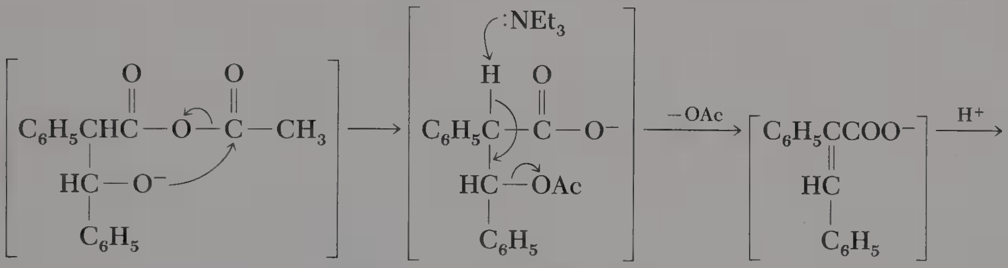
$$C_6H_5CH_2COOH + CH_3\overset{\overset{O}{\|}}{C}O\overset{\overset{O}{\|}}{C}CH_3 \longrightarrow C_6H_5CH_2\overset{\overset{O}{\|}}{C}O\overset{\overset{O}{\|}}{C}CH_3 \xrightarrow{Et_3N} \left[C_6H_5\bar{C}H\overset{\overset{O}{\|}}{C}O\overset{\overset{O}{\|}}{C}CH_3\right] \xrightarrow{C_6H_5\overset{\overset{O}{\|}}{C}-H}$$

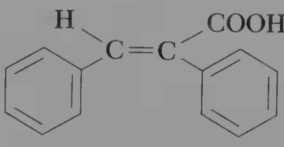
Phenylacetic acid
mp 77°, bp 265°,
MW 136.14

Acetic anhydride
bp 138–140°,
MW 102.09

Triethylamine
bp 89.5, den 0.729,
MW 101.19

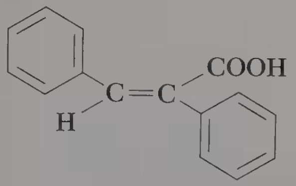
Benzaldehyde
bp 179°, den 1.046,
MW 106.12





E-*α*-Phenylcinnamic acid[10]
mp 174°, pK_a 6.1
MW 224.25

+



Z-*α*-Phenylcinnamic acid
mp 138°, pK_a 4.8
MW 224.25

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[9] Stereochemistry of alkenes can be designated by the *E*,*Z*-system of nomenclature [see J. L. Blackwood, C. L. Gladys, K. L. Loening, A. E. Petrarca, and J. E. Rush, *J. Am. Chem. Soc.*, **90**, 509 (1968)] in which the groups attached to the double bond are given an order of priority according to the Cahn, Ingold, and Prelog system [see R. S. Cahn, C. K. Ingold, and V. Prelog, *Experentia*, **12**, 81 (1956)]. The atom of highest atomic number attached directly to the alkene is given highest priority. If two atoms attached to the alkene are the same, one goes to the second or third atom, etc. away from the alkene carbons. When the two groups of highest priority are on adjacent sides of the double bond, the stereochemistry is designated as Z (German *zusammen*, together). When the two groups are on opposite sides of the double bond, the stereochemistry is designated *E* (German *entgegen*, opposed).

[10] pK_a measured in 60% ethanol.

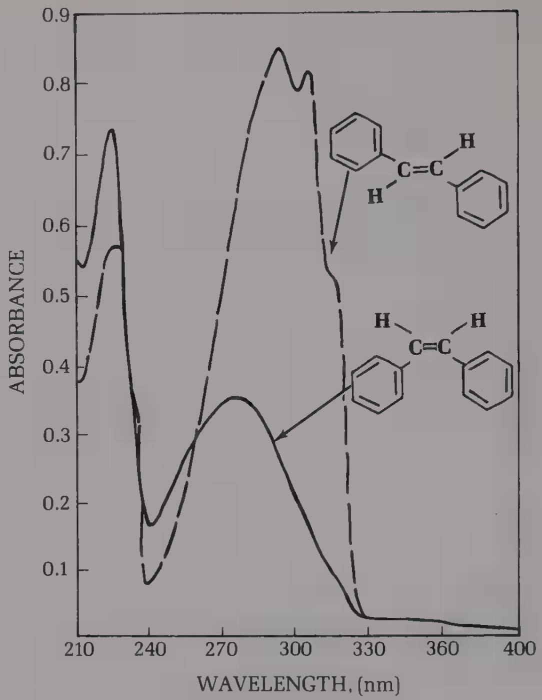

FIGURE 38.10 Ultraviolet spectra of Z- and E-stilbene. Z: $\lambda_{\max}^{\text{EtOH}}$ 224 nm ($\epsilon = 23{,}300$), 279 nm ($\epsilon = 11{,}100$); E: $\lambda_{\max}^{\text{EtOH}}$ 226 nm ($\epsilon = 18{,}300$), 295 nm ($\epsilon = 27{,}500$). Like the diacetates, steric hindrance and lack of coplanarity in these hydrocarbons cause the long wavelength absorption of the Z-isomer to be of diminished intensity relative to the E-isomer.

The reaction of benzaldehyde with phenylacetic acid to produce a mixture of the α-carboxylic acid derivatives of *Z*- and *E*-stilbene, a form of aldol condensation known as the Perkin reaction, is effected by heating a mixture of the components with acetic anhydride and triethylamine. In the course of the reaction the phenylacetic acid is probably present both as anion and as the mixed anhydride resulting from equilibration with acetic anhydride. A reflux period of 5 hrs specified in an early procedure has been shortened by a factor of 10 by restriction of the amount of the volatile acetic anhydride, use of an excess of the less expensive, high-boiling aldehyde component, and use of a condenser that permits some evaporation and consequent elevation of the reflux temperature.

E-Stilbene is a by-product of the condensation, but experiment has shown that neither the *E*- nor *Z*-acid undergoes decarboxylation under the conditions of the experiment.

At the end of the reaction the α-phenylcinnamic acids are present in part as the neutral mixed anhydrides, but these can be hydrolyzed by addition of excess hydrochloric acid. The organic material is taken up in ether and the acids extracted with alkali. Neutralization with acetic acid (pK_a 4.76) then causes precipitation of only the less acidic *E*-acid (see pK_a values under the formulas); the *Z*-acid separates on addition of hydrochloric acid.

Whereas *Z*-stilbene is less stable and lower melting than *E*-stilbene, the reverse is true of the α-carboxylic acids, and in this preparation the more stable, higher-melting *E*-acid is the predominant product. Evidently the steric inter-

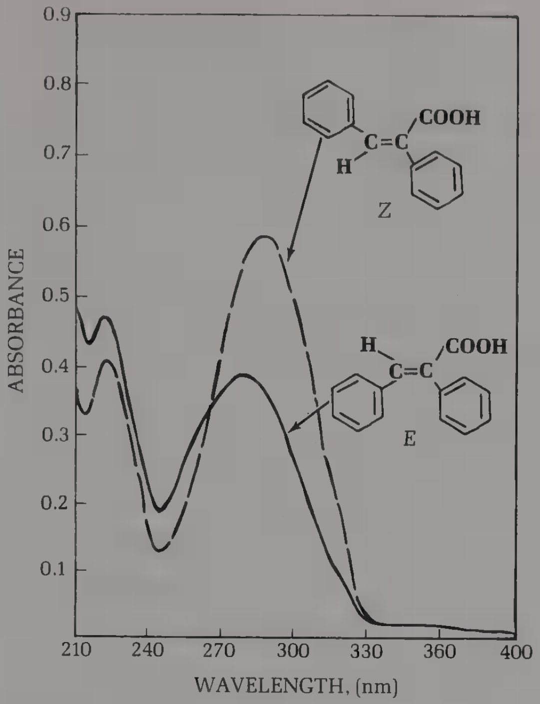

FIGURE 38.11 Ultraviolet spectra of *Z*- and *E*-α-phenylcinnamic acid, run at identical concentrations. *E*: $\lambda_{\text{max}}^{\text{EtOH}}$ 222 nm ($\epsilon = 18{,}000$), 282 nm ($\epsilon = 14{,}000$); *Z*: $\lambda_{\text{max}}^{\text{EtOH}}$ 222 nm ($\epsilon = 15{,}500$), 292 nm ($\epsilon = 22{,}300$).

ference between the carboxyl and phenyl groups in the Z-acid is greater than that between the two phenyl groups in the *E*-acid. Steric hindrance is also evident from the fact that the Z-acid is not subject to Fischer esterification whereas the *E*-acid is.

Procedure

▼
Reflux time: 35 min

Measure into a 25 × 150-mm test tube 2.5 g of phenylacetic acid, 3 ml of benzaldehyde, 2 ml of triethylamine, and 2 ml of acetic anhydride. Insert a boiling stone, mount a cold-finger condenser, and reflux the mixture for 35 min. Alternatively, carry out the reaction in a 25-ml round-bottomed flask equipped with a condenser. Cool the yellow melt, add 4 ml of concentrated hydrochloric acid, and swirl, where upon the mixture sets to a stiff paste. Add ether, warm to dissolve the bulk of the solid, and transfer to a separatory funnel with use of more ether. Wash the ethereal solution twice with water and then extract it with a mixture of 25 ml of water and 5 ml of 10% sodium hydroxide solution.[11] Repeat the extraction twice more and discard the dark-colored ethereal solution.[12] Acidify the combined, colorless alkaline extract to pH 6

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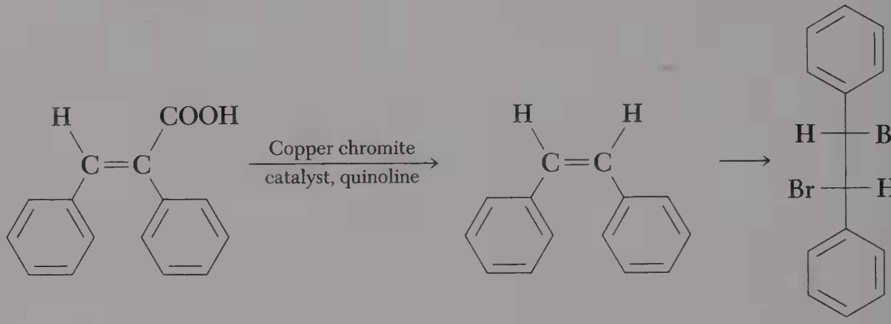
[11] If stronger alkali is used the sodium salt may separate.

[12] For isolation of stilbene, wash this ethereal solution with saturated sodium bisulfite solution for removal of benzaldehyde, dry, evaporate, and crystallize the residue from a little methanol. Large, slightly yellow spars, mp 122–124°, separate (90 mg).

by adding 5 ml of acetic acid, collect the Z-acid that precipitates, and save the filtrate and washings. The yield of Z-acid, mp 163–166°, is usually about 2.9 g. Crystallize 0.3 g of material by dissolving it in 8 ml of ether, adding 8 ml of petroleum ether (bp 30–60°), heating briefly to the boiling point, and letting the solution stand. Silken needles form, mp 173–174°.

Addition of 5 ml of concentrated hydrochloric acid to the aqueous filtrate from precipitation of the *E*-acid produces a cloudy emulsion which on standing for about one half hour coagulates to crystals of Z-acid: 0.3 g, mp 136–137°.[13]

9. Z-Stilbene and *dl*-Stilbene Dibromide



Z-α-Phenylcinnamic acid
MW 224.25

Z-Stilbene
mp 4°, bp 82–84°/0.4 mm,
MW 180.24

***dl*-Dibromide**
mp 114°,
MW 340.07

Decarboxylation of *E*-α-phenylcinnamic acid is effected by refluxing the acid in quinoline in the presence of a trace of copper chromite catalyst; both the basic properties and boiling point (237°) of quinoline make it a particularly favorable solvent. Z-Stilbene, a liquid at room temperature, can be characterized by *trans* addition of bromine to give the crystalline *dl*-dibromide. A little *meso*-dibromide derived from *E*-stilbene in the crude hydrocarbon starting material is easily separated by virtue of its sparing solubility.

Although free rotation is possible around the single bond connecting the asymmetric carbon atoms of the stilbene dibromides and hydrobenzoins, evidence from dipole-moment measurements indicates that the molecules tend to exist predominantly in the specific shape or conformation in which the two phenyl groups repel each other and occupy positions as far apart as possible. The optimal conformations of the *d*- or *l*-dibromide and the *meso*-dibromide are represented in Fig. 38.12 by Newman projection formulas, in which the molecules are viewed along the axis of the bond connecting the two asymmetric

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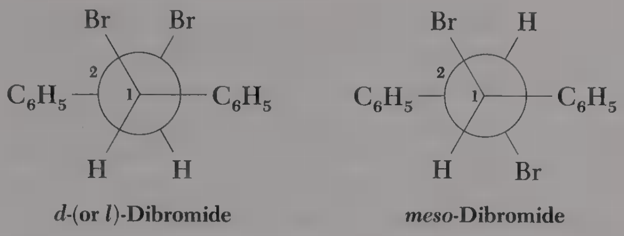
[13] The *E*-acid can be recrystallized by dissolving 0.3 g in 5 ml of ether, filtering if necessary from a trace of sodium chloride, adding 10 ml of petroleum ether (bp 30–60°), and evaporating to a volume of 5 ml; the acid separates as a hard crust of prisms, mp 138–139°.

FIGURE 38.12 Favored conformations of stilbene dibromide.



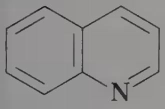
carbon atoms. The carbon atom nearest to the eye is numbered 1; the carbon atom to the rear is 2. In the *meso*-dibromide the two repelling phenyl groups are on opposite sides of the molecule, and so are the two large bromine atoms. Hence, the structure is much more symmetrical than that of the *d*- (or *l*-) dibromide. X-ray diffraction measurements of the dibromides in the solid state confirm the conformations indicated in Fig. 38.12. The Br-Br distances found are: *meso*-dibromide, 4.50Å; *dl*-dibromide, 3.85Å. The difference in symmetry of the two optically inactive isomers accounts for the marked contrast in properties:

	Mp	Solubility in ether (18°)
dl-Dibromide	114°	1 part in 3.7 parts
meso-Dibromide	237°	1 part in 1025 parts

Procedure

▼
Reaction time in first step: 10 min

Since a trace of moisture causes troublesome spattering, the reactants and catalyst are dried prior to decarboxylation. Stuff 2.5 g of crude, "dry" *E*-α-phenylcinnamic acid and 0.2 g of copper chromite catalyst[14] into a 20 × 150-mm test tube, add 3 ml of quinoline[15] (bp 237°), and let it wash down the solids. Make connection with a rubber stopper to the aspirator and turn it on full force. Make sure that you have a good vacuum (pressure gauge) and heat the tube strongly on the steam bath with most of the rings removed. Heat and evacuate for 5–10 min to remove all traces of moisture. Then wipe the outside walls of the test tube dry, insert a thermometer, clamp the tube over a microburner, raise the temperature to 230° and note the time. Then maintain a temperature close to 230° for 10 minutes. Cool the yellow solution containing suspended catalyst to 25°, add 30 ml of ether, and filter the solution by gravity (use more ether for rinsing). Transfer the solution to a separatory funnel and remove the quinoline by extraction twice with about 15 ml of water containing 3–4 ml of concentrated hydrochloric acid. Then shake the ethereal solution well with water containing a little sodium hydroxide solution, draw off the



Quinoline, bp 237°

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[14] The preparations described in *J. Am. Chem. Soc.*, **54**, 1138 (1932) and *ibid.*, **72**, 2626 (1950) are both satisfactory.

[15] Material that has darkened in storage should be redistilled over a little zinc dust.

alkaline liquor, and acidify it. A substantial precipitate will show that decarboxylation was incomplete, in which case the starting material can be recovered and the reaction repeated. If there is only a trace of precipitate, shake the ethereal solution with saturated sodium chloride solution for preliminary drying, dry the ethereal solution over sodium sulfate, remove the drying agent by filtration, and evaporate the ether. The residual brownish oil (1.3–1.8 g) is crude *Z*-stilbene containing a little *E*-isomer formed by rearrangement during heating.

▼

Second step requires about one half hour

Dissolve the crude *Z*-stilbene (e.g., 1.5 g) in 10 ml of acetic acid and, in subdued light, add double the weight of pyridinium hydrobromide perbromide (e.g., 3.0 g). Warm on the steam bath until the reagent is dissolved, and then cool under the tap and scratch to effect separation of a small crop of plates of the *meso*-dibromide (10–20 mg). Filter the solution by suction, dilute extensively with water, and extract with ether. Wash the solution twice with water and then with 5% sodium bicarbonate solution until neutral; shake with saturated sodium chloride solution, dry over sodium sulfate, and evaporate to a volume of about 10 ml. If a little more of the sparingly soluble *meso*-dibromide separates, remove it by gravity filtration and then evaporate the remainder of the solvent. The residual *dl*-dibromide is obtained as a dark oil that readily solidifies when rubbed with a rod. Dissolve it in a small amount of methanol and let the solution stand to crystallize. The *dl*-dibromide separates as colorless prismatic plates, mp 113–114°; yield about 0.6 g.

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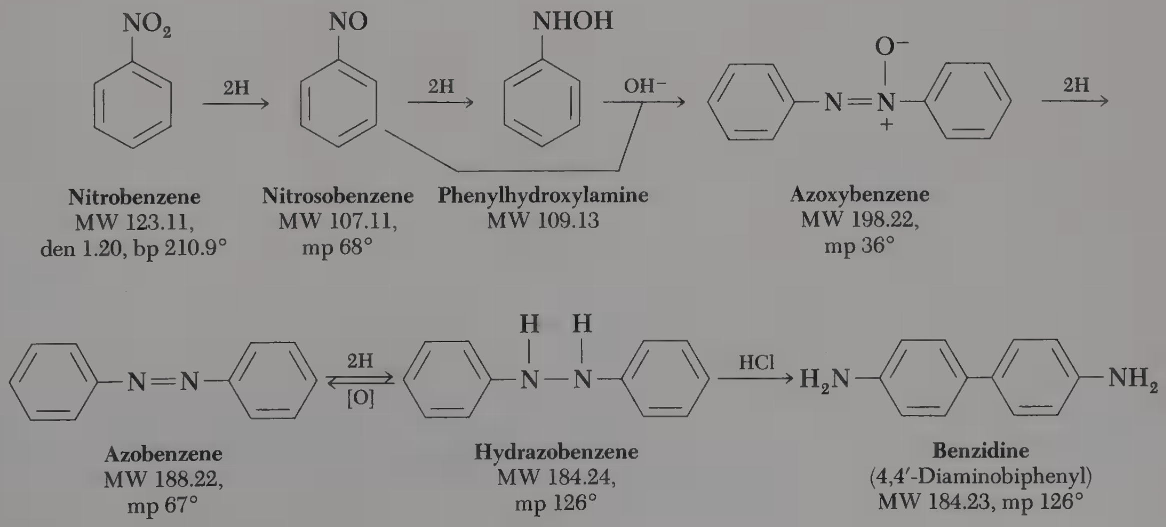
Azoxybenzene, Azobenzene, and Hydrazobenzene

KEYWORDS

Reducing agents
Zinc dust, triethylene glycol, diimide, stannous chloride

Hydrogen peroxide + hydrazine = diimide

Benzidine rearrangement



The formation of *E*-azobenzene by reduction of nitrobenzene in an alkaline medium involves reduction to nitrosobenzene and phenylhydroxylamine, aldol-like condensation of these reactants to azoxybenzene, and reductive removal of the oxygen function.

The classical procedure calls for heating a mixture of nitrobenzene, zinc dust, and aqueous-methanolic sodium hydroxide under reflux with mechanical stirring for 10 hr. However, the reaction period can be reduced to 35 min by use of a higher boiling alcohol with greater solvent power and a more effective alkali. Gentle heating of a mixture of nitrobenzene, zinc dust (2 equivalents), and potassium hydroxide in triethylene glycol initiates a strongly exothermic reaction which, if checked first at 80–85°, can be controlled and completed in 15 min at 135–140°. Surprisingly, very little zinc dust is consumed. The reducing agent is triethylene glycol, which is transformed into the corresponding aldehyde, isolable as the 2,4-dinitrophenylhydrazone.

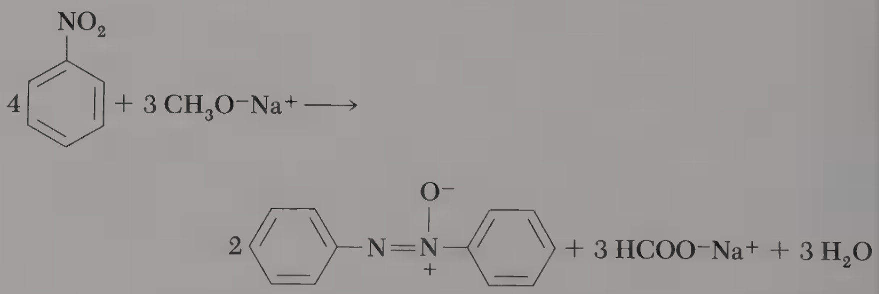
▼
Reduction by
$RCH_2OH \longrightarrow RCHO$

$$2\ C_6H_5NO_2 + 4\ HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH \longrightarrow C_6H_5N{=}NC_6H_5 + 4\ HOCH_2CH_2OCH_2CH_2OCH_2CHO$$

Azobenzene can be obtained in high yield from nitrobenzene, potassium hydroxide, and triethylene glycol without any zinc, but the period of heating at 135–140° must be extended from 15 min to 1 hr. Zinc is beneficial in accelerating this reaction if used in adequate quantity, but it is not effective in truly catalytic amount.

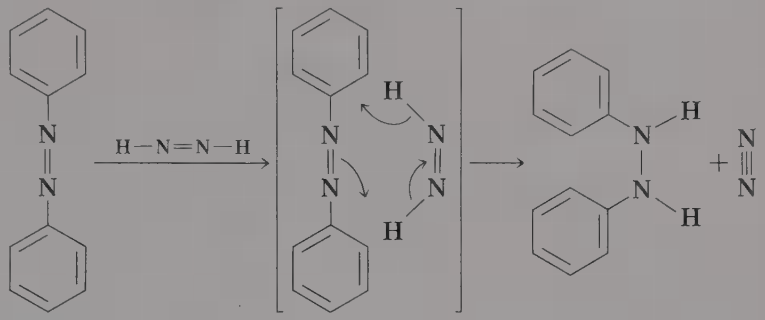
The intermediate, azoxybenzene, can be prepared by refluxing nitrobenzene with methanolic sodium hydroxide for three hours.

$$CH_3OH + NaOH \rightleftharpoons CH_3O^-Na^+ + H_2O$$



The reduction of orange colored azobenzene to colorless hydrazobenzene without reductive cleavage to aniline is accomplished efficiently by *in situ* generation of the highly reactive diimide by cupric ion-catalyzed oxidation of hydrazine with hydrogen peroxide. Diimide combines with azobenzene (or with an olefin) to form a cyclic transition state which collapses to nitrogen and hydrazobenzene.

$$\underset{\text{Hydrazine}}{H_2NNH_2} + H_2O_2 \xrightarrow{Cu^{2+}} \underset{\text{Diimide}}{H{-}N{=}N{-}H} + 2\,H_2O$$



The procedure which follows affords hydrazobenzene as colorless crystals of high purity. This substance is very susceptible to air oxidation and darkens rapidly in storage. It is easily transformed by acid-catalyzed rearrangement into the more stable benzidine by reaction with hydrochloric acid in methanol-water at 0°. However, benzidine has been found to be a potent carcinogen.

EXPERIMENTS

1. Azobenzene

▼
Do not allow nitrobenzene to come in contact with the skin.

▼
Reaction time: about 35 min

Weigh or measure the following reagents into a 250-ml Erlenmeyer flask: 100 ml of triethylene glycol, 16 g of zinc dust, 22 g of potassium hydroxide, and 10 ml of nitrobenzene. Insert a thermometer and, with vigorous swirling, heat the mixture on a 70-watt hot plate or over a steam bath in the course of about 5 min until the temperature rises to 80°. Remove the flask from the source of heat, continue to swirl, observe the temperature carefully, and avoid a rise above 85° by brief chilling under the tap. By appropriate heating or cooling, keep the greenish suspension in the range 80–85° for 10 min. Then, by heating and swirling, bring the temperature to 135° in the course of 3–5 min. Remove the flask from the hot plate, swirl, and prevent a rise above 140° by brief cooling. Then maintain a temperature of 135–140° for 15 min by appropriate heating or cooling; during this period alternately swirl the flask and stir the mixture using the thermometer. Note that the mixture soon reddens and gradually acquires a red color of maximal intensity.

Cool the mixture to 100° and add 50 ml of 95% ethanol, using it to rinse the thermometer. Then remove the zinc dust by filtering the hot solution by suction through an 80-mm Büchner funnel; use ethanol or methanol to rinse the flask and wash the filter cake. Chill the filtrate in an ice bath, add an equal volume of water, and collect the orange colored azobenzene by filtration, if it crystallizes, or by extraction with ether if it separates as an oil. Recrystallize the crude material from the least volume of methanol; collect the product after cooling the mixture in ice. Product collected by filtration need not be dried prior to this purification by crystallization. The yield of the crude azobenzene, mp 65–67°, is 7.5–8.0 g; the first crop of orange plates, mp 65–66°, weighs 5.5 g.

2. Azoxybenzene

▼
Reaction time: 3 hr

Add 10 g of sodium hydroxide pellets to 40 ml of methanol in a 100-ml round-bottomed flask equipped with a reflux condenser. Warm the flask and swirl to hasten solution of the hydroxide, then add 5 ml of nitrobenzene and reflux the solution (boiling chip) on the steam bath for 3 hr. Pour the reaction mixture onto a mixture of 20 g of ice and 20 ml of water in a 250-ml beaker. Stir until the azoxybenzene solidifies, then filter the product on a Büchner funnel with suction, wash well with water, and recrystallize the damp product from alcohol. Recrystallization is accomplished by dissolving the product in a small quantity of alcohol at room temperature and cooling the resulting solution well in ice with seeding and scratching, if necessary. A second crop of crystals can be collected by evaporating methanol from the mother liquor and repeating the crystallization process. Azoxybenzene forms pale yellow needles, mp 36°. Yield approximately 4 g.

3. Hydrazobenzene

▼
Reaction time: about 15 min

▼
Caution. H_2O_2 blisters the skin

▼
Caution! Hydrazine is reported to be a carcinogen. Conduct this experiment in the hood. Wear gloves when handling hydrazine solution.

In a 125-ml Erlenmeyer flask dissolve 3.0 g of the azobenzene prepared in the earlier experiment in 50 ml of 95% ethanol with heating on a steam bath and then cool the orange solution to room temperature. Add 6 ml of 95% hydrazine *(caution!)* and 1 ml of a 1% solution of copper(II) sulfate pentahydrate and swirl the mixture vigorously in an ice-water bath until a large crop of azobenzene crystallizes and the temperature is close to 0° (a low temperature minimizes air oxidation). Measure 3.0 ml of 30% hydrogen peroxide *(caution!)* in a graduate using a capillary dropping tube to obtain exactly 3.0 ml. The peroxide is to be added gradually to the azobenzene mixture in the course of 12–15 min while the flask is swirled vigorously in the ice bath, for example at the rate of one drop every 10 sec. The azobenzene dissolves as the reaction proceeds. Toward the end of the addition, the orange or yellow color should be almost completely discharged and colorless hydrazobenzene should begin to separate. If not, add 0.5 ml more hydrogen peroxide, gradually, as before. Heat the mixture on the hot plate to redissolve the product and filter the hot solution by gravity to remove a trace of copper oxide (even if the solution is yellow, do not use decolorizing charcoal as this catalyzes air oxidation). Reheat the filtrate, add water until crystallization starts (10–15 ml), and after a large crop of product has separated, cool in ice. Collect the colorless plates of hydrazobenzene. The dry weight of product, mp 125–126°, is usually about 2.7 g.

FIGURE 39.1 ^{13}C nmr spectrum of azobenzene.

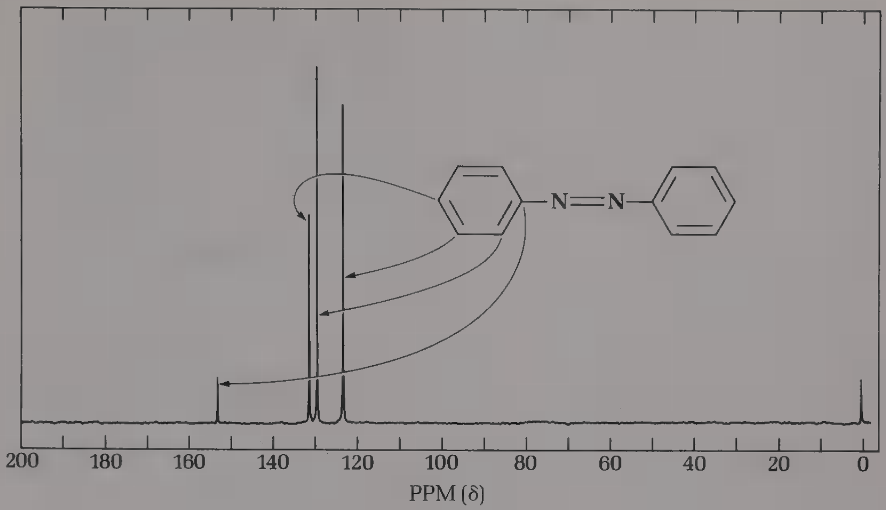
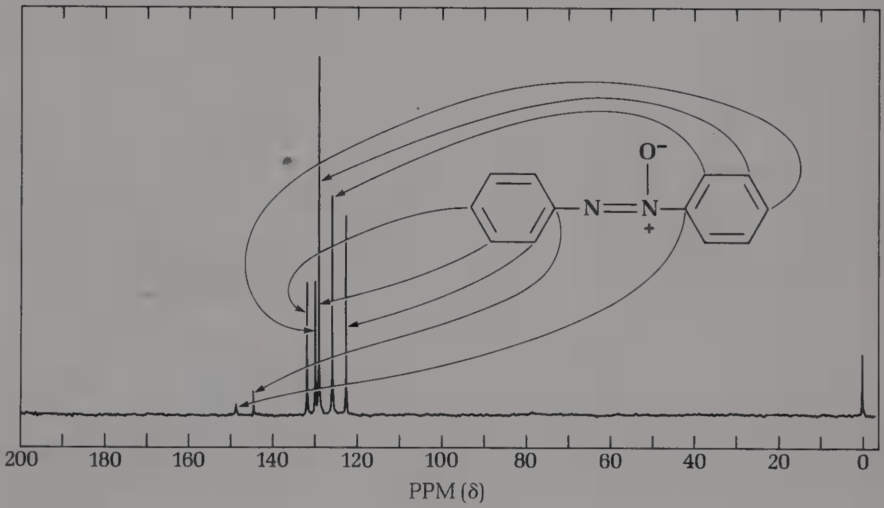


FIGURE 39.2 ^{13}C nmr spectrum of azoxybenzene.

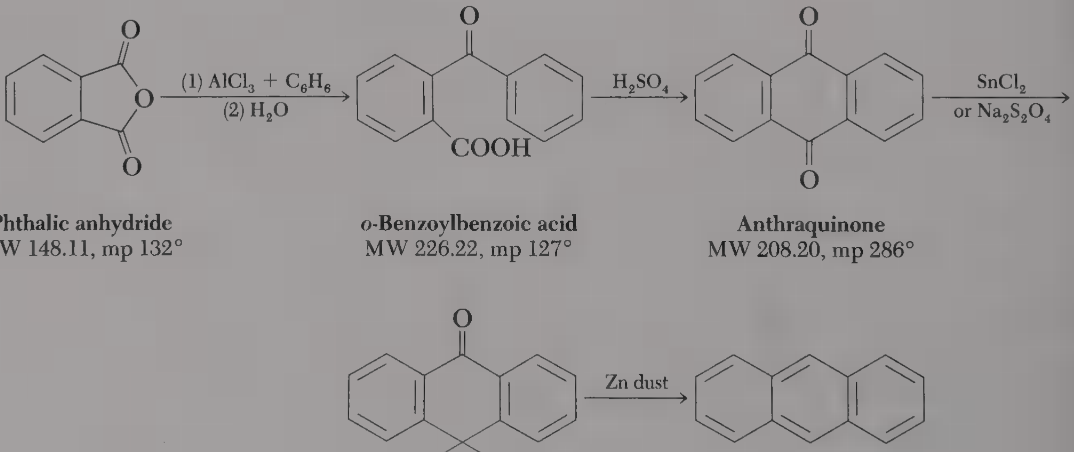


40

Anthraquinone and Anthracene

KEYWORDS

Friedel-Crafts acylation
o-Benzoylbenzoic acid
HCl trap
$AlCl_3$, two equivalents
Cyclodehydration, H_2SO_4
Reduction, stannous chloride
Sodium hydroxide, sodium hydrosulfite
Anthrone



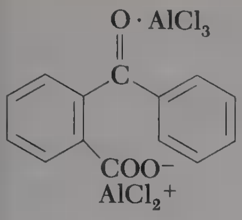
Phthalic anhydride
MW 148.11, mp 132°

***o*-Benzoylbenzoic acid**
MW 226.22, mp 127°

Anthraquinone
MW 208.20, mp 286°

Anthrone
MW 194.22, mp 156°

Anthracene
MW 178.22, mp 216°



Complex salt

The Friedel-Crafts reaction of phthalic anhydride with excess benzene as solvent and two equivalents of aluminum chloride proceeds rapidly and gives a complex salt of *o*-benzoylbenzoic acid in which one mole of aluminum chloride has reacted with the acid function to form the salt $RCO_2^-AlCl_2^+$ and a second mole is bound to the carbonyl group. On addition of ice and hydrochloric acid the complex is decomposed and basic aluminum salts are brought into solution.

Treatment of *o*-benzoylbenzoic acid with concentrated sulfuric acid effects cyclodehydration to anthraquinone, a pale-yellow high-melting compound of great stability. Since anthraquinone can be sulfonated only under forcing conditions, a high temperature can be used to shorten the reaction time without loss in yield of product; the conditions are so adjusted that anthraquinone separates from the hot solution in crystalline form favoring rapid drying.

▼
$Na_2S_2O_4$
Sodium hydrosulfite

Reduction of anthraquinone to anthrone can be accomplished rapidly on a small scale with tin(II) chloride in acetic acid solution. A second method, which involves refluxing anthraquinone with an aqueous solution of sodium hydroxide and sodium hydrosulfite, is interesting to observe because of the sequence of color changes: anthraquinone is reduced first to a deep red liquid containing anthrahydroquinone dianion; the red color then gives place to a yellow color characteristic of anthranol anion; as the alkali is neutralized by the conversion of $Na_2S_2O_4$ to 2 $NaHSO_3$, anthranol ketonizes to the more stable anthrone. The second method is preferred in industry, because sodium hydrosulfite costs less than half as much as tin(II) chloride and because water is cheaper than acetic acid and no solvent recovery problem is involved.

Reduction of anthrone to anthracene is accomplished by refluxing in aqueous sodium hydroxide solution with activated zinc dust. The method has the merit of affording pure, beautifully fluorescent anthracene.

EXPERIMENTS

1. o-Benzoylbenzoic Acid

This Friedel-Crafts reaction is conducted in a 500-ml round-bottomed flask equipped with a short condenser. A trap for collecting hydrogen chloride liberated is connected to the top of the condenser by rubber tubing of sufficient length to make it possible either to heat the flask on the steam bath or to plunge it into an ice bath. The trap is a suction flask half filled with water and fitted with a delivery tube inserted to within 1 cm of the surface of the water (see Fig. 12.1).

▼
Benzene is a mild carcinogen. Carry out this experiment in a hood. Avoid contact of the benzene with the skin.

Fifteen grams of phthalic anhydride and 75 ml of reagent grade benzene (*caution!*) are placed in the flask and this solution is cooled in an ice bath until the benzene begins to crystallize. Ice cooling serves to moderate the vigorous reaction which otherwise might be difficult to control. Thirty grams of anhydrous aluminum chloride[1] is added, the condenser and trap are connected,

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[1] This is best weighed in a stoppered test tube. The chloride should be from a freshly opened bottle.

and the flask is shaken well and warmed for a few minutes by the heat of the hand. If the reaction does not start, the flask is warmed *very gently* by holding it for a few seconds over the steam bath. At the first sign of vigorous boiling, or evolution of hydrogen chloride, the flask is held over the ice bath in readiness to cool it if the reaction becomes too vigorous. This gentle, cautious heating is continued until the reaction is proceeding smoothly enough to be refluxed on the steam bath. This point is reached in about 5 min. Continue the heating on the steam bath, swirl the mixture, and watch it carefully for sudden separation of the addition compound, since the heat of crystallization is such that it may be necessary to plunge the flask into the ice bath to moderate the process. Once the addition compound has separated as a thick paste, heat the mixture for 10 min more on the steam bath, remove the condenser, and swirl the flask in an ice bath until cold. (Should no complex separate, heat for 10 min more and then proceed as directed.) Take the flask and ice bath to the hood, weigh out 100 g of ice, add a few small pieces of ice to the mixture, swirl and cool as necessary, and wait until the ice has reacted before adding more. After the 100 g of ice have been added and the reaction of decomposition has subsided, add 20 ml of concentrated hydrochloric acid, 100 ml of water, swirl vigorously, and make sure that the mixture is at room temperature. Then add 50 ml of water, swirl vigorously, and again make sure the mixture is at room temperature. Add 50 ml of ether and, with a flattened stirring rod, dislodge solid from the neck and walls of the flask and break up lumps at the bottom. To further promote hydrolysis of the addition compound, extraction of the organic product, and solution of basic aluminum halides, stopper the flask with a cork and shake vigorously for several minutes.

▼
Short reaction period, high yield

When most of the solid has disappeared, pour the mixture through a funnel into a separatory funnel until the separatory funnel is nearly filled. Discard the lower aqueous layer. Pour the rest of the mixture into the separatory funnel, rinse the reaction flask with fresh ether, and again drain off the aqueous layer. To reduce the fluffy, dirty precipitate that appears at the interface add 10 ml of concentrated hydrochloric acid and 25 ml of water, shake vigorously for 2–3 min, and drain off the aqueous layer. If some interfacial dirty emulsion still persists, decant the benzene-ether solution through the mouth of the funnel into a filter paper for gravity filtration and use fresh ether to rinse the funnel. Clean the funnel and pour in the filtered benzene-ether solution. Shake the solution with a portion of dilute hydrochloric acid, and then isolate the reaction product by either of the following procedures:

▼
Alternative procedures

(a) Add 50 ml of 10% sodium hydroxide solution, shake thoroughly, and separate the aqueous layer.[2] Extract with a further 25-ml portion of aqueous alkali and combine the extracts. Wash with 10 ml of water, and add this aqueous solution to the 75 ml of aqueous extract already collected. Discard the benzene-ether solution. Acidify the 85 ml of combined alkaline extract with concentrated hydrochloric acid to pH 1–2 and, if the *o*-benzoylbenzoic acid separates

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[2] The nature of a yellow pigment that appears in the first alkaline extract is unknown; the impurity is apparently transient, for the final product dissolves in alkali to give a colorless solution.

as an oil, cool in ice and rub the walls of the flask with a stirring rod to induce crystallization of the hydrate; collect the product and wash it well with water. This material is the monohydrate: $C_6H_5COC_6H_4CO_2H \cdot H_2O$. To convert it into anhydrous *o*-benzoylbenzoic acid, put it in a tared, 250-ml round-bottomed flask, evacuate the flask at the full force of the aspirator, and heat it in the open rings of a steam bath covering the flask with a towel. Check the weight of the flask and contents for constancy after 45 min, 1 hr, and 1.25 hr. Yield 19–21 g, mp 126–127°.

▼
Drying time: about 1 hr

(b) Filter the benzene-ether solution through anhydrous sodium sulfate for superficial drying, put it into a 250-ml round-bottomed flask, and distil over the steam bath through a condenser into an ice-cooled receiver until the volume in the distilling flask is reduced to about 55 ml. Add ligroin slowly to slight turbidity and let the product crystallize at 25° and then at 5°. The yield of anhydrous, colorless, well-formed crystals, mp 127–128°, is about 18–20 g.

▼
Extinguish flames

Any *o*-benzoylbenzoic acid not used in the next preparation should be saved for the experiment of Chapter 41.

2. Anthraquinone

Place 5.0 g of *o*-benzoylbenzoic acid (anhydrous) in a 125-ml round-bottomed flask, add 25 ml of concentrated sulfuric acid, and heat on the steam bath with swirling until the solid is dissolved. Then clamp the flask over a microburner, insert a thermometer, raise the temperature to 150°, and heat to maintain a temperature of 150–155° for 5 min. Let the solution cool to 100°, remove the thermometer after letting it drain, and, with a capillary dropping tube, add 5 ml of water by drops with swirling to keep the precipitated material dissolved as long as possible so that it will separate as small, easily filtered crystals. Let the mixture cool further, dilute with water until the flask is full, again let cool, collect the product by suction filtration, and wash well with water. Then remove the filtrate and wash the filter flask, return the funnel to the filter flask but do not apply suction, and test the filter cake for unreacted starting material as follows: Dilute 10 ml of concentrated ammonia solution with 50 ml of water, pour the solution onto the filter and loosen the cake so that it is well leached. Then apply suction, wash the cake with water, and acidify a few ml of the filtrate. If there is no precipitate upon acidifying, the yield of anthraquinone should be close to the theory, since it is insoluble in water. Dry the product to constant weight but do not take the melting point since it is so high (mp 286°).

▼
Reaction time: 10 min

3. Anthrone

(a) Tin(II) Chloride Reduction

In a 125-ml round-bottomed flask provided with a reflux condenser put 5.0 g of anthraquinone, 40 ml of acetic acid, and a solution made by warming 13 g of tin(II) chloride dihydrate with 13 ml of concentrated hydrochloric acid.

▼
Procedure (a)

Add a boiling stone to the reaction mixture, note the time, and reflux gently until crystals of anthraquinone have completely disappeared (8–10 min); then reflux 15 min longer and record the total time. Disconnect the flask, heat it on the steam bath, and add water (about 12 ml) in 1-ml portions until the solution is saturated. Let the solution stand for crystallization. Collect and dry the product and take the melting point (156° given). The yield of pale yellow crystals is 4.3 g.

(b) Hydrosulfite Reduction

▼
Procedure (b)

In a 500-ml round-bottomed flask, which can be heated under reflux, put 5.0 g of anthraquinone, 6 g of sodium hydroxide, 15 g of sodium hydrosulfite, and 130 ml of water. Heat over a free flame and swirl for a few minutes to convert the anthraquinone into the deep red anthrahydroquinone anion. Note that particles of different appearance begin to separate even before the anthraquinone has all dissolved. Arrange for refluxing and reflux for 45 min; cool, filter the product, and wash it well and let dry. Note the weight of the product and melting point of the crude material and then crystallize it from 95% ethanol; the solution may require filtration to remove insoluble impurities. Record the approximate volume of solvent used and, if the first crop of crystals recovered is not satisfactory, concentrate the mother liquor and secure a second crop.

4. Comparison of Results

Compare your results with those obtained by neighbors using the alternative procedure with respect to yield, quality of product, and working time. Which is the better laboratory procedure? Then consider the cost of the three solvents concerned, the cost of the two reducing agents (current prices to be posted), the relative ease of recovery of the organic solvents, and the prudent disposal of by-products, and decide which method would be preferred as a manufacturing process.

5. Special Experiment: Fluorescent Anthracene

▼
Reflux time: 0.5 hr

Put 10 g of zinc dust into a 500-ml round-bottomed flask and activate the dust by adding 60 ml of water and 1 ml of copper(II) sulfate solution (Fehling solution I) and swirling for a minute or two. Add 4.0 g of anthrone, 10 g of sodium hydroxide, and 100 ml of water; attach a reflux condenser, heat to boiling, note the time, and start refluxing the mixture. Anthrone at first dissolves as the yellow anion of anthranol, but anthracene soon begins to separate as a white precipitate. In about 15 min the yellow color initially observed on the walls disappears, but refluxing should be continued for a full 30 min. Then remove the flame, use a water wash bottle to rinse down anthracene that has lodged in the condenser, and filter the still hot mixture on a large Büchner funnel. It usually is possible to decant from, and so remove, a mass of zinc. After liberal washing with water, blow or shake out the gray cake into a 400-ml beaker and rinse funnel and paper with water. To remove most of the zinc metal and zinc oxide, add 20 ml of concentrated hydrochloric acid, heat on the steam bath with stirring for 20–25 min when initial frothing due to liberated hydrogen should have ceased. Collect the now nearly white precipitate on a large Büchner funnel and, after liberal washing with water, release the suction, rinse the walls of the funnel with methanol, use enough more methanol to cover the cake, and then apply suction. Wash again with enough methanol to cover the cake and then remove solvent thoroughly by suction.

▼
Methanol removes water without dissolving much anthracene

The product need not be dried before crystallization from toluene. Transfer the methanol-moist material to a 125-ml Erlenmeyer and add 60 ml of toluene; a liberal excess of solvent is used to avoid crystallization in the funnel. Make sure there are no flames nearby and heat the mixture on the hot plate to bring the anthracene into solution but leaving a small residue of zinc. Filter by gravity by the usual technique to remove zinc. From the filtrate anthracene is obtained as thin, colorless, beautifully fluorescent plates; yield about 2.8 g. In washing the equipment with acetone, you should be able to observe the striking fluorescence of very dilute solutions. The fluorescence is quenched by a bare trace of impurity.

▼
Caution! Highly flammable solvent

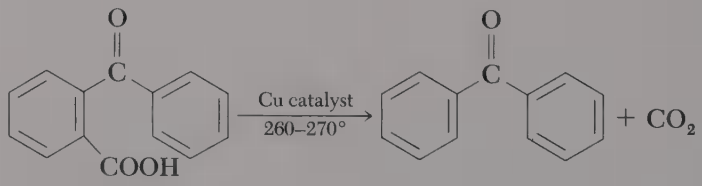
41

Benzophenone and Benzopinacol—A Photochemical Reaction

KEYWORDS

Photochemistry, photochemical reduction
Decarboxylation, Cu catalyst
Labile, allotropic
Electronically excited, triplet state
Radicals
Benzopinacol, alkaline cleavage
Benzhydrol, benzophenone
Pinacol-pinacolone rearrangement
Carbonium ion

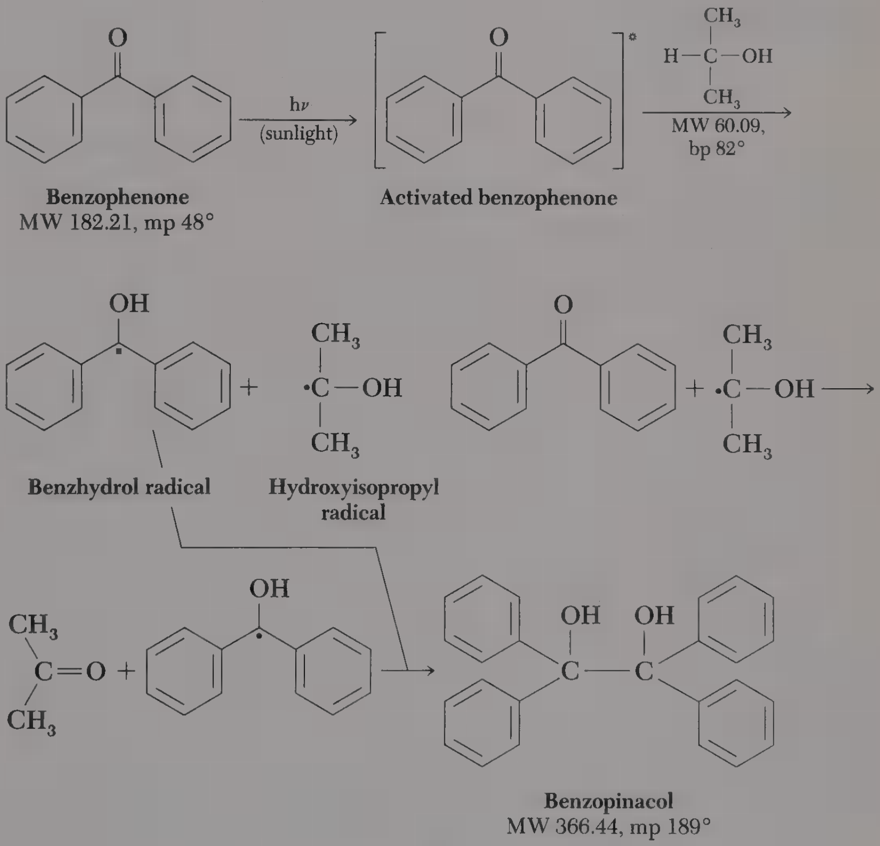
Of the several methods possible for the preparation of benzophenone, the one used here is decarboxylation of *o*-benzoylbenzoic acid in the presence of copper catalyst derived from copper carbonate.



o-Benzoylbenzoic acid **Benzophenone**

The reaction is conducted by heating the molten acid and catalyst to the high temperature required; in the absence of catalyst the acid is cyclized to anthraquinone. When decarboxylation is complete the product is isolated by distillation from the reaction mixture. Benzophenone exists in a labile form, mp 26°, and a stable allotropic form, mp 48°. Distillation usually gives the labile form as a liquid which, if let stand undisturbed in the absence of seed crystals, very slowly solidifies to transparent crystals. However, if the flask is scratched, if the liquid is inoculated with the stable form, or if the liquid is in an atmosphere carrying seed crystals, it suddenly changes to the stable form and solidifies.

Benzophenone is colorless, and like aliphatic α,β-unsaturated ketones it absorbs ultraviolet light with formation of an electronically excited state. This activated ketone, thought to be in the triplet state, abstracts a hydrogen atom from the solvent, isopropyl alcohol, to produce two radicals, benzhydrol and hydroxyisopropyl. The hydroxyisopropyl radical transfers a hydrogen atom to neutral benzophenone, giving another benzhydrol radical and a molecule of acetone. Combination of the benzhydrol radicals produces the product benzopinacol, which crystallizes from the solution in dramatic fashion when the reactants are exposed to bright sunlight. The experiment should be done when there is good prospect for long hours of bright sunshine for several days. The benzopinacol is cleaved by alkali to benzhydrol and benzophenone (Experiment 3) and it is rearranged in acid to benzopinacolone (Experiment 4).



EXPERIMENTS

1. Benzophenone

Place 15 g of anhydrous *o*-benzoylbenzoic acid and 0.5 g of basic copper(II) carbonate dihydroxide in a 50-ml round-bottomed flask and heat gently, with shaking, over a free flame to melt the acid and effect neutralization. Attach a glass tube through a rubber stopper, evacuate the flask and heat it on the steam bath to eliminate the water formed. Then support the flask for heating with a free flame, insert a thermometer with the bulb submerged in the melt, heat with a small flame, and raise the temperature to 265°, at which point evolution of carbon dioxide starts. Note the time and, by intermittent heating, keep the temperature close to 265° until, in 20–25 min, metallic copper separates from the clear solution. Continue to heat at 265° for 5 min longer (when evolution of CO_2 should stop), remove the flame, and withdraw the thermometer. Fit the flask with a stillhead, thermometer, and an air condenser (empty fractionating column); use a small tared Erlenmeyer as receiver and distil the benzophenone. The corrected boiling point is 306°; the uncorrected boiling point may be as low as 294°. Continue the distillation until there is a marked rise in boiling point (4–5°) or until the distillate becomes dark yellow.[1]

▼
Reaction time: 30 min

Record the weight of crude benzophenone (11–13 g) and see if it will solidify when cooled or rubbed with a stirring rod. One usually obtains at this point the labile form, which (particularly since it is not pure) does not solidify easily. If this is the case, fix a barely visible particle of ordinary benzophenone on the end of a stirring rod and rub it into the liquid against the side of the flask. When crystallization sets in, note the warming against the palm of the hand. Save a trace of seed. Benzophenone crystallizes well from ligroin, bp 60–90°, (alcohol leaves too much material in the mother liquor). Without removing the material from the original receiving flask, cover it with ligroin (1.5 ml per gram) and dissolve by heating on the steam bath. Without using charcoal or filtering, cool the solution in an ice bath until it becomes cloudy and the product oils out. Then remove the flask from the bath, allow the liquid to come to rest, and add a seed crystal. As crystallization progresses, return the flask to the ice bath and eventually stir the mixture and cool it thoroughly. Collect the crystals on a suction funnel and use 30–40 ml of fresh, ice-cold solvent (ligroin) to wash any yellow material into the mother liquor. Recrystallize the product if it is not white; very large crystals can be obtained by allowing the solution to cool slowly and without seeding; yield, 9.5–11.5 g, mp 47–48°.

2. Benzopinacol

In a 125-ml round-bottomed flask dissolve 10 g of benzophenone in 60–70 ml of isopropyl alcohol by warming on the steam bath, fill the flask to the neck

---

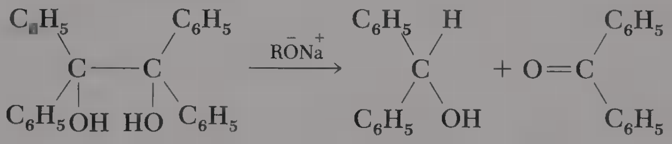
[1] The dark residue in the distillation flask can be loosened and the mass of copper dislodged by adding toluene and heating the flask on the steam bath for one half hour. The dark solution is decanted and if necessary the process is repeated.

with more of this alcohol, and add one drop of glacial acetic acid. (If the acid is omitted enough alkali may be derived from the glass of the flask to destroy the reaction product by the alkaline cleavage described in Experiment 3.) Stopper the flask with a well-rolled, tight-fitting cork, which is then wired in place. Invert the flask in a 100-ml beaker placed where the mixture will be most exposed to direct sunlight for some time. Since benzopinacol is but sparingly soluble in alcohol, its formation can be followed by the separation from around the walls of the flask of small, colorless crystals (benzophenone forms large, thick prisms). If the reaction mixture is exposed to direct sunlight throughout the daylight hours, the first crystals separate in about 5 hr and the reaction is practically complete (95% yield) in four days. In winter the reaction may take as long as two weeks, and any benzophenone which crystallizes must be brought into solution by warming on the steam bath. When the reaction appears to be over, chill the flask if necessary and collect the product. The material should be pure, mp 188–189°. If the yield is low, more material can be obtained by further exposure of the mother liquor to sunlight.

▼
Reaction time: 4 days to two weeks

3. Alkaline Cleavage

Suspend a small test sample of benzopinacol in alcohol and heat to boiling, making sure that the amount of solvent is not sufficient to dissolve the solid. Add one drop of sodium hydroxide solution, heat for a minute or two, and observe the result. The solution contains equal parts of benzhydrol and benzophenone, formed by the following reaction:



Benzopinacol Mp 189°

Benzhydrol Mp 68°

Benzophenone Mp 48°

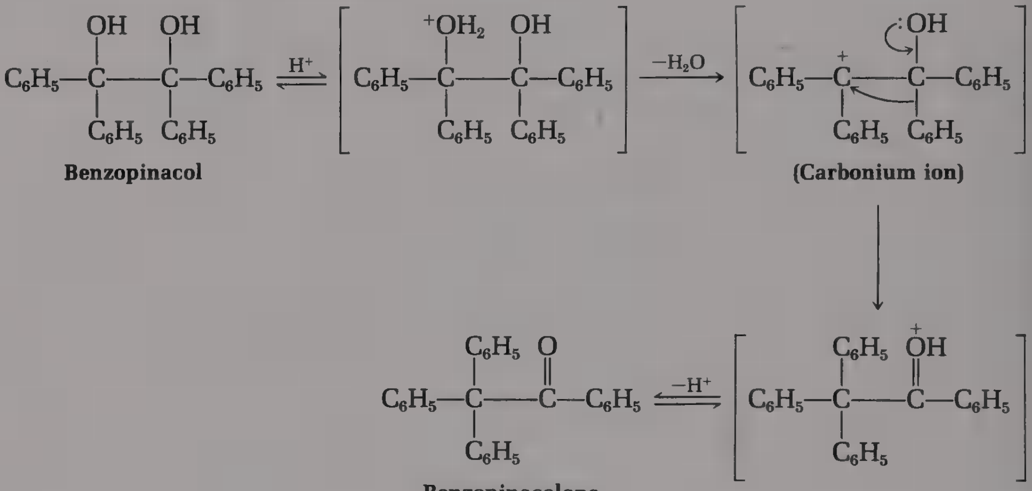
The low-melting products resulting from the cleavage are much more soluble than the starting material.

Benzophenone can be converted into benzhydrol in nearly quantitative yield by following the procedure outlined above for the preparation of benzopinacol, modified by addition of a very small piece of sodium (0.05 g) instead of the acetic acid. The reaction is complete when, after exposure to sunlight, the greenish-blue color disappears. To obtain the benzhydrol the solution is diluted with water, acidified, and evaporated. Benzopinacol is produced as before by photochemical reduction, but it is at once cleaved by the sodium alkoxide; the benzophenone formed by cleavage is converted into more benzopinacol, cleaved, and eventually consumed.

4. Pinacolone Rearrangement

This acid-catalyzed, carbonium ion rearrangement is characterized by rapidity and by the high yield.

In a 125-ml round-bottomed flask place 5 g of benzopinacol, 25 ml of acetic acid, and two or three very small crystals of iodine (0.05 g). Heat to the boiling point for a minute or two under a reflux condenser until the crystals are dissolved, and then reflux the red solution for 5 min. On cooling, the pinacolone separates as a stiff paste. Thin the paste with alcohol, collect the product, and wash it free from iodine with alcohol. The material should be pure; yield 95%.



Benzopinacol

(Carbonium ion)

Benzopinacolone
Mp 179–180°

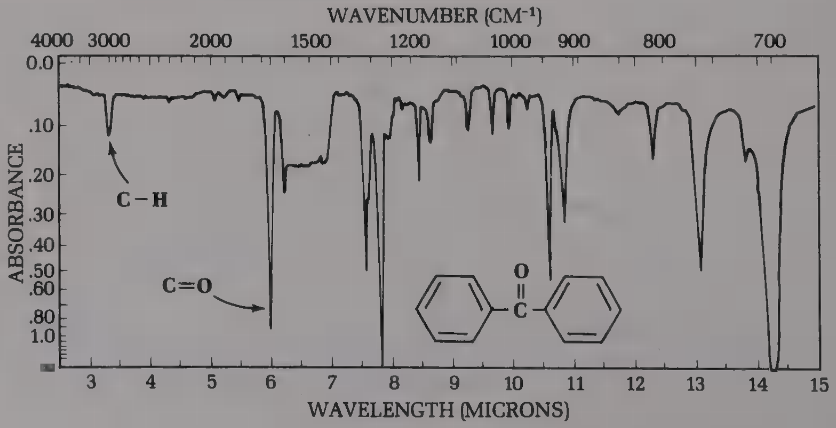


FIGURE 41.1 Infrared spectrum of benzophenone in CS_2.

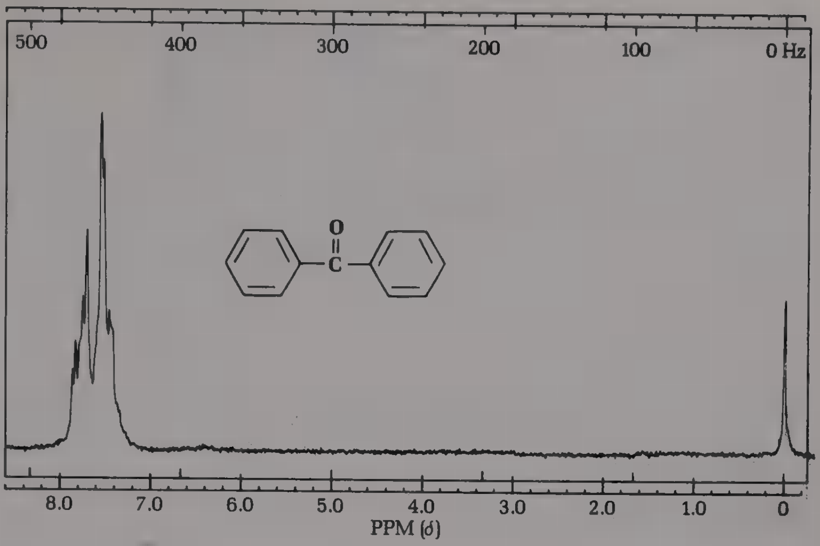


FIGURE 41.2 ^{1}H nmr spectrum of benzophenone.

QUESTION

Write a mechanism for the alkaline cleavage of benzopinacol to benzhydrol and benzophenone.

42

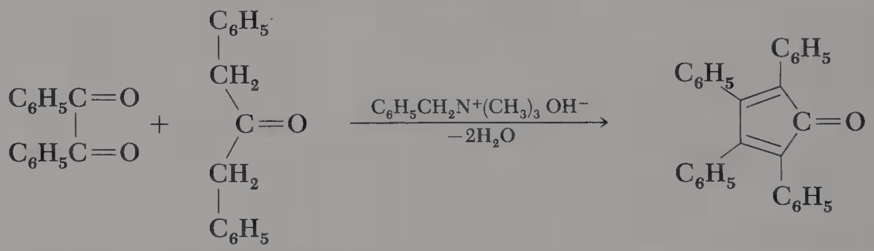
Tetraphenylcyclopentadienone

KEYWORDS

Base-catalyzed condensation reaction

Triethylene glycol, high boiling solvent

Benzyltrimethylammonium hydroxide, basic catalyst



Benzil
mp 96°,
MW 210.22

Dibenzyl ketone
mp 35°,
MW 210.26

Tetraphenylcyclopentadienone
mp 219°, MW 384.45

Cyclopentadienone is an elusive compound which has been sought for many years but with little success. Molecular orbital calculations predict that it should be highly reactive, and so it is; it exists only as the dimer. The tetraphenyl derivative of this compound is to be synthesized in this experiment. This derivative is stable, and reacts readily with dienophiles. It is used not only for the synthesis of highly aromatic, highly arylated compounds, but also for examination of the mechanism of the Diels-Alder reaction itself. Tetraphenylcyclopentadienone has been carefully studied by means of molecular orbital methods in attempts to understand its unusual reactivity, color, and dipole moment. In Chapter 44 this highly reactive molecule is used to trap the fleeting benzyne to form tetraphenylnaphthalene. Indeed this reaction constitutes evidence that benzyne does exist.

The literature procedure for condensation of benzil with dibenzyl ketone in ethanol with potassium hydroxide as basic catalyst suffers from the low boiling point of the alcohol and the limited solubility of both potassium hydroxide and the reaction product in this solvent. Triethylene glycol is a better solvent and permits operation at a higher temperature. In the procedure that follows, the glycol is used with benzyltrimethylammonium hydroxide, a strong base readily soluble in organic solvents, which serves as catalyst.

EXPERIMENT

▼
Short reaction period

Measure into a 25 × 150-mm test tube 2.1 g of benzil, 2.1 g of dibenzyl ketone, and 10 ml of triethylene glycol, using the solvent to wash the walls of the test tube. Support the test tube over a microburner, stir the mixture with a thermometer, and heat with a small flame until the benzil is dissolved and remove the flame. Measure 1 ml of a commercially available 40% solution of benzyltrimethylammonium hydroxide (Triton B) in methanol into a 10 × 75-mm test tube, adjust the temperature of the solution to exactly 100°, remove the flame, add the catalyst, and stir once to mix. Crystallization usually starts in 10–20 seconds. Let the temperature drop to about 80° and then cool under the tap, add 10 ml of methanol, stir to a thin crystal slurry, collect the product, and wash it with methanol until the filtrate is purple-pink, not brown. The yield of deep purple crystals is 3.3–3.7 g. If either the crystals are not well formed or if the melting point is low, place 1 g of material and 10 ml of triethylene glycol in a vertically supported test tube, stir with a thermometer, raise the temperature to 220° to bring the solid into solution, and let stand for crystallization (if initially pure material is recrystallized, the recovery is 92%).

QUESTION

Write a detailed mechanism for this reaction.

43

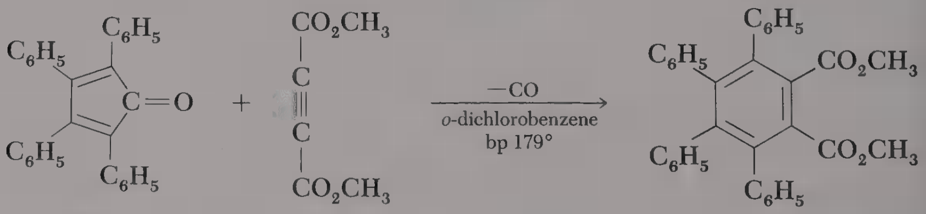
Derivatives of Tetraphenylcyclopentadienone

KEYWORDS

Diels-Alder reaction
Acetylenic dienophile
o-Dichlorobenzene, high boiling solvent
Carbon monoxide
Dienophile, diene
Diphenyl ether, high boiling recrystallization solvent
Soxhlet extractor
Cold finger condenser

EXPERIMENT

1. Dimethyl Tetraphenylphthalate



MW 384.45

Dimethyl acetylenedicarboxylate
Liq., MW 142.11

Dimethyl tetraphenylphthalate
mp 258°, MW 474.53

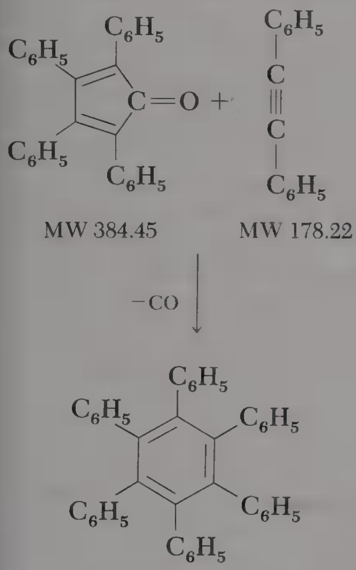
Tetraphenylcyclopentadienone undergoes Diels-Alder reaction with dienophiles with expulsion of carbon monoxide. An acetylenic dienophile is selected for this experiment because it affords an adduct which is directly aromatic. The solvent specified, *o*-dichlorobenzene, has a boiling point such that the reaction is over in a minute or two.

Procedure

▼
Caution! See footnotes

Measure into a 25 × 150-mm test tube 2 g of tetraphenylcyclopentadienone, 10 ml of *o*-dichlorobenzene,[1] and 1 ml (1.1 g) of dimethyl acetylenedicarboxylate.[2] Clamp the test tube over a microburner, insert a thermometer, and raise the temperature to the boiling point (180–185°). Boil gently until there is no further color change and let the rim of condensate rise just high enough to wash the walls of the tube. The pure adduct is colorless, and if the starting ketone is adequately pure the color changes from purple to pale tan. A 5-min boiling period should be sufficient. Cool to 100°, slowly stir in 15 ml of 95% ethanol, and let crystallization proceed. Cool under the tap, collect the product, and rinse the tube with methanol. Yield of colorless crystals should be in the range of 2.1–2.2 g.

▼
Reaction time about 5 min



Hexaphenylbenzene
MW 534.66, mp 465°

2. Hexaphenylbenzene

Diphenylacetylene is a less reactive dienophile than dimethyl acetylenedicarboxylate, but when heated with tetraphenylcyclopentadienone without solvent a temperature (*ca.* 380–400°) suitable for reaction can be attained. In the following procedure the dienophile is taken in large excess to serve as solvent. Since refluxing diphenylacetylene (bp about 300°) keeps the temperature below the melting point of the product, the excess diphenylacetylene is removed in order that the reaction mixture can be melted to ensure completion of the reaction.

Diphenyl ether (mp 27°, bp 259°) is selected for crystallization of the sparingly soluble product because of its high boiling point and its superior solvent power.

Procedure

▼
A hydrocarbon of very high mp

Place 0.5 g each of tetraphenylcyclopentadienone and diphenylacetylene in a 25 × 150-mm test tube supported in a clamp and heat the mixture strongly with the free flame of a microburner held in the hand (do not insert a thermometer into the test tube; the temperature will be too high). Soon after the reactants have melted with strong bubbling, white masses of the product become visible. Let the diphenylacetylene reflux briefly on the walls of the

---

[1] *o*-Dichlorobenzene is toxic and carbon monoxide is evolved during the reaction. Carry out this experiment in a hood.

[2] This ester is a powerful lachrymator and vesicant and should be dispensed from a bottle provided with a pipette and a pipetter. Even a trace of ester on the skin should be washed off with methanol, followed by soap and water.

tube and then remove some of the diphenylacetylene by letting it condense for a minute or two on a cold finger filled with water, but without fresh water running through it. Remove the flame, withdraw the cold finger, and wipe it with a towel. Repeat the operation until you are able, by strong heating, to melt the mixture completely. Then let the melt cool and solidify. Add 10 ml of diphenyl ether, using it to rinse the walls. Heat *carefully* over a free flame to dissolve the solid and then let the product crystallize. When cold, add 10 ml of toluene to thin out the mixture, collect the product, and wash with toluene. The yield of colorless plates, mp 465°, is 0.6–0.7 g.

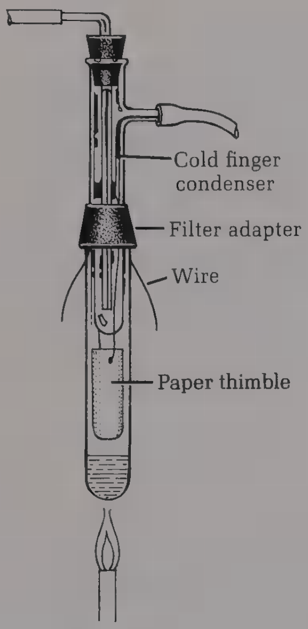


FIGURE 43.1 Soxhlet-type extractor.

Notes:

1. The melting point of the product can be determined with a Mel-Temp apparatus and a 500°-thermometer. To avoid oxidation, seal the sample in an evacuated capillary tube.
2. In case the hexaphenylbenzene is contaminated with insoluble material, crystallization from a filtered solution can be accomplished as follows: Place 10 ml of diphenyl ether in a 25 × 150-mm test tube and pack the sample into a 10-mm extraction thimble and suspend this in the test tube with two nichrome wires, as shown in Fig. 43.1. Insert a cold finger condenser supported by a filter adapter and adjust the condenser and the wires so that condensing liquid will drop into the thimble. Let the diphenyl ether reflux until the hexaphenylbenzene in the thimble is dissolved, and then let the product crystallize, add toluene, collect the product, and wash with toluene as described previously.

QUESTIONS

1. Draw the structures of the bicyclic compounds which must be intermediates in these Diels-Alder reactions.
2. What is the driving force for loss of carbon monoxide from these intermediates?

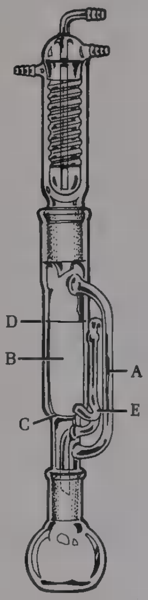


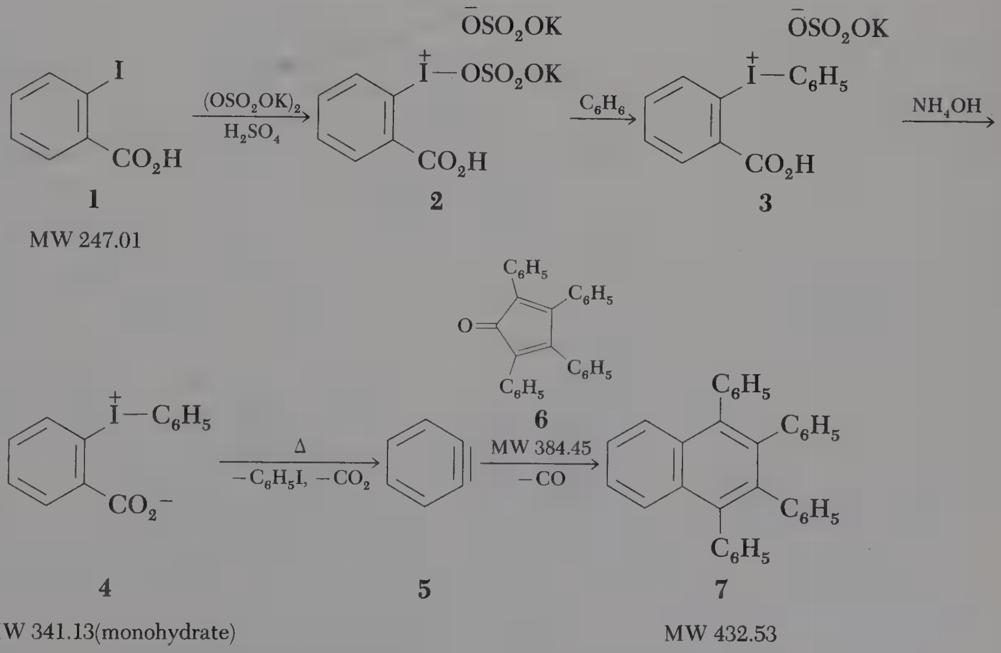
FIGURE 43.2 Research quality Soxhlet extractor. Solvent vapors from the flask rise through A and up into the condenser. As they condense the liquid just formed returns to B, where sample to be extracted is placed. (Bottom of B is sealed at C.) Liquid rises in B to level D at which time the automatic siphon, E, starts. Extracted material accumulates in the flask as more pure liquid vaporizes to repeat the process.

44

1,2,3,4-Tetraphenylnaphthalene via Benzyne

KEYWORDS

Benzyne
Trapping agent
Diels-Alder reaction
Iodonium salts
Potassium persulfate
o-Iodobenzoic acid synthesis
Anthranilic acid, diazotization



▼
Synthetic use of an intermediate not known as such

This synthesis of 1,2,3,4-tetraphenylnaphthalene (**7**) demonstrates the transient existence of benzyne (**5**), a hydrocarbon which has not been isolated as such. The precursor, diphenyliodonium-2-carboxylate (**4**), is heated in an inert solvent to a temperature at which it decomposes to benzyne, iodobenzene, and carbon dioxide in the presence of tetraphenylcyclopentadienone (**6**) as trapping agent. The preparation of the precursor **4** illustrates oxidation of a derivative of iodobenzene to an iodonium salt (**2**) and the Friedel-Crafts-like reaction of the substance with benzene to form the diphenyliodonium salt **3**. Neutralization with ammonium hydroxide then liberates the precursor, inner salt, **4**, which when obtained by crystallization from water is the monohydrate.

The procedure affords about twice the amount of precursor (**4**) required for the synthesis of (**7**). Hence samples of both (**4**) and (**7**) should be submitted for evaluation.

EXPERIMENTS

1. Diphenyliodonium-2-carboxylate monohydrate (4)

▼
$K_2S_2O_8$
Potassium persulfate
(Potassium peroxydisulfate)
MW 270.33

▼
1st step: about 20 min

Measure 8 ml of concentrated sulfuric acid into a 25-ml Erlenmeyer flask and place the flask in an ice bath to cool. Place 2.0 g of *o*-iodobenzoic acid[1] and 2.6 g of potassium persulfate[2] in a 125-ml Erlenmeyer flask, swirl the flask containing sulfuric acid vigorously in the ice bath for 2–3 min and then remove it and wipe it dry. Place the larger flask in the ice bath and pour the chilled acid down the walls to dislodge any particles of solid. Swirl the flask in the ice bath for 4–5 min to produce an even suspension and then remove it and note the time. The reaction mixture foams somewhat and acquires a succession of colors. After it has stood at room temperature for 20 min, swirl the flask vigorously in an ice bath for 3–4 min, add 2 ml of benzene (*caution!*) and swirl and cool until the benzene freezes. Then remove and wipe the flask and note the time at which the benzene melts. Warm the flask in the palm of the hand and swirl frequently at room temperature for 20 min to promote completion of reaction in the two-phase mixture.

▼
Caution! Benzene is a mild carcinogen. Carry out this experiment in the hood.

▼
2nd step: about 30 min

While the reaction is going to completion, place three 50-ml Erlenmeyer flasks in an ice bath to chill: one containing 19 ml of distilled water, another 23 ml of 29% ammonium hydroxide solution, and another 40 ml of dichloromethane (bp 40.8°). At the end of the 20-min reaction period, chill the reaction mixture thoroughly in an ice bath, mount a separatory funnel to deliver into the flask containing the reaction mixture in benzene, and place in the funnel the chilled 19 ml of water. Swirl the reaction flask vigorously while running in the water slowly. The solid that separates is **3**, the potassium bisulfate salt of diphenyliodonium-2-carboxylic acid. Pour the chilled ammonia solution into the funnel and pour the chilled dichloromethane into the reaction flask so that it will be available for efficient extraction of the reaction product (**4**) as it is

▼
Note for the instructor

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[1] See note at end of this chapter.

[2] The fine granular material suplied by Fisher Scientific Co. is satisfactory. Persulfate in the form of large prisms should be finely ground prior to use.

liberated from 3 on neutralization. While swirling the flask vigorously in the ice bath, run in the chilled ammonia solution during the course of about 10 min. The mixture must be alkaline (pH 9). If not, add more ammonia solution. Pour the mixture into a separatory funnel and rinse the flask with a little fresh dichloromethane. Let the layers separate and run the lower layer into a tared 125-ml Erlenmeyer flask, through a funnel fitted with a paper containing anhydrous sodium sulfate. Extract the aqueous solution with two 10-ml portions of dichloromethane and run the extracts through the drying agent into the tared flask. Evaporate the dried extracts to dryness on the steam bath and remove solvent from the residual cake of solid by connecting the flask, with a rubber stopper, to the aspirator and heating the flask on the steam bath until the weight is constant; yield about 2.4 g.

For crystallization, dislodge the bulk of the solid with a spatula and transfer it onto weighing paper and then into a 50-ml Erlenmeyer flask. Measure 28 ml of distilled water into a flask and use part of the water to dissolve the residual material in the tared 125 ml flask by heating the mixture to the boiling point over a free flame. Pour this solution into the 50-ml flask. Add the remainder of the 28 ml of water to the 50 ml flask and bring the solid into solution at the boiling point. Add a small portion of charcoal for decolorization of the pale tan solution, swirl, and filter at the boiling point through a funnel, preheated on the steam bath and fitted with moistened filter paper. Diphenyliodonium-2-carboxylate monohydrate (4), the benzyne precursor, separates in colorless, rectangular prisms, mp 219–220°, dec.; yield about 2.1 g.

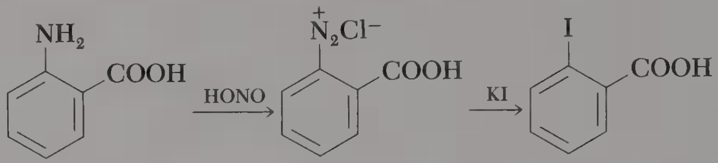
2. Preparation of 1,2,3,4-Tetraphenylnaphthalene (7)

▼
Diels-Alder reaction

Place 1.0 g of the diphenyliodonium-2-carboxylate monohydrate just prepared and 1.0 g of tetraphenylcyclopentadienone in a 25 × 150-ml test tube. Add 6 ml of triethylene glycol dimethyl ether (bp 222°) in a way that the solvent will rinse the walls of the tube. Support the test tube vertically, insert a thermometer, and heat with a microburner. When the temperature reaches 200° remove the burner and note the time. Then keep the mixture at 200–205° by intermittent heating until the purple color is discharged, the evolution of gas (CO_2 + CO) subsides, and a pale yellow solution is obtained. In case a purple or red color persists after 3 min at 200–205°, add additional small amounts of the benzyne precursor and continue to heat until all the solid is dissolved. Let the yellow solution cool to 90° while heating 6 ml of 95% ethanol to the boiling point on the steam bath. Pour the yellow solution into a 25-ml Erlenmeyer flask and use portions of the hot ethanol, drawn into a capillary dropping tube, to rinse the test tube. Add the remainder of the ethanol to the yellow solution and heat at the boiling point. If shiny prisms do not separate at once, add a few drops of water by drops at the boiling point of the ethanol until prisms begin to separate. Let crystallization proceed at room temperature and then at 0°. Collect the product and wash it with methanol. The yield of colorless prisms is 0.8–0.9 g. The pure hydrocarbon (7) exists in two crystalline forms (allotropes) and has a double melting point, the first of which is

▼
Use hood; CO is evolved.

in the range 196–199°. Let the bath cool to about 195°, remove the thermometer and let the sample solidify. Then determine the second melting point which, for the pure hydrocarbon, is 203–204°.



Synthesis of *o*-Iodobenzoic Acid

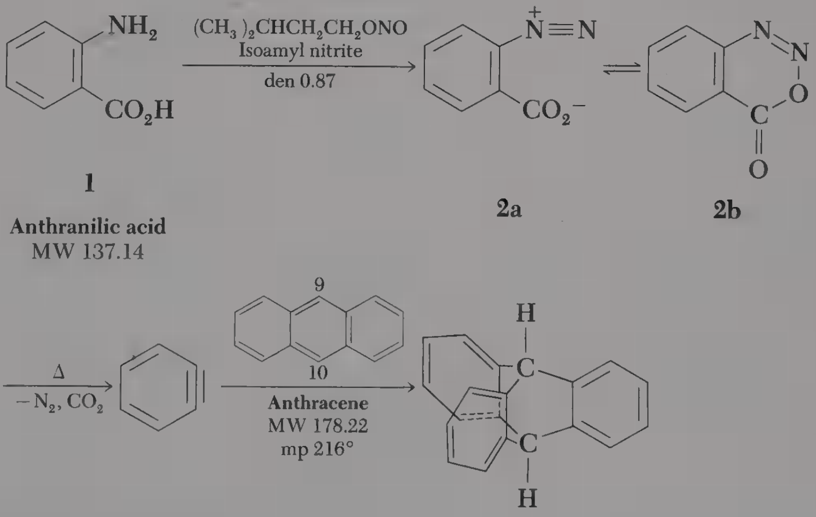
Commercial *o*-iodobenzoic acid is expensive, but the reagent can be prepared easily by diazotization of anthranilic acid followed by a Sandmeyer-like reaction with iodide ion. A 500-ml round-bottomed flask containing 13.7 g of anthranilic acid, 100 ml of water, and 25 ml of concentrated hydrochloric acid is heated until the solid is dissolved. The mixture is then cooled in ice while bubbling in nitrogen to displace the air. When the temperature reaches 0–5° a solution of 7.1 g of sodium nitrite is added slowly. After 5 min a solution of 17 g of potassium iodide in 25 ml of water is added, when a brown complex partially separates. The mixture is let stand without cooling for 5 min (under nitrogen) and then warmed to 40°, at which point a vigorous reaction ensues (gas evolution, separation of a tan solid). After reacting for 10 min the mixture is heated on the steam bath for 10 min and then cooled in ice. A pinch of sodium bisulfite is added to destroy any iodine present and the granular tan product collected and washed with water. The still moist product is dissolved in 70 ml of 95% ethanol, 35 ml of hot water is added, and the brown solution treated with decolorizing charcoal, filtered, diluted at the boiling point with 35–40 ml of water, and let stand. *o*-Iodobenzoic acid separates in large, slightly yellow needles of satisfactory purity (mp 164°) for the experiment; yield approximately 17.6 g (71%).

45

Triptycene via Benzyne

KEYWORDS

Benzyne, anthracene
Diazotization, anthranilic acid
Isoamyl nitrite
Aprotic solvent
Maleic anhydride adduct
Triglyme, $(CH_3OCH_2CH_2OCH_2)_2$
Optional project



1
Anthranilic acid
MW 137.14

2a

2b

3
Benzyne

4
Triptycene
MW 254.31, mp 225°

▼
Aprotic means "no protons"; HOH *and* ROH *are protic*

▼
Removal of anthracene

This interesting cage-ring hydrocarbon results from 9,10-addition of benzyne to anthracene. In one procedure presented in the literature benzyne is generated under nitrogen from *o*-fluorobromobenzene and magnesium in the presence of anthracene, but the work-up is tedious and the yield only 24%. Diazotization of anthranilic acid to benzenediazonium-2-carboxylate (**2a**) or to the covalent form (**2b**), gives another benzyne precursor, but the isolated substance can be kept only at a low temperature and is sometimes explosive. However, isolation of the precursor is not necessary. On slow addition of anthranilic acid to a solution of anthracene and isoamyl nitrite in an aprotic solvent, the precursor (**2**) reacts with anthracene as fast as the precursor is formed. If the anthranilic acid is all present at the start, a side reaction of this substance with benzyne drastically reduces the yield. A low-boiling solvent (CH_2Cl_2, bp 41°) is used, in which the desired reaction goes slowly, and a solution of anthranilic acid is added dropwise over a period of 4 hours. To bring the reaction time into the limits of a laboratory period, the higher boiling solvent 1,2-dimethoxyethane (bp 83°, water soluble) is specified in this procedure and a large excess of anthranilic acid and isoamyl nitrite is used. Treatment of the dark reaction mixture with alkali removes acidic by-products and most of the color, but the crude product inevitably contains anthracene. However, brief heating with maleic anhydride at a suitable temperature leaves the triptycene untouched and converts the anthracene into its maleic anhydride adduct. Treatment of the reaction mixture with alkali converts the adduct into a water-soluble salt and affords colorless, pure triptycene.

EXPERIMENT

▼
Technique for slow addition of a solid

▼
Reaction time: 1 hr

Place 2 g of anthracene,[1] 2 ml of isoamyl nitrite, and 20 ml of 1,2-dimethoxyethane in a 125-ml round-bottomed flask mounted over a microburner and fitted with a short reflux condenser. Insert a filter paper into a 55-mm short stem funnel, moisten the paper with 1,2-dimethoxyethane, and rest the funnel in the top of the condenser. Weigh 2.6 g of anthranilic acid on a folded paper, scrape the acid into the funnel with a spatula, and pack it down. Bring the mixture in the reaction flask to a gentle boil and note that the anthracene does not all dissolve. Measure 20 ml of 1,2-dimethoxyethane into a graduate and use a capillary droping tube to add small portions of the solvent to the anthranilic acid in the funnel, to slowly leach the acid into the reaction flask. If you make sure that the condenser is exactly vertical and the top of the funnel is centered, it should be possible to arrange for each drop to fall free into the flask and not touch the condenser wall. Once dripping from the funnel has started, add fresh batches of solvent to the acid but only 2–3 drops at a time. Plan to complete leaching the first charge of anthranilic acid in a period of not less than 20 min, using about 10 ml of solvent. Then add a second 2.6-g portion of anthranilic acid to the funnel, remove the burner, and by lifting the funnel up a little run in 2 ml of isoamyl nitrite through the condenser. Replace the funnel, resume

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[1] Eastman Practical Grade anthracene and anthranilic acid are satisfactory.

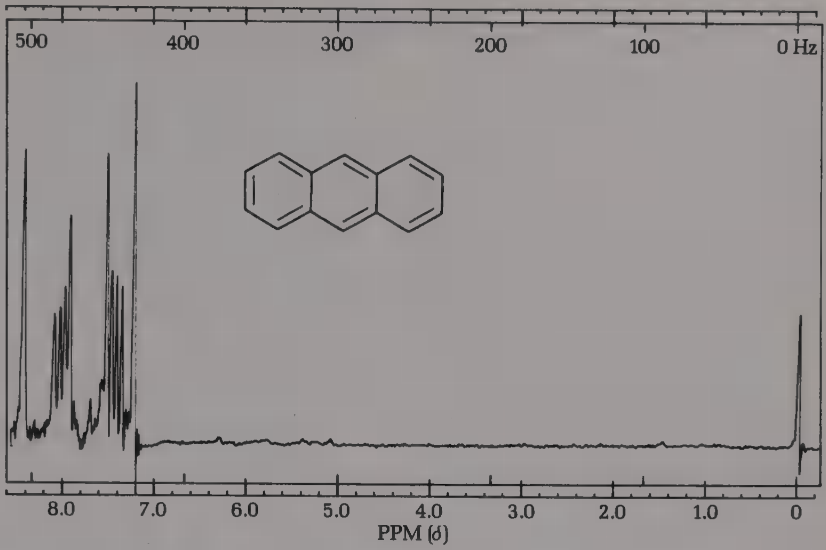


FIGURE 45.1 ^{1}H nmr spectrum of anthracene.

heating, and leach the anthranilic acid as before in about 20 min time. Reflux for 10 min more and then add 10 ml of 95% ethanol and a solution of 3 g of sodium hydroxide in 40 ml of water to produce a suspension of solid in a brown alkaline liquor. Cool thoroughly in ice, and also cool a 4:1 methanol-water mixture for rinsing. Collect the solid on a small Büchner funnel and wash it with the chilled solvent to remove brown mother liquor. Transfer the moist, nearly colorless solid to a tared 125-ml round-bottomed flask and evacuate on the steam bath until the weight is constant; the anthracene-triptycene mixture (mp about 190–230°) weighs 2.1 g. Add 1 g of maleic anhydride and 20 ml of triethylene glycol dimethyl ether ("triglyme," bp 222°), heat the mixture to the bp under reflux, and reflux for 5 min. Cool to about 100°, add 10 ml of 95% ethanol and a solution of 3 g of sodium hydroxide in 40 ml of water; then cool in ice, along with 25 ml of 4:1 methanol-water for rinsing. Triptycene separates as nearly white crystals from the slightly brown alkaline liquor. The washed and dried product weighs 1.5 g and melts at 255°. It will appear to be colorless, but it contains a trace of black insoluble material. Dissolve the material in excess dichloromethane (10 ml/g), filter to remove the specks of black solid, and rinse the flask and filter paper with a liberal amount of dichloromethane. Then add a boiling stone, heat on the hot plate to boiling, slowly add about two volumes of methanol (bp 65°) for each volume of dichloromethane present, and boil on the hot plate to eliminate the solvent of lower bp (41°) and higher solvent power. Concentrate the solution until crystals just begin to separate, and let stand for crystallization. Better formed crystals can be obtained by recrystallization from methylcyclohexane (23 ml/g). Crystals thus produced are flat, rectangular, laminated prisms.

Optional Project

In Chapter 44 diphenyliodonium-*o*-carboxylate is utilized as a benzyne precursor in the synthesis of 1,2,3,4-tetraphenylnaphthalene. For reasons unknown, the reaction of anthracene with benzyne generated in this way proceeds very poorly and gives only a trace of triptycene. What about the converse proposition? Would benzyne generated by diazotization of anthranilic acid in the presence of tetraphenylcyclopentadienone afford 1,2,3,4-tetraphenylnaphthalene in satisfactory yield? In case you are interested in exploring this possibility, plan and execute a procedure and see what you can discover.

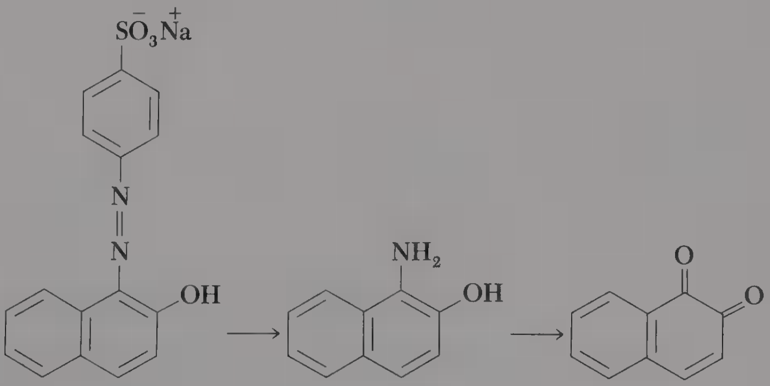
46

Quinones

KEYWORDS

Orange II, reduction
ortho-Quinone
Vitamin K_1, antihemorrhagic
Oxidation, chromic acid
1,4-Naphthoquinone, yellow
Reduction, hydroquinone
Phytol, allylic alcohol
Condensation
Phthiocol

In the first experiment of this chapter the dye Orange II is reduced in aqueous solution with sodium hydrosulfite to water-soluble sodium sulfanilate and 1-amino-2-naphthol, which precipitates. This intermediate is purified as the hydrochloride and oxidized to 1,2-naphthoquinone.

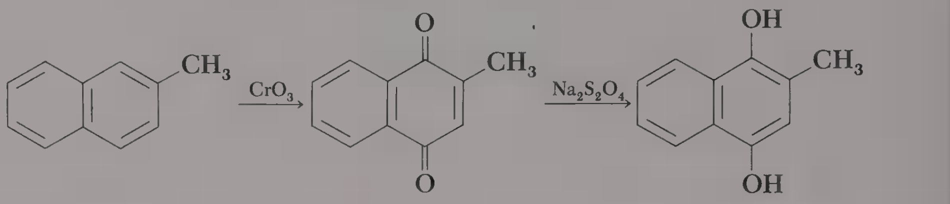


1
Orange II
MW 350.34

2
1-Amino-2-naphthol
MW 159.18

3
1,2-Naphthoquinone
MW 158.15, mp 145–147° dec

The next three experiments of this chapter are a sequence of steps for the synthesis of the antihemorrhagic Vitamin K_1 (or an analogue) starting with a coal-tar hydrocarbon.

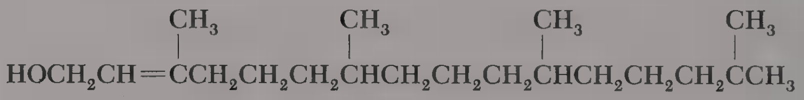


4
2-Methylnaphthalene
MW 142.19, mp 35°

5
2-Methyl-1,4-naphthoquinone
MW 172.17, mp 105–106°

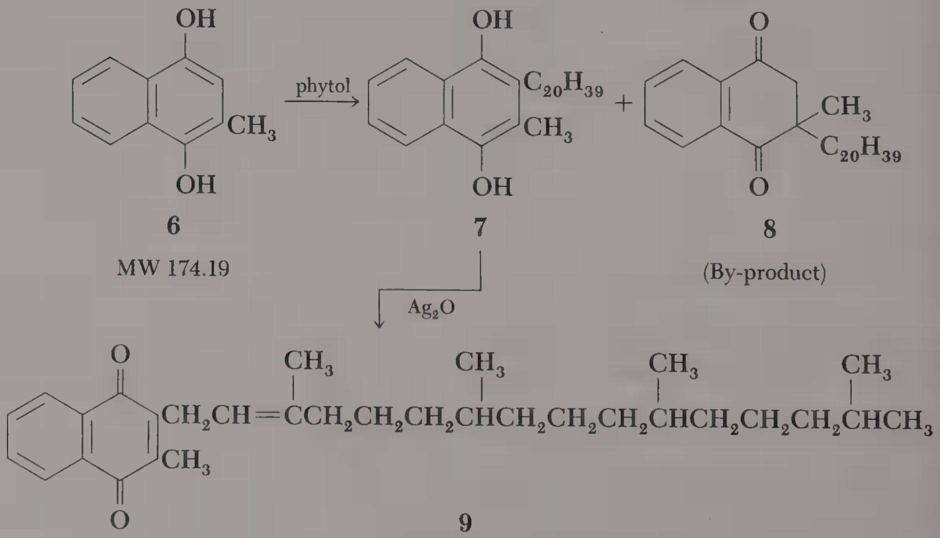
6
2-Methyl-1,4-naphthohydroquinone
MW 174.19

2-Methylnaphthalene is oxidized with chromic acid to 2-methyl-1,4-naphthoquinone, the yellow quinone is purified and reduced to its hydroquinone by shaking an ethereal solution of the substance with aqueous hydrosulfite solution, the colorless hydroquinone is condensed with phytol, and the substituted hydroquinone oxidized to vitamin K_1.



$$HOCH_2CH{=}\overset{CH_3}{\overset{|}{C}}CH_2CH_2CH_2\overset{CH_3}{\overset{|}{C}}HCH_2CH_2CH_2\overset{CH_3}{\overset{|}{C}}HCH_2CH_2CH_2\overset{CH_3}{\overset{|}{C}}CH_3$$

Phytol
MW 296.52, bp 145° at 0.03–0.04 torr



6
MW 174.19

7

8
(By-product)

9
Vitamin K_1
MW 450.68

An additional or alternative experiment is conversion of 2-methyl-1,4-naphthoquinone through the oxide into the 3-hydroxy compound phthiocol, which has been isolated from human tubercle bacilli after saponification, probably as a product of cleavage of vitamin K_1.

EXPERIMENTS

1. 1,2-Naphthoquinone (3)

▼
Two short-time reactions

In a 125-ml Erlenmeyer flask dissolve 3.9 g of the dye Orange II in 50 ml of water and warm the solution to 40–50°. Add 4.5 g of sodium hydrosulfite dihydrate and swirl until the red color is discharged and a cream-colored or pink precipitate of 1-amino-2-naphthol separates. To coagulate the product, heat the mixture nearly to boiling until it begins to froth, then cool in an ice bath, collect the product on a suction filter, and wash the residue with water. Prepare a solution of 1 ml of concentrated hydrochloric acid, 20 ml of water, and an estimated 50 mg of tin(II) chloride (antioxidant); transfer the precipitate of aminonaphthol to this solution and wash in material adhering to the funnel. Swirl, warm gently, and when all but a little fluffy material has dissolved, filter the solution by suction through a thin layer of decolorizing charcoal. Transfer the filtered solution to a clean flask, add 4 ml of concentrated hydrochloric acid, heat over a free flame until the precipitated aminonaphthol hydrochloride has been brought into solution, and then cool thoroughly in an ice bath. Collect the crystalline, colorless hydrochloride and wash it with a mixture of 1 ml of concentrated hydrochloric acid and 4 ml of water. Leave the air-sensitive crystalline product in the funnel while preparing a solution for its oxidation. Dissolve 5.5 g of iron(III) chloride crystals ($FeCl_3 \cdot 6H_2O$) in 2 ml of concentrated hydrochloric acid and 10 ml of water by heating, cool to room temperature, and filter by suction.

Wash the crystalline aminonaphthol hydrochloride into a beaker, stir, add more water, and warm to about 35° until the salt is dissolved. Filter the solution quickly by suction from a trace of residue and stir in the iron(III) chloride solution. 1,2-Naphthoquinone separates at once as a voluminous precipitate and is collected on a suction filter and washed thoroughly to remove all traces of acid. The yield from pure, salt-free Orange II is 75%.

1,2-Naphthoquinone, highly sensitive and reactive, does not have a well-defined melting point but decomposes at about 145–147°. Suspend a sample in hot water and add concentrated hydrochloric acid. Dissolve a small sample in cold methanol and add a drop of aniline; the red product is 4-anilino-1,2-naphthoquinone.

2. 2-Methyl-1,4-naphthoquinone (5)

In the hood, clamp a separatory funnel in place to deliver into a 600-ml beaker which can be cooled in an ice bath when required. The oxidizing solution to be placed in the funnel is prepared by dissolving 50 g of chromium(VI) oxide (CrO_3, chromic anhydride), in 35 ml of water and diluting the dark red solution

▼ *Reaction time: 1.25 hr*

with 35 ml of acetic acid.[1] In the beaker prepare a mixture of 14.2 g of 2-methylnaphthalene and 150 ml of acetic acid, and without cooling run in small portions of the oxidizing solution. Stir with a thermometer until temperature rises to 60°. At this point ice cooling will be required to prevent a further rise in temperature. By alternate addition of reagent and cooling, the temperature is maintained close to 60° throughout the addition, which can be completed in about 10 min. When the temperature begins to drop spontaneously the solution is heated gently on the steam bath (85–90°) for one hour to complete the oxidation.

Dilute the dark green solution with water nearly to the top of the beaker, stir well for a few minutes to coagulate the yellow quinone, collect the product on a Büchner funnel, and wash it thoroughly with water to remove chromium(III) acetate. The crude material can be crystallized from methanol (40 ml) while still moist (without filtering), and gives 6.5–7.3 g of satisfactory 2-methyl-1,4-naphthoquinone, mp 105–106°. The product is to be saved for the preparation of **6**, **9**, and **11**. This quinone must be kept away from light, which converts it into a pale yellow, sparingly soluble polymer.

3. 2-Methyl-1,4-naphthohydroquinone (6)

▼ *Short-time reaction*

In an Erlenmeyer flask dissolve 2 g of 2-methyl-1,4-naphthoquinone (**5**) in 35 ml of ether by warming on a steam bath, pour the solution into a separatory funnel, and shake with a fresh solution of 4 g of sodium hydrosulfite in 30 ml of water. After passing through a brown phase (quinhydrone) the solution should become colorless or pale yellow in a few minutes; if not, add more hydrosulfite solution. After removing the aqueous layer, shake the ethereal solution with 25 ml of saturated sodium chloride solution and 1–2 ml of saturated hydrosulfite solution to remove the bulk of the water. Filter the ethereal layer by gravity through a filter paper moistened with ether and about one-third filled with anhydrous sodium sulfate. Evaporate the filtrate on the steam bath until nearly all the solvent has been removed, cool, and add petroleum ether. The hydroquinone separating as a white or grayish powder is collected, washed with petroleum ether, and dried; yield 1.9 g (the substance has no sharp mp).

▼ *Aspirator tube*

4. Vitamin K$_1$ (2-Methyl-3-phytol-1,4-naphthoquinone, 9)

▼ *Explanation*

Phytol, being an allylic alcohol, is reactive enough to condense with 2-methyl-1,4-naphthohydroquinone (**6**) under mild conditions of acid catalysis as specified in the following procedure. Over-heating must be avoided or the alcohol is dehydrated to phytadiene. The reaction mixture is diluted with water and extracted with ether and unchanged starting material removed by extraction

---

[1] The chromium(VI) oxide is hygroscopic; weigh it quickly and do not leave the bottle unstoppered. The substance dissolves very slowly in acetic acid-water mixtures, and solutions are prepared by adding the acetic acid only after the substance has been completely dissolved in water.

with aqueous alkali containing hydrosulfite to protect the hydroquinones from oxidation by air. The hydroquinone of Vitamin K_1 (7) has a methyl group adjacent to one hydroxyl group and the long phytol side chain adjacent to the other; it is a cryptophenol (hidden phenolic properties), insoluble in aqueous alkali. It is separated from the nonhydroxylic by-product 8 by crystallization from petroleum ether and oxidized to Vitamin K_1 (9).

▼
***Caution!** Dioxane is a mild carcinogen. Carry out this experiment in a hood.*

Place 1.5 g of phytol[2] and 10 ml of dioxane *(caution!)* in a 50-ml Erlenmeyer flask and warm to 50° on a hot plate. Prepare a solution of 1.5 g of 2-methyl-1,4-naphthohydroquinone (6) and 1.5 ml of boron trifluoride etherate in 10 ml of dioxane, and add this in portions with a capillary dropper in the course of 15 min with constant swirling and while maintaining a temperature of 50° (do not overheat). Continue in the same way for 20 min longer. Cool to 25°, wash the solution into a separatory funnel with 40 ml of ether, and wash the orange-colored ethereal solution with two 40-ml portions of water to remove boron trifluoride and dioxane. Extract the unchanged hydroquinone with a freshly prepared solution of 2 g of sodium hydrosulfite in 40 ml of 2% aqueous sodium hydroxide and 10 ml of a saturated sodium chloride solution (which helps break the resulting emulsion). Shake vigorously for a few minutes, during which time any red color should disappear and the alkaline layer should acquire a bright yellow color. After releasing the pressure through the stopcock, allow the layers to separate, keeping the funnel stoppered as a precaution against oxidation. Draw off the yellow liquor and repeat the extraction a second and a third time, or until the alkaline layer remains practically colorless. Separate the faintly colored ethereal solution, dry it over anhydrous sodium sulfate, filter into a tared flask, and evaporate the filtrate on the steam bath—eventually with evacuation at the aspirator. The total oil, which becomes waxy on cooling, amounts to 1.7–1.9 g.

Add 10 ml of petroleum ether (bp 20–40°) and boil and manipulate with a spatula until the brown mass has changed to a white paste. Wash the paste into small centrifuge tubes with 10–20 ml of fresh petroleum ether, make up the volume of paired tubes to the same point, cool well in ice, and centrifuge. Decant the brown supernatant liquor into the original tared flask, fill the tubes with fresh solvent, and stir the white sludge to an even suspension. Then cool, centrifuge, and decant as before. Evaporation of the liquor and washings gives 1.1–1.3 g of residual oil. Dissolve the portions of washed sludge of Vitamin K_1 hydroquinone in a total of 10–15 ml of absolute ether, and add a little decolorizing charcoal for clarification, if the solution is pink or dark. Add 1 g of silver oxide and 1 g of anhydrous sodium sulfate. Shake for 20 min, filter into a tared flask, and evaporate the clear yellow solution on the steam bath, removing traces of solvent at the water pump. Undue exposure to light should be avoided when the material is in the quinone form. The residue is a light

▼
Note for the instructor

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[2] Suppliers: Matheson, Coleman, and Bell Co.; Aldrich Chemical Co. Geraniol can be used instead of phytol, it reacts with 2-methyl-1,4-naphthohydroquinone to give a product similar in chemical and physical properties to the natural vitamin and with pronounced antihemorrhagic activity.

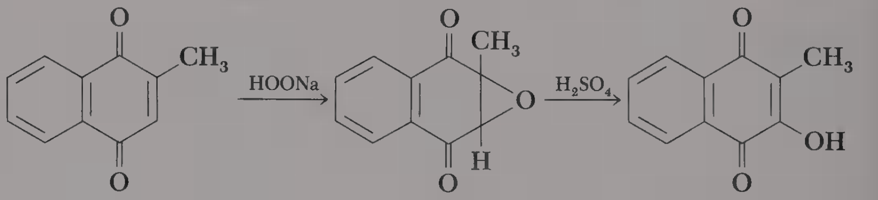
yellow, rather mobile oil consisting of pure Vitamin K_1; yield 0.6–0.9. A sample for preservation is transferred with a capillary dropper to a small specimen vial wrapped in metal foil or black paper to exclude light.

To observe a characteristic color reaction, transfer a small bit of vitamin on the end of a stirring rod to a test tube, stir with 1 ml of alcohol, and add 1 ml of 10% alcoholic potassium hydroxide solution; the end pigment responsible for the red color is phthiocol.

5. Phthiocol (2-Methyl-3-hydroxy-1,4-naphthoquinone, 11)

▼
Two short-time reactions

Dissolve 1 g of 2-methyl-1,4-naphthoquinone (**5**) in 10 ml of alcohol by heating, and let the solution stand while the second reagent is prepared by dissolving 0.2 g of anhydrous sodium carbonate in 5 ml of water and adding (cold) 1 ml of 30% hydrogen peroxide solution. Cool the quinone solution under the tap until crystallization begins, add the peroxide solution all at once, and cool the mixture. The yellow color of the quinone should be discharged immediately. Add about 100 ml of water, cool in ice, and collect the colorless, crystalline oxide, **10**; yield 0.97 g, mp 93.5–94.5° (pure: 95.5–96.5°).



5

10
Oxide
MW 188.17, mp 93.5–94.5°

11
Phthiocol
MW 188.17, mp 172–173°

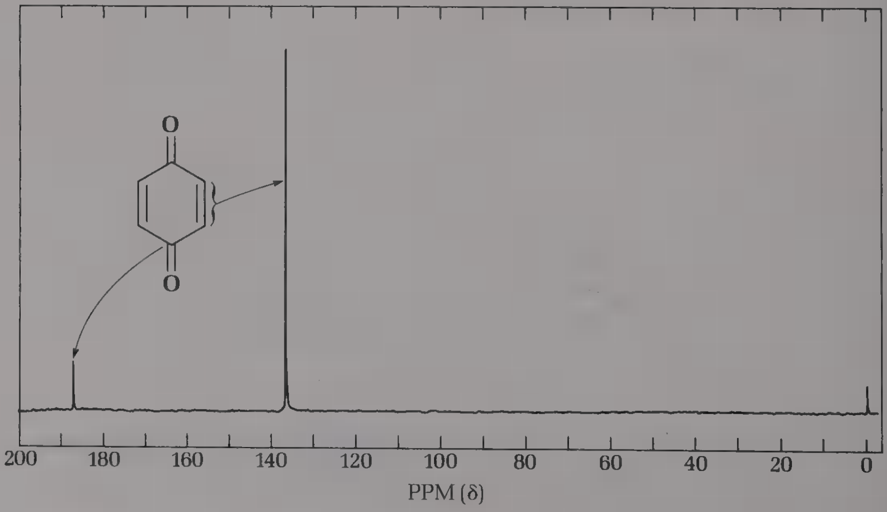


FIGURE 46.1 ^{13}C nmr spectrum of quinone.

To 1 g of **10** in a 25-ml Erlenmeyer flask add 5 ml of concentrated sulfuric acid; stir if necessary to produce a homogeneous deep red solution, and after 10 min cool this in ice and slowly add 20 ml of water. The precipitated phthiocol can be collected, washed, and crystallized by dissolving in methanol (25 ml), adding a few drops of hydrochloric acid to give a pure yellow color, treating with decolorizing charcoal, concentrating the filtered solution, and diluting to the saturation point. Alternatively, the yellow suspension is washed into a separatory funnel and the product extracted with a mixture of 25 ml each of benzene and ether. The organic layer is dried over anhydrous sodium sulfate and evaporated to a volume of about 10 ml for crystallization. The total yield of pure phthiocol (**11**) mp 172–173°, is about 0.84–0.88 g.

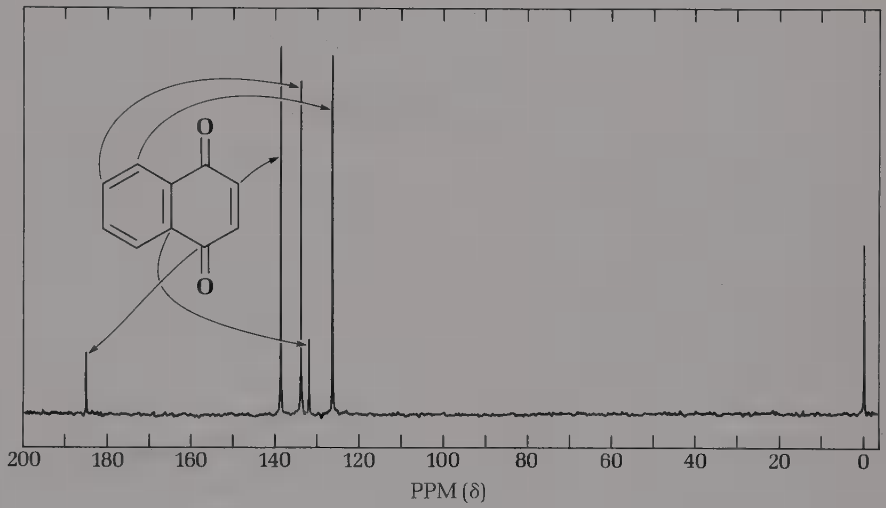


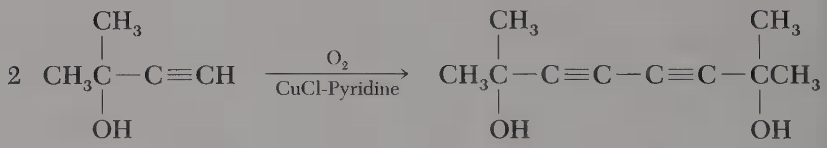
FIGURE 46.2 ^{13}C nmr spectrum of 1,4-naphthoquinone.

47

2,7-Dimethyl-3,5-octadiyn-2,7-diol; Oxidative Coupling of Alkynes

KEYWORDS

2-Methyl-3-butyn-2-ol
Oxidative coupling, Glaser reaction
Copper(I) chloride–pyridine complex (catalyst)
Pressure balloon



$$2\ CH_3\overset{CH_3}{\underset{OH}{C}}-C\equiv CH \xrightarrow[\text{CuCl-Pyridine}]{O_2} CH_3\overset{CH_3}{\underset{OH}{C}}-C\equiv C-C\equiv C-\overset{CH_3}{\underset{OH}{C}}CH_3$$

2-Methyl-3-butyn-2-ol
MW 84.11, den 0.868, bp 103°

2,7-Dimethyl-3,5-octadiyn-2,7-diol
MW 166.21, mp 130°

The starting material, 2-methyl-3-butyn-2-ol, is made commercially from acetone and acetylene and is convertible into isoprene. This experiment illustrates the oxidative coupling of a terminal acetylene to produce a diacetylene, the Glaser reaction. The catalyst, a copper(I) chloride-pyridine complex, probably functions by converting the acetylene into a copper derivative, which is oxidized by oxygen to the diacetylene and copper(I) oxide. The reaction time is shortened by use of excess catalyst and by supplying oxygen under the pressure of a balloon; the state of the balloon provides an index of the course of the reaction.

EXPERIMENT

The reaction vessel is a 125-ml filter flask or Erlenmeyer flask with side tube with a rubber bulb[1] secured to the side arm with copper wire, evident from

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[1] White rubber pipette bulb for medicine dropper, Will Corp. No. 23266.

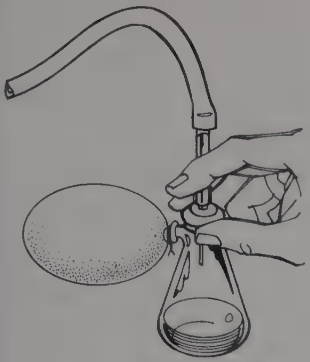

FIGURE 47.1 Balloon technique of oxygenation. About 10 lb oxygen pressure are needed to inflate the pipette bulbs.

FIGURE 47.2 Appearance of pipette bulb after oxygenation.

Figs. 47.1 or 47.2. Add 10 ml of 2-methyl-3-butyn-2-ol, 10 ml of methanol, 3 ml of pyridine, and 0.5 g of copper(I) chloride. Before going to the oxygen station,[2] practice capping the flask with a large serum stopper[3] until you can do this quickly. You are to flush out the flask with oxygen and cap it before air can diffuse in; the reaction is about twice as fast in an atmosphere of oxygen as in air. Insert the oxygen delivery syringe into the flask with the needle under the surface of the liquid, open the valve and let oxygen bubble through the solution in a brisk stream for 2 min. Close the valve and quickly cap the flask and wire the cap. Next thrust the needle of the delivery syringe through the center of the serum stopper, open the valve, and run in oxygen until you have produced a sizeable inflated bulb (see Fig. 47.1). Close the valve and withdraw the needle (the hole is self-sealing), note the time and start swirling the reaction mixture. The rate of oxygen uptake depends on the efficiency of mixing of the liquid and gas phases. By vigorous and continuous swirling it is possible to effect deflation of the balloon to a 5-cm sphere (see Fig. 47.2) in 20–25 min. The reaction mixture warms up and becomes deep green. Introduce a second charge of oxygen of the same size as the first, note the time, and swirl. In 25–30 min the balloon reaches a constant volume (*e.g.*, a 5-cm sphere), and the reaction is complete. A pair of calipers is helpful in recognizing the constant size of the balloon and thus the end of the reaction.[4]

Open the reaction flask, cool if warm, add 5 ml of concentrated hydrochloric acid to neutralize the pyridine and keep copper compounds in solution, and cool again; the color changes from green to yellow. Use a spatula to dislodge copper salts adhering to the walls, leaving it in the flask. Then add 25 ml of saturated sodium chloride solution to precipitate the diol, and stir and cool the thick paste that results. Scrape out the paste onto a small Büchner funnel with the spatula; press down the material to an even cake. Rinse out the flask with water and wash the filter cake with enough more water to remove all the color from the cake and leave a colorless product. Since the moist product dries slowly, drying is accomplished in ether solution, and the operation is combined with recovery of diol retained in the mother liquor. Transfer the moist product to a 125-ml Erlenmeyer flask, extract the mother liquor in the filter flask with one portion of ether, wash the extract once with water, and run the ethereal solution into the flask containing the solid product. Add enough more ether to dissolve the product, transfer the solution to a separatory funnel, drain off the water layer, wash with saturated sodium chloride solution, filter through anhydrous sodium sulfate into a tared flask. The solvent is evaporated on the steam bath and the solid residue is heated and evacuated until the weight is constant. Crystallization from toluene then gives colorless needles of the diol, mp 129–130°. The yield of crude product is 7–8 g. In the crystallization, the recovery is practically quantitative.

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[2] The valves of a cylinder of oxygen should be set to deliver gas at a pressure of 10 lbs/sq in. when the terminal valve is opened. The barrel of a 2.5 ml plastic syringe is cut off and thrust into the end of a $\frac{1}{4} \times \frac{3}{16}$-in. rubber delivery tube.

[3] Red rubber serum stopper (large), Scientific Glass Co., R7950.

[4] A magnetic stirrer does not materially shorten the reaction time.

48

Oleic Acid from Olive Oil

KEYWORDS

Saponification, olive oil
Oleic acid, linoleic acid
High boiling solvent, triethylene glycol
Urea inclusion compounds
Host-guest ratio
Filter thimble

Saponification is the name given to alkaline hydrolysis of fats and oils to give glycerol and the alkali metal salt of a long chain fatty acid (a soap). In this experiment saponification of olive oil is accomplished in a few minutes by use of a solvent permitting operation at 160°.[1] Of the five acids found in olive oil, listed in Table 48.1, three are unsaturated and two are saturated. Oleic acid and linoleic acid are considerably lower melting and more soluble in organic solvents than the saturated components, and when a solution of the hydrolyzate in acetone is cooled to −15°, about half of the material separates as a crystallizate containing the two saturated compounds, stearic acid and palmitic acid.

Table 48.1 Acids of Split Olive Oil (Typical Composition)

Acid	Formula	%	MW	Mp
Oleic	$C_{18}H_{34}O_2$	64	282.45	13°, 16°
Linoleic	$C_{18}H_{32}O_2$	16	280.44	−5°
Linolenic	$C_{18}H_{30}O_2$	2	278.42	liquid
Stearic	$C_{18}H_{36}O_2$	4	284.07	69.9°
Palmitic	$C_{16}H_{32}O_2$	14	256.42	62.9°

▼
Note for the instructor

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[1] The experiment gains in interest if students are given the choice of olive oil from two sources (for example, Italy and Israel) and instructed to compare notes.

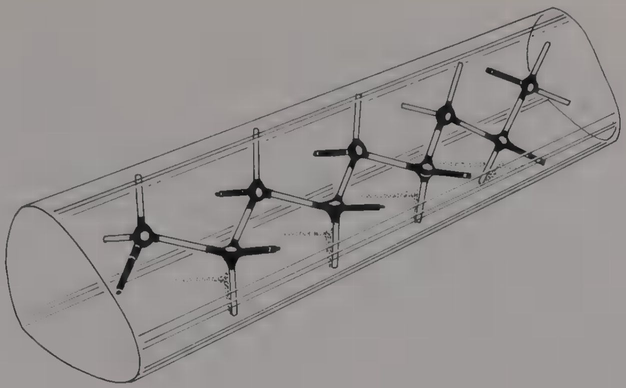


FIGURE 48.1 Model of *n*-nonane molecule in a plastic cylinder, 14.3 cm in diameter.

▼
Urea inclusion complexes

The unsaturated acid fraction is then recovered from the filtrate and treated with urea in methanol to form the urea inclusion complex. Normal alkanes having seven or more carbon atoms form complexes with urea in which hydrogen-bonded urea molecules are oriented in a helical crystal lattice in such a way as to leave a cylindrical channel in which a straight-chain hydrocabon fits. The guest molecule (hydrocarbon) is not bonded to the host (urea), but merely trapped in the channel. The cylindrical channel is of such a diameter (5.3 Å) as to accommodate a normal alkane, but not a thick, branched-chain hydrocarbon such as 2,2,4-trimethylpentane. In Fig. 48.1 a model of *n*-nonane to the scale 0.2 cm = 1 Å constructed from plastic-metal atoms[2] is inserted into a tightly fitting cellulose acetate cylinder, 14.3 cm in diameter, which defines the space occupied by the resting guest molecule. Table 48.2 shows that as the hydrocarbon chain is lengthened, more urea molecules are required to extend the channel but that the host-guest relationship is not stoichiometric. The higher saturated fatty acids form urea-inclusion complexes in which the ratio of host to guest molecules is about the same as for the corresponding alkanes:

$$\left.\begin{array}{l}\text{Myristic acid } (C_{14})\\ \text{Palmitic acid } (C_{16})\\ \text{Stearic acid } (C_{18})\end{array}\right\}\text{requires}\left\{\begin{array}{c}11.3\\ 12.8\\ 14.2\end{array}\right\}\text{moles of urea}$$

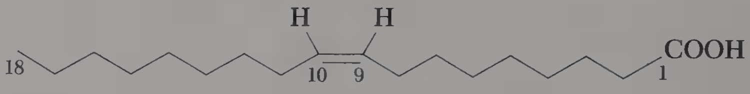
Table 48.2 Molecules of Urea per Molecule of *n*-Alkane

Alkane	Ratio	Alkane	Ratio
C_6	No complex	C_{11}	8.7
C_7	6.1	C_{12}	9.7
C_8	7.0	C_{16}	12.3
C_9	7.3	C_{24}	18.0
C_{10}	8.3	C_{28}	21.2

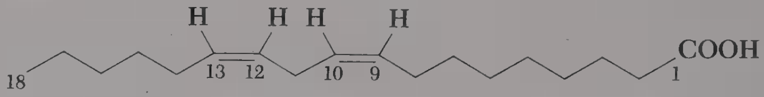
---

[2] L. F. Fieser, *J. Chem. Ed.*, **40,** 457 (1963); *ibid.*, **42,** 408 (1965).

With the elimination of saturated acids from the olive oil hydrolyzate by crystallization from acetone, the problem remaining in isolation of oleic acid is to remove the doubly unsaturated linoleic acid. Models and cylinders show that the introduction of just one *cis* double bond is enough to widen the molecule to the extent that it can no longer be inserted into the 14.3-cm wide channel which accommodates *n*-alkanes (Fig. 48.1). However, a model of 3-nonyne likewise fails to fit into the 14.3-cm channel, and the fact that this acetylenic hydrocarbon nevertheless forms a urea complex[3] indicates that the channel is subject to some stretching, namely to a diameter of 16.2 cm, as in Fig. 48.2. From examination of the oleic acid model together with the 16.2-cm cylinder, it is evident that when the carbon atoms are arranged in the particular manner shown in Fig. 48.3 the oleic acid molecule can be accommodated in this channel. Careful study of the drawing in Fig. 48.4 will show that an attempt to insert the linoleic acid model into the same 16.2-cm cylinder meets with failure; the carboxy half of the molecule is accommodated, including the 9,10-



Oleic acid



Linoleic acid

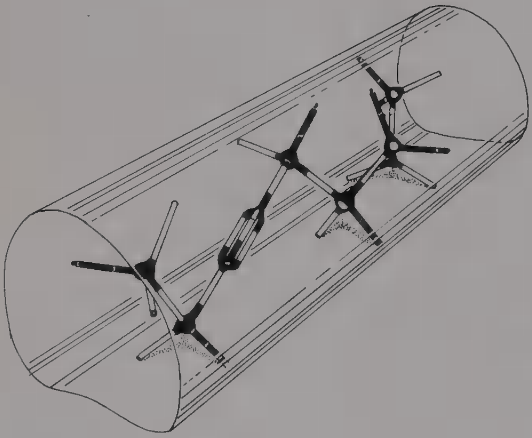


FIGURE 48.2 3-Nonyne molecular model in a plastic cylinder, 16.2 cm in diameter.

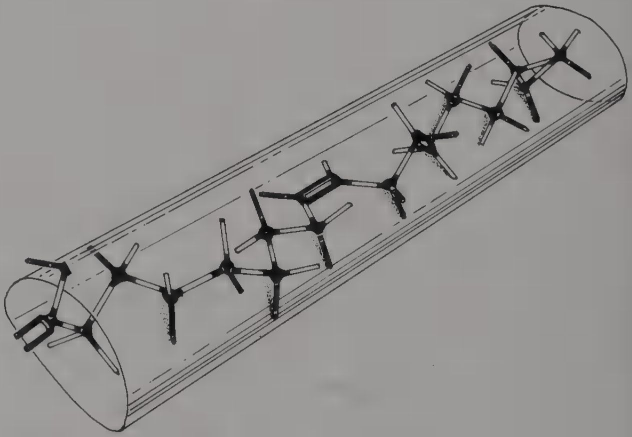


FIGURE 48.3 Oleic acid model in a plastic cylinder, 16.2 cm in diameter.

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[3] J. Radell, J. W. Connolly, and L. D. Yuhas, *J. Org. Chem.*, 26, 2022 (1961).

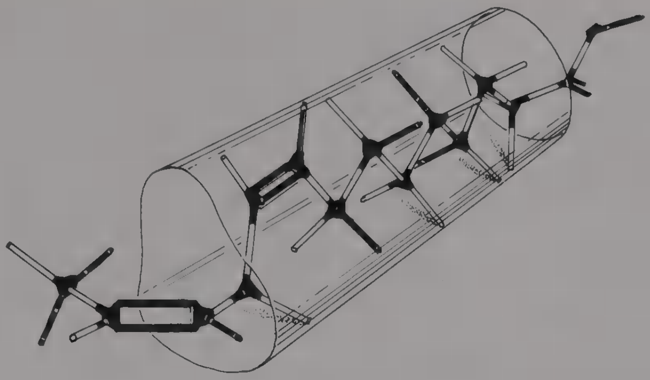

FIGURE 48.4 Model of linoleic acid molecule does not have adequate space in the 16.2-cm cylinder.

double bond, but the 12,13-double bond imposes a stoppage and leaves a five-carbon tail projecting. Thus, when a solution of the two acids in hot methanol is treated with urea and let cool, crystals of the oleic acid complex separate and the linoleic acid is retained in the mother liquor.

▼
Predict the host/guest ratio

The experiment has two objectives. One is to isolate pure oleic acid; the other is to determine the number of molecules of urea in the inclusion complex per molecule of the fatty acid. Make a prediction in advance.

EXPERIMENT

▼
Rapid saponification

Pour about 10 g of olive oil into a tared 125-ml Erlenmeyer flask and adjust the weight to exactly 10.0 g using a capillary dropping tube. Add 2.3 g of potassium hydroxide pellets and 20 ml of triethylene glycol, insert a thermometer, and bring the temperature to 160° by heating over a free flame at first and then on a hot plate; the two layers initially observed soon merge. Then, by removing the flask from the hot plate and replacing it as required, keep the temperature at 160° for 5 min to insure complete hydrolysis, and cool the thick yellow sirup to room temperature. Add 50 ml of water, using it to rinse the thermometer, and acidify the soapy solution with 10 ml of concentrated hydrochloric acid. Cool to room temperature, extract the oil with ether, wash the ether with saturated sodium chloride solution, and dry the mixture over anhydrous sodium sulfate. Filter, to remove the drying agent, into a tared 125-ml Erlenmeyer filter flask. Evaporate the solution on the steam bath and evacuate at the aspirator until the weight is constant (9–10 g of acid mixture).

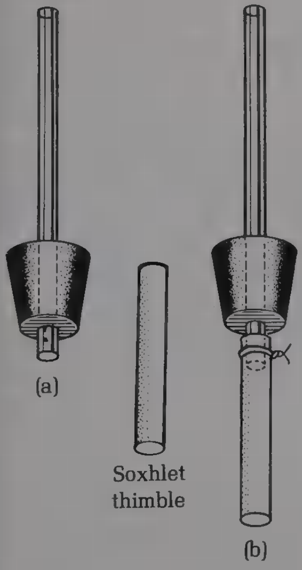

FIGURE 48.5 Filter thimble.

Add 75 ml of acetone to dissolve the oil in the filter flask and place the flask in an ice bath to cool while making preparations for quick filtration. An internal filter for the filter flask is made by moistening the hole of a No. 4 one-hole fusiform rubber stopper (Fig. 2.6) with glycerol and thrusting a 10-cm section of 9-mm glass tubing through it until the tube projects about 8 mm from the smaller end of the stopper (Fig. 48.5a). A 10 × 50-mm Soxhlet extraction thimble is then wired onto the projecting end (b).

When a mixture of oleic acid crystals and mother liquor has been prepared as described below, the internal filtering operation is performed as follows: The side arm of a filter flask is connected with 5-mm rubber tubing to a rubber

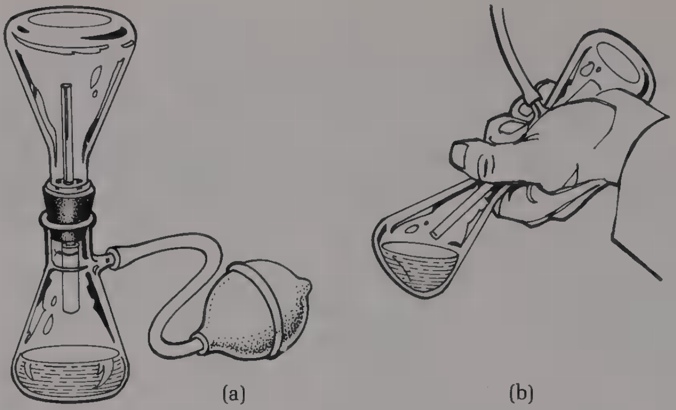

FIGURE 48.6 Apparatus for filtering crystals from mother liquor. (*a*) Flasks with pressure bulb and filter thimble assembly; (*b*) position of flasks when filtering.

pressure bulb with valve,[4] the stopper carrying the filter is inserted tightly into the flask, and a receiving flask (125 ml) is inverted and rested on the rubber stopper (Fig. 48.6a). The two flasks are grasped in the left hand, with the thumb and forefinger pressing down firmly on the rim of the empty flask to keep the stopper of the other flask in place. While operating the pressure bulb constantly with the right hand, turn the assembly slowly to the left until the side arm of the filter flask is slanting up and the delivery tube is slanting down (Fig. 48.6b). Squeeze the bulb constantly until the bulk of the liquid has been filtered and then stop. Remove the filtrate and place the filter flask in the normal position.

▼
Quick filtration at $-15°$

Crystallization of the saturated acids requires cooling in a dry ice-acetone bath at $-15°$ for about 15 min and this is done most conveniently in a beaker half-filled with acetone and mounted on a magnetic stirrer and provided with a toluene low temperature thermometer. Add crushed dry ice a little at a time to bring the bath temperature to $-15°$. The filter flask containing the acetone solution of acids is fitted with the rubber pressure bulb, but the stopper carrying the filter is not put in place until later. Place the filter flask in the cooling bath and swirl the mixture occasionally. Add more dry ice as required to maintain a bath temperature of $-15°$. After the first crystals appear, as a white powder, swirl and cool the solution an additional 10 min to let the whole mixture acquire the temperature of the bath. Then introduce the filter and inverted receiver and filter quickly by the technique described previously. Stop the process as soon as the bulk of liquid has been collected, for the solid will melt rapidly.[5]

Evaporate the solvent from the filtrate on the steam bath and evacuate the residual fraction of unsaturated acids at the aspirator until the weight is constant. The yield of a mixture of stearic and palmitic acids is 5–7 g. In a second 125-ml Erlenmeyer flask dissolve 11 g of urea in 50 ml of methanol and pour

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[4] Will Scientific, Inc. 23276.

[5] Alternative technique: Do the crystallization in an Erlenmeyer flask cooled in a salt-ice bath to $-15°$ and filter by suction on a small Büchner funnel that has been chilled outdoors in winter weather or in a refrigerator freezing compartment.

the solution onto the unsaturated acid fraction. Reheat to dissolve any material that separates and let the solution stand until a large crop of needles has separated. Then cool thoroughly in an ice bath with swirling, collect the product, and rinse the flask with filtrate. Press down the crystals and drain to promote rapid drying and spread out the needles on a large filter paper. After a few minutes, transfer the crystals to a fresh paper. The yield of colorless oleic acid-urea inclusion complex is 10–12 g. The complex does not have a characteristic melting point.

▾ *Determine the host/guest ratio*

When the complex is fully dry, bottle a small sample, note carefully the weight of the remainder and place it in a separatory funnel. Add 25 ml of water, swirl, and note the result. Then extract with ether for recovery of oleic acid. Wash the extract with saturated sodium chloride solution, filter the solution through sodium sulfate into a tared 125-ml Erlenmeyer flask, evaporate the solution on the steam bath, and pump out to constant weight on the steam bath. The yield of pure oleic acid is 2–3 g. From the weights of the complex and of the acid, calculate the number of moles of urea per mole of acid in the complex.

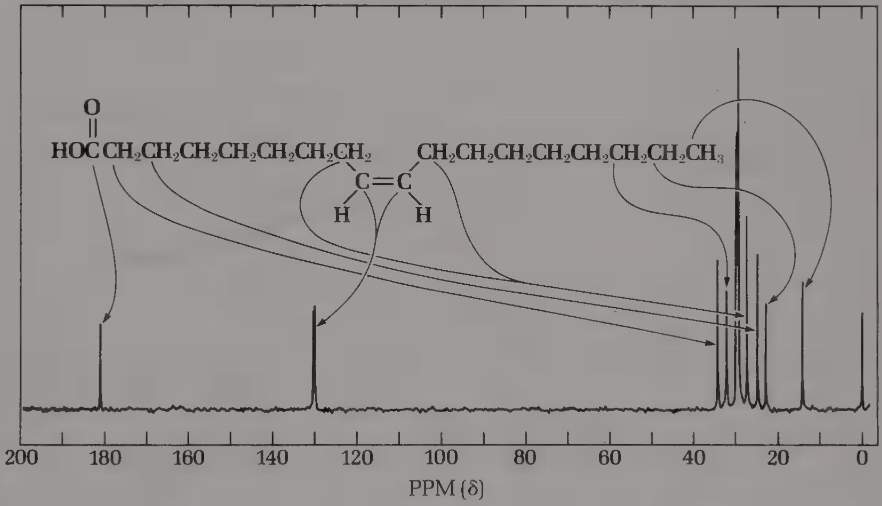


FIGURE 48.7 ^{13}C nmr spectrum of oleic acid.

49

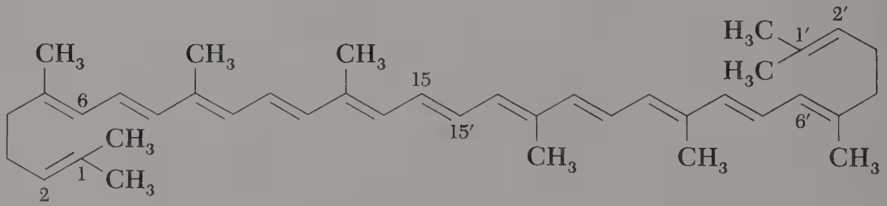
Isolation of Lycopene and *β*-Carotene

KEYWORDS

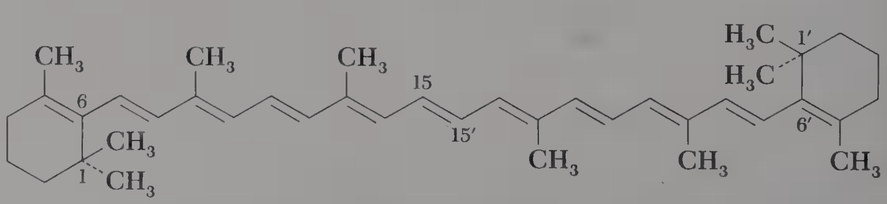
Carotenoid, isoprene units
Chromophore, conjugated double bonds

Tomato, lycopene
Carrot, *β*-carotene

Photochemical air oxidation
Column chromatography



Lycopene ($C_{40}H_{56}$)
MW 536.85,
mp 173°, λ_{max}^{hexane} 475 nm



***β*-Carotene** ($C_{40}H_{56}$)
mp 183°, λ_{max}^{hexane} 451 nm

Lycopene, the red pigment of the tomato, is a C_{40}-carotenoid made up of eight isoprene units. β-Carotene, the yellow pigment of the carrot, is an isomer of lycopene in which the double bonds at $C_1—C_2$ and $C_1'—C_2'$ are replaced by bonds extending from C_1 to C_6 and from C_1' to C_6' to form rings. The chromophore in each case is a system of eleven all-*trans* conjugated double bonds; the closing of the two rings renders β-carotene less highly pigmented than lycopene.

The colored hydrocarbons are materials for an interesting experiment in thin layer chromatography (Chapter 16), but commercial samples are extremely expensive and subject to deterioration on storage. The isolation procedure in this chapter affords amounts of pigments that are unweighable, except on a microbalance, but more than adequate for the thin layer chromatography experiment. It is suggested that half the students isolate lycopene and the other half β-carotene.

▼
Lycopene and β-carotene from tomato paste and strained carrots

Fresh tomato fruit contains about 96% of water, and R. Willstätter and H. R. Escher isolated from this source 20 mg of lycopene per kg of fruit. They then found a more convenient source in commercial tomato paste, from which seeds and skin have been eliminated and the water content reduced by evaporation in vacuum to a content of 26% solids, and isolated 150 mg of lycopene per kg of paste. The expected yield in the present experiment is 0.75 mg.

▼
As an interesting variation, try extraction of lycopene from commercial catsup

A jar of strained carrots sold as baby food serves as a convenient source of β-carotene. The German investigators isolated 1 g of β-carotene per kg of "dried" shredded carrots of unstated water content.

The following procedure calls for dehydration of tomato or carrot paste with ethanol and extraction with dichloromethane, an efficient solvent for lipids.

EXPERIMENT

A 5-g sample of tomato or carrot paste is transferred to the bottom of a 25 × 150-mm test tube with the aid of a paste dispenser,[1] made by connecting an 18-cm section of 11-mm glass tubing by means of a No. 00 rubber stopper and a short section of 5 mm glass tubing to a 50-cm length of 3-mm rubber tubing. The open end of the glass tube is calibrated to deliver 5 g of paste and marked with a file scratch.

These carotenoids are very sensitive to photochemical air oxidation. Protect solutions and solid from undue exposure to light.

Thrust the dispenser into a can of fruit paste and "suck up" paste to the scratch mark on the glass tube. Then wipe the glass tube with a facial tissue, insert it into a 25 × 150-mm test tube, and blow out the paste onto the bottom of the receiver. Wash the dispenser tube clean with water and leave it upright to drain for the next user.

▼
Dehydration of fruit paste

Add 10 ml of 95% ethanol, insert a cold finger condenser, heat to boiling, and reflux for 5 min. Then filter the hot mixture on a small Hirsch funnel. Scrape out the test tube with a spatula, let the tube drain thoroughly, and

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[1] To be supplied to each section of the class, along with a can opener.

squeeze liquid out of the solid residue in the funnel with a spatula. Pour the yellow filtrate into a 125-ml Erlenmeyer flask. Then return the solid residue, with or without adhering filter paper, to the original test tube, add 10 ml of dichloromethane to effect an extraction, insert the condenser, and reflux the mixture for 3–4 min. Filter the yellow filtrate and add the filtrate to the storage flask. Repeat the extraction with two or three further 10-ml portions of dichloromethane, pour the combined extracts into a separatory funnel, add water and sodium chloride solution (to aid in layer separation), and shake. Dry the colored lower layer over anhydrous sodium sulfate, filter the solution into a dry flask and evaporate to dryness (aspirator tube or rotary evaporator).

▼
CH_2Cl_2, bp 41° den 1.336

The crude carotenoid is to be chromatographed on a 12-cm column of acid-washed alumina (Merck), prepared with petroleum ether (37–53°) as solvent. Run out excess solvent, or remove it from the top of the chromatography column with a suction tube, dissolve the crude carotenoid in a few milliliters of toluene, and then transfer the solution onto the chromatographic column with a capillary dropping tube. Elute the column with petroleum ether, discard the initial colorless eluate, and collect all yellow or orange eluates together in a 50-ml Erlenmeyer flask. Place a drop of solution on a microscope slide and evaporate the rest to dryness (rotary evaporator or aspirator tube). Examination of the material spotted on the slide may reveal crystallinity. Then put a drop of concentrated sulfuric acid beside the spot and mix with a stirring rod. Compare the color of your test with that of a test on the other carotenoid.

▼
Color test

Finally, dissolve the sample obtained by evaporating the petroleum ether in the least amount of dichloromethane, hold the Erlenmeyer in a slanting position for drainage into a small area, and transfer the solution with a capillary dropping tube to a 10 × 75-mm test tube or a 3-ml centrifuge tube. Add a boiling stone, hold the tube over a hot plate in a slanting position, and evaporate to dryness. Evacuate the tube at the aspirator, shake out the boiling stone, cork and label the test tube, and keep it in a dark place.

Look up the current prices of commercial lycopene[2] and β-carotene.[3] Note that β-carotene is in demand as a source of vitamin A and is manufactured by an efficient synthesis. No uses for lycopene have been found.

▼
Notes for the instructor

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[2] Pfaltz and Bauer, Flushing, N.Y. 11368.

[3] Aldrich Chemical Co., Milwaukee, Wis. 53233.

50

Synthesis of Carpanone

KEYWORDS

Lignan
Stereospecific synthesis
Five asymmetric carbons
Biosynthetic-like synthesis
Williamson ether synthesis
Claisen rearrangement
Oxidative dimerization
Diels-Alder cyclization

Carpanone is a lignan from a tree, native to the South Pacific island of Bougainville. Lignans, which can be extracted from wood with organic solvents, are related to the lignins, the insoluble, noncarbohydrate polymers which bind cellulose fibers together in wood.

The structure of carpanone was determined primarily by an analysis of its nmr spectrum.[1] The synthesis[2] might, at first glance, seem a formidable task. Carpanone has no element of symmetry and five contiguous asymmetric carbon atoms. A rational total synthesis of a compound of this type is the ultimate test of its structure determination. In the course of such a synthesis, new organic reactions might be invented to circumvent particular problems, and the synthesis might provide clues to biosynthetic pathways. In this case, the ease with which the five asymmetric centers are formed in the last reaction, all with the correct stereochemistry, may mimic the way this compound is formed in nature.

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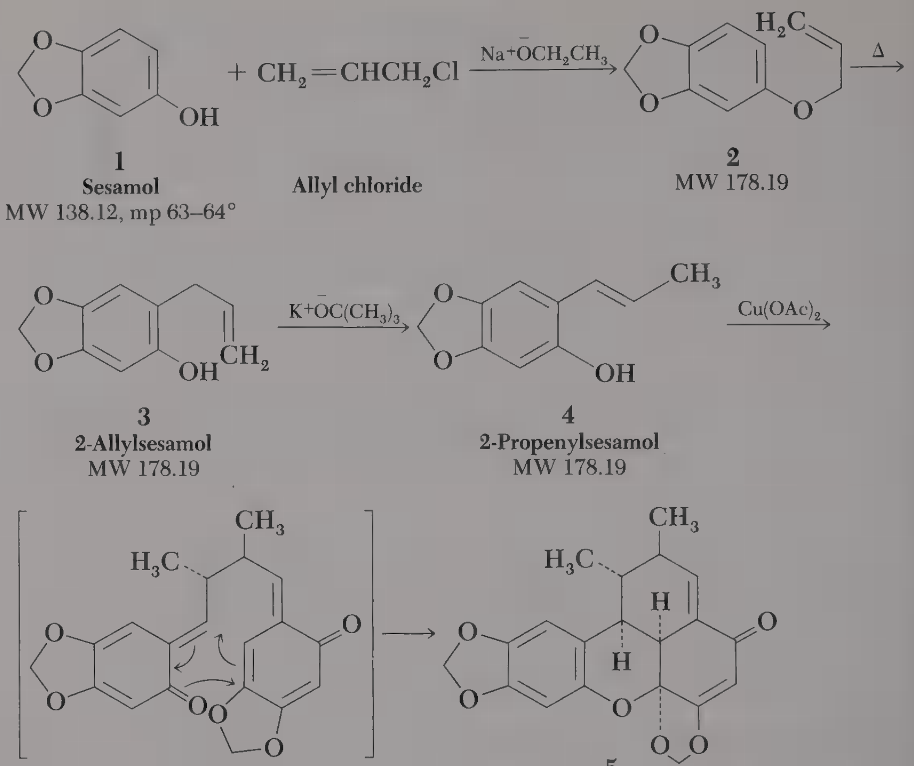
[1] G. C. Brophy, J. Mohandas, M. Slaytor, S. Sternhall, T. R. Watson and L. A. Wilson, *Tetrahedron Lett.* 5159 (1969).

[2] O. L. Chapman, M. R. Engel, J. P. Springer, and J. C. Clardy, *J. Am. Chem. Soc.*, **93**, 6696 (1971).



1
Sesamol
MW 138.12, mp 63–64°

Allyl chloride

2
MW 178.19

3
2-Allylsesamol
MW 178.19

4
2-Propenylsesamol
MW 178.19

5
Carpanone, MW 354.36, mp 210–212°
Carpanone $\cdot CCl_4$, MW 508.20, mp 185°

The first reaction, addition of allyl chloride to sesamol (**1**), is an example of the Williamson ether synthesis. The phenyl allyl ether, (**2**), undergoes the Claisen rearrangement on thermolysis to 2-allylsesamol (**3**), which, in turn, is isomerized by strong base to the conjugated olefin, 2-propenylsesamol (**4**). Under catalysis by copper(II) ion 2-propenylsesamol undergoes oxidative dimerization and cyclization, via a Diels-Alder type reaction, to carpanone, with the formation of five asymmetric centers. A study of models will clarify this reaction, as well as the stereochemistry of the product.

EXPERIMENTS

1. Sesamol allyl ether (2)

Add 0.84 g of sodium pellets to 25 ml of anhydrous ethanol in a 125-ml round-bottomed flask equipped with condenser and calcium chloride drying tube. After the sodium has dissolved, add 5.0 g of sesamol in 6 ml of anhydrous ethanol and then, with swirling, 3.4 g of allyl chloride. Reflux the solution 2 hr, cool to room temperature, pour into 200 ml of water, and extract three times with 35-ml portions of diethyl ether. Wash the combined ether extracts with 100-ml portions of 5% aqueous sodium hydroxide, 5% aqueous sulfuric acid, water, and saturated sodium chloride solutions. Dry the ether solution of the product over 5 g of anhydrous magnesium sulfate, remove the drying agent by filtration or careful decantation, and evaporate the diethyl ether on the steam bath (boiling chip, aspirator tube), or on a rotary evaporator. The product is a brown liquid.

2. 2-Allylsesamol (3)

Place the crude sesamol allyl ether (2) from the previous experiment in a 50-ml round-bottomed flask equipped with a condenser. Heat the flask in an oil bath at 170° for 2 hr. This is most easily done in a large Petri dish containing cottonseed oil and a thermometer placed atop an electric hot plate. The oil bath is large enough for several reactions to be carried out at the same time. The product will solidify to a dark-brown mass on cooling to room temperature. Remove a small sample of the product and sublime it. This is done by heating a few mg of the compound in an inclined evacuated test tube. Crystals of the product should appear on the cool upper portions of the tube. The crude product is, however, pure enough to use in the next step.

3. 2-Propenylsesamol (4)

Equip a 150-ml three-necked flask with a gas inlet tube, a thermometer, a Teflon-covered magnetic stirring bar, and a condenser leading to a bubbler (Fig. 50.1). Add to the flask 50 ml of dimethyl sulfoxide, 5 g of potassium *t*-butoxide[3] and the 2-allylsesamol (3) prepared in the previous experiment.

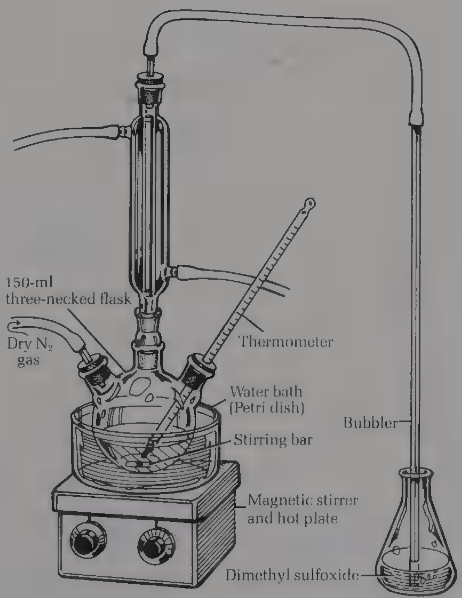


FIGURE 50.1 Inert atmosphere reaction flask.

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[3] Potassium *t*-butoxide is very sensitive to moisture. Weigh rapidly and keep container tightly closed. Alternatively, the laboratory instructor may prepare a stock solution containing 5 g of *t*-butoxide per 50 ml of dimethyl sulfoxide.

Pass a slow stream of nitrogen through the flask for 5 min and then reduce the flow to just a few bubbles per minute. Start the stirrer and heat the reaction mixture in a water bath at 80° for 30 min. Cool the mixture to room temperature and then pour into a 250-ml beaker filled with ice. Stir the slurry and make the solution acidic to Congo Red paper with concentrated hydrochloric acid (about 10 ml). Extract the suspension with three 35-ml portions of diethyl ether, wash the combined ether extracts three times with 100-ml portions of water and once with 100 ml of saturated aqueous sodium chloride solution. Dry the ether extracts over anhydrous magnesium sulfate, remove the drying agent, and evaporate to give 2-propenylsesamol, which will solidify on standing.

4. Carpanone (5)

Dissolve 1.0 g of the 2-propenylsesamol (4) prepared in the previous step in a solution of 5 ml of methanol and 10 ml of water. Add 3 g of sodium acetate and then a solution of 1 g of copper(II) acetate in 5 ml of water dropwise with stirring. Stir for an additional 5 min and filter the resulting suspension. Dilute the filtrate with twice its volume of water and extract with three 35-ml portions of ether. Wash the combined ether extracts with two 50-ml portions of saturated sodium chloride solution and dry the extracts over anhydrous magnesium sulfate. Remove the drying agent by filtration and evaporate the ether. Take up the residue in 2 ml of warm carbon tetrachloride, stopper the flask, and set aside to crystallize. If crystals have not formed overnight, add a few seed crystals and let the solution stand for several hours. After crystallization, decant the solvent with a micropipette and allow the crystals to dry. Since each molecule of carpanone crystallizes with a molecule of carbon tetrachloride the theoretical yield is 1.4 g, mp 185°.

▼

***Caution.** Carbon tetrachloride is toxic. Handle with care in the hood.*

51

Dichlorocarbene

KEYWORDS

Carbene, bivalent carbon, electrophile
Cyclopropanes
Sodium trichloroacetate
Trichloromethyl anion
Dichlorocarbene
1,5-Cyclooctadiene
Diglyme
Phase transfer catalysis
Benzyltriethylammonium chloride

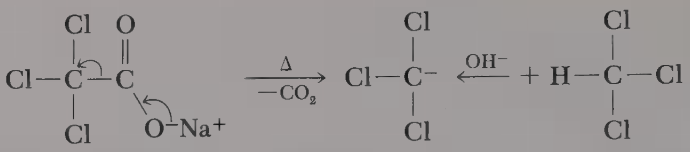
Dichlorocarbene is a highly reactive intermediate of bivalent carbon with only six valence electrons around the carbon. It is electrically neutral and a powerful electrophile. As such it reacts with alkenes forming cyclopropane derivatives by *cis*-addition to the double bond.

Of the dozen or so ways by which dichlorocarbene may be generated we shall investigate two. In the first experiment thermal decomposition of anhydrous sodium trichloroacetate in an aprotic solvent in the presence of *cis*, *cis*-1,5-cyclooctadiene gives 1. In the second experiment, reaction of chloroform with aqueous hydroxide ion in the presence of a phase transfer catalyst and cyclohexene gives 3.

EXPERIMENTS

1. Thermal Decomposition of Sodium Trichloroacetate; Reaction of Dichlorocarbene with 1,5-Cyclooctadiene

The thermal decomposition of sodium trichloroacetate initially gives the trichloromethyl anion (Eq. 1). In the presence of a proton-donating solvent (or moisture) this anion gives chloroform; in the absence of these reagents the anion decomposes by loss of chloride ion to give dichlorocarbene (Eq. 2).



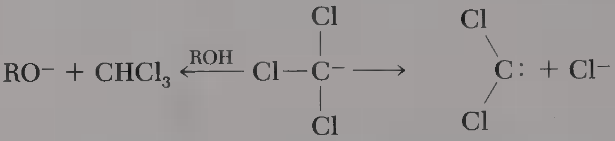
$$\mathrm{Cl_3C{-}CO_2^-Na^+} \xrightarrow[-CO_2]{\Delta} \mathrm{Cl_3C^-} \xleftarrow{OH^-} + \mathrm{H{-}CCl_3}$$

Eq. 1

Sodium trichloroacetate MW 185.39

Trichloromethyl anion

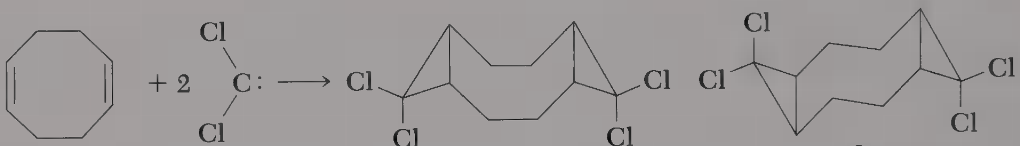
Chloroform MW 119.39, bp 61°



$$RO^- + CHCl_3 \xleftarrow{ROH} Cl_3C^- \longrightarrow Cl_2C{:} + Cl^-$$

Eq. 2

Dichlorocarbene



Eq. 3

1

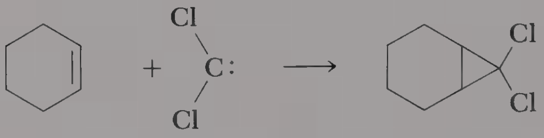
2

***cis,cis*-1,5-Cyclooctadiene** MW 108.14, bp 149–150°

cis, mp 176°

trans, mp 230° dec

5,5,10,10-Tetrachlorotricyclo[7.1.0.0^{4,6}]decane MW 274.03



Eq. 4

3

Cyclohexene MW 82.14, bp 83°

7,7-Dichlorobicyclo[4.1.0]heptane MW 165.06, bp 198°

▼ $CH_3OCH_2CH_2OCH_2CH_2OCH_3$ **Diglyme** MW 134.17, bp 161°, miscible with water

The conventional method for carrying out the reaction is to add the salt portionwise, during 1–2 hr, to a magnetically stirred solution of the olefin in diethylene glycol dimethyl ether ("diglyme") at a temperature maintained in a bath at 120°. Under these conditions the reaction mixture becomes almost black, isolation of a pure product is tedious, and the yield is low.

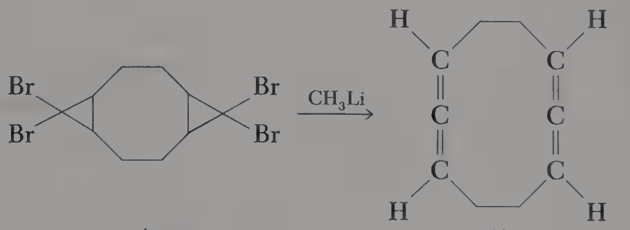
Tetrachloroethylene, a nonflammable solvent widely used in the dry cleaning industry, boils at 121° and is relatively inert toward electrophilic dichlorocarbene. On generation of dichlorocarbene from either chloroform or sodium trichloroacetate in the presence of tetrachloroethylene the yield of hexachlorocyclopropane (mp 104°) is only 0.2–10% (W. R. Moore, 1963; E. K. Fields,

▼
Rationale of the procedure

1963). The first idea for simplifying the procedure[1] was to use tetrachloroethylene to control the temperature to the desired range, but sodium trichloroacetate is insoluble in this solvent and no reaction occurs. Diglyme, or an equivalent, is required to provide some solubility. The reaction proceeds better in a 7:10 mixture of diglyme to tetrachloroethylene than in diglyme alone, but the salt dissolves rapidly in this mixture and has to be added in several small portions and the reaction mixture becomes very dark. The situation is vastly improved by the simple expedient of decreasing the amount of diglyme to a 2.5:10 ratio. The salt is so sparingly soluble in this mixture that it can be added at the start of the experiment and it dissolves slowly as the reaction proceeds. The boiling and evolution of carbon dioxide provide adequate stirring, the mixture can be left unattended, and what little color develops is eliminated by washing the crude product with methanol.

▼
X-ray analysis

The main reaction product crystallizes from ethyl acetate in beautiful prismatic needles, mp 177–179°, and this was the sole product encountered in runs made in the solvent mixture recommended. In earlier runs made in diglyme with manual control of temperature, the ethyl acetate mother liquor material on repeated crystallization from toluene afforded small amounts of a second isomer, mp 230°, dec. Analyses checked for a pair of *cis-trans* isomers and both gave negative permanganate tests. To distinguish between them, the junior author of the paper cited[1] undertook an X-ray analysis which showed that the lower melting isomer is *cis* and the higher melting isomer is *trans*.



4

5,5,10,10-Tetrabromotricyclo-[7.1.0.0^{4,6}]decane

5

Cyclodeca-1,2,6,7-tetraene

The bis adduct (4) of *cis,cis*-1,5-cyclooctadiene with dibromocarbene is described as melting at 174–180° and may be a *cis-trans* mixture. Treatment of the substance with methyl lithium at −40° gave a small amount of the ring-expanded bisallene (5).

Procedure

▼
A striking reaction of a bis dihalocarbene adduct

Place 12.8 g of trichloroacetic acid in a 125-ml filter flask, dissolve 3.2 g of sodium hydroxide pellets in 12 ml of water in a 50-ml Erlenmeyer, cool the

---

[1] L. F. Fieser and David H. Sachs, *J. Org. Chem.* **29**, 1113 (1964).

solution thoroughly in an ice bath, and swirl the flask containing the acid in the ice bath while slowly pouring in about nine-tenths of the alkali solution. Then add a drop of 0.04% Bromocresol Green solution to produce a faint yellow color, visible when the flask is dried and placed on white paper. With a capillary dropping tube (Pasteur pipette), titrate the solution to an endpoint where a single drop produces a change from yellow to blue. If the endpoint is overshot, add a few crystals of acid and titrate more carefully. Close the flask with a rubber stopper, connect the side arm to an aspirator, and place the flask within the rings of the steam bath and wrap a towel around both for maximum heat. Turn on the water at full force for maximum suction. The evaporation requires no further attention and should be complete in 15–20 min. When you have an apparently dry white solid, scrape it out with a spatula and break up the large lumps. If you see any evidence of moisture, or in case the weight exceeds the theory, place the solid in a 25 × 150-mm test tube and rest this on its side on a drying tray mounted 5 cm above the base of a 70-watt hot plate and let the drier operate overnight.

▼
Make sure that the salt is completely dry

Place 18.1 g of dry sodium trichloroacetate in a 250-ml round-bottomed flask mounted over a microburner and add 20 ml of tetrachloroethylene, 5 ml of diglyme, and 5 ml (4.4 g) of *cis,cis*-1,5-cyclooctadiene. Attach a reflux condenser and in the top opening of the condenser insert a rubber stopper carrying a glass tube connected by rubber tubing to a short section of glass tube which can be inserted below the surface of 2–3 ml of tetrachloroethylene in a 20 × 150 mm test tube mounted at a suitable level. This bubbler will show when the evolution of carbon dioxide ceases; the solvent should be the same as that of the reaction mixture, should there be a suckback. Heat to boiling, note the time, and reflux gently until the reaction is complete. You will notice foaming, due to liberated carbon dioxide, and separation of finely divided sodium chloride. Inspection of the bottom of the flask will show lumps of undissolved sodium trichloroacetate which gradually disappear. A large flask is specified because it will serve later for removal of tetrachloroethylene by steam distillation. Make advance preparation for this operation.

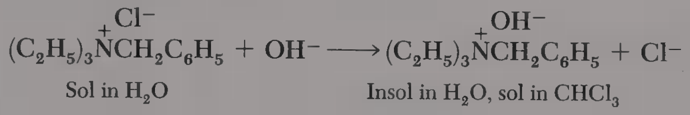
▼
Reflux time: about 75 min

▼
Easy workup

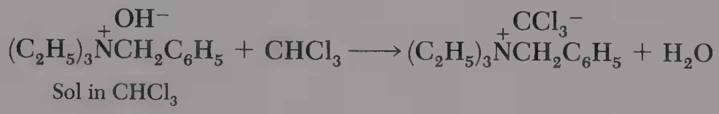
When the reaction is complete add 75 ml of water to the hot mixture, heat with the small flame of a microburner, and steam distil until the tetrachloroethylene is eliminated and the product separates as an oil or semisolid. Cool the flask to room temperature, decant the supernatant liquid into a separatory funnel and extract with dichloromethane. Run the extract into the reaction flask and use enough more dichloromethane to dissolve the product; use a capillary dropping tube to rinse down material adhering to the adapter. Run the lower layer into an Erlenmeyer through a cone of anhydrous sodium sulfate in a funnel and evaporate the solvent (bp 41°). The residue is a tan or brown solid (8 g). Cover it with methanol, break up the cake with a flattened stirring rod, and crush the lumps. Cool in ice, collect, and wash the product with methanol. The yield of colorless, or nearly colorless, 5,5,10,10-tetrachlorotricyclo[7.1.0.$0^{4,6}$]-decane is 3.3 g. This material, mp 174–175°, consists almost entirely of the *cis* isomer. Dissolve it in ethyl acetate (15–20 ml) and let the solution stand undisturbed for crystallization at room temperature. The pure *cis* isomer separates in large, prismatic needles, mp 175–176°.

2. Phase Transfer Catalysis—Reaction of Dichlorocarbene with Cyclohexene

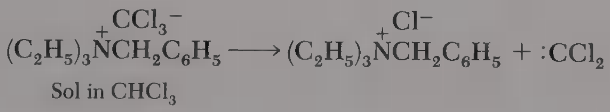
Ordinarily water must be scrupulously excluded from carbene generating reactions. Both the intermediate trichloromethyl anion and dichlorocarbene react with water. But in the presence of a phase transfer catalyst[2] (a quaternary ammonium salt such as benzyltriethylammonium chloride) it is possible to carry out the reaction in aqueous medium. Chloroform, 50% aqueous sodium hydroxide, and the olefin, in the presence of a catalytic amount of the quaternary ammonium salt, are stirred for a few minutes to produce an emulsion, and after the exothermic reaction is complete (about 30 min) the product is isolated. Apparently the water-soluble benzyltriethylammonium chloride reacts with hydroxide ion to give the water insoluble quaternary ammonium hydroxide ion (Eq. 5), which passes into the chloroform layer and generates the trichloromethyl anion and water (Eq. 6).

 Eq. 5

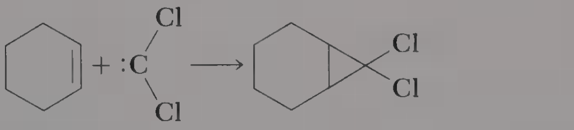
$$\underset{\text{Sol in }H_2O}{(C_2H_5)_3\overset{+\ Cl^-}{N}CH_2C_6H_5} + OH^- \longrightarrow \underset{\text{Insol in }H_2O\text{, sol in }CHCl_3}{(C_2H_5)_3\overset{+\ OH^-}{N}CH_2C_6H_5} + Cl^-$$

 Eq. 6

$$\underset{\text{Sol in }CHCl_3}{(C_2H_5)_3\overset{+\ OH^-}{N}CH_2C_6H_5} + CHCl_3 \longrightarrow (C_2H_5)_3\overset{+\ CCl_3^-}{N}CH_2C_6H_5 + H_2O$$

 Eq. 7

$$\underset{\text{Sol in }CHCl_3}{(C_2H_5)_3\overset{+\ CCl_3^-}{N}CH_2C_6H_5} \longrightarrow (C_2H_5)_3\overset{+\ Cl^-}{N}CH_2C_6H_5 + :CCl_2$$



Eq. 8

The trichloromethyl anion decomposes in the organic layer to give dichlorocarbene and chloride ion (Eq. 7). The carbene reacts with the olefin to form the product (Eq. 8) and the chloride ion regenerates the quaternary ammonium hydroxide ion which migrates back to the water layer to start the process anew.

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[2] E. Y. Dehmlow, *Angew. Chem., Int. Ed.*, 13, 170 (1974).

Procedure

▼
Caution! Chloroform is a mild carcinogen and should be handled in the hood. Avoid all contact with the skin.

To a mixture of 8.2 g of cyclohexene, 12.0 g of chloroform (*caution!*), and 20 ml of 50% aqueous sodium hydroxide in a 125 ml Erlenmeyer flask containing a thermometer add 0.2 g of benzyltriethylammonium chloride.[3] Swirl the mixture to produce a thick emulsion. The temperature of the reaction will rise gradually at first and then markedly accelerate. As it approaches 60° prepare to immerse the flask in an ice bath. With the flask both in and out of the ice bath, stir the thick paste and maintain the temperature between 50 and 60°. After the exothermic reaction is complete (about 10 min) allow the mixture to cool spontaneously to 35° and then dilute with 50 ml of water. Separate the layers (test to determine which is the organic layer), extract the aqueous layer once with 10 ml of ether, and wash the combined organic layer and ether extract once with 20 ml of water. Dry the cloudy organic layer by shaking it with anhydrous sodium sulfate until the liquid is clear. Decant into a 50-ml round-bottomed flask and distil the mixture (boiling chip), first from a steam bath to remove ether and some unreacted cyclohexene and chloroform, then over a free flame. Collect 7,7-dichlorobicyclo[4.1.0]heptane over the range 195–200°. Yield about 9 g. A purer product will result if the distillation is carried out under reduced pressure (Chapter 59).

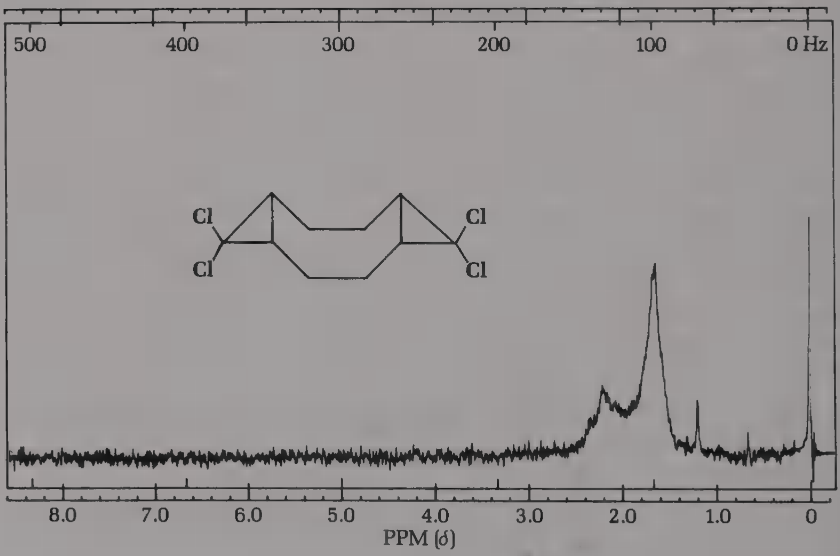

FIGURE 51.1 **^{1}H nmr spectrum of 5,5,10,10-tetrachlorotricyclo-[7,1,0,$0^{4,6}$]decane.**

▼
Note for the instructor

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[3] Prepared by adding 20.2 g (0.2 mole) of triethylamine to 25.3 g (0.2 mole) of benzyl chloride (α-chlorotoluene) in 50 ml of benzene (caution! mild carcinogen) with stirring. The benzene is removed at the water aspirator and the dry product pulverized in a mortar and stored in a desiccator over anhydrous calcium chloride and paraffin (to remove traces of benzene). Many other quaternary ammonium salts, commercially available, work equally well (e.g., cetyltrimethylammonium bromide).

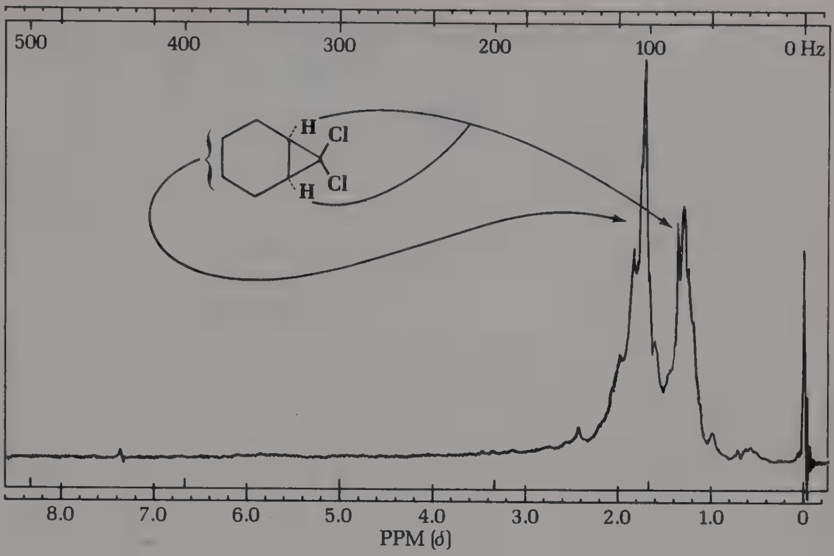


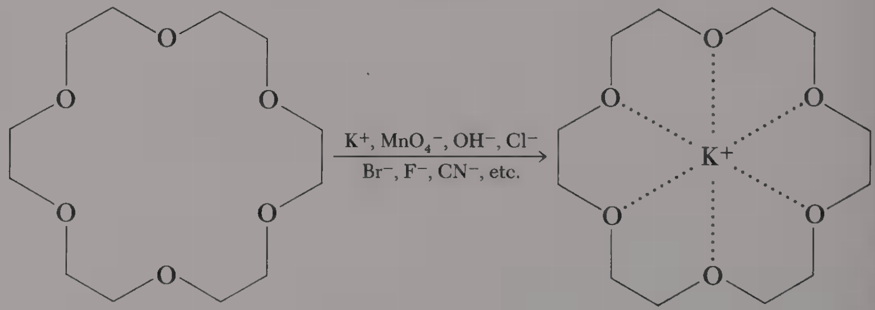
FIGURE 51.2 ^{1}H nmr spectrum of 7,7-dichlorobicyclo[4.1.0]-heptane.

52

Crown Ethers—18-Crown-6

KEYWORDS

Macrocyclic polyether, crown ether
Host–guest chemistry, molecular complex
Naked anions, powerful nucleophiles
Triethylene glycol



1
18-Crown-6
(1,4,7,10,13,16-Hexaoxacyclooctadecane)
MW 264.32, mp 37–38°

2
Potassium ion complex
(soluble in organic solvents)

Macrocyclic polyethers of the type exemplified by 18-crown-6 have the extraordinary ability to form stable molecular complexes with cations, owing to the efficient coordination of the cation by the ether oxygens.[1] The better

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[1] For a discussion of the complexes see C. J. Pederson and H. H. Frensdorff, *Angew. Chem., Internat. Edit.*, 11, 16 (1972).

▼
Host–guest chemistry

the cation (the guest) fits into the cavity of the crown ether (the host), the more stable the complex. The particular crown ether to be synthesized in this experiment, 18-crown-6 (18-membered ring, 6 oxygen atoms), has a cavity diameter estimated to be between 2.6Å and 3.2Å. It will, therefore, complex most strongly with Rb^+, NH_4^+, and K^+, which have ionic diameters of 2.94A, 2.86A, and 2.66A, respectively. It will not, however, complex with Na^+ (diameter of Na^+, 2.2A). When a complex is formed, the cation will be in the hole and the anion will be associated with the cation; the complex is soluble in organic solvents.

The anion is not highly solvated in organic solvents, as it would be in water, and consequently this "naked" anion is highly reactive. For instance, the 18-crown-6 complex with potassium fluoride is a highly reactive nucleophile in benzene solutions. Primary alkyl fluorides can be prepared from primary alkyl bromides in >90% yield employing this potassium fluoride complex.[2] Under the usual conditions (e.g., KF in diethylene glycol) the yields are about 40%. The crown ether complex of potassium hydroxide will displace the chloride ion from aryl chlorides to form phenols in organic solvents. In many of these reactions the crown ether can be used in catalytic amounts to solubilize the solid inorganic compound in an aprotic solvent like benzene. As such, it functions as a phase transfer catalyst between the solid and liquid phases [compare the phase transfer catalyst used to prepare dichlorocarbene (Chapter 51)]. 18-Crown-6 is the simplest member of this interesting new class of compounds, the crown ethers. Other crown ethers form complexes with ions of different sizes; asymmetric crown ethers will selectively complex one enantiomer in a racemic mixture, facilitating simple resolutions of, for example, amino acids.[3] A study of such host-guest chemistry may shed some light on the complexation-decomplexation reactions between enzymes and their substrates. The crown ethers bear a resemblance to the macrocyclic antibiotics in their ability to complex metal ions, and to effect ion transport and are, therefore, of interest to biochemists.

The procedure to be followed in this experiment involves initial conversion of the inexpensive solvent triethylene glycol to the corresponding 1,8-dichloro compound, **4**, followed by condensation of this halide with an equimolar amount of triethylene glycol in the presence of potassium hydroxide (an example of the Williamson ether synthesis) to give the product, 18-crown-6 (**1**).

EXPERIMENTS

1. 1,8-Dichloro-3,6-dioxaoctane (4)

▼
***Caution!** Benzene is a mild carcinogen. Carry out this entire experiment in a hood.*

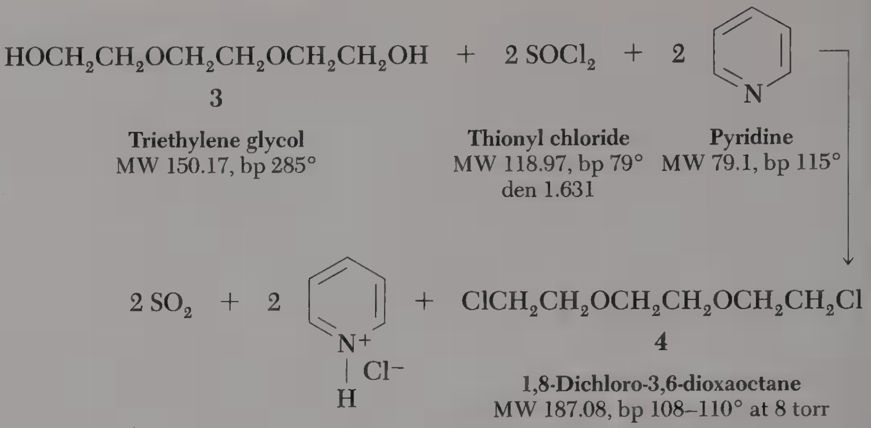
To a mixture of 10g of triethylene glycol, 70 ml of benzene (*caution!*), and 11.9 g of pyridine in a 250-ml, round-bottomed flask add 10.8 ml of thionyl chloride[4] dropwise with swirling. Reflux the mixture in the hood (SO_2 evolution) for 30 min, then cool the reaction mixture, pour it into a separatory

---

[2] C. L. Liotta and H. P. Harris, *J. Am. Chem. Soc.*, **96**, 2250 (1974).

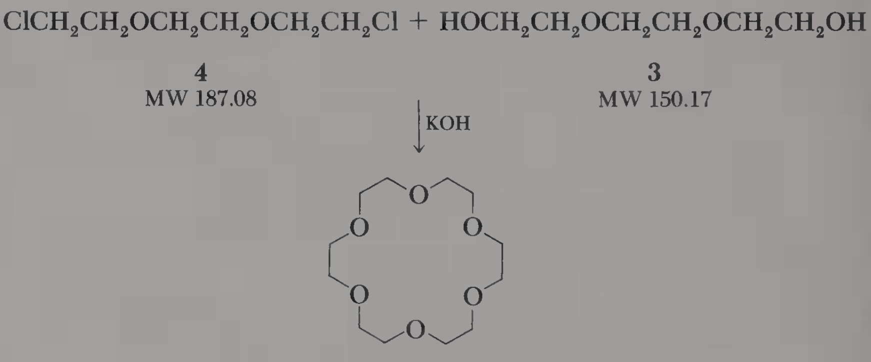
[3] R. C. Helgeson, K. Koga, J. M. Timko, and D. J. Cram, *J. Am. Chem. Soc.*, **95**, 3023 (1973).

[4] Conveniently and safely dispensed from a burette in the hood.



funnel, and shake it with 25 ml of 10% hydrochloric acid. Separate the layers, filter the organic layer through a cone of anhydrous sodium sulfate, and evaporate the benzene on a rotary evaporator (any residual water will evaporate as an azeotrope with the benzene). The residual light-yellow 1,8-dichloro-3,6-dioxaoctane (4) [also known as 1,2-bis(2-chloroethoxy)ethane] should weigh 12.9 g (100% yield).

2. 18-Crown-6 (1)



1

In a 25-ml Erlenmeyer flask held with a clamp add 2.2 g of potassium hydroxide pellets to 2.26 g of triethylene glycol. Heat the mixture to boiling over a microburner to dissolve all of the potassium hydroxide. Boil for one minute after the potassium hydroxide dissolves, then allow the mixture to cool slightly while rotating the flask to coat the wall and bottom with a uniformly thick layer of glycol–KOH paste. While the flask is still quite warm add 2.8 g of the dichloroether, 4, prepared in the previous experiment. With a stainless steel spatula scrape and stir the mixture in the flask. After approximately one minute a vigorous exothermic reaction will begin. Be prepared to lower the

▼
Do not scale up this reaction. *It is vigorously exothermic.*

flask into a pan of water to control the reaction. Continue to scrape and stir the paste until it begins to cool, then warm the mixture to boiling over a microburner for 3 min to complete the reaction. Cool the mixture under the tap, add 20 ml of dichloromethane, and remove the potassium chloride by filtration on a small Büchner funnel, using 5 ml more of dichloromethane to complete the transfer. Dry the dichloromethane solution over anhydrous sodium sulfate, remove the drying agent by filtration, and evaporate the solvent to leave crude 18-crown-6 (**1**) as a light brown oil. This oil can be used directly for the test that follows or several preparations can be pooled and distilled (bp 130–133° at 0.3 torr) to give a semisolid oil.

▼
Caution! *The toxicological effects of the macrocyclic polyethers are uncertain. Do not inhale the vapors of 18-crown-6 ether or allow it to contact the skin.*[7]

The semisolid oil can also be purified as its acetonitrile complex.[5] To do this, dissolve each gram of crude crown ether in 2.5 ml of hot acetonitrile. Cool the solution to room temperature and then in an ice-salt mixture before collecting the complex on a Büchner or Hirsch funnel. The complex melts at 63.5–65.5° and can be converted to the pure crown ether by pumping off the acetonitrile at 0.5 to 1.0 torr, while heating the complex at 40° in a small round-bottomed flask. The resulting 18-crown-6 has mp 36.5–38°.

3. Potassium permanganate complex of 18-crown-6[6]

▼
Caution! *Benzene is a mild carcinogen. Carry out this experiment in a hood.*

In each of four 13 × 100-mm test tubes place small quantities (about 10 mg) of finely powdered (mortar and pestle) potassium permanganate. Add 3 ml of benzene (*caution!*) to each tube and then, to the first, add two drops of your crude 18-crown-6 prepared in Section 2; to the second, add two drops of the dichloro compound (**4**) prepared in Section 1; and to the third, add two drops of triethylene glycol. To the fourth add nothing. Stopper the tubes, shake them vigorously for three min, and allow the permanganate to settle for one min before recording your observations. Finally, add a drop of cyclohexene to the first tube and note any apparent reaction.

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[5] C. L. Liotta, H. P. Harris, F. L. Cook, G. W. Gokel, and D. J. Cram, *J. Org. Chem.*, **39**, 2445 (1974).

[6] D. J. Sam and H. E. Simmons, *J. Am. Chem. Soc.*, **94**, 4024 (1972).

[7] See also *C&EN*, Jan. 27, 1975, p. 5.

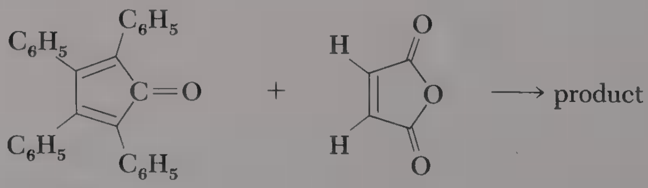
53

Order of Reaction

KEYWORDS

Tetraphenylcyclopentadienone
Maleic anhydride
Rate dependent on concentrate of dienophile?
CO given off?
Product structure?
Constant-temperature bath
Refluxing *o*-dichlorobenzene
Tetraethylene glycol, heat exchange fluid
Dewar condenser
Pyridinium bromide perbromide, aromatization

The second experiment of Chapter 44 involves a Diels-Alder reaction of tetraphenylcyclopentadienone with an acetylenic dienophile with loss of carbon monoxide and formation of an aromatic product. In the present experiment a pair of students is to study the reaction of tetraphenylcyclopentadienone with maleic anhydride at two concentrations of this dienophile and, from the results, to infer the nature of the adduct and to decide whether or not the reaction rate is dependent upon the concentration of the dienophile. Note that if the rate is indeed dependent upon concentration the time required for utilization of a given amount of colored dienone can be shortened by use of excess dienophile.



Tetraphenylcyclopentadienone
MW 384.45, mp 219°
(the diene)

Maleic anhydride
MW 98.06, mp 53°
(the dienophile)

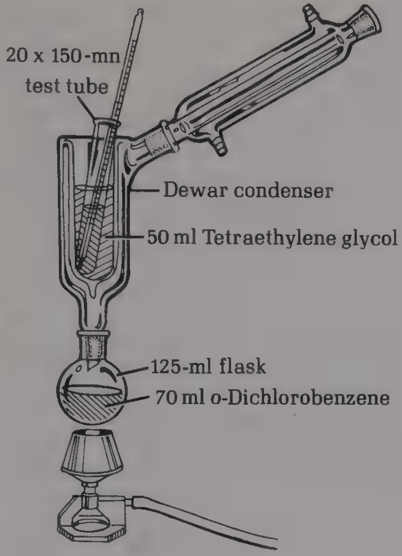


FIGURE 53.1 Constant temperature apparatus.

▼ *Constant-temperature boiler*

For significant comparison of reaction times at two concentrations of one reactant, it is necessary that the two runs be made at the same temperature. This condition is not fulfilled by carrying out the reactions in a given refluxing solvent, for the *liquid* temperature will be higher in the more concentrated of the two solutions because of greater elevation of the boiling point. An elegant constant-temperature bath (Fig. 53.1) is constructed from a Dewar condenser fitted with a 24/40 condenser and attached to a 125-ml round-bottomed flask containing 70 ml of *o*-dichlorobenzene (bp 179°). The reaction vessel is a 20 × 150-mm test tube placed in a heating bath containing 50 ml of the nonvolatile, high-boiling tetraethylene glycol as heat-exchange fluid.

You are to make runs at two concentrations of the dienophile that are sufficiently different to show whether or not the reaction rate is dependent upon the concentration of the dienophile. You are advised against use of only 1.0 equivalent of the dienophile, as the reaction will be inordinately slow.[2] For 1.0 g of tetraphenylcyclopentadienone and 5 ml of *o*-dichlorobenzene, it is suggested that in the first run you use 4 equivalents (1.02 g) of maleic anhydride. You can then decide on the amount to be used in the second run, for example, 3.0, 2.0, or 1.5 equivalents.

EXPERIMENT

Place 1.0 g of tetraphenylcyclopentadienone and 5 ml of *o*-dichlorobenzene in a 20 × 150-mm test tube, introduce a thermometer, note the time, place the tube in the heating bath of the *o*-dichlorobenzene boiler, and reflux vigorously until the purple solution acquires a constant temperature (which may be a few degrees below the true boiling point). The heatup period is about

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[1] O. Grummit, *Org. Syn., Coll. Vol.* 3, 807 (1945), mixed 0.094 mole of tetraphenylcyclopentadienone with 0.095 mole of maleic anhydride and 25 ml of bromobenzene (bp 155.5°) and refluxed the mixture for 3.5 hr; yield 88%.

18–20 min. A 1-g sample of maleic anhydride is then added and rinsed down the walls with a few drops of *o*-dichlorobenzene and the time is noted. Keep a time-temperature record and note particularly the time required to reach an end point, ie., a stage which you think you can recognize and reproduce in a second run, for example, a pale yellow solution with reduction in the rate of bubbling. For isolation of the product, remove the test tube from the boiler, dilute the mixture with toluene, cool in ice, and use ligroin to rinse the tube.

QUESTIONS

1. Is the reaction attended with elimination of carbon monoxide and what is the structure of the crystalline product? The reaction product is sparingly soluble in acetic acid, but a small sample (0.1 g) can be dissolved by refluxing it with this solvent. Addition to the hot solution of pyridinium hydrobromide perbromide (Chapter 12) will disclose unsaturation (and effect aromatization).
2. On the assumption that the reaction rate is dependent upon the concentration of the dienone, is the reaction:

$$\text{1st order, rate} = k_1\,[\text{dienone}] \text{ or}$$
$$\text{2nd order, rate} = k_2\,[\text{dienone}][\text{dienophile}]?$$

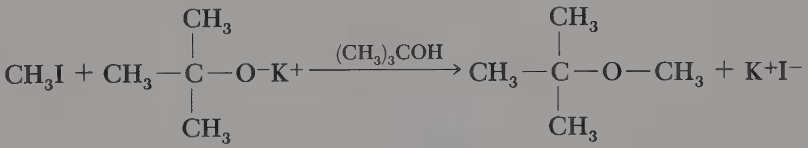
Note: The heating chamber shown in Fig. 53.1 is large enough to hold five 13 × 100-mm test tubes, each of which can easily accommodate 5 ml of solution. Hence, as many as 5 runs on the scale suggested can be made at the same time.

54

Reaction Kinetics: Williamson Ether Synthesis

KEYWORDS

Williamson ether synthesis
Rate constant, *k*
First order reaction
Second order reaction
Potassium *t*-butoxide
BASIC computer program
Slope
Time-zero



$$CH_3I + CH_3-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-O^-K^+ \xrightarrow{(CH_3)_3COH} CH_3-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{C}}-O-CH_3 + K^+I^-$$

The formation of *t*-butyl methyl ether from methyl iodide and potassium *t*-butoxide is an example of the Williamson ether synthesis. By analyzing this reaction as it proceeds you can determine the rate of the reaction as well as the order. In this way you also may be able to infer a mechanism for the reaction.

Known quantities of reactants are allowed to react for known lengths of time; from time to time, samples of the reaction mixture are analyzed quantitatively. You can follow either the disappearance of the reactants or the appearance of the products. Analysis of the reaction might include gas chromatography, infrared or nmr spectroscopy (methyl iodide decrease or ether increase), or reaction with aqueous silver nitrate to precipitate insoluble silver iodide. We shall use still another method—titration of the unreacted base, potassium *t*-butoxide, with an acid. When using this method aliquots (known fractions) of the reaction mixture are removed and immediately diluted with ice water to stop the reaction. Unreacted *t*-butoxide ion will react with the water to form hydroxide ion and *t*-butyl alcohol; the base is titrated to a phenolphthalein end point with standard perchloric acid.

The rate of the reaction depends on the temperature. Ideally the reaction should be run in a constant temperature bath; otherwise wrap the reaction flask with a towel to shield it from drafts, keep it stoppered, and assure that all reactants and apparatus are at room temperature (which should be above 25.5°, the freezing point of *t*-butyl alcohol).

EXPERIMENT

Procedure

Pipet 200 ml of a 0.110 *M* solution of methyl iodide in *t*-butyl alcohol into a 300 ml Erlenmeyer flask, quickly followed by exactly 20.0 ml of *ca.* 0.5 *M* potassium *t*-butoxide in *t*-butyl alcohol (note the exact concentration of the potassium *t*-butoxide on the label). Swirl to insure thorough mixing, and place the flask in the constant temperature bath (note exact temperature). Immediately withdraw a 10-ml aliquot ($^1/_{22}$ of the reaction mixture) with a pipet and run it into 20 ml of an ice and water mixture in a 250-ml Erlenmeyer flask. Between aliquots rinse the pipet with water and a few drops of acetone and dry by sucking air through it with an aspirator. It is not necessary to carry out the titration immediately, so label the flask. During the first hour withdraw 10-ml aliquots at about 10-min intervals (note the exact time in seconds when the pipet is half empty), then at 20-min intervals during the second hour. By the end of the second hour well over half of the reactants will have reacted to give the product. Add a drop of phenolphthalein indicator to each quenched aliquot and titrate with standardized perchloric acid to a very faint pink color when seen against a white background. Note the exact concentration of the perchloric acid; it should be near 0.025 *M*.

Mathematics of Rate Studies

In studying a reaction you must first determine what the products are. It has previously been found that the reaction of methyl iodide and potassium *t*-butoxide gives only the ether, but the equation tells you nothing about how the rate of the reaction is affected by the relative concentrations of the reactants, nor does it tell you anything about the mechanism of the reaction.

(a) First Order Reaction. The rate of a first order reaction depends only upon the concentration of one reactant, A. For example, if

$$2\text{A} + 3\text{B} \longrightarrow \text{products}$$

and the reaction is first order in A, despite what the equation seems to say the rate equation is

$$\text{Rate} = \frac{-d[\text{A}]}{dt} = k[\text{A}]$$

where [A] is the concentration of A, $-d[\mathrm{A}]/dt$ is the rate of disappearance of A with time, and k is a proportionality constant known as "the rate constant." Experimentally we determine

a = concentration of A at time zero
x = concentration of A which has reacted by time t
$a - x$ = concentration of A remaining at time t

The rate expression is then

$$\frac{dx}{dt} = k(a - x)$$

which upon integration gives

$$-\ln(a - x) = kt - \ln(a)$$

Changing signs and changing from base e to base 10 logarithms gives

$$\log(a - x) = \frac{-k}{2.303}t + \log(a)$$

This equation has the form of the equation for a straight line,

$$y = mx + b$$

If the reaction under study is first order, a straight line will be obtained when the concentration of A at various times is plotted against time. The slope of the line is $-k/2.303$, from which the value of the rate constant can be found. It will have units of $(\text{time})^{-1}$.

(b) Second Order Reaction. The rate of a second order reaction depends on the concentration of two reactants. For example if

$$2\mathrm{A} + 3\mathrm{B} \longrightarrow \text{products}$$

the rate equation would be

$$\text{Rate} = -\frac{d[\mathrm{A}]}{dt} = -\frac{d[\mathrm{B}]}{dt} = k_2[\mathrm{A}][\mathrm{B}]$$

Experimentally we determine

a = concentration of A at time zero
b = concentration of B at time zero
x = concentration of A (and concentration of B) which has reacted by time t
$a - x$ = concentration of A remaining at time t
$b - x$ = concentration of B remaining at time t

The rate equation now becomes

$$\frac{dx}{dt} = k_2(a - x)(b - x)$$

which on integration gives

$$k_2t = \frac{1}{a - b} \ln \frac{b(a - x)}{a(b - x)}$$

which upon rearrangement and conversion to base ten logarithms gives

$$k_2t = \frac{2.303}{a - b} \log \frac{a - x}{b - x} + \frac{2.303}{a - b} \log \frac{b}{a}$$

The final term is a constant. Rearrangement into the form of an equation for a straight line gives

$$\log \frac{a - x}{b - x} = k_2 \left(\frac{a - b}{2.303}\right) t + \text{constant}$$

If the reaction being studied is second order, a plot of log $(a - x/b - x)$ against time will give a straight line with slope of $k_2(a - b/2.303)$. The value of the rate constant is given by

$$k_2 = \text{slope} \left(\frac{2.303}{a - b}\right)$$

Treatment of Data

The fact that some reaction occurred before the first aliquot was withdrawn is immaterial. It is only necessary to know the concentrations of both reactants at the time of withdrawal of the first aliquot (which we shall call time-zero). The amount of base present at time-zero is determined directly by titration. The amount of methyl iodide present is equal to the concentration originally present after mixing minus that which reacted before the first aliquot was withdrawn. The amount which reacted before the first aliquot was withdrawn is equal to the amount of butoxide which reacted between the time of mixing and the time the first aliquot was withdrawn.

Make a rough plot of the concentration of methyl iodide and the concentration of potassium *t*-butoxide against time. If either gives a straight line relationship, the reaction is first order. If not, calculate the quantity log $(a - x/b - x)$ for each aliquot and plot against time in seconds. If this plot gives a straight line (within your experimental error) for this reaction, you now have strong evidence that the reaction is second order, i.e., first order in each reactant. Obtain the rate constant, *k*, for this reaction at this temperature, from the slope of your plot. The units of k_2 are moles $\text{sec}^{-1}\ 1^{-1}$.

Computer Analysis of Data

The repeated calculation of log $(a - x/b - x)$ for each aliquot from titration data is, to say the least, tedious. A computer program can do this simple job. After a little experience you will be able to write a program that would take as input the concentrations of all reactants, ml of acid, the time, and then print out k. There are standard computer programs ("library programs") available which will accept your various values of ln $(a - x/b - x)$ and the corresponding times, calculate the mathematically "best" straight line through the points (the "least squares" line) and print out the slope and intercept of the line.

A program written in the BASIC computer language to calculate the value of ln $(a - x/b - x)$ for various values of x looks like this:

```
10  READ A, B
20  DATA 0.098, 0.479
30  READ X
40  DATA 0.007, 0.019, 0.025, 0.037, 0.049, 0.058, 0.062
50  LET Y = LOG((A − X)/(B − X))
60  PRINT A,B,X,Y
70  GO TO 30
80  END
```

In this example, $a = 0.098$, $b = 0.479$, and $x = 0.007, 0.019$, etc. The computer will READ for "a" the value 0.098, because that is the first number it finds in the data printed in line 20. It reads 0.479 for "b" because that is the second number in line 20. The next command (line 30) says READ X and the computer reads the number 0.007 (line 40). We are trying to calculate ln $(a - x/b - x)$ so we set this equal to Y. The LET statement tells the computer to carry out the calculation substituting 0.098 for A, 0.479 for B, and 0.007 for X. LOG means natural logarithms to the computer so it carries out the computation and prints out the values of A, B, and X, which we supplied, and Y, which it calculates. The next command (line 70) says go back to line 30 and pick up new values for X, the values for A and B remaining the same. This the computer does, but this time it automatically substitutes 0.019 in the equation, ignoring 0.007 which it has already used. Again it carries out the computation, prints out A, B, X, and Y, and keeps going through the cycle until it runs out of data in line 40.

55

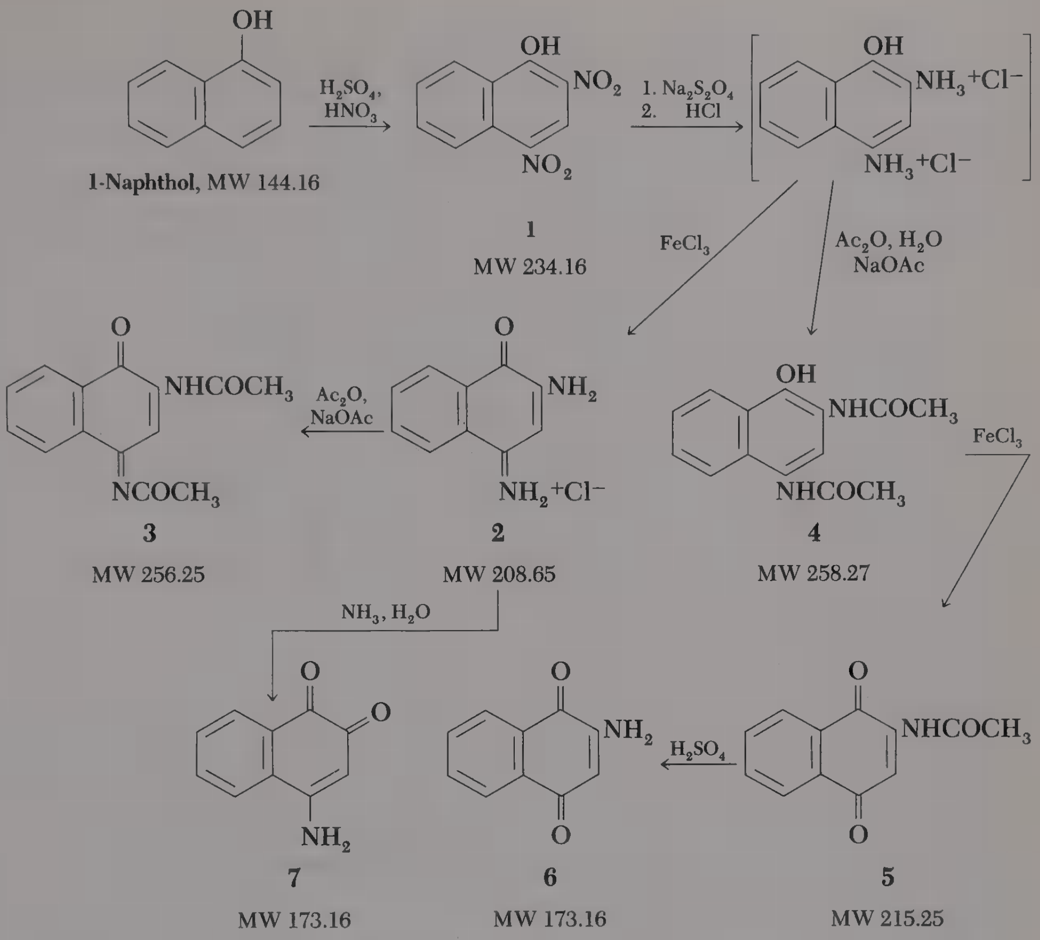
Martius Yellow

KEYWORDS

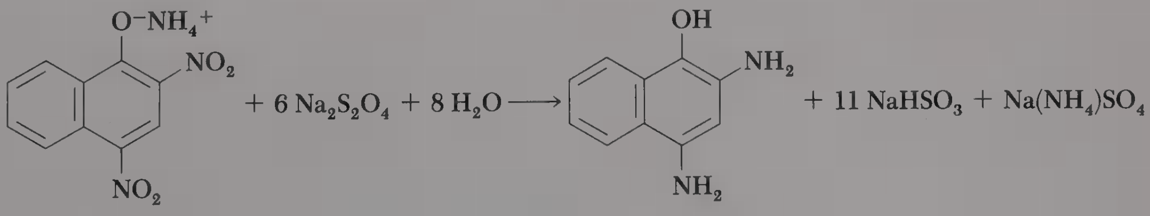
Rapid laboratory work, 3–4 hr
Small samples, seven crystalline compounds
A laboratory contest
Martius Yellow, dye
Sulfonation, nitration
Sodium hydrosulfite, reduction
Stable imine
Quinone
Acetylation

The series of experiments described here provides some experience in the rapid handling of small quantities of materials. Starting with 5 g of 1-naphthol, a skilled operator familiar with the procedures can prepare pure samples of the seven compounds in 3–4 hours. In a first trial of the experiment, a particularly competent student, who plans his work in advance, can complete the program in two laboratory periods (6 hr).

The first compound of the series, Martius Yellow, a mothproofing dye for wool (1 g of Martius Yellow dyes 200 g of wool) discovered in 1868 by Karl Alexander von Martius, is the ammonium salt of 2,4-dinitro-1-naphthol (**1**). Compound **1** in the series of reactions is obtained by sulfonation of 1-naphthol with sulfuric acid and treatment of the resulting disulfonic acid with nitric acid in aqueous medium. The exchange of groups occurs with remarkable ease, and it is not necessary to isolate the disulfonic acid. The advantage of introducing the nitro groups in this indirect way is that 1-naphthol is very sensitive to oxidation and would be partially destroyed on direct nitration. Martius Yellow is prepared by reaction of the acidic phenolic group of **1** with ammonia to form the ammonium salt. A small portion of this salt (Martius Yellow) is



converted by acidification and crystallization into pure 2,4-dinitro-1-naphthol (**1**), a sample of which is saved. The rest is suspended in water and reduced to diaminonaphthol with sodium hydrosulfite according to the equation:



Martius Yellow

2,4-Diamino-1-naphthol

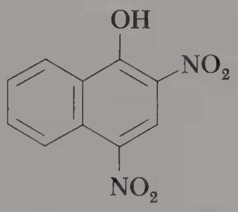
The diaminonaphthol separates in the free condition, rather than as an ammonium salt, because the diamine, unlike the dinitro compound, is a very weak acidic substance.

Since 2,4-diamino-1-naphthol is exceedingly sensitive to air oxidation as the free base, it is at once dissolved in dilute hydrochloric acid. The solution of diaminonaphthol dihydrochloride is clarified with decolorizing charcoal and divided into equal parts. One part on oxidation with iron(III) chloride affords the fiery red 2-amino-1,4-naphthoquinonimine hydrochloride (**2**). Since this substance, like many other salts, has no melting point, it is converted for identification to the yellow diacetate, **3**. Compound **2** is remarkable in that it is stable enough to be isolated. On hydrolysis it affords the orange 4-amino-1,2-naphthoquinone (**7**).

The other part of the diaminonaphthol dihydrochloride solution is treated with acetic anhydride and then sodium acetate; the reaction in aqueous solution effects selective acetylation on the amino groups and affords 2,4-diacetylamino-1-naphthol (**4**). Oxidation of **4** is attended with cleavage of the acetylamino group at the 4-position and the product is 2-acetylamino-1,4-naphthoquinone (**5**). This yellow substance is hydrolyzed by sulfuric acid to the red 2-amino-1,4-naphthoquinone (**6**), the last member of the series. The reaction periods are brief and the yields high; however, remember to scale down quantities of reagents and solvents if the quantity of starting material is less than that called for.[1]

EXPERIMENTS

1. Preparation of 2,4-Dinitro-1-naphthol(1)



1

Place 5 g of pure 1-naphthol[2] in a 125-ml Erlenmeyer flask, add 10 ml of concentrated sulfuric acid, and heat the mixture with swirling on the steam bath for 5 min, when the solid should have dissolved and an initial red color should be discharged. Cool in an ice bath, add 25 ml of water, and cool the solution rapidly to 15°. Measure 6 ml of concentrated nitric acid into a test tube and transfer it with a Pasteur pipette in small portions (0.5 ml) to the chilled aqueous solution while keeping the temperature in the range 15–20° by swirling the flask vigorously in the ice bath. When the addition is complete and the exothermic reaction has subsided (1–2 min), warm the mixture gently to 50° (1 min), when the nitration product should separate as a stiff yellow paste. Apply the full heat of the steam bath for 1 min more, fill the flask

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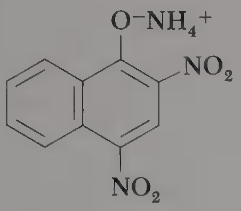
[1] This series of reactions lends itself to a laboratory competition, the rules for which might be as follows: (1) No practice or advance preparation is allowable except collection of reagents not available at the contestant's bench (ammonium chloride, sodium hydrosulfite, iron(III) chloride solution, acetic anhydride). (2) The time scored is the actual working time, including that required for bottling the samples and cleaning the apparatus and bench; labels can be prepared out of the working period. (3) Time is not charged during an interim period (overnight) when solutions are let stand to crystallize or solids are let dry, on condition that during this period no adjustments are made and no cleaning or other work is done. (4) Melting point and color test characterizations are omitted. (5) Successful completion of the contest requires preparation of authentic and macroscopically crystalline samples of all seven compounds. (6) Judgment of the winners among the successful contestants is based upon quality and quantity of samples, technique and neatness, and working time. (Superior performance: 3–4 hr.)

[2] If the 1-naphthol is dark it can be purified by distillation at atmospheric pressure. The colorless distillate is most easily pulverized before it has completely cooled and hardened.

▼
Avoid contact of the yellow product and its orange NH_4^+ *salt with the skin*

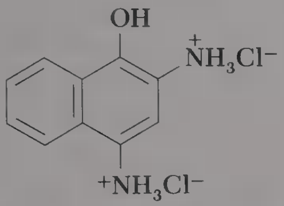
with water, break up the lumps and stir to an even paste, collect the product (1) on a Büchner funnel, wash it well with water, and then wash it into a 600-ml beaker with water (100 ml). Add 150 ml of hot water and 5 ml of concentrated ammonia solution (den 0.90), heat to the boiling point, and stir to dissolve the solid. Filter the hot solution by suction if it is dirty, add 10 g of ammonium chloride to the filtrate to salt out the ammonium salt (Martius Yellow), cool in an ice bath, collect the orange salt, and wash it with water containing 1–2% of ammonium chloride. The salt does not have to be dried (dry weight 7.7 g, 88.5%).



Martius Yellow

Set aside an estimated 0.3 g of the moist ammonium salt. This sample is to be dissolved in hot water, the solution acidified (HCl), and the free 2,4-dinitro-1-naphthol (1) crystallized from methanol or ethanol (use decolorizing charcoal if necessary); it forms yellow needles, mp 138°.

Preparation of Unstable 2,4-Diamino-1-naphthol

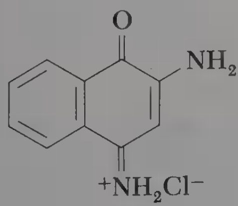


2,4-Diamino-1-naphthol dihydrochloride

Wash the rest of the ammonium salt into a beaker with a total of about 200 ml of water, add 40 g of sodium hydrosulfite, stir until the original orange color has disappeared and a crystalline tan precipitate has formed (5–10 min), and cool in ice. Make ready a solution of 1–2 g of sodium hydrosulfite in 100 ml of water for use in washing and a 400-ml beaker containing 6 ml of concentrated hydrochloric acid and 25 ml of water. In collecting the precipitate by suction filtration, use the hydrosulfite solution for rinsing and washing, avoid even briefly sucking air through the cake after the reducing agent has been drained away, and wash the solid at once into the beaker containing the dilute hydrochloric acid and stir to convert all the diamine to the dihydrochloride.

The acid solution, often containing suspended sulfur and filter paper, is clarified by filtration by suction through a moist charcoal bed made by shaking 2 g of the decolorizing carbon with 25 ml of water in a stoppered flask to produce a slurry and pouring this on the paper of an 85-mm Büchner funnel. Pour the water out of the filter flask and then filter the solution of dihydrochloride. Divide the pink or colorless filtrate into approximately two equal parts and at once add the reagents for conversion of one part to **2** and the other part to **4**.

2. Preparation of 2-Amino-1,4-naphthoquinonimine Hydrochloride (2)



2

To one half of the diamine dihydrochloride solution add 25 ml of 1.3 *M* iron(III) chloride solution,[3] cool in ice, and, if necessary, initiate crystallization by scratching. Rub the liquid film with a glass stirring rod at a single spot slightly above the surface of the liquid. If efforts to induce crystallizations are unsuccessful, add more hydrochloric acid. Collect the red product and wash with dilute HCl. Dry weight 2.4–2.7 g.

▼
Note for the instructor

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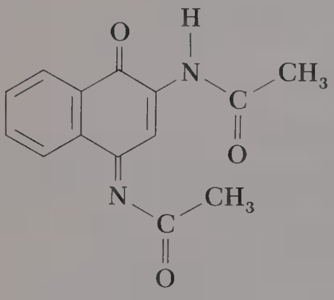
[3] Dissolve 90 g of $FeCl_3 \cdot 6H_2O$ (MW 270.32) in 100 ml of water and 100 ml of concentrated hydrochloric acid by warming, cool and filter (248 ml of solution).

Divide the moist product into three equal parts and spread out one part to dry for conversion to 3. The other two parts can be used while still moist for conversion to **7** and for recrystallization. Dissolve the other two parts by gentle warming in a little water containing 2–3 drops of hydrochloric acid, shake for a minute or two with decolorizing charcoal, filter, and add concentrated hydrochloric acid to decrease the solubility.

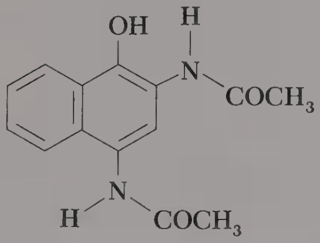
3. Preparation of 2-Amino-1,4-naphthoquinonimine Diacetate (3)



3

A mixture of 0.5 g of the dry quinonimine hydrochloride (**2**), 0.5 g of sodium acetate (anhydrous), and 3 ml of acetic anhydride is stirred in a test tube and warmed gently on a hot plate or steam bath. With thorough stirring the red salt should soon change into yellow crystals of the diacetate. The solution may appear red, but as soon as particles of red solid have disappeared the mixture can be poured into about 10 ml of water. Stir until the excess acetic anhydride has either dissolved or become hydrolyzed, collect and wash the product (dry weight 0.5 g), and (drying is unnecessary) crystallize it from ethanol or methanol; yellow needles, mp 189°.

4. Preparation of 2,4-Diacetylamino-1-naphthol (4)



4

To one half of the diaminonaphthol dihydrochloride solution saved from Section 1 add 3 ml of acetic anhydride, stir vigorously, and add a solution of 3 g of sodium acetate (anhydrous) and about 100 mg of sodium hydrosulfite in 20–30 ml of water. The diacetate may precipitate as a white powder or it may separate as an oil that solidifies when chilled in ice and rubbed with a rod. Collect the product and, to hydrolyze any triacetate present, dissolve it in 5 ml of 10% sodium hydroxide and 50 ml of water by stirring at room temperature. If the solution is colored, a pinch of sodium hydrosulfite may bleach it. Filter by suction and acidify by gradual addition of well-diluted hydrochloric acid (2 ml of concentrated acid). The diacetate tends to remain in supersaturated solution and hence, either to initiate crystallization or to insure maximum separation, it is advisable to stir well, rub the walls with a rod, and cool in ice. Collect the product, wash it with water, and divide it into thirds (dry weight 2.1–2.6 g).

Two thirds of the material can be converted without drying into **5** and the other third used for preparation of a crystalline sample. Dissolve the third reserved for crystallization (moist or dry) in enough hot acetic acid to bring about solution, add a solution of a small crystal of tin(II) chloride in a few drops of dilute hydrochloric acid to inhibit oxidation, and dilute gradually with 5–6 volumes of water at the boiling point. Crystallization may be slow, and cooling and scratching may be necessary. The pure diacetate forms colorless prisms, mp 224°, dec.

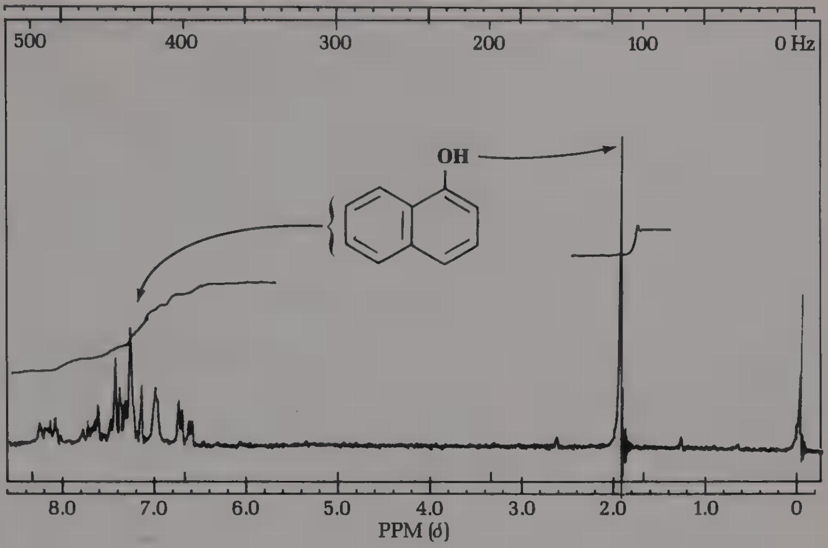


FIGURE 55.1 ^{1}H nmr spectrum of α-naphthol.

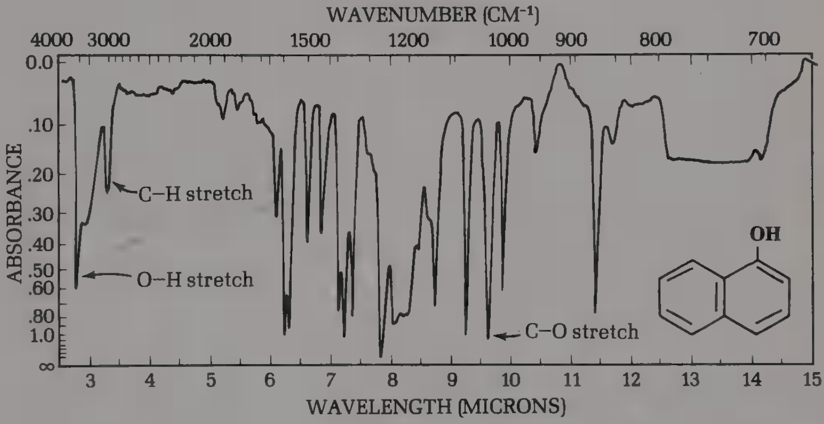
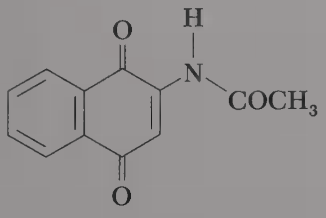


FIGURE 55.2 Infrared spectrum of α-naphthol.

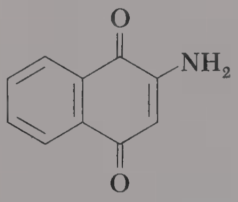


5

5. Preparation of 2-Acetylamino-1,4-naphthoquinone (5)

Dissolve 1.5 g of the moist diacetylaminonaphthol (4) in 10 ml of acetic acid (hot), dilute with 20 ml of hot water, and add 10 ml of 0.13 *M* iron(III) chloride solution. The product separates promptly in flat, yellow needles, which are collected (after cooling) and washed with a little alcohol; yield 1.2 g. Dry one-half of the product for conversion to 6 and crystallize the rest from 95% ethanol; mp 204°.

6. Preparation of 2-Amino-1,4-naphthoquinone (6)



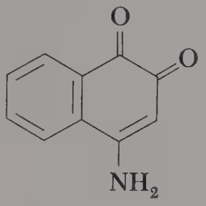
6

To 0.5 g of dry 2-acetylamino-1,4-naphthoquinone (5) contained in a 25-ml Erlenmeyer flask add 2 ml of concentrated sulfuric acid and heat the mixture on the steam bath with swirling to promote rapid solution (1–2 min). After 5 min cool the deep red solution, dilute extensively with water, and collect the precipitated product; wash it with water and crystallize the moist sample (dry weight 0.37 g) from alcohol or alcohol-water; red needles, mp 206°.

7. Preparation of 4-Amino-1,2-naphthoquinone (7)

▼
Moist 2 is satisfactory

Dissolve 1 g of the aminonaphthoquinonimine hydrochloride (2) reserved from Experiment 2 in 25 ml of water, add 2 ml of concentrated ammonia solution (den 0.90), and boil the mixture for 5 min. The free quinonimine initially precipitated is hydrolyzed to a mixture of the aminoquinone 7 and the isomer 6. Cool, collect the precipitate, and suspend it in about 50 ml of water, and add 25 ml of 10% sodium hydroxide solution. Stir well, remove the small amount of residual 2-amino-1,4-naphthoquinone (6) by filtration and acidify the filtrate with acetic acid. The orange precipitate of 7 is collected, washed, and crystallized while still wet from 500–600 ml of hot water (the separation is slow). The yield of orange needles, dec about 270°, is 0.4 g.



7

56

Dyes and Dyeing[1]

KEYWORDS

- Acetate rayon, Dacron, Nylon, cotton, wool, Orlon
- Azo dyes, diazotize, diazonium salt
- Indicators, Methyl Orange, Orange II
- Direct dyes, Martius Yellow, picric acid, eosin
- Substantive dyes, Congo Red
- Mordants, triphenylmethane dyes, Crystal Violet
- Developed dyes, ingrain dyes
- Vat dyes
- Leuco derivatives, indigo
- Disperse dyes, fiber reactive dyes
- Brighteners-fluorescent dyes

Since prehistoric times man has been dyeing cloth. The "wearing of the purple" has long been synonymous with royalty, attesting to the cost and rarity of Tyrian purple, a dye derived from the sea snail *Murex brandaris*. The organic chemical industry originated with William Henry Perkin's discovery of the first synthetic dye, Perkin's Mauve, in 1856.

In this experiment several dyes will be synthesized and these and other dyes will be used to dye a representative group of natural and man-made fibers.

You will receive several 2½″ squares of Multifiber Fabric 10A[2] which have six strips of different fibers woven into it: spun acetate rayon, cotton, a spun polyamide (Nylon 6,6), silk, viscose rayon, and wool.

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[1] For a detailed discussion of the chemistry of dyes and dyeing see *Topics in Organic Chemistry*. by Louis F. Fieser and Mary Fieser, Reinhold Publishing Corp., New York, 1963. p. 357–417.

[2] Obtained from Testfabrics, Inc., P.O. Box 118, 200 Blackford Ave., Middlesex, N.J. 08846. Cut into 2½″ squares which include all six fibers.

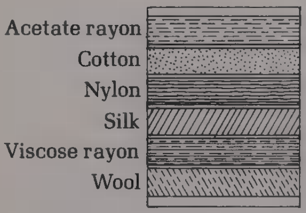
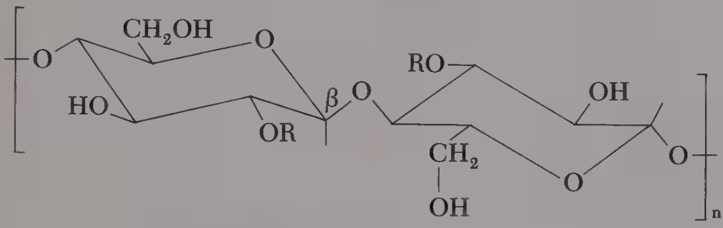


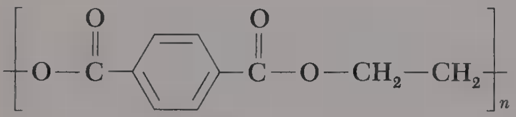
FIGURE 56.1 Multifiber fabric.

Acetate rayon is cellulose (from any source) in which about two of the hydroxyl groups in each unit have been acetylated. This renders the polymer soluble in acetone from which it can be spun into fiber. The smaller number of hydroxyl groups in acetate rayon compared to cotton makes direct dyeing of rayon more difficult than cotton.

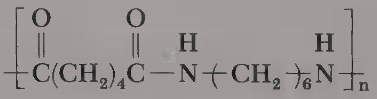
Cotton is pure cellulose. Nylon is a polyamide and made by polymerizing adipic acid and hexamethylenediamine. The nylon polymer chain can be prepared with one acid and one amine group at the termini, or with both acids or both amines. Except for these terminal groups, there are no polar centers in nylon and consequently it is difficult to dye. Similarly Dacron, a polyester made by polymerizing ethylene glycol and terephthalic acid, has few polar centers within the polymer, and consequently is difficult to dye. Even more difficult to dye is Orlon, a polymer of acrylonitrile. Wool and silk are polypeptides cross-linked with disulfide bridges. The acidic and basic amino acids (e.g., glutamic acid and lysine) provide many polar groups in wool and silk to which a dye can bind, making these fabrics easy to dye. In this experiment note the marked differences in shade produced by the same dye on different fibers.



Cellulose (Cotton, R=H)
Acetylated Cellulose (Acetate rayon, R=OAc)

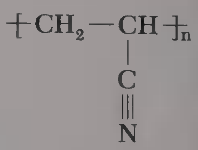


Polyethyleneglycol terephthalate
(Dacron)



$$\left[\overset{O}{\overset{\|}{C}}(CH_2)_4\overset{O}{\overset{\|}{C}}-\overset{H}{N}-(CH_2)_6-\overset{H}{N}\right]_n$$

Nylon



$$\left[CH_2-\underset{\underset{N}{\underset{|||}{C}}}{\underset{|}{CH}}\right]_n$$

Polyacrylonitrile
(Orlon)

Part 1. Dyes

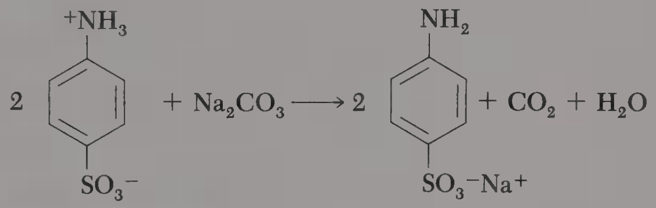
The most common dyes are the azo dyes, formed by coupling diazotized amines to phenols. The dye can be made in bulk, or, as we shall see, the dye molecule can be developed on and in the fiber by combining the reactants in the presence of the fiber.

One dye, Orange II, is made by coupling diazotized sulfanilic acid with 2-naphthol in alkaline solution; another, Methyl Orange, is prepared by coupling the same diazonium salt with N,N-dimethylaniline in a weakly acidic solution. Methyl Orange is used as an indicator as it changes color at pH 3.2–4.4. The change in color is due to transition from one chromophore (azo group) to another (quinonoid system).

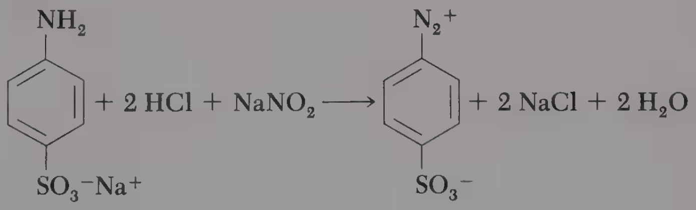
You are to prepare one of these two dyes and then exchange samples with a neighbor and do the tests with both dyes. Both substances dye wool, silk, and skin, and you must work carefully to avoid getting them on your hands or clothes. The dye will eventually wear off your hands or they can be cleaned by soaking them in warm, slightly acidic (H_2SO_4) permanganate solution until heavily stained with manganese dioxide and then removing the stain in a bath of warm, dilute bisulfite solution.

EXPERIMENTS

1. Diazotization of Sulfanilic Acid

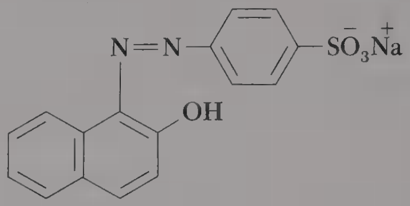


Sulfanilic acid
MW 173.19



In a 125-ml Erlenmeyer flask dissolve, by boiling, 4.8 g of sulfanilic acid monohydrate in 50 ml of 2.5% sodium carbonate solution (or use 1.33 g of anhydrous sodium carbonate and 50 ml of water). Cool the solution under the tap, add 1.9 g of sodium nitrite, and stir until it is dissolved. Pour the solution into a flask containing about 25 g of ice and 5 ml of concentrated hydrochloric acid. In a minute or two a powdery white precipitate of the diazonium salt should separate and the material is then ready for use. The product is not collected but is used in the preparation of the dye Orange II while in suspension. It is more stable than most diazonium salts and will keep for a few hours.

2. Orange II (1-*p*-Sulfobenzeneazo-2-naphthol Sodium Salt)



Orange II

▼
Choice of 2 or 3

In a 400-ml beaker dissolve 3.6 g of 2-naphthol in 20 ml of cold 10% sodium hydroxide solution and pour into this solution, with stirring, the suspension of diazotized sulfanilic acid from Section 1 (rinse). Coupling occurs very rapidly and the dye, being a sodium salt, separates easily from the solution because a considerable excess of sodium ion from the carbonate, the nitrite, and the alkali is present. Stir the crystalline paste thoroughly to effect good mixing and, after 5–10 min, heat the mixture until the solid dissolves. Add 10 g of sodium chloride to further decrease the solubility of the product, bring this all into solution by heating and stirring, set the beaker in a pan of ice and water, and let the solution cool undisturbed. When near room temperature, cool further by stirring and collect the product on a Büchner funnel. Use saturated sodium chloride solution rather than water for rinsing the material out of the beaker and for washing the filter cake free of the dark-colored mother liquor. The filtration is somewhat slow.[3]

The product dries slowly and it contains about 20% of sodium chloride. The crude yield is thus not significant, and the material need not be dried before being purified. This particular azo dye is too soluble to be crystallized from water; it can be obtained in a fairly satisfactory form by adding saturated sodium chloride solution to a hot, filtered solution in water and cooling, but the best crystals are obtained from aqueous ethanol. Transfer the filter cake to a beaker, wash the material from the filter paper and funnel with water, and bring the cake into solution at the boiling point. Avoid a large excess of water, but use enough to prevent separation of solid during filtration (use about 50 ml). Filter by suction through a Büchner funnel that has been preheated on the steam bath. Pour the filtrate into an Erlenmeyer flask (wash), estimate the volume, and if greater than 60 ml, evaporate by boiling. Cool to 80°, add 100–125 ml of ethanol, and allow crystallization to proceed. Cool the solution well before collecting the product. Rinse the beaker with mother liquor and wash finally with a little ethanol. The yield of pure, crystalline material

▼
Extinguish flames!

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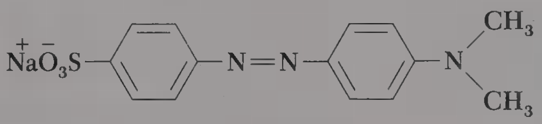
[3] If the filtration must be interrupted, fill the funnel, close the rubber suction tubing (while the pump is still running) with a screw pinchclamp placed close to the filter flask, and then disconnect the tubing from the pump. Set the unit aside; thus, suction will be maintained, and filtration will continue.

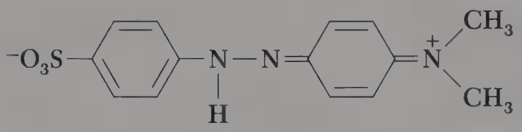
is 6.8 g. Orange II separates from aqueous alcohol with two molecules of water of crystallization and allowance for this should be made in calculating the yield. If the water of hydration is eliminated by drying at 120° the material becomes fiery red.

3. Methyl Orange (*p*-Sulfobenzeneazo-4-dimethylaniline Sodium Salt)

In a test tube, thoroughly mix 3.2 ml of dimethylaniline and 2.5 ml of glacial acetic acid. To the suspension of diazotized sulfanilic acid contained in a 400-ml beaker add, with stirring, the solution of dimethylaniline acetate (rinse). Stir and mix thoroughly and within a few minutes the red, acid-stable form of the dye should separate. A stiff paste should result in 5–10 min and 35 ml of 10% sodium hydroxide solution is then added to produce the orange sodium salt. Stir well and heat the mixture to the boiling point, when a large part of the dye should dissolve. Place the beaker in a pan of ice and water and allow the solution to cool undisturbed.



Methyl Orange
(alkali-stable form)



Methyl Orange
(acid-stable form)

When cooled thoroughly, collect the product on a Büchner funnel, using saturated sodium chloride solution rather than water to rinse the flask and to wash the dark mother liquor from the filter cake.

The crude product need not be dried but can be crystallized from water after making preliminary solubility tests to determine the proper conditions. The yield is 5–6 g.

Tests

Solubility and Color. Compare the solubility in water of Orange II and Methyl Orange and account for the difference in terms of structure. Treat the first solution with alkali and note the change in shade due to salt formation; to the other solution alternately add acid and alkali.

Reduction. Characteristic of an azo compound is the ease with which the molecule is cleaved at the double bond by reducing agents to give two amines. Since amines are colorless, the reaction is easily followed by the color change. The reaction is of use in preparation of hydroxyamino and similar compounds, in analysis of azo dyes by titration with a reducing agent, and in identification of azo compounds from an examination of the cleavage products.

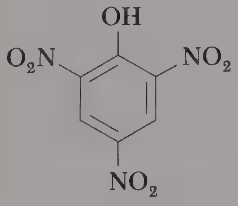
Dissolve about 0.5 g of tin(II) chloride in 1 ml of concentrated hydrochloric acid, add a small quantity of the azo compound (0.1 g), and heat. A colorless solution should result and no precipitate should form on adding water. The aminophenol or the diamine derivative is present as the soluble hydrochloride; the other product of cleavage, sulfanilic acid, is sufficiently soluble to remain in solution.

Part 2. Dyeing

EXPERIMENTS

1. Direct Dyes

The sulfonate groups on the Methyl Orange and Orange II molecules are polar and thus enable these dyes to combine with polar sites in the fibers. Wool and silk have many polar sites on their polypeptide chains and hence bind strongly to a dye of this type. Martius Yellow, picric acid, and eosin are also highly polar dyes and thus dye directly to wool and silk.

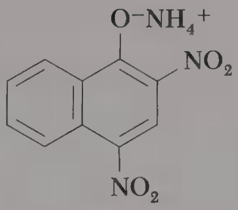


Picric Acid

Orange II or Methyl Orange

The dye bath is prepared from 0.5 g of Orange II or Methyl Orange, 5 ml of sodium sulfate solution, 300 ml of water, and 5 drops of concentrated sulfuric acid. Place a piece of test fabric in the bath for 5 min at a temperature near the boiling point. Remove fabric from the bath and let cool.

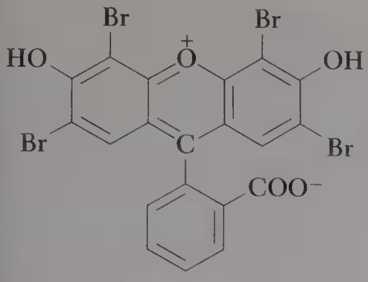
Replace one-half of the dyed fabric in the bath, make the solution alkaline with sodium carbonate, and add sodium hydrosulfite ($Na_2S_2O_4$) until the color of the bath is discharged. Account for the result.



Martius Yellow

Picric Acid or Martius Yellow

Dissolve 0.5 g of one of these acidic dyes in a little hot water to which a few drops of dilute sulfuric acid have been added. Heat a piece of test fabric in this bath for one minute, then remove it with a stirring rod, rinse well, wring, and dry. Describe the results.



Eosin A
(λ_{max} 516,483 nm)

Eosin

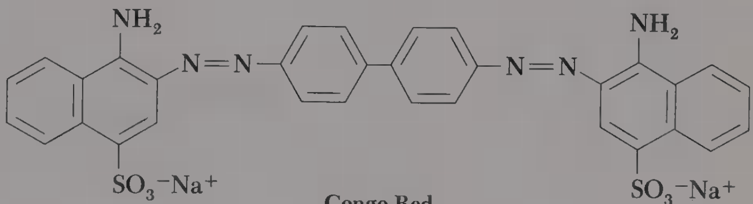
Dissolve 0.1 g of sodium eosin in 200 ml of water and dye a piece of test fabric by heating it with the solution for about 10 min. Eosin is the dye used in red ink.

2. Substantive Dyes

Cotton and the rayons do not have the anionic and cationic carboxyl and amine groups of wool and silk and hence do not dye well with direct dyes, but they can be dyed with substances of rather high molecular weight showing colloidal properties; such dyes probably become fixed to the fiber by hydrogen bonding. Such a dye is Congo Red, a substantive dye.

Congo Red, a Benzidine Dye

Dissolve 0.1 g of Congo Red in 400 ml of water, add about 1 ml each of 10% solutions of sodium carbonate and sodium sulfate, heat to a temperature just below the boiling point, and introduce a piece of test fabric. At the end of 10 min remove the fabric and wash in warm water as long as the dye is removed. Place pieces of the dyed material in very dilute hydrochloric acid solution and observe the result. Rinse and wash the material with soap.



Congo Red
(λ_{max} 497 nm)

3. Mordant Dyes

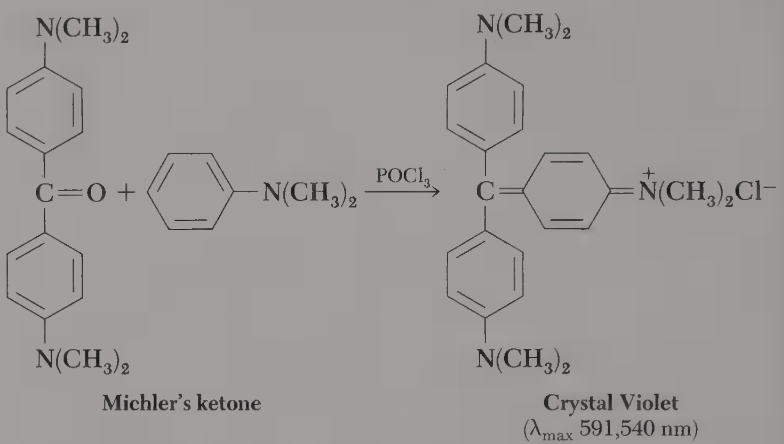
One of the oldest known methods of producing wash-fast colors involves the use of metallic hydroxides, which form a link, or mordant (L. *mordere,* to bite), between the fabric and the dye. Other substances, such as tannic acid, also function as mordants. The color of the final product depends on both the dye used and the mordant. For instance, the dye Turkey Red is red with an aluminum mordant, violet with an iron mordant, and brownish-red with a chromium mordant. Important mordant dyes are those possessing a structure based on triphenylmethane, as exemplified by Crystal Violet and Malachite Green.

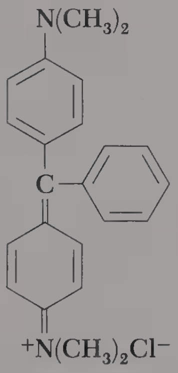
Synthesis of Crystal Violet, a Triphenylmethane Dye

Place 0.1 g of Michler's ketone, 5 drops of dimethylaniline, and 2 drops of phosphorus oxychloride in a test tube, and heat the tube in boiling water for

0.5 hr. Add 10 ml of water and stir. Add several drops of this solution to 20 ml of water and treat with a few drops of ammonium hydroxide solution. Let stand until the color has disappeared and then add dilute hydrochloric acid. Account for the color changes noted.

If the original solution is allowed to stand overnight, crystals of Crystal Violet should separate.





Malachite Green
(λ_{max} 617 nm)

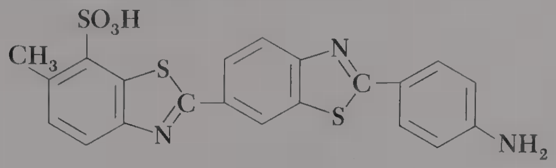
Dyeing with a Triphenylmethane Dye and a Mordant

Mordant pieces of cotton cloth by allowing them to stand in a hot solution of 0.5 g of tannic acid in 500 ml of water for 5 min. The mordant must now be fixed to the cloth, otherwise it would wash out. For this purpose, transfer the cloth to a hot bath made from 0.2 g of tartar emetic (potassium antimonyl tartrate) in 200 ml of water. After 5 min, wring the cloth. A dye bath is prepared by dissolving 0.1 g of either Crystal Violet or Malachite Green in 200 ml of water (boiling). Dye the mordanted cloth in this bath for 5–10 min at a temperature just below the boiling point. Try further dyeings with the test fabric.

Note: The stains on glass produced by triphenylmethane dyes can be removed with concentrated hydrochloric acid and washing with water, as HCl forms a di- or trihydrochloride more soluble in water than the original monosalt.

4. Developed Dyes

A superior method of applying azo dyes to cotton, patented in England in 1880, is that in which cotton is soaked in an alkaline solution of a phenol and then in an ice cold solution of a diazonium salt; the azo dye is developed directly on the fiber. The reverse process (ingrain dyeing) of impregnating cotton with an amine, which is then diazotized and developed by immersion in a solution of the phenol, was introduced in 1887. The first ingrain dye was Primuline Red, obtained by coupling the sulfur dye Primuline, after application to the cloth and diazotization, with 2-naphthol. Primuline (substantive to cotton) is a complex thiazole, prepared by heating *p*-toluidine with sulfur and then introducing a solubilizing sulfonic acid group.

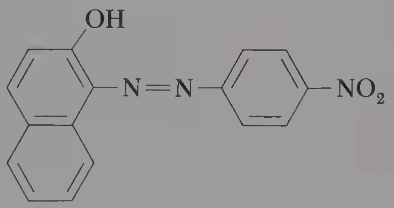


Primuline

Primuline Red

Dye three pieces of cotton cloth in a solution of 0.2 g of Primuline and 5 ml of sodium carbonate solution in 500 ml of water, at a temperature just below the boiling point for 15 min. Wash the cloth twice in about 500 ml of water. Prepare a diazotizing bath by dissolving 0.2 g of sodium nitrite in 500 ml of water containing a little ice and, just before using the bath, add 5 ml of concentrated hydrochloric acid. Allow the cloth dyed with Primuline to stay in this diazotizing bath for about 5 min. Now prepare three baths for the coupling reaction. Dissolve 0.1 g of 2-naphthol in 2 ml of 5% sodium hydroxide solution and dilute with 100 ml of water; prepare similar baths from phenol, resorcinol, Naphthol AS, or other phenolic substances.

Transfer the cloth from the diazotizing bath to a beaker containing about 500 ml of water and stir. Put one piece of cloth in each of the developing baths and allow them to stay for 5 min.



Para Red

Para Red, an Ingrain Color

Prepare a solution of *p*-nitrobenzenediazonium chloride as follows: dissolve 1.4 g of *p*-nitroaniline in a mixture of 30 ml of water and 6 ml of 10% hydrochloric acid by heating. Cool the solution in ice (the hydrochloride of the amine may crystallize), add all at once a solution of 0.7 g of sodium nitrite in about 5 ml of water and filter the solution by suction. The material to be dyed is first soaked in a solution prepared by suspending 0.5 g of 2-naphthol in 100 ml of water, stirring well and adding alkali, a drop at a time, until the naphthol all dissolves. This solution may also be painted onto the cloth. The cloth is then dried and dipped into the solution of the diazotized amine, after diluting the latter with about 300 ml of water.

Good results can be obtained by substituting Naphthol-AS for 2-naphthol; in this case, it is necessary to warm the Naphthol-AS with alkali and to break the lumps with a flattened stirring rod in order to bring the naphthol into solution.

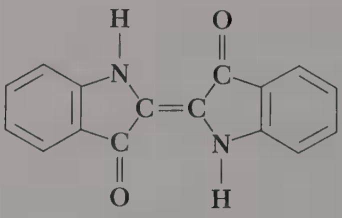
5. Vat Dyes

Vat dyeing depends upon the fact that some dyes (e.g., indigo) can be reduced to a colorless, or leuco, derivative, which is soluble in dilute alkali. If fabric is immersed in this alkaline solution, the leuco compound is adsorbed by hydrogen bonding. On exposure to air the leuco compound is oxidized to the dye, which remains fixed to the cloth. Vat dyes are all quinonoid substances that are readily reduced to hydroquinonoid compounds reoxidizable by oxygen in the air.

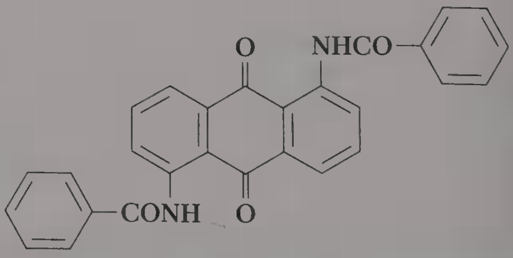
The indigo so formed is very insoluble in all solvents. However, it is not covalently bound to the cotton, only adhering to the surface of the fiber. Hence, it is subject to removal by abrasion. This explains why the knees and other parts of blue jeans (dyed exclusively with indigo) subject to wear will gradually turn white.

Dyeing with a Vat Dye

Use 0.2 g of one of the following dyes: Indigo, Indanthrene Brilliant Violet, Indanthrene Yellow. If one of the dyes is available in the form of a paste use as much as will adhere to about 1 cm of the end of a stirring rod. Boil the dye with 100–200 ml of water, 5 ml of 10% sodium hydroxide solution, and about 1 g of sodium hydrosulfite until the dye is reduced. Introduce a piece of cloth and boil the solution gently for 10 min. Rinse the cloth well in water and allow it to dry.



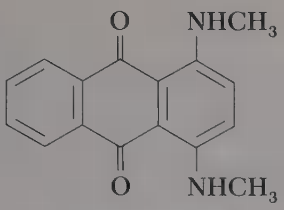
Indigo



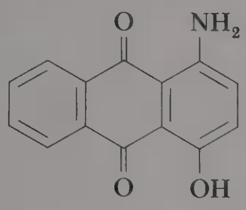
Indanthrene Yellow

6. Disperse Dyes

Fibers such as Dacron, acetate rayon, Nylon, and polypropylene are difficult to dye with conventional dyes because they contain so few polar groups. These fibers are dyed with substances which are insoluble in water but which at elevated temperatures (pressure vessels) are soluble in the fiber as true solutions. They are applied to the fiber in the form of a dispersion of finely divided dye, hence the name. The Cellitons are typical disperse dyes.



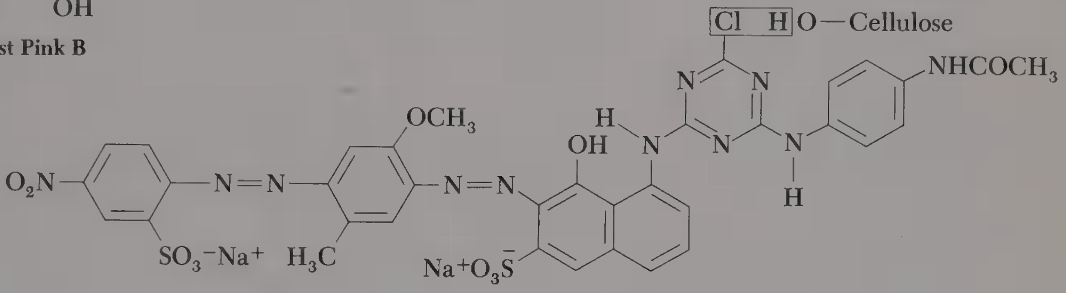
Celliton Fast Blue B



Celliton Fast Pink B

7. Fiber Reactive Dyes

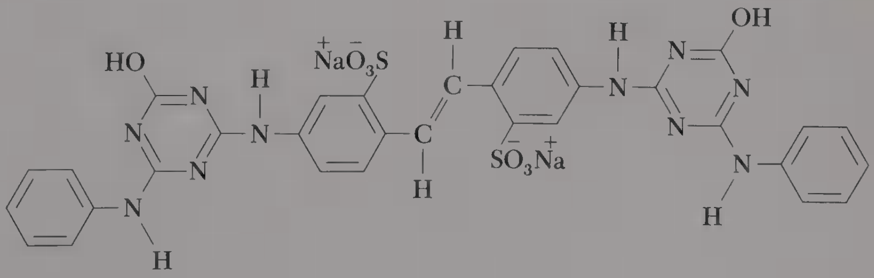
Among the newest of the dyes are the fiber reactive compounds which form a covalent link to the hydroxyl groups of cellulose. The reaction involves an amazing and little understood nucleophilic displacement of a chloride ion from the triazine part of the molecule by the hydroxyl groups of cellulose, yet the reaction occurs in aqueous solution.



Chlorantin Light Blue 8G

8. Optical Brighteners—Fluorescent White Dyes

Most modern detergents contain a blue-white fluorescent dye which is adsorbed on the cloth during the washing process. These dyes fluoresce, that is, absorb ultraviolet light and reemit light in the visible blue region of the spectrum. This blue color counteracts the pale yellow color of white goods, which develops because of a buildup of lipid soil. The modern day use of optical brighteners has replaced a past custom of using bluing (ferriferrocyanide).



Blankophor B
an optical brightener

Dyeing with Detergents

Immerse a piece of test fabric in a hot solution (0.5 g of detergent, 200 ml of water) of a commercial laundry detergent which you suspect may contain an optical brightener (e.g., *Tide* and *New Blue Cheer*) for 15 min. Rinse the fabric thoroughly, dry, and compare with an untreated fabric sample under an ultraviolet lamp.

57

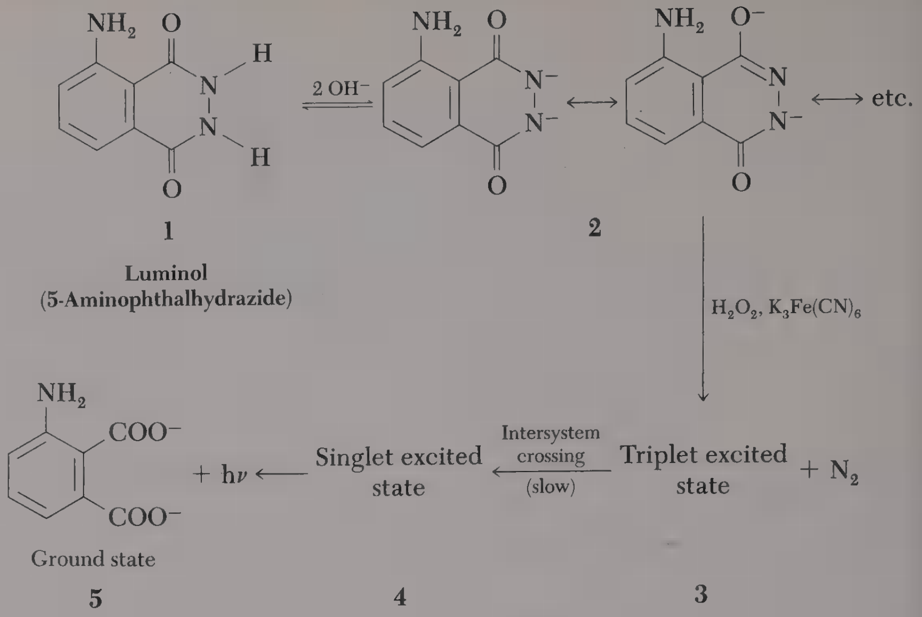
Luminol

KEYWORDS

Chemiluminescence, fluorescence, phosphorescence
Singlet, triplet, intersystem crossing
Excited state, ground state
Phthalic acid, hydrazine
Triethylene glycol, high bp solvent
Reduction, sodium hydrosulfite

The oxidation of 5-aminophthalhydrazide, **1**, commonly known as luminol, is attended with a striking emission of blue-green light. Most exothermic chemical reactions produce energy in the form of heat, but a few produce light and release little or no heat. This phenomenon, chemiluminescence, is usually an oxidation reaction. In the case of luminol an alkaline solution of the compound is allowed to react with a mixture of hydrogen peroxide and potassium ferricyanide. The dianion **2** is oxidized to the triplet excited state (two unpaired electrons of like spin) of the amino phthalate ion, **3**. This slowly undergoes intersystem crossing to the singlet excited state (two unpaired electrons of opposite spin) **4**, which decays to the ground state ion, **5**, with the emission of one quantum of light (a photon) per molecule. Very few molecules are more efficient in chemiluminescence than luminol.

Luminol, **1**, is made by reduction of the nitro derivative, **8**, formed on thermal dehydration of a mixture of 3-nitrophthalic acid, **6**, and hydrazine, **7**. An earlier procedure for effecting the first step called for addition of hydrazine sulfate to an alkaline solution of the acid, evaporation to dryness, and baking the resulting mixture of the hydrazine salt and sodium sulfate at 165°, and required a total of 4.5 hr for completion. This working time can be drastically reduced by adding high-boiling (bp 290°) triethylene glycol to an aqueous solution of the hydrazine salt, distilling the excess water, and raising the



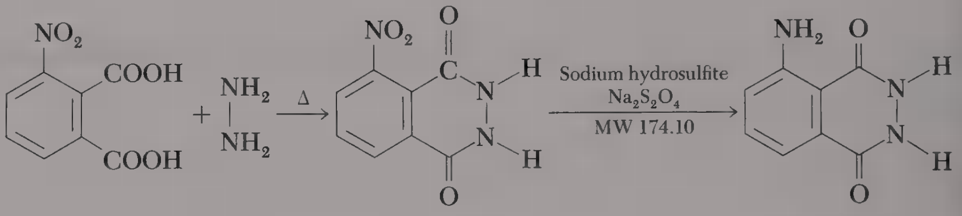
1
Luminol
(5-Aminophthalhydrazide)

2

5

4

3

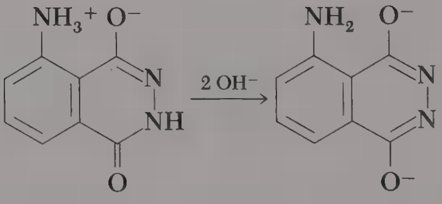


6
3-Nitrophthalic acid
Mp 222°,
MW 211.13

7
Hydrazine

8

1
Luminol
Mp 332°,
MW 177.16



9

10

temperature to a point where dehydration to 8 is complete within a few minutes. Nitrophthalhydrazide, 8, is insoluble in dilute acid, but soluble in alkali, by virtue of enolization, and is conveniently reduced to luminol, 1, by sodium hydrosulfite (sodium dithionite) in alkaline solution. In dilute, weakly acidic or neutral solution luminol exists largely as the dipolar ion 9 which exhibits beautiful blue fluorescence.[1]

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[1] Several methods of demonstrating the chemiluminescence of luminol are described by E. H. Huntress, L. N. Stanley, and A. S. Parker, *J. Chem. Ed.*, 11, 142 (1934). The mechanism of the reaction has been investigated by Emil H. White and co-workers. (*J. Am. Chem. Soc.*, 86, 940 and 942 (1964)).

An alkaline solution contains the doubly enolized anion **10** and displays particularly marked chemiluminescence when oxidized with a combination of hydrogen peroxide and potassium ferricyanide.

EXPERIMENT

▼
Caution! Hydrazine is a carcinogen. Handle with care. Wear gloves and carry out this experiment in the hood.

First heat a flask containing 15 ml of water on the steam bath. Then heat a mixture of 1 g of 3-nitrophthalic acid and 2 ml of an 8% aqueous solution of hydrazine[2] (*caution!*) in a 20 × 150-mm test tube over a free flame until the solid is dissolved, add 3 ml of triethylene glycol, and clamp the tube in a vertical position above a microburner. Insert a thermometer, a boiling chip, and an aspirator tube connected to an aspirator, and boil the solution vigorously to distil the excess water (110–130°). Let the temperature rise rapidly until (3–4 min) it reaches 215°. Remove the burner, note the time, and by intermittent gentle heating maintain a temperature of 215–220° for 2 min. Remove the tube, cool to about 100° (crystals of the product often appear), add the 15 ml of hot water, cool under the tap, and collect the light yellow granular nitro compound (**8**). (Dry weight, 0.7 g).[3]

▼
The two-step synthesis of a chemiluminescent substance can be completed in 25 min

$Na_2S_2O_4 \cdot 2H_2O$
Sodium hydrosulfite dihydrate
MW 210.15

The nitro compound need not be dried and can be transferred at once, for reduction, to the uncleaned test tube in which it was prepared. Add 5 ml of 10% sodium hydroxide solution, stir with a rod, and to the resulting deep brown-red solution add 3 g of sodium hydrosulfite dihydrate. Wash the solid down the walls with a little water. Heat to the boiling point, stir, and keep the mixture hot for 5 min, during which time some of the reduction product may separate. Then add 2 ml of acetic acid, cool under the tap, and stir; collect the resulting precipitate of light yellow luminol (**1**). The filtrate on standing overnight usually deposits a further crop of luminol (0.1–0.2 g).

▼
The light-producing reaction

Dissolve the first crop of moist luminol (dry weight, 0.2–0.3 g) in 10 ml of 10% sodium hydroxide solution and 90 ml of water; this is stock solution A. Prepare a second stock B by mixing 20 ml of 3% aqueous potassium ferricyanide, 20 ml of 3% hydrogen peroxide, and 160 ml of water. Now dilute 25 ml of solution A with 175 ml of water and, in a relatively dark corner or cupboard, pour this solution and solution B simultaneously into a funnel resting in the neck of a large Erlenmeyer flask. Swirl the flask and, to increase the brilliance, gradually add further small quantities of alkali and ferricyanide crystals.

Ultrasonic sound can also be used to promote this reaction. Prepare stock solutions A and B again but omit the hydrogen peroxide. Place the combined solutions in an ultrasonic cleaning bath or immerse an ultrasonic probe into the reaction mixture. Spots of light are seen where the ultrasonic vibrations produce hydroxyl radicals.

And the sanguinary can mix solutions A and B, omitting the ferricyanide from solution B. Light can be generated by adding blood dropwise to the reaction mixture.

---

[2] Dilute 31.2 g of the commercial 64% hydrazine solution to a volume of 250 ml.

[3] The reason for adding hot water and then cooling rather than adding cold water is that the solid is then obtained in more easily filterable form.

58

Qualitative Organic Analysis

Identification and characterization of the structures of unknown substances are an important part of organic chemistry. It is sometimes possible to establish the structure of a compound on the basis of spectra alone (IR, UV, and nmr), but these spectra must usually be supplemented with other information about the unknown: physical state, elementary analysis, solubility, and confirmatory tests for functional groups. Conversion of the unknown to a solid derivative of known melting point will often provide final confirmation of structure.

PROCEDURES

1. Physical State

Check for sample purity. Distill or recrystallize as necessary. Constant bp and sharp mp are indicators, but beware of azeotropes and eutectics. Check homogeneity by TLC, gas or paper chromatography.

Note the color. Common colored compounds include nitro and nitroso compounds (yellow), α-diketones (yellow), quinones (yellow to red), azo compounds (yellow to red), and polyconjugated olefins and ketones (yellow to red). Phenols and amines are often brown to dark-purple because of traces of air oxidation products.

Note the odor. Some liquid and solid amines are recognizable by their fishy odors; esters are often pleasantly fragrant. Alcohols, ketones, aromatic hydrocarbons, and aliphatic olefins have characteristic odors. On the unpleasant side are thiols, isonitriles, and low MW carboxylic acids.

Make an ignition test. Heat a small sample on a spatula; first hold the sample near the side of a microburner to see if it melts normally and then burns. Heat it in the flame. If a large ashy residue is left after ignition, the unknown is probably a metal salt. Aromatic compounds often burn with a smoky flame.

2. Spectra

Obtain infrared and nuclear magnetic resonance spectra following the procedures of Chapters 7 and 8.[1] If these spectra indicate the presence of conjugated double bonds, aromatic rings, or conjugated carbonyl compounds obtain the ultraviolet spectrum following the procedures of Chapter 9. Interpret the spectra as fully as possible by reference to the sources cited at the end of the various spectroscopy chapters.

▼
Explanation

3. Elementary Analysis, Sodium Fusion

This method for detection of nitrogen, sulfur, and halogen in organic compounds depends upon the fact that fusion of substances containing these elements with sodium yields NaCN, Na_2S, and NaX ($X = Cl, Br, I$). These products can, in turn, be readily identified. The method has the advantage that the most usual elements other than C, H, and O present in organic compounds can all be detected following a single fusion, although the presence of sulfur sometimes interferes with the test for nitrogen. Unfortunately, even in the absence of sulfur the test for nitrogen is sometimes unsatisfactory (nitro compounds in particular).

▼
***Caution!** Manipulate sodium with a knife and forceps; never touch it with the fingers. Wipe it free of kerosene with a dry towel or filter paper; return scraps to the bottle or destroy scraps with methyl or ethyl alcohol, never with water. Safety glasses!*

▼
Do not use $CHCl_3$ or CCl_4 as samples in sodium fusion. They react extremely violently.

▼
Notes for the instructor

Place a 3-mm cube of sodium[2] (30 mg, no more)[3] in a 10 × 75-mm Pyrex test tube and support the tube in a vertical position (Fig. 58.1). Have a microburner with small flame ready to move under the tube, place an estimated 20 mg of solid on a spatula or knife blade, put the burner in place, and heat until the sodium first melts and then vapor rises 1.5–2.0 cm in the tube. Remove the burner and at once drop the sample onto the hot sodium. If the substance is a liquid add 2 drops of it. If there is a flash or small explosion the fusion is complete; if not, heat briefly to produce a flash or a charring. Then let the tube cool to room temperature, be sure it is cold, add a drop of methanol, and let it react (heat effect). Repeat until 10 drops have been added. With a stirring rod break up the char to uncover sodium. When you are sure that all sodium has reacted, empty the tube into a 13 × 100-mm test tube, hold the small tube pointing away from you or a neighbor, and pipette into it 1 ml of

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[1] Kits of unknowns with IR and nmr spectra may be purchased from the Aldrich Chemical Co. The properties of all unknowns in kits A, B and C are included in the tables at the end of this chapter.

[2] Sodium spheres $\frac{1}{16}''$ to $\frac{1}{4}''$ (CB1035) from Matheson, Coleman, and Bell are convenient.

[3] A dummy 3 mm cube of rubber can be attached to the sodium bottle to indicate the correct amount.

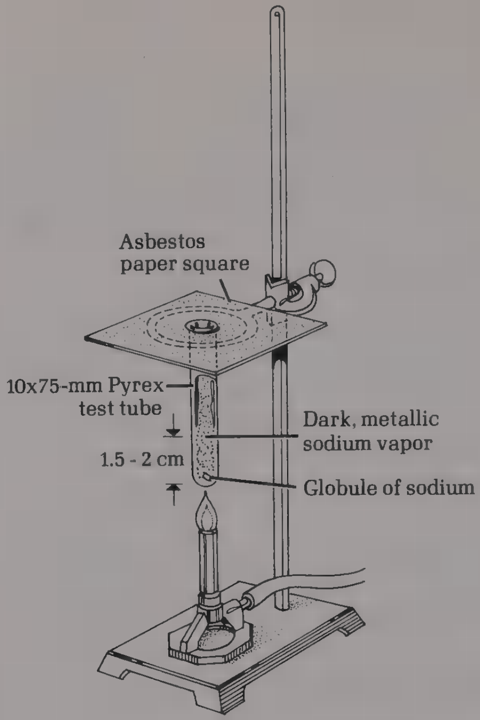


FIGURE 58.1 Sodium fusion, just prior to addition of sample.

water. Boil and stir the mixture and pour the water into the larger tube; repeat with 1 ml more water. Then transfer the solution with a capillary dropper to a 2.5-cm funnel (fitted with a fluted filter paper) resting in a second 13 × 100-mm test tube. Portions of the alkaline filtrate are used for the tests that follow:

▼ *Run each test on a known and an unknown*

(a) Nitrogen. The test is done by boiling the alkaline solution with iron(II) sulfate and then acidifying. Sodium cyanide reacts with iron(II) sulfate to produce ferrocyanide, which combines with iron(III) salts, inevitably formed by air oxidation in the alkaline solution, to give Prussian Blue, $NaFe^{+3}[Fe^{+2}(CN)_6]$. Iron(II) and iron(III) hydroxides precipitate along with the blue pigment but dissolve on acidification.

Place 50 mg of powdered iron(II) sulfate (this is a large excess) in a 13 × 100-mm test tube, add 0.5 ml of the alkaline solution from the fusion, heat the mixture gently with shaking to the boiling point, and then—without cooling—acidify with dilute sulfuric acid (hydrochloric acid is unsatisfactory). A deep blue precipitate indicates the presence of nitrogen. If the coloration is dubious, filter through a 2.5-cm funnel and see if the paper shows blue pigment.

▼ $Na_2(NO)Fe(CN)_6 \cdot 2H_2O$ *Sodium nitroprusside*

(b) Sulfur. (1) Dilute one drop of the alkaline solution with 1 ml of water and add a drop of sodium nitroprusside; a purple coloration indicates the presence of sulfur. (2) Prepare a fresh solution of sodium plumbite by adding 10% sodium hydroxide solution to 0.2 ml of 0.1 *M* lead acetate solution until the precipitate just dissolves, and add 0.5 ml of the alkaline test solution. A black precipitate or a colloidal brown suspension indicates the presence of sulfur.

Differentiation of the halogens

(c) Halogen. Acidify 0.5 ml of the alkaline solution from the fusion with dilute nitric acid (indicator paper) and, if nitrogen or sulfur has been found present, boil the solution (hood) to expel HCN or H_2S. On addition of a few drops of silver nitrate solution, halide ion is precipitated as silver halide. Filter with minimum exposure to light on a 2.5-cm funnel, wash with water, and then with 1 ml of concentrated ammonia solution. If the precipitate is white and readily soluble in ammonium hydroxide solution it is AgCl; if it is pale yellow and difficulty soluble it is AgBr; if yellow and insoluble it is AgI. Fluorine is not detected in this test since silver fluoride is soluble in water.

4. Beilstein Test for Halogens

Heat the tip of a copper wire in a burner flame until no further coloration of the flame is noticed. Allow the wire to cool slightly then dip it into the unknown (solid or liquid) and again heat it in the flame. A green flash is indicative of chlorine, bromine, and iodine; fluorine is not detected since copper fluoride is not volatile. The Beilstein test is very sensitive; halogen-containing impurities may give misleading results. Run the test on a compound known to contain halogen for comparison with your unknown.

It is good practice to run tests on knowns in parallel with unknowns for all qualitative organic reactions. In this way, interpretations of positive reactions are clarified and defective test reagents can be identified and replaced.

---

5. Solubility Tests

Like dissolves like; a substance is most soluble in that solvent to which it is most closely related in structure. This statement serves as a useful classification scheme for all organic molecules. The solubility measurements are done at room temperature with 1 drop of a liquid, or 5 mg of a solid (finely crushed), and 0.2 ml of solvent. The mixture should be rubbed with a rounded stirring rod and shaken vigorously. Lower members of a homologous series are easily classified, higher members become more like the hydrocarbons from which they are derived.

If a very small amount of the sample fails to dissolve when added to some of the solvent, it can be considered insoluble, and, conversely, if several portions dissolve readily in a small amount of the solvent, the substance is obviously soluble.

If an unknown seems to be more soluble in dilute acid or base than in water, the observation can be confirmed by neutralization of the solution; the original material will precipitate if it is less soluble in a neutral medium.

If both acidic and basic groups are present, the substance may be amphoteric and therefore soluble in both acid and base. Aromatic aminocarboxylic acids are amphoteric, like aliphatic ones, but they do not exist as inner salts. They are soluble in both dilute hydrochloric acid and sodium hydroxide, but not in bicarbonate solution. Aminosulfonic acids exist as inner salts; they are soluble in alkali but not in acid.

The solubility tests are not infallible and many borderline cases are known.

Carry out the tests according to the scheme of Fig. 58.2 and tentatively assign the unknown to one of the groups I-X.

6. Classification Tests

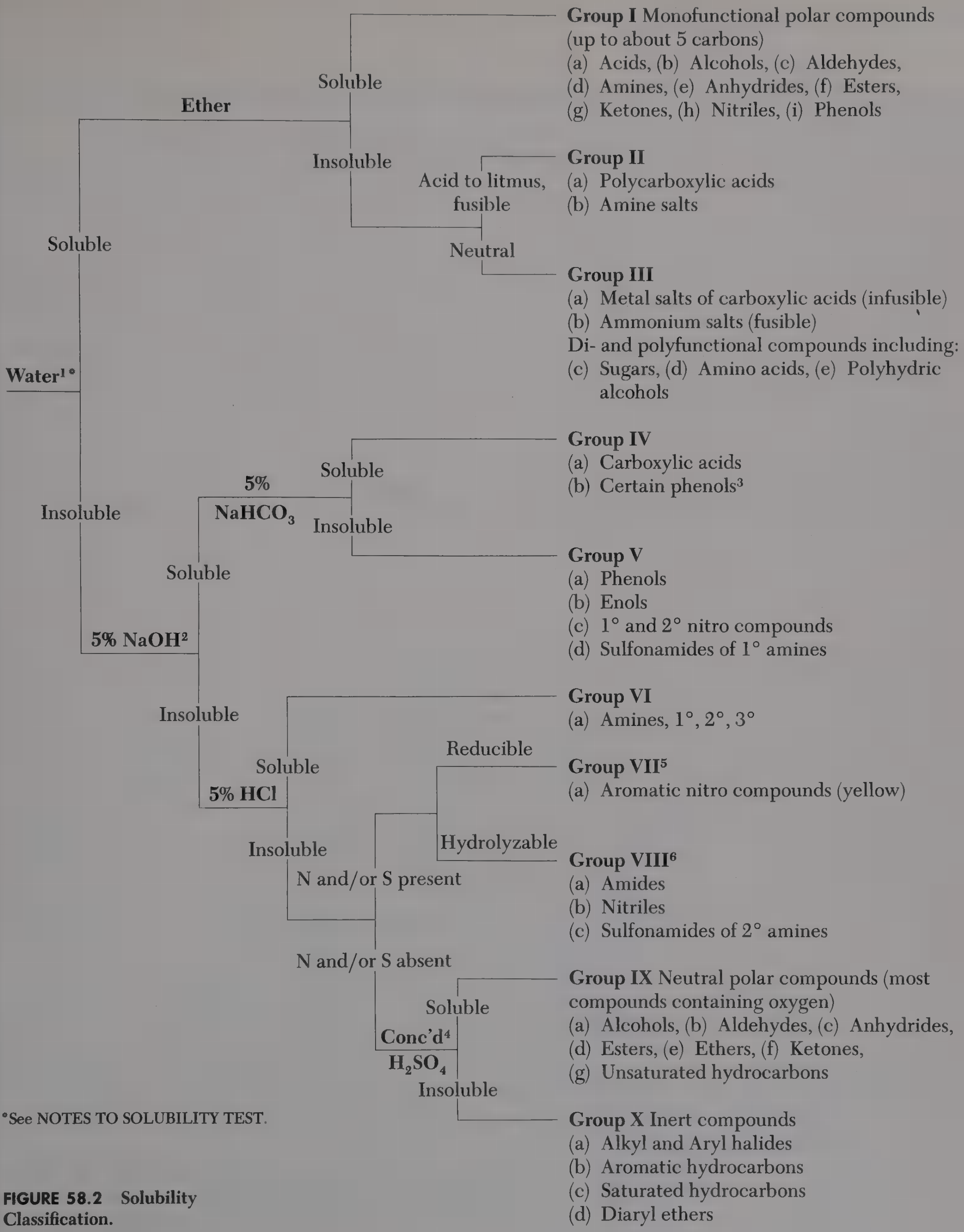
After the unknown is assigned to one of the solubility groups (Fig. 58.2) on the basis of solubility tests, the possible type should be further narrowed by application of classification tests, e.g., for alcohols, or methyl ketones, or esters.

7. Complete Identification—Preparation of Derivatives

Once the unknown has been classified by functional group, the physical properties should be compared with those of representative members of the group (see Tables at the end of this chapter). Usually, several possibilities present themselves, and the choice can be narrowed by preparation of derivatives. Select derivatives that distinguish most clearly among the possibilities.

NOTES TO SOLUBILITY TESTS

1. Groups I, II, III (soluble in water). Test the solution with pH paper. If the compound is not easily soluble in cold water, treat it as water-insoluble but test with indicator paper.
2. If the substance is insoluble in water but dissolves partially in 5% sodium hydroxide, add more water; the sodium salts of some phenols are less soluble in alkali than in water. If the unknown is colored, be careful to distinguish between the *dissolving* and the *reacting* of the sample. Some quinones (colored) *react* with alkali and give highly colored solutions. Some phenols (colorless) *dissolve and then* become oxidized to give colored solutions. Some compounds (e.g., benzamide) are hydrolyzed with such ease that careful observation is required to distinguish them from acidic substances.
3. Nitrophenols (yellow), aldehydophenols, and polyhalophenols are sufficiently strongly acidic to react with sodium bicarbonate.
4. Oxygen- and nitrogen-containing compounds form oxonium and ammonium ions in concentrated sulfuric acid and dissolve. The test distinguishes phenyl ethers from hydrocarbons.
5. On reduction in the presence of hydrochloric acid these compounds form water-soluble amine hydrochlorides. Dissolve 0.5 g of tin(II) chloride in 1 ml of concentrated hydrochloric acid, add 0.1 g of the unknown, and warm. The material should dissolve with the disappearance of the color and give a clear solution when diluted with water.
6. Most amides can be hydrolyzed by short boiling with 10% sodium hydroxide solution; the acid dissolves with evolution of ammonia. Reflux 0.2 g of the sample and 10% sodium hydroxide solution for 15–20 min under a cold finger condenser. Test for the evolution of ammonia, which confirms the elementary analysis for nitrogen and establishes the presence of a nitrile or amide.



*See NOTES TO SOLUBILITY TEST.

FIGURE 58.2 Solubility Classification.

CLASSIFICATION TESTS

Group 1 Monofunctional Polar Compounds (up to ca. 5 carbons)

(a) Acids. (Table 58.1; Derivatives, page 361)
No classification test is necessary. Carboxylic and sulfonic acids are detected by testing aqueous solutions with litmus. Acid halides may hydrolyze during the solubility test.

(b) Alcohols. (Table 58.2; Derivatives, page 361)

Jones' Oxidation. Follow the procedure of Chapter 18, for the oxidation of cholesterol with chromic acid but using one-tenth the listed quantities of reagents and solvent. Run a control test on the acetone solvent. A positive test is formation of a green color within 5 sec upon addition of the orange-yellow reagent to a primary or secondary alcohol. Aldehydes also give a positive test. Tertiary alcohols are readily dehydrated to olefins with acids.

▼
Caution!
Dioxane is a mild carcinogen.

Cerium(IV) Nitrate Test. (For alcohols with ten or fewer carbons.) Dissolve 4 or 5 drops (or 25–30 mg) of the unknown in water or dioxane. Add 0.5 ml of the reagent and shake the mixture. Alcohols cause the reagent to change from yellow to red.

Reagent: Dissolve 90 g of cerium(IV) ammonium nitrate, $Ce(NH_4)_2(NO_3)_6$, in 225 ml of 2 *N* nitric acid.

(c) Aldehydes. (Table 58.3; Derivatives, page 362)

2,4-Dinitrophenylhydrazones. All aldehydes and ketones readily form bright yellow to dark red 2,4-dinitrophenylhydrazones. Yellow derivatives are formed from isolated carbonyl groups and orange-red to red derivatives from aldehydes or ketones conjugated with double bonds or aromatic rings.

Add 1 or 2 drops (20 mg) of the unknown to 2 ml of ethanol followed by 3 ml of 2,4-dinitrophenylhydrazine reagent. Shake and let sit for a few minutes. A yellow to red precipitate is a positive test.

Reagent: Dissolve 3 g of 2,4-dinitrophenylhydrazine in 15 ml of concentrated sulfuric acid. Add this solution, with stirring, to a mixture of 20 ml of water and 70 ml of ethanol.

Schiff Test. Add 3 drops of the unknown to 2 ml of Schiff's reagent. A magenta color will appear within ten minutes with aldehydes. Compare the color of your unknown with that of a known aldehyde.

Reagent: Prepare 100 ml of a 0.1 percent aqueous solution of *p*-rosaniline hydrochloride (fuchsia). Add 4 ml of a saturated aqueous solution of sodium bisulfite. After 1 hr add 2 ml of concentrated hydrochloric acid.

Bisulfite Test. Follow the procedure on page 112, Chapter 14. Nearly all aldehydes and most methyl ketones form solid, water soluble bisulfite addition products.

Tollens Test. Follow the procedure on page 111, Chapter 14. A positive test, deposition of a silver mirror, is given by most aldehydes, but not by ketones.

(d) Amines. (Tables 58.5 and 58.6; Derivatives, pages 368, 369)

Hinsberg Test. Follow the procedure on page 186, Chapter 28, using benzenesulfonyl chloride to distinguish between primary, secondary, and tertiary amines.

(e) Anhydrides and Acid Halides. (Table 58.7; Derivatives, page 370) Anhydrides and acid halides will react with water to give acidic solutions, detectable with litmus paper.

Acidic Iron(III) Hydroxamate Test. With iron(III) chloride alone a number of substances give a color which can interfere with this test. Dissolve 2 or 3 drops (or about 30 mg) of the unknown in 1 ml of ethanol and add 1 ml of 1 *N* hydrochloric acid followed by 1 drop of 10% aqueous iron(III) chloride solution. If any color except yellow appears you will find it difficult to interpret the results from the following test.

Add 2 or 3 drops (or about 30 mg) of the unknown to 0.5 ml of a 1 *N* solution of hydroxylamine hydrochloride in alcohol. Add 2 drops of 6 *M* hydrochloric acid to the mixture, warm it slightly for 2 min, and boil it for a few seconds. Cool the solution and add 1 drop of 10 percent ferric chloride solution. A red-blue color is a positive test.

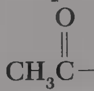
(f) Esters. (Table 58.8, page 370. Derivatives prepared from component acid and alcohol obtained on hydrolysis)
Esters, unlike anhydrides and acid halides, do not react with water to give acidic solutions and do not react with acidic hydroxylamine hydrochloride. They do, however, react with alkaline hydroxylamine.

Alkaline Iron(III) Hydroxamate Test. First test the unknown with iron(III) chloride alone. (See under Group I(e), Acidic Iron(III) Hydroxamate Test.)

To a solution of two drops (or about 30 mg) of the unknown in 1 ml of 0.5 *N* hydroxylamine hydrochloride in ethanol add four drops of 20% sodium hydroxide solution. Heat the solution to boiling, cool slightly, and add 2 ml of 1 *N* hydrochloric acid. If cloudiness develops add up to 2 ml of ethanol. Add 10% iron(III) chloride solution dropwise with swirling. A red-blue color is a positive test. Compare your unknown with a known ester.

(g) Ketones. (Table 58.14; Derivatives, page 374)

2,4-Dinitrophenylhydrazone. See under Group I(c), Aldehydes. All ketones react with 2,4-dinitrophenylhydrazine reagent.

Iodoform Test for Methyl Ketones. Follow the procedure on page 112, Chapter 14. A positive iodoform test is given by substances containing the  $CH_3\overset{\overset{\displaystyle O}{\|}}{C}-$ group or by compounds easily oxidized to this group, e.g., CH_3COR, CH_3CHOHR, CH_3CH_2OH, CH_3CHO, $RCOCH_2COR$. The test is negative for compounds of the structure CH_3COOR, CH_3CONHR, and other compounds of similar structure which give acetic acid on hydrolysis. It is also negative for $CH_3COCH_2CO_2R$, CH_3COCH_2CN, $CH_3COCH_2NO_2$.

Bisulfite Test. Follow the procedure on page 112, Chapter 14. Aliphatic methyl ketones and unhindered cyclic ketones form bisulfite addition products. Methyl aryl ketones, such as acetophenone, $C_6H_5COCH_3$, fail to react.

(h) Nitriles. (Table 58.15, page 375. Derivatives prepared from the carboxylic acid obtained by hydrolysis)

At high temperature nitriles (and amides) are converted to hydroxamic acids by hydroxylamine: $RCN + 2\ H_2NOH \longrightarrow RCONHOH + NH_3$. The hydroxamic acid forms a red-blue complex with iron(III) ion. The unknown must first give a negative test with hydroxylamine at lower temperature (Group I(f), Alkaline Hydroxamic Acid Test) before trying this test.

Hydroxamic Acid Test for Nitriles (and Amides). To 2 ml of a 1 *M* hydroxylamine hydrochloride solution in propylene glycol add one drop (30 mg) of the unknown dissolved in the minimum amount of propylene glycol. Then add one ml of 1 *N* potassium hydroxide in propylene glycol and boil the mixture for 2 min. Cool the mixture and add 0.25 to 0.5 ml of 10% iron(III) chloride solution. A red-blue color is a positive test for almost all nitrile and amide groups, although benzanilide fails to give a positive test.

(i) Phenols. (Table 58.17, page 376)

Iron(III) Chloride Test. Dissolve one drop (30 mg) of the unknown compound in a ml of water or water-alcohol mixture and add up to three drops of 1% iron(III) chloride solution. A red, blue, green or purple color is a positive test.

A more sensitive test for phenols consists of dissolving or suspending 30 mg of the unknown in 1 ml of chloroform and adding 2 drops of a solution made by dissolving 1 g of iron(III) chloride in 100 ml of chloroform. (*Caution!* $CHCl_3$ is a carcinogen.) Addition of a drop of pyridine, with stirring, will produce a color if phenols or enols are present.

Group II Water-soluble Acidic Salts, Insoluble in Ether

(a) Amine salts. [Table 58.5 (1° and 2° amines); Tables 58.6 (3° amines)] The free amine can be liberated by addition of base and extraction into ether. Following evaporation of the ether the Hinsberg test, Group I(d), can be applied to determine if the compound is a primary, secondary, or tertiary amine.

The acid iron(III) hydroxamate test, Group I(d), can be applied directly to the amine salt.

Group III Water-soluble Neutral Compounds, Insoluble in Ether

(a) Metal Salts of Carboxylic Acids. (Table 58.1, carboxylic acids; Derivatives, page 364)
The free acid can be liberated by addition of acid and extraction into an appropriate solvent, after which the carboxylic acid can be characterized by mp or bp before proceeding to prepare a derivative.

(b) Ammonium Salts. (Table 58.1, carboxylic acids; Derivatives, page 364)
Ammonium salts on treatment with alkali liberate ammonia, which can be detected by its odor and the fact it will turn damp red litmus, blue. A more sensitive test utilizes the copper(II) ion, which is blue in the presence of ammonia [see Group VIII a(i)]. Ammonium salts will not give a positive hydroxamic acid test (Ih) as given by amides.

(c) Sugars. See page 111, Chapter 14, for Tollens test and page 195, Chapter 29, for phenylosazone formation.

(d) Amino Acids. See Chapter 31, paper chromatography of amino acids and color reaction with ninhydrin.

(e) Polyhydric Alcohols. (Table 58.2; Derivatives, page 366)

Periodic Acid Test for vic-Glycols.[4] Vicinal glycols (hydroxyl groups on adjacent carbon atoms) can be detected by reaction with periodic acid. In addition to 1,2-glycols a positive test is given by α-hydroxy aldehydes, α-hydroxy ketones, α-hydroxy acids, and α-amino alcohols, as well as 1,2-diketones.

To 2 ml of periodic acid reagent add one drop (no more) of concentrated nitric acid and shake. Then add one drop or a small crystal of the unknown. Shake for 15 sec, and add 1 or 2 drops of 5% aqueous silver nitrate solution. Instantaneous formation of a white precipitate is a positive test.

Reagent: Dissolve 0.5 g of paraperiodic acid (H_5IO_6) in 100 ml of water.

Group IV Certain Carboxylic Acids, Certain Phenols, and Sulfonamides of 1° Amines

(a) Carboxylic Acids. Solubility in both 5% sodium hydroxide and sodium bicarbonate is usually sufficient to characterize this class of compounds. Addition of mineral acid should regenerate the carboxylic acid. The neutralization equivalent can be obtained by titrating a known quantity of the acid (ca. 0.15 g) dissolved in water-ethanol with 0.1 *N* sodium hydroxide to a phenolphthalein end point.

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[4] R. L. Shriner, R. C. Fuson, and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, 5th ed., John Wiley & Sons, Inc., New York, 1964, p. 145.

(b) Phenols. Negatively substituted phenols such as nitrophenols, aldehydophenols, and polyhalophenols are sufficiently acidic to dissolve in 5% sodium bicarbonate. See Group I(i), page 356, for the iron(III) chloride test for phenols; however this test is not completely reliable for these acidic phenols.

Group V Acidic Compounds, Insoluble in Bicarbonate

(a) Phenols. See Group I(i).

(b) Enols. See Group I(i).

(c) 1° and 2° Nitro Compounds. (Table 58.16; Derivatives, page 375)

Iron(II) Hydroxide Test. In a small test tube add 1 drop (or 20 mg) of the unknown to 1.5 ml of a freshly prepared 5% aqueous solution of iron(II) ammonium sulfate. Add one drop of 3 *N* sulfuric acid and 1 ml of 2 *N* methanolic potassium hydroxide solution. Quickly stopper the tube and shake it. The appearance of a red-brown precipitate of iron(III) hydroxide within 1 min is a positive test. Almost all nitro compounds give a positive test within 30 sec.

(d) Sulfonamides of 1° amines. An extremely sensitive test for sulfonamides (Feigl, *Spot Tests in Organic Analysis*) consists of placing a drop of a suspension or solution of the unknown on sulfonamide test paper followed by a drop of 0.5% hydrochloric acid. A red color is a positive test for sulfonamides.

The test paper is prepared by dipping filter paper into a mixture of equal volumes of a 1% aqueous solution of sodium nitrite and a 1% methanolic solution of *N,N*-dimethyl-1-naphthylamine. Allow the filter paper to dry in the dark. *Caution! Handle the naphthylamine with great care. It is carcinogenic.*

Group VI Basic Compounds, Insoluble in Water, Soluble in Acid

(a) Amines. See Group I(d).

Group VII Reducible, Neutral N- and S-Containing Compounds

(a) Aromatic Nitro Compounds. See Group V(c).

Group VIII Hydrolyzable, Neutral N- and S-Containing Compounds (Identified through the acid and amine obtained on hydrolysis)

(a) Amides. Unsubstituted amides are detected by the hydroxamic acid test, Group I(h).

(1) **Unsubstituted Amides.** Upon hydrolysis, unsubstituted amides liberate ammonia, which can be detected by reaction with cupric ion (Group III(b)).

To 2 ml of 20% sodium hydroxide solution add 50 mg of the unknown. Cover the mouth of the test tube with a piece of filter paper moistened with a few drops of 10% copper(II) sulfate solution. Boil for 1 min. A blue color on the filter paper is a positive test for ammonia.

(2) **Substituted Amides.** The identification of substituted amides is not easy. There are no completely general tests for the substituted amide groups and hydrolysis is often difficult.

Hydrolyze the amide by refluxing 0.5 g with 5 ml of 20% sodium hydroxide for 20 min. Isolate the primary or secondary amine produced, by extraction into ether, and identify as described under Group I(d). Liberate the acid by acidification of the residue and isolate by filtration or extraction and characterize by bp or mp and the mp of an appropriate derivative.

(3) **Anilides.** Add 100 mg of the unknown to 3 ml of concentrated sulfuric acid. Carefully stopper the test tube with a rubber stopper and shake vigorously. **Caution.** Add 50 mg of finely powdered potassium dichromate. A blue-pink color is a positive test for an anilide which does not have substituents on the ring (e.g., acetanilide).

(b) Nitriles. See Group I(h).

(c) Sulfonamides. See Group V(d), page 358.

Group IX Neutral Polar Compounds, Insoluble in Dilute Hydrochloric Acid, Soluble in Concentrated Sulfuric Acid. (Most compounds containing oxygen.)

(a) Alcohols. See Group I(b).

(b) Aldehydes. See Group I(c).

(c) Anhydrides. See Group I(e).

(d) Esters. See Group I(f).

(e) Ethers. (Table 58.9, page 371)

Ethers are very unreactive. Care must be used to distinguish ethers from those hydrocarbons which are soluble in concentrated sulfuric acid.

$Fe[Fe(SCN)_6]$
Iron(III) hexathiocyanatoferrate(III)

Ferrox Test. In a dry test tube grind together, with a stirring rod, a crystal of iron(III) ammonium sulfate (or iron(III) chloride) and a crystal of potassium thiocyanate. Iron(III) hexathiocyanatoferrate(III) will adhere to the stirring rod. In a clean tube place 3 or 4 drops of a liquid unknown or a saturated toluene solution of a solid unknown and stir with the rod. The salt will dissolve if the unknown contains oxygen to give a red to red-purple color, but it will not dissolve in hydrocarbons or halocarbons. Diphenyl ether does not give a positive test.

Alkyl ethers are generally soluble in concentrated hydrochloric acid; alkyl aryl and diaryl ethers are not soluble.

(f) Ketones. See Group I(g).

(g) Unsaturated Hydrocarbons. (Table 58.12, page 372)

Bromine in Carbon Tetrachloride. Dissolve 3–4 drops (40–60 mg) of the unknown in 2 ml of carbon tetrachloride (*caution! toxic*). Add a 2% solution of bromine in carbon tetrachloride dropwise with shaking. If more than 2 drops of bromine solution are required to give a permanent red color, unsaturation is indicated.

Potassium Permanganate Solution. Dissolve 2 or 3 drops (30 mg) of the unknown in reagent grade acetone and add a 1% aqueous solution of potassium permanganate dropwise with shaking. If more than one drop of reagent is required to give a purple color to the solution, unsaturation or an easily oxidized functional group is present. Run parallel tests on pure acetone and, as usual, a compound known to be an alkene.

Group X Inert Compounds. Insoluble in Concentrated Sulfuric Acid

(a) Alkyl and Aryl Halides. (Table 58.10, page 371)

Alcoholic Silver Nitrate. Add one drop of the unknown (or saturated solution of 20 mg of unknown in ethanol) to 1 ml of a saturated solution of silver nitrate. A precipitate which forms within 2 min is a positive test for an alkyl bromide, or iodide, or a tertiary alkyl chloride, as well as allyl halides.

If no precipitate forms within 2 min, heat the solution to boiling. A precipitate of silver chloride will form from primary and secondary alkyl chlorides. Aryl halides and vinyl halides will not react.

(b) Aromatic Hydrocarbons. (Table 58.13; Derivatives, page 373) Aromatic hydrocarbons are best identified and characterized by ultraviolet and nmr spectroscopy, but the Friedel-Crafts reaction produces a characteristic color with certain aromatic hydrocarbons.

Friedel-Crafts Test. Heat a test tube containing about 0.1 g of anhydrous aluminum chloride in a hot flame to sublime the salt up onto the sides of the tube. Add a solution of about 20 mg of the unknown dissolved in 0.1 ml of chloroform to the cool tube in such a way that it comes into contact with the sublimed aluminum chloride. Note the color that appears.

Nonaromatic compounds fail to give a color with aluminum chloride, benzene and its derivatives give orange or red colors, naphthalenes a blue or purple color, biphenyls a purple color, phenanthrene a purple color, and anthracene a green color.

(c) Saturated Hydrocarbons. Saturated hydrocarbons are best characterized by nmr and IR spectroscopy, but they can be distinguished from aromatic hydrocarbons by the Friedel-Crafts test (Group X(b)).

(d) Diaryl Ethers. Because they are so inert, diaryl ethers are difficult to detect and may be mistaken for aromatic hydrocarbons. They do not give a positive Ferrox Test, Group IX(e), for ethers, and do not dissolve in concentrated sulfuric acid. Their infrared spectra, however, are characterized by an intense C—O single-bond, stretching vibration in the region 1270–1230 cm^{-1}.

DERIVATIVES

1. Acids. (Table 58.1)

p-Toluidides and Anilides. Reflux a mixture of the acid (0.5 g) and thionyl chloride (2 ml) under a cold finger condenser for 0.5 hr. Cool the reaction mixture and add 1 g of aniline or *p*-toluidine in 30 ml of benzene (*caution!*). Warm the mixture on the steam bath for 2 min, transfer the benzene solution to a separatory funnel, and wash with 5-ml portions of water, 5% hydrochloric acid, 5% sodium hydroxide and water. The benzene is filtered through a cone of anhydrous sodium sulfate and evaporated; the derivative is recrystallized from water or ethanol-water.

▼
Caution! Benzene is a mild carcinogen. Carry out test in a hood.

Amides. Reflux a mixture of the acid (0.5 g) and thionyl chloride (2 ml) under a cold finger condenser for 0.5 hr. Pour the cool reaction mixture into 7 ml of ice-cold concentrated ammonia. Stir until reaction is complete, collect the product by filtration, and recrystallize it from water or water-ethanol.

2. Alcohols. (Table 58.2)

3,5-Dinitrobenzoates. Gently boil 0.3 g of 3,5-dinitrobenzoyl chloride and 0.5 ml of the alcohol for 5 min. Cool the mixture, pulverize any solid that forms, and add 5 ml of 2% sodium carbonate solution. Continue to grind and stir the solid with the sodium carbonate solution (to remove 3,5-dinitrobenzoic acid) for about a minute, filter, and wash the crystals with water. Dissolve the product in about 10 ml of hot ethanol, add water to the cloud point, and allow crystallization to proceed. Wash the 3,5-dinitrobenzoate with water-alcohol and dry.

$$\begin{array}{l} C_6H_5{-}N{=}C{=}O + ROH \\ \qquad\downarrow \\ C_6H_5{-}NHCOOR \end{array}$$

Phenylurethanes. Mix 0.5 ml of anhydrous alcohol (or phenol) and 0.5 ml of phenyl isocyanate (or α-naphthylurethane) and heat on the steam bath for 5 min. (If the unknown is a phenol add 2 or 3 drops of pyridine to the reaction mixture.) Cool, add about 6 ml of ligroin, heat to dissolve the product, filter hot to remove a small amount of diphenylurea which usually forms, and cool the filtrate in ice, with scratching, to induce crystallization.

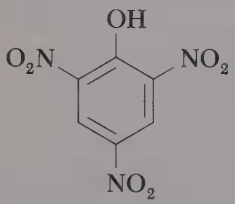
3. *Aldehydes.* (Table 58.3)

Semicarbazones. See page 111, Chapter 14. Use 0.5 ml of the stock solution and an estimated 1 millimole of the unknown aldehyde (or ketone).

2,4-Dinitrophenylhydrazones. See page 109, Chapter 14. Use 10 ml of the stock solution of 0.1 *M* 2,4-dinitrophenylhydrazine and an estimated 1 millimole of the unknown aldehyde (or ketone).

4. *Primary and Secondary Amines.* (Table 58.5)

Benzamides. Add about 0.5 g of benzoyl chloride in small portions with vigorous shaking and cooling to a suspension of 1 millimole of the unknown amine in 1 ml of 10% aqueous sodium hydroxide solution. After about 10 min of shaking the mixture is made pH 8 (pH paper) with dilute hydrochloric acid. The lumpy product is removed by filtration, washed thoroughly with water, and recrystallized from ethanol-water.



Picric acid
(2,4,6-Trinitrophenol)

Picrates. Add a solution of 0.15 g of the unknown in 5 ml of ethanol (or 5 ml of a saturated solution of the unknown) to 5 ml of a saturated solution of picric acid (2,4,6-trinitrophenol, a strong acid) in ethanol, and heat the solution to boiling. Cool slowly, remove the picrate by filtration, and wash with a small amount of ethanol. Recrystallization is not usually necessary; in the case of hydrocarbon picrates the product is often too unstable to be recrystallized.

Acetamides. Reflux about 1 millimole of the unknown with 0.4 ml of acetic anhydride for 5 min, cool, and dilute the reaction mixture with 5 ml of water. Initiate crystallization by scratching, if necessary. Remove the crystals by filtration and wash thoroughly with dilute hydrochloric acid to remove unreacted amine. Recrystallize the derivative from alcohol-water. Amines of low basicity, e.g., *p*-nitroaniline, should be refluxed for 30 to 60 min with 2 ml of pyridine as a solvent. The pyridine is removed by shaking the reaction mixture with 10 ml of 2% sulfuric acid solution; the product is isolated by filtration and recrystallized.

5. *Tertiary Amines.* (Table 58.6)

Picrates. See under Primary and Secondary Amines.

$$\begin{array}{c} R_3N + CH_3I \\ \downarrow \\ R_3\overset{+}{N}CH_3I^- \end{array}$$

Methiodides. Reflux 0.3 ml of the amine and 0.3 ml of methyl iodide under a cold finger condenser for 5 min on the steam bath. Cool, scratch to induce crystallization, and recrystallize the product from ethyl alcohol or ethyl acetate.

6. *Anhydrides and Acid Chlorides.* (Table 58.7)

Acids. Reflux 0.2 g of the acid chloride or anhydride with 5 ml of 5% sodium carbonate solution for 20 min or less. Extract unreacted starting material with 5 ml of ether, if necessary, and acidify the reaction mixture with dilute sulfuric acid to liberate the carboxylic acid.

Amides. Since the acid chloride (or anhydride) is already present, simply mix the unknown (0.5 g) and 7 ml of ice-cold concentrated ammonia until reaction is complete, collect the product by filtration, and recrystallize it from water or ethanol-water.

▼
Caution! Benzene is a mild carcinogen. Carry out preparation in a hood.

Anilides. Reflux 0.2 g of the acid halide or anhydride with 0.5 g of aniline in 10 ml of benzene (*caution!*) for 10 min. Transfer the benzene solution to a separatory funnel and wash with 5 ml portions each of water, 5% hydrochloric acid, 5% sodium hydroxide and water. The benzene solution is filtered through a cone of anhydrous sodium sulfate and evaporated; the anilide is recrystallized from water or ethanol-water.

7. Aryl Halides. (Table 58.11)

Nitration. Add 2 ml of concentrated sulfuric acid to 0.5 g of the aryl halide (or aromatic compound) and stir. Add 2 ml of concentrated nitric acid dropwise with stirring and shaking while cooling the reaction mixture in water. Then heat and shake the reaction mixture in a water bath at about 50° for 15 min, pour into 10 ml of cold water, and collect the product by filtration. Recrystallize from methanol to constant melting point.

To nitrate unreactive compounds use fuming nitric acid in place of concentrated nitric acid.

Sidechain Oxidation Products. Dissolve 1 g of sodium dichromate in 3 ml of water and add 2 ml of concentrated sulfuric acid. Add 0.25 g of the unknown and boil for 30 min. Cool, add 2 or 3 ml of water and then remove the carboxylic acid by filtration. Wash the crystals with water and recrystallize from methanol-water.

8. Hydrocarbons: Aromatic. (Table 58.13)

Nitration. See preceding, under Aryl Halides.

Picrates. See preceding, under Primary and Secondary Amines.

9. Ketones. (Table 58.14)

Semicarbazones and 2,4-dinitrophenylhydrazones. See preceding directions under Aldehydes.

10. Nitro Compounds. (Table 58.16)

Reduction to Amines. Place 0.5 g of the unknown in a 25-ml round-bottomed flask, add 1 g of tin, and then—in portions—10 ml of 10% hydrochloric acid. Reflux for 30 min, add 5 ml of water, then add slowly, with good cooling, sufficient 40% sodium hydroxide solution to dissolve the tin hydroxide. Extract the reaction mixture with three 5-ml portions of ether, dry the ether extract over anhydrous sodium sulfate, and evaporate the ether to leave the amine. Determine the bp or mp of the amine and then convert it into a benzamide or acetamide as described under Primary and Secondary Amines.

11. Phenols. (Table 58.17)

α-Naphthylurethane. Follow the procedure for preparation of a phenylurethane under Alcohols, page 361.

Bromo Derivative. In a 10-ml Erlenmeyer flask dissolve 0.8 g of potassium bromide in 5 ml of water. *Carefully* add 0.5 g of bromine. In a separate flask dissolve 0.1 g of the phenol in 1 ml of methanol and add 1 ml of water. Add about 1.5 ml of the bromine solution with swirling (hood); continue the addition of bromine until the yellow color of unreacted bromine persists. Add 3 to 4 ml of water to the reaction mixture and shake vigorously. Remove the product by filtration and wash well with water. Recrystallize from methanol-water.

Table 58.1 Acids

Bp	Mp	Name of Compound	Derivatives		
			p-Toluidide[a]	Anilide[b]	Amide[c]
			Mp	*Mp*	*Mp*
101		Formic acid	53	47	43
118		Acetic acid	126	106	79
139		Acrylic acid	141	104	85
141		Propionic acid	124	103	81
162		*n*-Butyric acid	72	95	115
163		Methacrylic acid		87	102
165		Pyruvic acid	109	104	124
185		Valeric acid	70	63	106
186		2-Methylvaleric acid	80	95	79
194		Dichloroacetic acid	153	118	98
202–203		Hexanoic acid	75	95	101
237		Octanoic acid	70	57	107
254		Nonanoic acid	84	57	99
	31–32	Decanoic acid	78	70	108
	43–45	Lauric acid	87	78	100
	47–49	Bromoacetic acid		131	91
	47–49	Hydrocinnamic acid	135	92	105
	54–55	Myristic acid	93	84	103
	54–58	Trichloroacetic acid	113	97	141
	61–62	Chloroacetic acid	162	137	121
	61–62.5	Palmitic acid	98	90	106
	67–69	Stearic acid	102	95	109
	68–69	3,3-Dimethylacrylic acid		126	107
	71–73	Crotonic acid	132	118	158
	77–78.5	Phenylacetic acid	136	118	156
	101–102	Oxalic acid dihydrate		257	400 (dec)
	98–102	Azelaic acid (nonanedioic)	164 (di)	107 (mono) 186 (di)	93 (mono) 175 (di)
	103–105	*o*-Toluic acid	144	125	142

Table 58.1 Acids (cont.)

Bp	Mp	Name of Compound	Derivatives		
			p-Toluidide[a]	Anilide[b]	Amide[c]
			Mp	*Mp*	*Mp*
	108–110	*m*-Toluic acid	118	126	94
	119–121	DL-Mandelic acid	172	151	133
	122–123	Benzoic acid	158	163	130
	127–128	2-Benzoylbenzoic acid		195	165
	129–130	2-Furoic acid	107	123	143
	131–133	DL-Malic acid	178 (mono) 207 (di)	155 (mono) 198 (di)	 163 (di)
	131–134	Sebacic acid	201	122 (mono) 200 (di)	170 (mono) 210 (di)
	134–135	*E*-Cinnamic acid	168	153	147
	134–136	Maleic acid	142 (di)	198 (mono) 187 (di)	260 (di)
	135–137	Malonic acid	86 (mono) 253 (di)	132 (mono) 230 (di)	
	138–140	2-Chlorobenzoic acid	131	118	139
	140–142	3-Nitrobenzoic acid	162	155	143
	144–148	Anthranilic acid	151	131	109
	147–149	Diphenylacetic acid	172	180	167
	152–153	Adipic acid	239	151 (mono) 241 (di)	125 (mono) 220 (di)
	153–154	Citric acid	189 (tri)	199 (tri)	210 (tri)
	157–159	4-Chlorophenoxyacetic acid		125	133
	158–160	Salicylic acid	156	136	142
	163–164	Trimethylacetic acid		127	178
	164–166	5-Bromosalicylic acid		222	232
	166–167	Itaconic acid		190	191 (di)
	171–174	*d*-Tartaric acid		180 (mono) 264 (di)	171 (mono) 196 (di)
	179–182	3,4-Dimethoxybenzoic acid		154	164
	180–182	4-Toluic acid	160	145	160
	182–185	4-Anisic acid	186	169	167
	187–190	Succinic acid	180 (mono) 255 (di)	143 (mono) 230 (di)	157 (mono) 260 (di)
	201–203	3-Hydroxybenzoic acid	163	157	170
	203–206	3,5-Dinitrobenzoic acid		234	183
	210–211	Phthalic acid	150 (mono) 201 (di)	169 (mono) 253 (di)	149 (mono) 220 (di)
	214–215	4-Hydroxybenzoic acid	204	197	162
	225–227	2,4-Dihydroxybenzoic acid		126	228
	236–239	Nicotinic acid	150	132	128
	239–241	4-Nitrobenzoic acid	204	211	201
	299–300	Fumaric acid		233 (mono) 314 (di)	270 (mono) 266 (di)
	>300	Terephthalic acid		334	

[a] For preparation, see page 361.
[b] For preparation, see page 361.
[c] For preparation, see page 361.

Table 58.2 Alcohols

Bp	Mp	Name of Compound	Derivatives	
			3,5-Dinitrobenzoate[a]	Phenylurethane[b]
			Mp	*Mp*
65		Methanol	108	47
78		Ethanol	93	52
82		2-Propanol	123	88
83		*t*-Butyl alcohol	142	136
96–98		Allyl alcohol	49	70
97		1-Propanol	74	57
98		2-Butanol	76	65
102		2-Methyl-2-butanol	116	42
104		2-Methyl-3-butyn-2-ol	112	
108		2-Methyl-1-propanol	87	86
114–115		Propargyl alcohol		63
114–115		3-Pentanol	101	48
118		1-Butanol	64	61
118–119		2-Pentanol	62	
123		3-Methyl-3-pentanol	96(62)	43
129		2-Chloroethanol	95	51
130		2-Methyl-1-butanol	70	31
132		4-Methyl-2-pentanol	65	143
136–138		1-Pentanol	46	46
139–140		Cyclopentanol	115	132
140		2,4-Dimethyl-3-pentanol		95
146		2-Ethyl-1-butanol	51	
151		2,2,2-Trichloroethanol	142	87
157		1-Hexanol	58	42
160–161		Cyclohexanol	113	82
170		Furfuryl alcohol	80	45
176		1-Heptanol	47	60 (68)
178		2-Octanol	32	oil
178		Tetrahydrofurfuryl alcohol	83	61
183–184		2,3-Butanediol		201 (di)
183–186		2-Ethyl-1-hexanol		34
187		1,2-Propanediol		153 (di)
194–197		Linaloöl		66
195		1-Octanol	61	74
196–198		Ethylene glycol	169	157 (di)
204		1,3-Butanediol		122
203–205		Benzyl alcohol	113	77
204		1-Phenylethanol	95	92
219–221		2-Phenylethanol	108	78
230		1,4-Butanediol		183 (di)
231		1-Decanol	57	59
259		4-Methoxybenzyl alcohol		92
	33–35	Cinnamyl alcohol	121	90
	38–40	1-Tetradecanol	67	74
	48–50	1-Hexadecanol	66	73
	58–60	1-Octadecanol	77 (66)	79
	66–67	Benzhydrol	141	139
	147	Cholesterol	195	168

[a] For preparation, see page 361.

[b] For preparation, see page 361.

Table 58.3 Aldehydes

Bp	Mp	Name of Compound	Derivatives	
			Semicarbazone[a]	2,4-Dinitrophenylhydrazone[b]
			Mp	*Mp*
21		Acetaldehyde	162	168
46–50		Propionaldehyde	89(154)	148
63		Isobutyraldehyde	125(119)	187(183)
75		Butyraldehyde	95(106)	123
90–92		3-Methylbutanal	107	123
98		Chloral	90	131
104		Crotonaldehyde	199	190
117		2-Ethylbutanal	99	95(130)
153		Heptaldehyde	109	108
162		2-Furaldehyde	202	212(230)
163		2-Ethylhexanal	254	114(120)
179		Benzaldehyde	222	237
195		Phenylacetaldehyde	153	121(110)
197		Salicylaldehyde	231	248
204–205		4-Tolualdehyde	234(215)	232
209–215		2-Chlorobenzaldehyde	146(229)	213
247		2-Ethoxybenzaldehyde	219	
248		4-Anisaldehyde	210	253
250–252		*E*-Cinnamaldehyde	215	255
	33–34	1-Naphthaldehyde	221	
	37–39	2-Anisaldehyde	215	254
	42–45	3,4-Dimethoxybenzaldehyde	177	261
	44–47	4-Chlorobenzaldehyde	230	254
	57–59	3-Nitrobenzaldehyde	246	293
	81–83	Vanillin	230	271

[a] For preparation see page 111.

[b] For preparation see page 109.

Table 58.4 Amides

Bp	Mp	Name of Compound	Mp	Name of Compound
153		*N,N*-Dimethylformamide	127–129	Isobutyramide
164–166		*N,N*-Dimethylacetamide	128–129	Benzamide
210		Formamide	130–133	Nicotinamide
243–244		*N*-Methylformanilide	177–179	4-Chloroacetanilide
	26–28	*N*-Methylacetamide		
	79–81	Acetamide		
	109–111	Methacrylamide		
	113–115	Acetanilide		
	116–118	2-Chloroacetamide		

Table 58.5 Primary and Secondary Amines

Bp	Mp	Name of Compound	Derivatives		
			Benzamide[a]	Picrate[b]	Acetamide[c]
			Mp	*Mp*	*Mp*
33–34		Isopropylamine	71	165	
46		*tert*-Butylamine	134	198	
48		*n*-Propylamine	84	135	
53		Allylamine		140	
55		Diethylamine	42	155	
63		*sec*-Butylamine	76	139	
64–71		Isobutylamine	57	150	
78		*n*-Butylamine	42	151	
84		Di-isopropylamine		140	
87–88		Pyrrolidine	oil	112	
106		Piperidine	48	152	
111		Di-*n*-propylamine		75	
118		Ethylenediamine	244(di)	233	172(di)
129		Morpholine	75	146	
137–139		Di-isobutylamine		121	86
145–146		Furfurylamine		150	
149		*N*-Methylcyclohexylamine	85	170	
159		Di-*n*-butylamine		59	
182–185		Benzylamine	105	199	60
184		Aniline	163	198	114
196		*N*-Methylaniline	63	145	102
199–200		2-Toluidine	144	213	110
203–204		3-Toluidine	125	200	65
205		*N*-Ethylaniline	60	138(132)	54
208–210		2-Chloroaniline	99	134	87
210		2-Ethylaniline	147	194	111
216		2,6-Dimethylaniline	168	180	177
218		2,4-Dimethylaniline	192	209	133
218		2,5-Dimethylaniline	140	171	139
221		*N*-Ethyl-*m*-toluidine	72		
225		2-Anisidine	60(84)	200	85
230		3-Chloroaniline	120	177	72(78)
231–233		2-Phenetidine	104		79
241		4-Chloro-2-methylaniline	142		140
242		3-Chloro-4-methylaniline	122		105
250		4-Phenetidine	173	69	137
256		Dicyclohexylamine	153(57)	173	103
	35–38	*N*-Phenylbenzylamine	107	48	58
	41–44	4-Toluidine	158	182	147
	49–51	2,5-Dichloroaniline	120	86	132
	52–54	Diphenylamine	180	182	101
	57–60	4-Anisidine	154	170	130
	57–60	2-Aminopyridine	165(di)	216(223)	
	60–62	*N*-Phenyl-1-naphthylamine	152		115

Table 58.5 Primary and Secondary Amines (cont.)

Bp	Mp	Name of Compound	Derivatives		
			Benzamide[a]	Picrate[b]	Acetamide[c]
			Mp	*Mp*	*Mp*
	62–65	2,4,5-Trimethylaniline	167		162
	64–66	3-Phenylenediamine	125(mono) 240(di)	184	87(mono) 191(di)
	66	4-Bromoaniline	204	180	168
	68–71	4-Chloroaniline	192	178	179(172)
	71–73	2-Nitroaniline	110(98)	73	92
	97–99	2,4-Diaminotoluene	224(di)		224(di)
	100–102	2-Phenylenediamine	301	208	185
	104–107	2-Methyl-5-nitroaniline	186		151
	107–109	2-Chloro-4-nitroaniline	161		139
	112–114	3-Nitroaniline	157(150)	143	155(76)
	115–116	4-Methyl-2-nitroaniline	148		99
	117–119	4-Chloro-2-nitroaniline			104
	120–122	2,4,6-Tribromoaniline	198(204)		232
	131–133	2-Methyl-4-nitroaniline			202
	138–140	2-Methoxy-4-nitroaniline	149		
	138–142	4-Phenylenediamine	128(mono) 300(di)		162(mono) 304(di)
	148–149	4-Nitroaniline	199	100	215
	162–164	4-Aminoacetanilide			304
	176–178	2,4-Dinitroaniline	202(220)		120

[a] For preparation see page 362. [b] For preparation see page 362. [c] For preparation see page 362.

Table 58.6 Tertiary Amines

Bp	Name of Compound	Derivatives	
		Picrate[a]	Methiodide[b]
		Mp	*Mp*
85–91	Triethylamine	173	280
115	Pyridine	167	117
128–129	2-Picoline	169	230
143–145	2,6-Lutidine	168(161)	233
144	3-Picoline	150	92(36)
145	4-Picoline	167	149
155–158	Tri-*n*-propylamine	116	207
159	2,4-Lutidine	180	113
183–184	*N,N*-Dimethylbenzylamine	93	179
216	Tri-*n*-butylamine	105	186
217	*N,N*-Diethylaniline	142	102
237	Quinoline	203	133(72)

[a] For preparation see page 362. [b] For preparation see page 362.

Table 58.7 Anhydrides and Acid Chlorides

Bp	Mp	Name of Compound	Derivatives			
			Acid[a]		Amide[b]	Anilide[c]
			Bp	*Mp*	*Mp*	*Mp*
52		Acetyl chloride	118		82	114
77–79		Propionyl chloride	141		81	106
102		Butyryl chloride	162		115	96
138–140		Acetic anhydride	118		82	114
167		Propionic anhydride	141		81	106
198–199		Butyric anhydride	162		115	96
198		Benzoyl chloride		122	130	163
225		3-Chlorobenzoyl chloride		158	134	122
238		2-Chlorobenzoyl chloride		142	142	118
	32–34	*cis*-1,2-Cyclohexanedicarboxylic anhydride		192		
	35–37	Cinnamoyl chloride		133	147	151
	39–40	Benzoic anhydride		122	130	163
	54–56	Maleic anhydride		130	181(mono) 266(di)	173(mono) 187(di)
	72–74	4-Nitrobenzoyl chloride		241	201	211
	119–120	Succinic anhydride		186	157(mono) 260(di)	148(mono) 230(di)
	131–133	Phthalic anhydride		206	149(mono) 220(di)	170(mono) 253(di)
	254–258	Tetrachlorophthalic anhydride		250		
	267–269	1,8-Naphthalic anhydride		274		250–282(di)

[a] For preparation see page 362.
[b] For preparation see page 362.
[c] For preparation see page 362.

Table 58.8 Esters

Bp	Mp	Name of Compound	Bp	Mp	Name of Compound
34		Methyl formate	169–170		Methyl acetoacetate
52–54		Ethyl formate	180–181		Dimethyl malonate
72–73		Vinyl acetate	181		Ethyl acetoacetate
77		Ethyl acetate	185		Diethyl oxalate
79		Methyl propionate	198–199		Methyl benzoate
80		Methyl acrylate	206–208		Ethyl caprylate
85		Isopropyl acetate	208–210		Ethyl cyanoacetate
93		Ethyl chloroformate	212		Ethyl benzoate
94		Isopropenyl acetate	217		Diethyl succinate
98		Isobutyl formate	218		Methyl phenylacetate
98		*t*-Butyl acetate	218–219		Diethyl fumarate
99		Ethyl propionate	222		Methyl salicylate
99		Ethyl acrylate	225		Diethyl maleate

Table 58.8 Esters (cont.)

Bp	Mp	Name of Compound	Bp	Mp	Name of Compound
100		Methyl methacrylate	229		Ethyl phenylacetate
101		Methyl trimethylacetate	234		Ethyl salicylate
102		*n*-Propyl acetate	268		Dimethyl suberate
106–113		*s*-Butyl acetate	271		Ethyl cinnamate
120		Ethyl butyrate	282		Dimethyl phthalate
127		*n*-Butyl acetate	298–299		Diethyl phthalate
128		Methyl valerate	298–299		Phenyl benzoate
130		Methyl chloroacetate	340		Dibutyl phthalate
131–133		Ethyl isovalerate		56–58	Ethyl *p*-nitrobenzene
142		*n*-Amyl acetate		88–90	Ethyl *p*-aminobenzoate
142		Isoamyl acetate		94–96	Methyl *p*-nitrobenzoate
143		Ethyl chloroacetate		95–98	*n*-Propyl *p*-hydroxybenzoate
154		Ethyl lactate		116–118	Ethyl *p*-hydroxybenzoate
168		Ethyl caproate (ethyl hexanoate)		126–128	Methyl *p*-hydroxybenzoate

Table 58.9 Ethers

Bp	Mp	Name of Compound	Bp	Mp	Name of Compound
32		Furan	215		4-Bromoanisole
33		Ethyl vinyl ether	234–237		Anethole
65–67		Tetrahydrofuran	259		Diphenyl ether
94		*n*-Butyl vinyl ether	273		2-Nitroanisole
154		Anisole	298		Dibenzyl ether
174		4-Methylanisole		50–52	4-Nitroanisole
175–176		3-Methylanisole		56–60	1,4-Dimethyoxybenzene
198–203		4-Chloroanisole		73–75	2-Methoxynaphthalene
206–207		1,2-Dimethoxybenzene			

Table 58.10 Halides

Bp	Name of Compound	Bp	Name of Compound
34–36	2-Chloropropane	100–105	1-Bromobutane
40–41	Dichloromethane	105	Bromotrichloromethane
44–46	Allyl chloride	110–115	1,1,2-Trichloroethane
57	1,1-Dichloroethane	120–121	1-Bromo-3-methylbutane
59	2-Bromopropane	121	Tetrachloroethylene
68	Bromochloromethane	123	3,4-Dichloro-1-butene
68–70	2-Chlorobutane	125	1,3-Dichloro-2-butene
69–73	Iodoethane	131–132	1,2-Dibromoethane
70–71	Allyl bromide	140–142	1,2-Dibromopropane
71	1-Bromopropane	142–145	1-Bromo-3-chloropropane
72–74	2-Bromo-2-methylpropane	146–150	Bromoform

Table 58.10 Halides (cont.)

Bp	Name of Compound	Bp	Name of Compound
74–76	1,1,1-Trichloroethane	147	1,1,2,2-Tetrachloroethane
81–85	1,2-Dichloroethane	156	1,2,3-Trichloropropane
87	Trichloroethylene	161–163	1,4-Dichlorobutane
88–90	2-Iodopropane	167	1,3-Dibromopropane
90–92	1-Bromo-2-methylpropane	177–181	Benzyl chloride
91	2-Bromobutane	197	(2-Chloroethyl)benzene
94	2,3-Dichloro-1-propene	219–223	Benzotrichloride
95–96	1,2-Dichloropropane	238	1-Bromodecane
96–98	Dibromomethane		

Table 58.11 Aryl Halides

Bp	Mp	Name of Compound	Derivatives			
			Nitration Product[a]		Oxidation Product[b]	
			Position	*Mp*	*Name*	*Mp*
132		Chlorobenzene	2,4	52		
156		Bromobenzene	2,4	70		
157–159		2-Chlorotoluene	3,5	63	2-Chlorobenzoic acid	141
162		4-Chlorotoluene	2	38	4-Chlorobenzoic acid	240
172–173		1,3-Dichlorobenzene	4,6	103		
178		1,2-Dichlorobenzene	4,5	110		
196–203		2,4-Dichlorotoluene	3,5	104	2,4-Dichlorobenzoic acid	164
201		3,4-Dichlorotoluene	6	63	3,4-Dichlorobenzoic acid	206
214		1,2,4-Trichlorobenzene	5	56		
279–281		1-Bromonaphthalene	4	85		
	51–53	1,2,3-Trichlorobenzene	4	56		
	54–56	1,4-Dichlorobenzene	2	54		
	66–68	1,4-Bromochlorobenzene	2	72		
	87–89	1,4-Dibromobenzene	2,5	84		
	138–140	1,2,4,5-Tetrachlorobenzene	3	99		
			3,6	227		

[a] For preparation, see page 363.

[b] For preparation, see page 363.

Table 58.12 Hydrocarbons: Alkenes

Bp	Name of Compound	Bp	Name of Compound
34	Isoprene	149–150	1,5-Cyclooctadiene
83	Cyclohexene	152	*dl*-α-Pinene
116	5-Methyl-2-norbornene	160	Bicyclo[4.3.0]nona-3,7-diene
122–123	1-Octene	165–167	$(-)$-β-Pinene
126–127	4-Vinyl-1-cyclohexene	165–169	α-Methylstyrene

Table 58.12 Hydrocarbons: Alkenes (cont.)

Bp	Name of Compound	Bp	Name of Compound
132–134	2,5-Dimethyl-2,4-hexadiene	181	1-Decene
141	5-Vinyl-2-norbornene	181	Indene
143	1,3-Cyclooctadiene	251	1-Tetradecene
145	4-Butylstyrene	274	1-Hexadecene
145–146	Cycloctene	349	1-Octadecene
145–146	Styrene		

Table 58.13 Hydrocarbons: Aromatic

Bp	Mp	Name of Compound	Melting Point of Derivatives		
			Nitro[a]		Picrate[b]
			Position	*Mp*	*Mp*
80		Benzene	1,3	89	84
111		Toluene	2,4	70	88
136		Ethylbenzene	2,4,6	37	96
138		*p*-Xylene	2,3,5	139	90
138–139		*m*-Xylene	2,4,6	183	91
143–145		*o*-Xylene	4,5	118	88
145		4-*t*-Butylstyrene	2,4	62	
145–146		Styrene			
152–154		Cumene	2,4,6	109	
163–166		Mesitylene	2,4	86	97
			2,4,6	235	
165–169		α-Methylstyrene			
168		1,2,4-Trimethylbenzene	3,5,6	185	97
176–178		*p*-Cymene	2,6	54	
189–192		4-*t*-Butyltoluene			
197–199		1,2,3,5-Tetramethylbenzene	4,6	181(157)	
203		*p*-Diisopropylbenzene			
204–205		1,2,3,4-Tetramethylbenzene	5,6	176	92
207		1,2,3,4-Tetrahydronaphthalene	5,7	95	
240–243		1-Methylnaphthalene	4	71	142
	34–36	2-Methylnaphthalene	1	81	116
	50–51	Pentamethylbenzene	6	154	131
	69–72	Biphenyl	4,4′	237(229)	
	80–82	1,2,4,5-Tetramethylbenzene	3,6	205	
	80–82	Naphthalene	1	61(57)	149
	90–95	Acenaphthene	5	101	161
	99–101	Phenanthrene			144(133)
	112–115	Fluorene	2	156	87(77)
			2,7	199	
	214–217	Anthracene			138

[a] For preparation see page 363.

[b] For preparation see page 362.

Table 58.14 Ketones

Bp	Mp	Name of Compound	Derivatives	
			Semicarbazone[a]	2,4-Dinitrophenylhydrazone[b]
			Mp	*Mp*
56		Acetone	187	126
80		2-Butanone	136,186	117
88		2,3-Butanedione	278	315
100–101		2-Pentanone	112	143
102		3-Pentanone	138	156
106		Pinacolone	157	125
114–116		4-Methyl-2-pentanone	132	95
124		2,4-Dimethyl-3-pentanone	160	88,95
128–129		5-Hexen-2-one	102	108
129		4-Methyl-3-penten-2-one	164	205
130–131		Cyclopentanone	210	146
133–135		2,3-Pentanedione	122(mono) 209(di)	209
145		4-Heptanone	132	75
145		5-Methyl-2-hexanone	147	95
145–147		2-Heptanone	123	89
146–149		3-Heptanone	101	
156		Cyclohexanone	166	162
162–163		2-Methylcyclohexanone	195	137
169		2,6-Dimethyl-4-heptanone	122	66,92
169–170		3-Methylcyclohexanone	180	155
173		2-Octanone	122	58
191		Acetonylacetone	185(mono) 224(di)	257(di)
202		Acetophenone	198	238
216		Phenylacetone	198	156
217		Isobutyrophenone	181	163
218		Propiophenone	182	191
226		4-Methylacetophenone	205	258
231–232		2-Undecanone	122	63
232		*n*-Butyrophenone	188	
232		4-Chloroacetophenone	204	236
235		Benzylacetone	142	127
	35–37	4-Chloropropiophenone	176	223
	35–39	4-Phenyl-3-buten-2-one	187	227
	36–38	4-Methoxyacetophenone	198	228
	48–49	Benzophenone	167	238
	53–55	2-Acetonaphthone	235	262
	60	Desoxybenzoin	148	204
	76–78	3-Nitroacetophenone	257	228

Table 58.14 Ketones (cont.)

Bp	Mp	Name of Compound	Derivatives	
			Semicarbazone[a]	2,4-Dinitrophenylhydrazone[b]
			Mp	*Mp*
	78–80	4-Nitroacetophenone		257
	82–85	9-Fluorenone	234	283
	134–136	Benzoin	206	245
	147–148	4-Hydroxypropiophenone		240

[a] For preparation, see page 109. [b] For preparation, see page 111.

Table 58.15 Nitriles

Bp	Mp	Name of Compound	Bp	Mp	Name of Compound
77		Acrylonitrile	212		3-Tolunitrile
83–84		Trichloroacetonitrile	217		4-Tolunitrile
97		Propionitrile	233–234		Benzyl cyanide
107–108		Isobutyronitrile	295		Adiponitrile
115–117		*n*-Butyronitrile		30.5	4-Chlorobenzyl cyanide
174–176		3-Chloropropionitrile		32–34	Malononitrile
191		Benzonitrile		38–40	Stearonitrile
205		2-Tolunitrile		46–48	Succinonitrile
				71–73	Diphenylacetonitrile

Table 58.16 Nitro Compounds

Bp	Mp	Name of Compound	Amine Obtained by Reduction of Nitro Groups			
					Acetamide[a]	Benzamide[b]
			Bp	*Mp*	*Mp*	*Mp*
210–211		Nitrobenzene	184		114	160
225		2-Nitrotoluene	200		110	146
225		2-Nitro-*m*-xylene	215		177	168
230–231		3-Nitrotoluene	203		65	125
245		3-Nitro-*o*-xylene	221		135	189
245–246		4-Ethylnitrobenzene	216		94	151
	34–36	2-Chloro-6-nitrotoluene	245		157(136)	173
	36–38	4-Chloro-2-nitrotoluene		21	139(131)	
	40–42	3,4-Dichloronitrobenzene		72	121	
	43–50	1-Chloro-2,4-dinitrobenzene		91	242(di)	178(di)
	52–54	4-Nitrotoluene		45	147	158
	55–56	1-Nitronaphthalene		50	159	160

Table 58.16 Nitro Compounds (cont.)

Bp	Mp	Name of Compound	Amine Obtained by Reduction of Nitro Groups			
					Acetamide[a]	Benzamide[b]
			Bp	*Mp*	*Mp*	*Mp*
	83–84	1-Chloro-4-nitrobenzene		72	179	192
	88–90	*m*-Dinitrobenzene		63	87(mono) 191(di)	125(mono) 240(di)

[a] For preparation, see page 362.
[b] For preparation, see page 362.

Table 58.17 Phenols

Bp	Mp	Name of Compound	Derivatives	
			α-Naphthylurethane[a]	Bromo[b]
			Mp	*Mp*
175–176		2-Chlorophenol	120	48(mono) 76(di)
181	42	Phenol	133	95(tri)
202	32–34	*p*-Cresol	146	49(di) 108(tetra)
203		*m*-Cresol	128	84(tri)
228–229		3,4-Dimethylphenol	141	171(tri)
	32–33	*o*-Cresol	142	56(di)
	42–43	2,4-Dichlorophenol		68
	42–45	4-Ethylphenol	128	
	43–45	4-Chlorophenol	166	90(di)
	44–46	2,6-Dimethylphenol	176	79
	44–46	2-Nitrophenol	113	117(di)
	49–51	Thymol	160	55
	62–64	3,5-Dimethylphenol		166(tri)
	64–68	4-Bromophenol	169	95(tri)
	74	2,5-Dimethylphenol	173	178(tri)
	92–95	2,3,5-Trimethylphenol	174	
	95–96	1-Naphthol	152	105(di)
	98–101	4-*t*-Butylphenol	110	50(mono) 67(di)
	104–105	Catechol	175	192(tetra)
	109–110	Resorcinol	275	112(tri)
	112–114	4-Nitrophenol	150	142(di)
	121–124	2-Naphthol	157	84
	133–134	Pyrogallol	173	158(di)

[a] For preparation, see pages 361, 364.
[b] For preparation, see page 364.

59

Vacuum Distillation

KEYWORDS

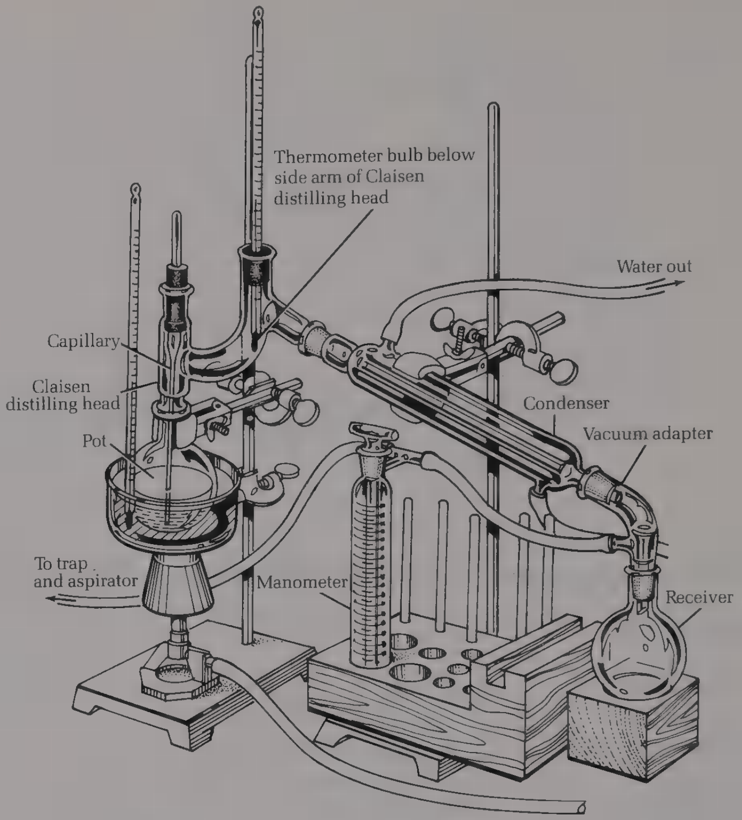
Claisen distilling head
Pot
Bumping, hair-fine capillary
Changing fractions
Mercury manometer
Vigreaux distilling column, flooding
Hold up
Fraction collector, cow
Vacuum distillation head
Water aspirator vacuum, 15 mm Hg
Rotary oil pump, 0.1 mm Hg
Mercury diffusion pump, 10^{-8} mm Hg
McLeod gauge
Dry ice trap
Distilling solids
Sublimation

Many substances cannot be distilled satisfactorily in the ordinary way, either because they boil at such high temperatures that decomposition occurs or because they are sensitive to oxidation. In such cases purification can be accomplished by distillation at diminished pressure. A few of the many pieces of apparatus for vacuum distillation are described on the following pages. Round-bottomed Pyrex ware and thick-walled suction flasks are not liable to collapse, but even so safety glasses should be worn at all times when carrying out this type of distillation.

1. Vacuum Distillation Assemblies

A typical vacuum distillation apparatus is illustrated in Fig. 59.1. It is constructed of a round-bottomed flask (often called the "pot") containing the material to be distilled, a Claisen distilling head fitted with a hair-fine capillary mounted through a rubber tubing sleeve, and a thermometer with the bulb extending below the side arm opening. The condenser fits into a vacuum

FIGURE 59.1 Vacuum distillation apparatus.



▼
Prevention of bumping

adapter which is connected to the receiver and, via heavy-walled rubber tubing, to a mercury manometer and thence to the trap and water aspirator.

Liquids usually bump vigorously when boiled at reduced pressure and most boiling stones lose their activity in an evacuated system; it is therefore essential to make special provision for controlling the bumping. This is done by allowing a fine stream of air bubbles to be drawn into the boiling liquid through a glass tube drawn to a hair-fine capillary. The capillary should be so fine that even under vacuum only a few bubbles of air are drawn in each second; smooth boiling will be promoted and the pressure will remain low. The capillary should extend to the very bottom of the flask and it should be slender and flexible so that it will whip back and forth in the boiling liquid. Another method used to prevent bumping, when small quantities of material are being distilled, is to introduce sufficient glass wool into the flask to fill a part of the space above the liquid.

▼
Making a hair-fine capillary

The capillary is made in three operations. First, a six-inch length of 6-mm glass tubing is rotated and heated in a small area over a *very hot* flame to collapse the glass and thicken the side walls as seen in Fig. 59.2a. The tube is removed from the flame, allowed to cool slightly and then drawn into a thick-walled, coarse diameter capillary (Fig. 59.2b). This coarse capillary is heated at point X over the wing top of a Bunsen burner turned 90°. When the glass is very soft, but not so soft as to collapse the tube entirely, the tubing is lifted from the flame and without hesitation drawn smoothly and rapidly into a hair-fine capillary by stretching the hands as far as they will reach (about 2 m) (Fig. 59.2c). The two capillaries so produced can be snapped off to the desired length. To ascertain that there is indeed a hole in the capillary, place the end beneath a low viscosity liquid such as acetone or ether and blow in the large end. A stream of very small bubbles should be seen. Should the right-hand capillary of Fig. 59.2c break when in use, it can be fused to a scrap of glass (for use as a handle) and heated again at point Y (Fig. 59.2c). In this way the capillary can be redrawn many times.

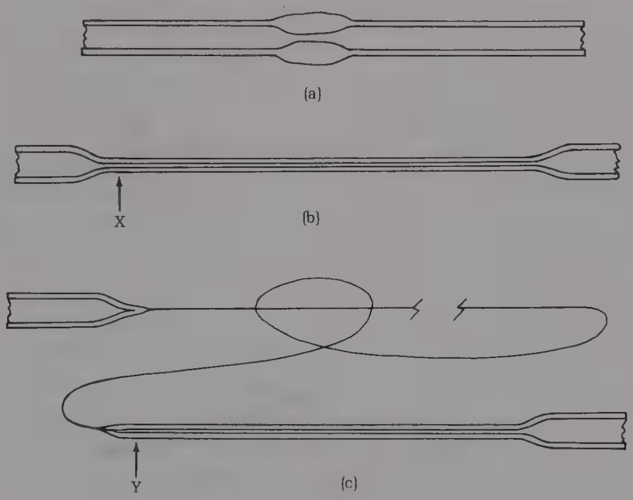


FIGURE 59.2 Capillary for vacuum distillation.

▼
Heating baths

The pot is heated with a heating bath rather than a free flame to promote even boiling and make possible accurate determination of the boiling point. The bath is filled with a suitable heat transfer liquid (water, cottonseed oil, silicone oil, or molten metal) and heated to a temperature about 20° higher than that at which the substance in the flask distils. The bath temperature is kept constant throughout the distillation. The surface of the liquid in the flask should be below that of the heating medium, for this condition lessens the tendency to bump. Heating of the flask is begun only after the system has been evacuated to the desired pressure; otherwise the liquid might boil too suddenly on reduction of the pressure.

▼
Changing fractions

To change fractions the following must be done in sequence: Remove the source of heat, release the vacuum, change the receiver, restore the vacuum to the former pressure, resume heating.

The pressure of the system is measured with a closed-end mercury manometer. The manometer (Fig. 59.3) is connected to the system by turning the stopcock until the V-groove in the stopcock is aligned with the side arm. To

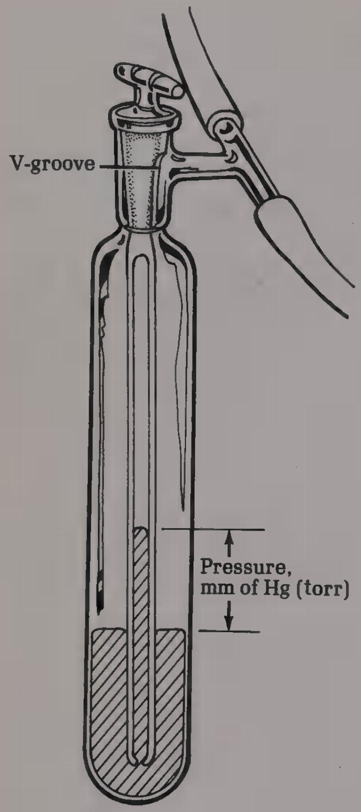


FIGURE 59.3 Closed end mercury manometer.

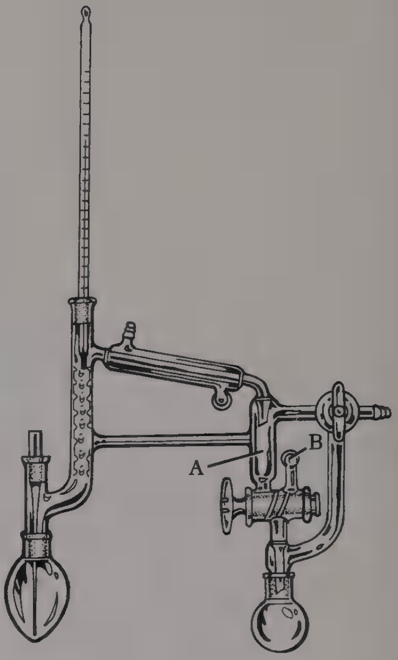


FIGURE 59.4 Vacuum distillation apparatus with Vigreaux column and fraction collector.

▼
Mercury manometer

avoid contamination of the manometer it should be connected to the system only when a reading is being made. [The pressure in the system can be monitored continuously by observing the rubber-tubing pressure gauge on the trap (Fig. 1.11)]. The pressure, in mm Hg, is given by the height, in mm, of the central mercury column above the reservoir of mercury and represents the difference in pressure between the nearly perfect vacuum in the center tube (closed at the top, open at the bottom) and the large volume of the manometer, which is at the pressure of the system.

A better vacuum distillation apparatus is shown in Fig. 59.4. The distillation neck of the Claisen adapter is longer than other adapters and has a series of indentures made from four directions, so that the points nearly meet in the center. These indentations increase the surface area over which rising vapor can come to equilibrium with descending liquid and it then serves as a fractionating column (a Vigreaux column). A column packed with a metal sponge has a great tendency to become filled with liquid (flood) at reduced pressure. The apparatus illustrated in Fig. 59.4 also has a fraction collector, which allows the removal of a fraction without disturbing the vacuum in the system. While the receiver is being changed the distillate collects in the small reservoir A. The clean receiver is evacuated by another aspirator at tube B before being connected again to the system.

If only a few milliliters of a liquid are to be distilled, the apparatus shown in Fig. 59.5 has the advantage of low hold-up, that is, not much liquid is lost wetting the surface area of the apparatus. The fraction collector illustrated is

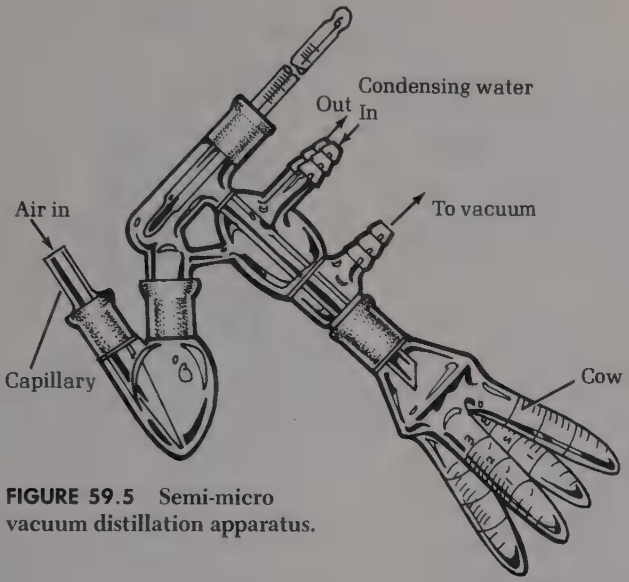


FIGURE 59.5 Semi-micro vacuum distillation apparatus.

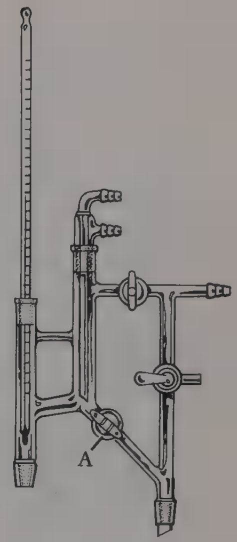


FIGURE 59.6 Vacuum distillation head.

known as a "cow." Rotation of the cow about the standard taper joint will allow four fractions to be collected without interrupting the vacuum.

A distillation head of the type shown in Fig. 59.6 allows fractions to be removed without disturbing the vacuum, and it also allows control of the reflux ratio (Chapter 2) by manipulation of the condenser and stopcock A. These can be adjusted to remove all material which condenses or only a small fraction, with the bulk of the liquid being returned to the distilling column to establish equilibrium between descending liquid and ascending vapor. In this way liquids with small boiling-point differences can be separated.

2. The Water Aspirator in Vacuum Distillation

A water aspirator in good order gives a vacuum nearly corresponding to the vapor pressure of the water flowing through it. Polypropylene aspirators give good service and are not subject to corrosion as are the brass ones. If a manometer is not available, and the assembly is free of leaks and the trap and lines clean and dry, an approximate estimate of the pressure can be made by measuring the water temperature and reading the pressure from Table 59.1.

Table 59.1 Vapor Pressure of Water at Different Temperatures

t, °C	p, mm Hg	t, °C	p, mm Hg	t, °C	p, mm Hg	t, °C	p, mm Hg
0°	4.58	20°	17.41	24°	22.18	28°	28.10
5°	6.53	21°	18.50	25°	23.54	29°	29.78
10°	9.18	22°	19.66	26°	24.99	30°	31.55
15°	12.73	23°	20.88	27°	26.50	35°	41.85

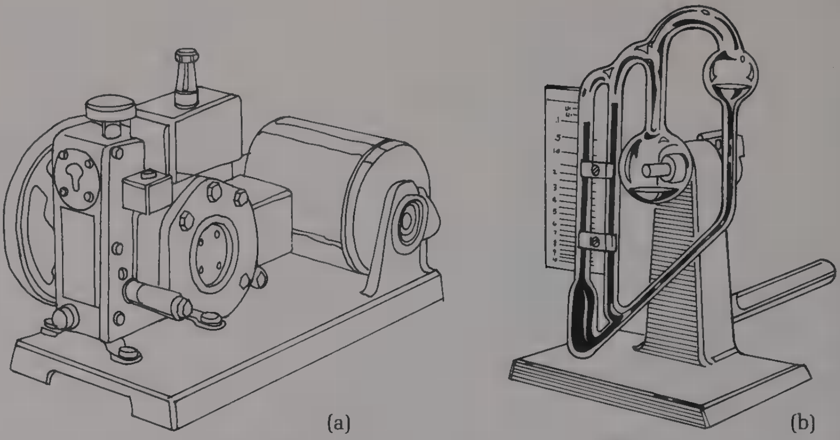


FIGURE 59.7 (a) Rotary oil pump, (b) Tilting McLeod gauge.

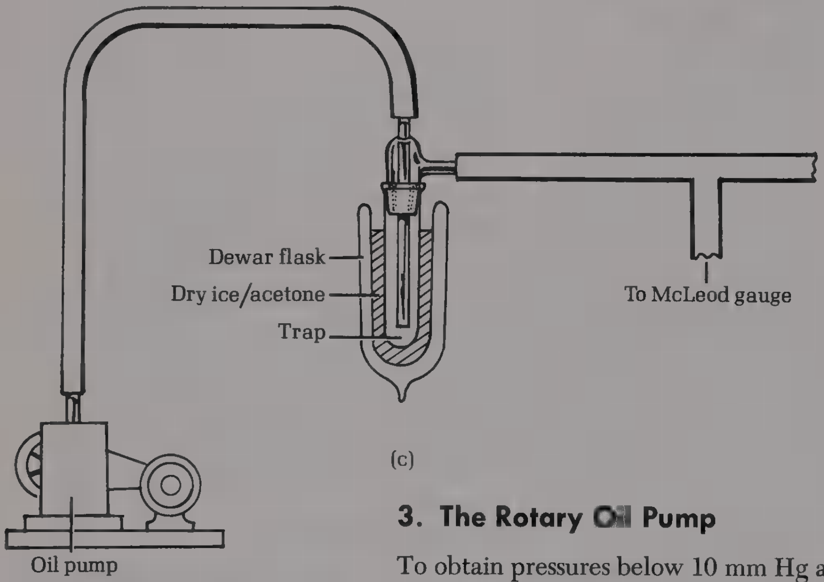


FIGURE 59.7 (c) Vacuum system.

3. The Rotary Oil Pump

To obtain pressures below 10 mm Hg a mechanical vacuum pump of the type illustrated in Fig. 59.7a is used. A pump of this type in good condition can give pressures as low as 0.1 mm Hg. These low pressures are measured with a tilting type McLeod gauge (Fig. 59.7b). When a reading is being made the gauge is tilted to the vertical position shown and the pressure is read as the difference between the heights of the two columns of mercury. Between readings the gauge is rotated clockwise 90°.

Never use a mechanical vacuum pump before placing a mixture of dry ice and acetone in a Dewar flask (Fig. 59.7c) around the trap and never pump corrosive vapors (e.g., HCl gas) into the pump. With care, it will give many years of good service. The dry ice trap condenses organic vapors and water vapor, both of which would otherwise contaminate the vacuum pump oil and exert enough vapor pressure to destroy a good vacuum.

For an exceedingly high vacuum (5×10^{-8} mm Hg) a high-speed three-stage mercury diffusion pump is used (Fig. 59.8).

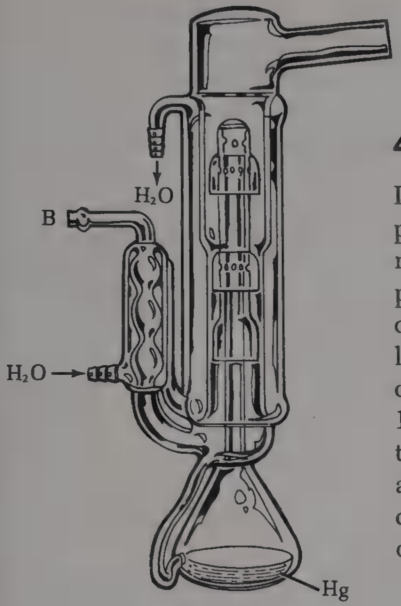


FIGURE 59.8 High speed three-stage mercury diffusion pump capable of producing a vacuum of 5 × 10^{-8} mm Hg. Mercury is boiled in the flask, the vapor rises in the center tube, is deflected downward in the inverted cups and entrains gas molecules which diffuse in from the space to be evacuated, A. The mercury condenses to a liquid and is returned to the flask, the gas molecules are removed at B by an ordinary rotary vacuum pump.

4. Relationship between Boiling Point and Pressure

It is not possible to calculate the boiling point of a substance at some reduced pressure from a knowledge of the boiling temperature at 760 mm Hg, for the relationship between boiling point and pressure varies from compound to compound and is somewhat unpredictable. It is true, however, that boiling point curves for organic substances have much the same general disposition, as illustrated by the two lower curves in Fig. 59.9. These are similar and do not differ greatly from the curve for water. For substances boiling in the region 150–250° at 760 mm Hg, the boiling point at 20 mm Hg is 100–120° lower than at 760 mm Hg. Benzaldehyde, which is very sensitive to air oxidation at the normal boiling point of 178°, distils at 76° at 20 mm Hg, and the concentration of oxygen in the rarefied atmosphere is just $^{20}/_{760}$, or 3% of that in an ordinary distillation.

The curves all show a sharp upward inclination in the region of very low pressure. The lowering of the boiling point attending a reduction in pressure is much more pronounced at low than at high pressures. A drop in the atmospheric pressure of 10 mm Hg lowers the normal boiling point of an ordinary liquid by less than a degree, but a reduction of pressure from 20 to 10 mm Hg causes a drop of about 15° in the boiling point. The effect at pressures below 1 mm is still more striking, and with development of practical forms of the highly efficient oil vapor or mercury vapor diffusion pump, distillation at a pressure of a few thousandths or ten thousandths of a millimeter has become a

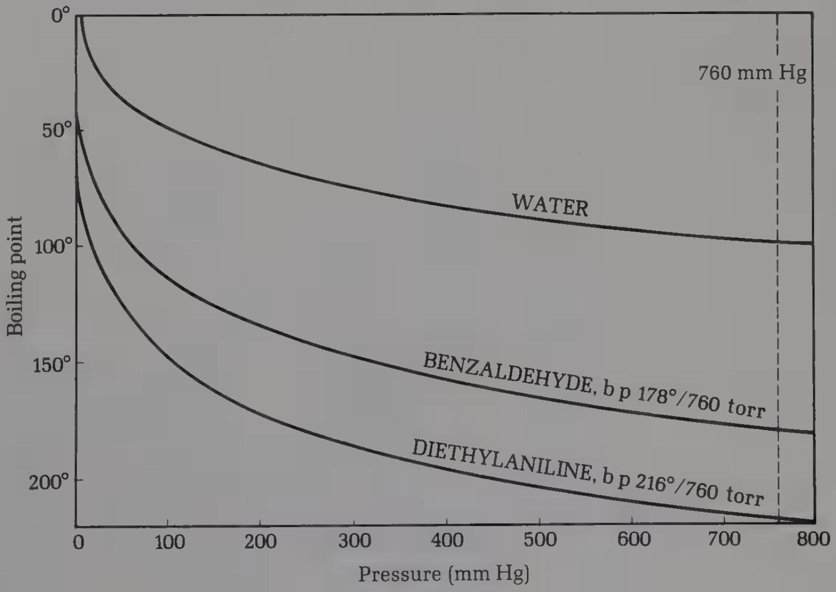


FIGURE 59.9 Boiling point curves.

standard operation in many research laboratories. High vacuum distillation, that is at a pressure below 1 mm Hg, affords a useful means of purifying extremely sensitive or very slightly volatile substances. Table 59.2 indicates the order of magnitude of the reduction in boiling point attainable by operating in different ways and illustrates the importance of keeping vacuum pumps in good repair.

Table 59.2 Distillation of a (Hypothetical) Substance at Various Pressures

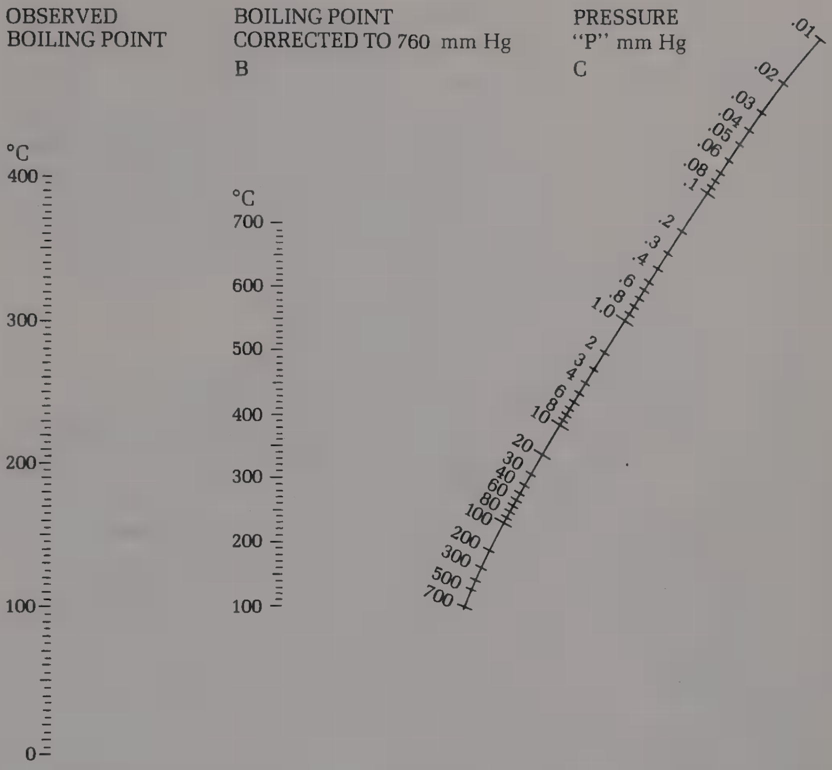
Method		Pressure (mm Hg)	Bp
Ordinary distillation		760	250°
Aspirator	summer	25	144°
	winter	15	134°
Rotary oil pump	poor condition	10	124°
	good condition	3	99°
	excellent condition	1	89°
Mercury vapor pump		0.01	30°

The boiling point of a substance at various pressures can be estimated from a pressure-temperature nomograph such as the one shown in Fig. 59.10. If the boiling point of a substance at 760 mm Hg is known, *e.g.*, 300° (Column B), and the new pressure measured, *e.g.*, 10 mm Hg (Column C), then a straight line connecting these values on Column B and C when extended intersects Column A to give the observed bp of 160°. Conversely, a substance observed to boil at 50° (Column A) at 1.0 mm Hg (Column C) will boil at approximately 212° at atmospheric pressure (Column B).

Vacuum distillation is not confined to the purification of substances liquid at ordinary temperatures but often can be used to advantage for solid substances. The operation is conducted for a different purpose and by a different technique. A solid is seldom distilled to cause a separation of constituents of different degrees of volatility but rather to purify the solid. It is often possible in one vacuum distillation to remove foreign coloring matter and tar without appreciable loss of product, whereas several wasteful crystallizations might be required to attain the same purity. It is often good practice to distil a crude product and then to crystallize it. Time is saved in the latter operation because the hot solution usually requires neither filtration nor clarification. The solid must be dry and a test should be made to determine if it will distil without decomposition at the pressure of the pump available. That a compound lacks the required stability at high temperatures is sometimes indicated by the structure, but a high melting point should not be taken as an indication that distillation will fail. Substances melting as high as 300° have been distilled with success at the pressure of an ordinary rotary vacuum pump.

It is not necessary to observe the boiling point in distillations of this kind because the purity and identity of the distillate can be checked by melting point determinations. The omission of the customary thermometer simplifies the technique. A simple and useful assembly is shown in Fig. 59.11. A rather

FIGURE 59.10 Pressure-temperature nomograph.



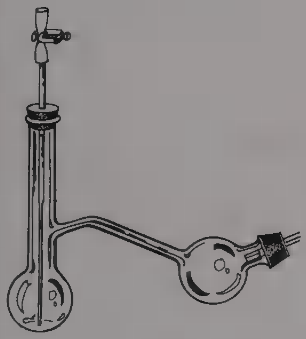


FIGURE 59.11 Two-bulb flask for the distillation of solids.

stout capillary tube carrying an adjustable vent at the top is fitted into the neck of the two-bulb flask by means of a rubber stopper and the suction pump is connected through a trap at the other bulb. It is not necessary to insert in the mouth a rubber stopper of just the right size; a somewhat larger stopper may be put on backwards as shown, as it will be held in place by atmospheric pressure. The same scheme can be used for the other stopper. Water cooling is unnecessary. If some cooling of the receiving bulb is required it is best to use an air blast. Since the connection between the distilling and the receiving flask is of glass, any material that solidifies and tends to plug the side arm can be melted with a free flame. A heating bath should not be used: it is best to heat the flask with a rather large flame. Hold the burner in the hand and play the flame in a rotary motion around the side walls of the flask. This allows less bumping than when the flask is heated from the bottom. If there is much frothing at the start of the heating, direct the flame upon the upper walls and the neck of the flask. If the liquid froths over into the receiving bulb, the flask is tilted to such a position that this bulb can drain through the connecting tube back into the distillation bulb when suitably warmed.

At the end of the distillation the vacuum is broken by carefully opening the pinchcock, and the contents of the receiving bulb melted and poured out. This method of emptying the bulb is sometimes inadvisable because the hot, molten material may be susceptible to air oxidation. In such a case, the material is allowed to solidify and cool completely before the vacuum is broken. The solid is then chipped out with a clean knife or with a strong nickel spatula and the last traces recovered with the solvent to be used in the crystallization. The tar usually remaining in the distillation bulb is best removed by adding small quantities of concentrated nitric and acid sulfuric acids, mixing the layers well, and heating the mixture while the flask is supported by a clamp under the hood. After cooling and pouring the acid mixture down the drain, loose char is removed with water and a brush and the process repeated.

5. Sublimation

When the quantity of solid is small or the thermal stability is such as to preclude distillation, sublimation is used to purify the solid. In an apparatus such as that illustrated in Fig. 59.12a, the solid to be sublimed is placed in the lower flask and connected via a lubricant free rubber "O" ring to the condenser, which in turn is connected to a vacuum pump. The lower flask is immersed in an oil bath at the appropriate temperature and the product sublimed and condensed on the cool walls of the condenser. The parts of the apparatus are gently separated and the condenser inverted; the vacuum connection serves as a convenient funnel for product removal. For large scale work the sublimator of Fig. 59.12b is used. The inner well is filled with a coolant (ice or dry ice). The sublimate clings to this cool surface, from which it can be removed by scraping and dissolving in an appropriate solvent.

Some substances, *e.g.* ferrocene (Chapter 23), can be sublimed at atmospheric pressure. The substance to be sublimed is placed on a watch glass on a hot plate and covered with an inverted stemless funnel.

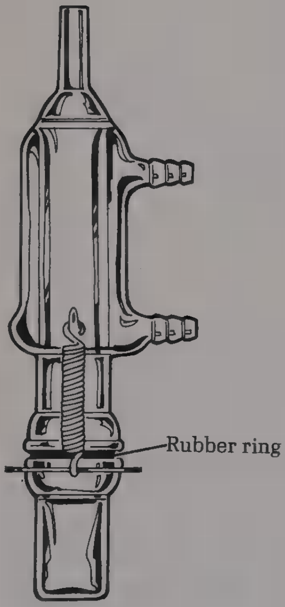


FIGURE 59.12 (a) Mallory sublimator.

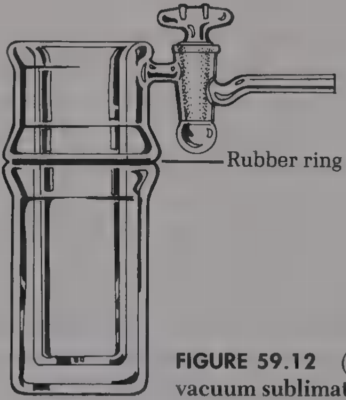


FIGURE 59.12 (b) Large vacuum sublimator.

60

Glass Blowing

KEYWORDS

Pyrex, borosilicate glass
Soft lime glass
Cutting
Fire polishing
Bending
Flaring
Straight seal
Test tube end
T-seal
Ring seal
Blowing a bulb
Annealing
Squaring

A knowledge of elementary glass blowing techniques is useful to every chemist and scientist, and glass blowing can be an enjoyable pasttime as well.

In the laboratory you will encounter primarily hard or Pyrex glass, a borosilicate glass with a low coefficient of thermal expansion which gives it remarkable resistance to thermal shock. It cannot easily be worked in a Bunsen burner flame because it has a working temperature of 820°. Consequently many laboratories stock soft or lime glass tubing and rod which, with a softening point of about 650°, can be worked in a Bunsen burner. However, except for very simple bends and joints, soft glass is not used for laboratory glass blowing because of the ease with which it breaks on being subjected to a thermal gradient. Since the two types of glass are not compatible it is important to be able to distinguish between them. This is easily done by immersing the glass object in a solution having exactly the same refractive index as the glass. In such a solution the glass will seem to disappear, whereas a glass of different refractive index will be plainly visible. A solution of 14 parts (by volume) of methanol and 86 parts of toluene has a refractive index of 1.474, which is the same as that of Pyrex 7740 glass, the most common of the various Pyrexes. In this solution Pyrex will not be visible. Store the solution in a wide-mouth jar with a close-fitting cap. It will keep indefinitely.

Glass Tubing

▼
Break by pulling

To cut a glass tube, first make a fine straight scratch, extending about a quarter of the way around the tube, with a glass scorer. This is done by applying firm pressure on the scorer and rotating the glass tube slightly (Fig. 60.1). Only one scratch should be made; in no case should you try to saw a groove in the tube. The tube is then grasped with the scratch away from the body and the thumbs pressed together at the near side of the tube just opposite the scratch, with the arms pressed tightly against the body (Fig. 60.2). A straight, clean break will result when *slight* pressure is exerted with the thumbs and a strong force applied to pull the tube apart. It is a matter of 90% pull and 10% bend.

Fire Polishing. The sharp edges that result from breaking a glass rod or tube will cut both you and the corks and rubber stoppers and tubing being fitted over them. Remove these sharp edges by holding the end of the rod (or tube) in a Bunsen burner flame and rotating the rod until the sharp edges melt and disappear. This fire polishing process can be done even for Pyrex glass if the flame is hot enough. Open the air inlet at the bottom of the burner to its maximum; the hottest part of the flame is about 7 mm above the inner blue cone. A stirring rod with a flattened head, useful for crushing lumps of solid against the bottom of a flask, is made by heating a glass rod until a short section at the end is soft and quickly pressing the end onto a smooth metal surface.

▼
Rotate constantly

Bends. The secret to successful glass working is to have the glass thoroughly and uniformly heated before an operation. Since Pyrex glass softens at 820° and soft glass at 650°, the best way to work Pyrex is with a gas-oxygen torch, but with patience it can be satisfactorily heated over an ordinary Bunsen burner with a wing top attached (Fig. 60.3). Stopper the tube at the left-hand end, grasp in the left hand with palm down and in the right hand with palm up so you can swing the open end of the tube into position for blowing without interruption of the synchronous rotation of the two ends. Adjust the air intake of the burner for the maximum amount of air possible (too much will blow out the flame) and rotate the tube constantly, holding it about 7 mm above the inner blue cone. A bit of coordination is needed to rotate both ends at the same speed once the glass begins to soften; when the flame is thoroughly tinged with yellow (from sodium ions escaping from the hot glass) and the tube begins to sag, remove the tube from the flame and bend it in the vertical plane with the ends upward and the bend at the bottom. Should the tube become constricted at the bend, blow into the open end immediately upon completion of the bend to expand the glass to its full size.

The Gas-Oxygen Torch

The following operations are best carried out using Pyrex tubing and a gas–oxygen torch. To light the torch turn on the gas first to give a large luminous

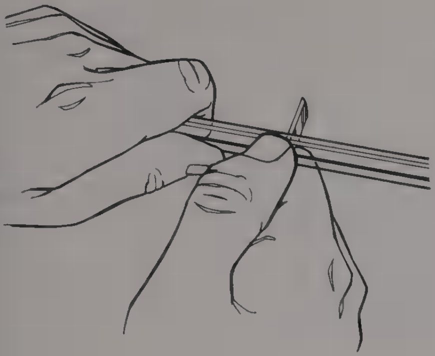


FIGURE 60.1 Scratching glass tubing with glass scorer prior to breaking. The scratch is about one-fourth the tube's circumference in length.

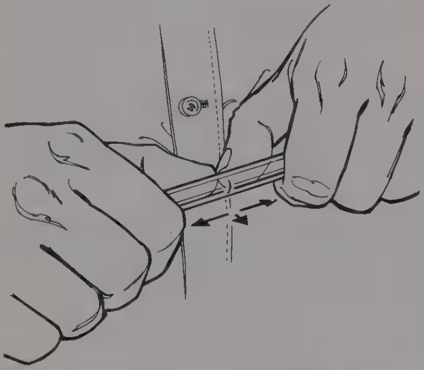


FIGURE 60.2 Breaking glass tubing. Thumbs are opposite the scratch. Pull, about 90%; bend, about 10%.

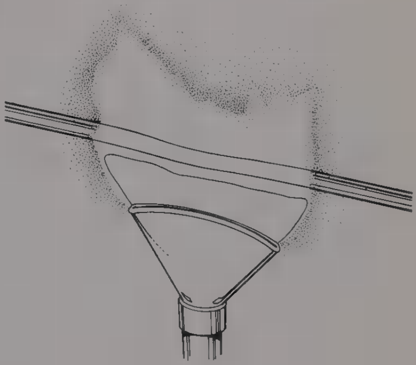


FIGURE 60.3 Heating glass tubing prior to bending. The wing top produces a broad flame that heats enough of the tubing to allow a good bend to be made. The tubing is held about 7 mm above the inner blue flame.

flame. *Gradually* turn on the oxygen until a long thin blue flame with a clearly defined inner blue cone is formed. The hottest part of the flame is at the tip of the inner blue cone. To turn off the flame always turn off the oxygen first. Wear glass blower's didymium goggles to protect the eyes from the blinding glare of hot Pyrex.

Flaring. It will often be necessary to flare the end of a tube in order to make a joint. Heat the end of the tube until the glass begins to sag, then remove the glass from the fire. While rotating the tube, insert a tool such as the tine of a file or a carbon rod and press it sufficiently to form the flare (Fig. 60.4).

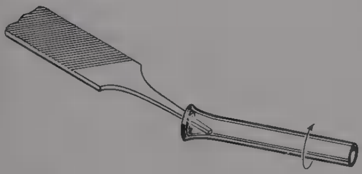


FIGURE 60.4 Flaring a glass tube.

Test Tube Ends. To close a tube heat it strongly at some convenient point while rotating both ends simultaneously. When the glass is soft remove the tube from the flame and pull the ends rapidly for a few inches while maintaining the rotation. Allow the glass to cool slightly, then heat the tube at A

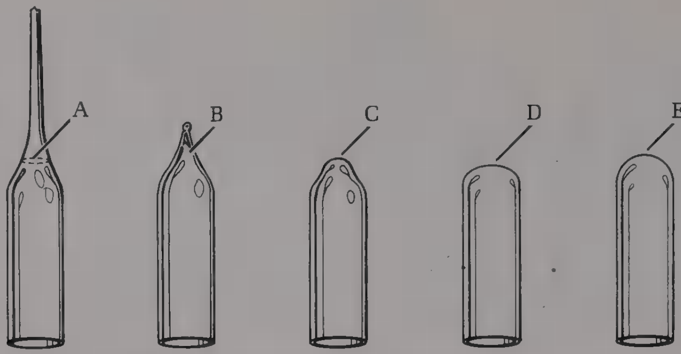


FIGURE 60.5 Blowing a test tube end.

(Fig. 60.5) and pull the tube into two pieces. Heat the point B with a sharp flame to collapse it and blow it out slightly as in C. Heat the whole end of the tube until it shrinks as in D and finally blow it to a uniform hemisphere as in E. Maintain uniform rotation while carrying out all of these operations.

Straight Seal. To join two tubes end-on, cork one and hold it with the palm down in the left hand. Hold the open tube in the right hand, palm up. Rotate both tubes simultaneously in the flame so that the glass becomes soft just at the end and forms a rounded edge, but does not constrict. Move the tubes to a cool part of the flame, press the ends lightly and evenly together on the same axis, and then with no hesitation pull the tubes apart slightly to reduce the thickness of the glass (Fig. 60.6). While rotating the tubing with both hands, heat the joint and cause it to shrink to about half its original diameter. Remove the tubing from the flame and blow gently to expand the joint slightly larger than the tubing diameter. Heat the joint once more and pull the tubing if necessary to restore the glass thickness to that of the original tubing. The joint when finished should resemble ordinary tubing closely.

T-Seal. Cork the end of a tube and heat a small spot on the side of the tube with a sharp flame (Fig. 60.7). Blow a small bulge on the tube. Reheat this bulge carefully at its tip and blow sharply to form a very thin walled bulb which is broken off. Cork the other end of the tube, heat uniformly the edges of the opening as well as the end of the side tube which has previously been slightly flared. After the edges of the openings are fairly soft remove the tubes from the flame, press them together, and then pull slightly as soon as complete contact has been made. Blow slightly to remove any irregularities. If necessary reheat, shrink, and blow until all irregularities are removed. Finally, heat the whole joint to obtain the correct angles between the tubes.

Ring Seals. Ring seals can be made in two different ways. In one method (Fig. 60.8) a flared tube of the appropriate length is dropped inside a test tube and centered with a smaller diameter tube through a cork (Fig. 60.8A). Heat the bottom of the test tube until the inner tube forms a seal, then blow out a bulb as in Fig. 60.8B. Cork the guide tube and attach another flared tube to give the finished seal as seen in Fig. 60.8C.

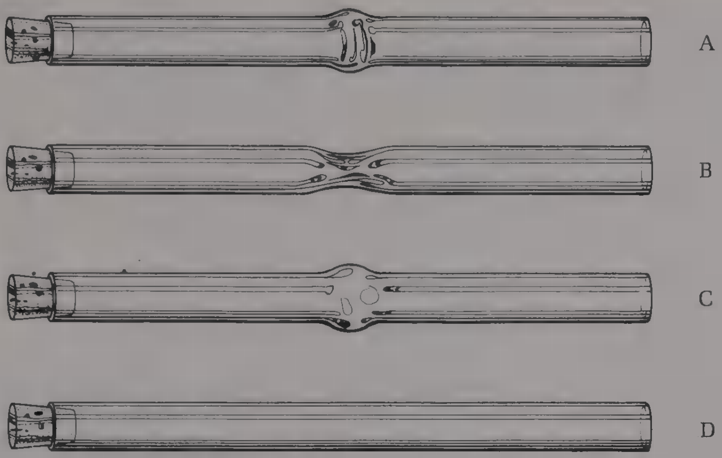


FIGURE 60.6 Making a straight seal.

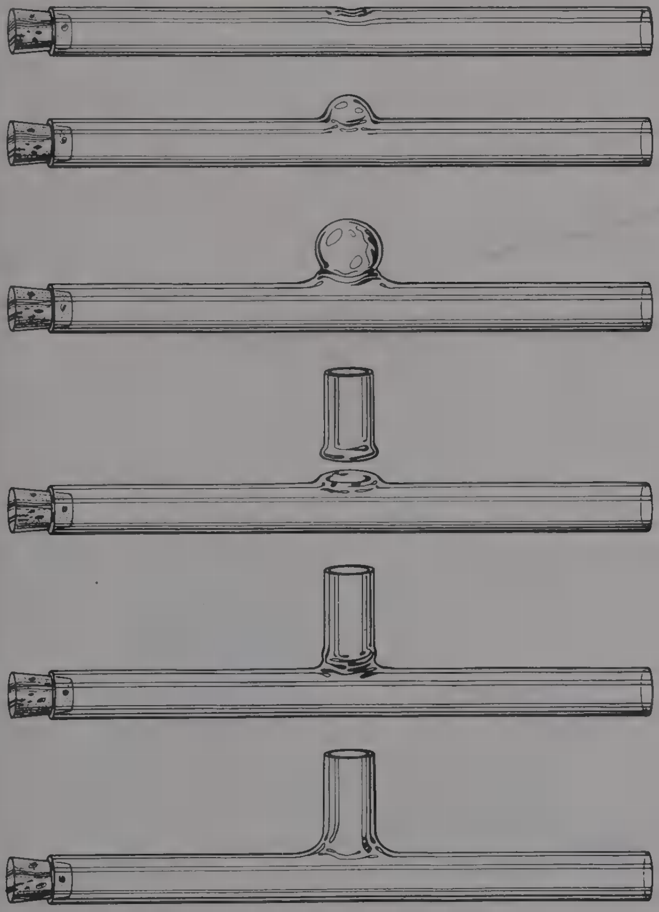


FIGURE 60.7 Making a T-seal.

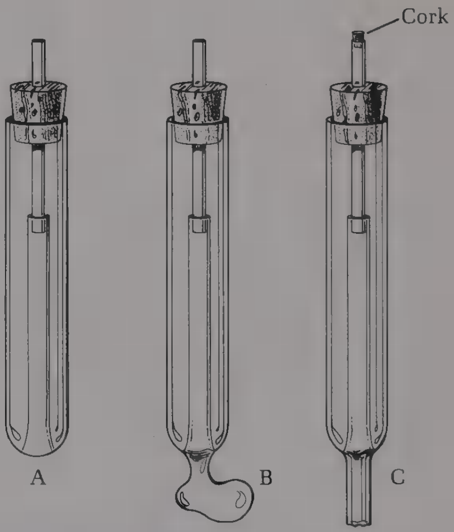


FIGURE 60.8 Making a ring seal, first method.

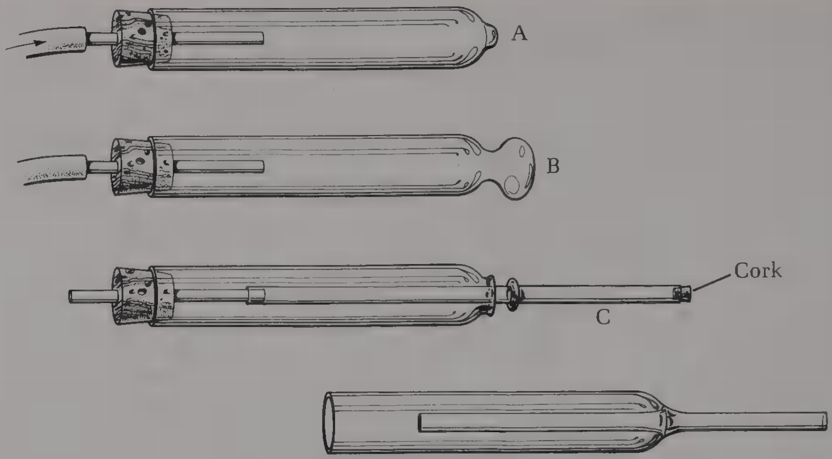


FIGURE 60.9 Making a ring seal, second method.

In the second method for making ring seals a test tube is heated at the bottom and a small bulge is blown (Fig. 60.9A). This is heated again and a thin bulb blown out (Fig. 60.9B) and broken off. The resulting hole should be the same size at the tube to be sealed in. At an appropriate place in the tube to be sealed in, blow a small bulge and assemble the two pieces (Fig. 60.9C). Heat the joint with a small flame until it is completely sealed then blow and shrink the glass alternately until irregularities are removed (Fig. 60.9D). These devices can be used as air locks in fermentation (See Fig. 61.6A).

Blowing a Bulb in a Tube. Cork one end of a 6-mm tube, grasp it left hand palm down, right hand palm up, and heat it uniformly over a length of about 25 mm while maintaining constant rotation. Surface tension will cause the glass to thicken as the ends of the tube are brought closer. When the tube has the appearance of Fig. 60.10A carefully blow a bulb (Fig. 60.10B). The success of this procedure is governed by the first operation. It is necessary to have enough hot glass gathered in the hot section such that the bulb, when blown, will have a wall thickness equal to that of the tubing.

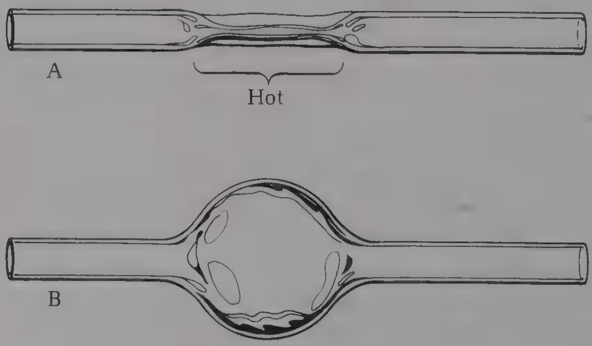


FIGURE 60.10 Blowing a bulb.

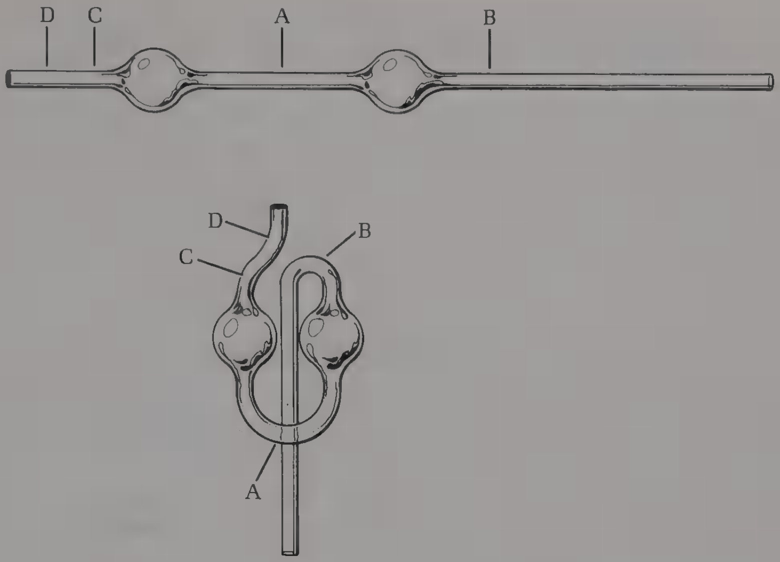


FIGURE 60.11 Fermentation air lock.

An exercise in bulb blowing and glass bending is to make a fermentation air lock (See Chapter 61). Blow bulbs 17 cm and 26 cm from the end of a 6-mm diameter tube which is 40 cm long (Fig. 60.11). Make the bend at A first, followed by the bend at B, and finally the two small bends at C and D. Trim the tubing to length and fire polish the ends.

Annealing. The rapid cooling of hot glass will put strains in the glass. In the case of soft glass these strains will often cause the glass to crack. With Pyrex the strains will make the glass mechanically weak where they occur, even if the glass does not crack. These strains are relieved by cooling the glass slowly from the molten state. On a small scale this can be done by turning off the oxygen of the burner and holding the hot glass joint in the relatively cool luminous flame until the joint is coated with an even layer of soot. During the minute or two this requires many of the strains will be relieved. Large and complex pieces are annealed in an oven with a controlled temperature drop.

Squaring a Jagged Break. It is often desired to square the end of a tube that has a jagged end prior to carrying out the fire polishing operation. This can be accomplished by stroking the glass with a 13-cm square of wire screen over a waste container (Fig. 60.12). The glass is removed as very small chips and dust. Much potentially hazardous apparatus can be repaired in this way, followed by fire polishing of the resulting opening.

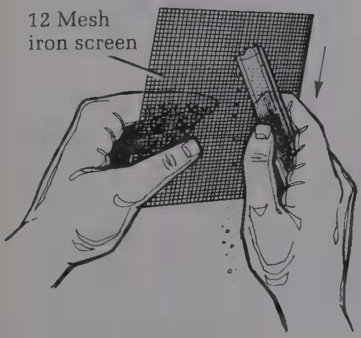


FIGURE 60.12 Squaring the end of ■ jagged tube.

Addendum: The Chemistry of Winemaking; The Biosynthesis of Ethanol

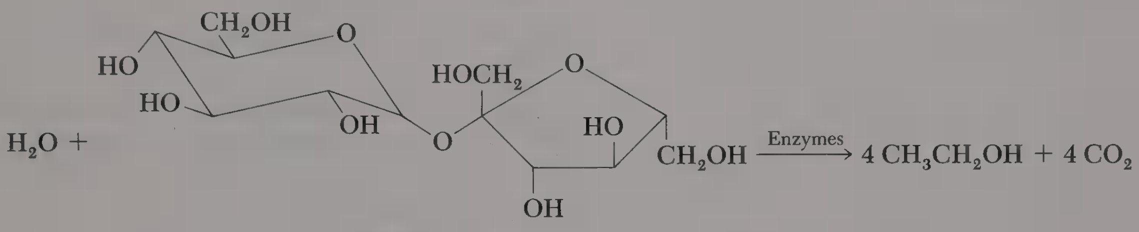
Man has been producing alcoholic beverages, specifically wine, for more than 10,000 years. By 4000 BC grape vines of the species *Vitis vinifera* were being cultivated as they are today. The conversion of sugar to alcohol is, as we shall see, a complex biochemical process, yet man at an early age, with no real understanding of the process, learned to produce wine instead of vinegar.

The list of scientists who sought to put the process of fermentation on a firm scientific basis is long and illustrious: Paracelsus, Robert Boyle, Lavoisier, Gay-Lussac, Dumas, Helmholz, and Liebig. All failed. It was Louis Pasteur, one of the enduring geniuses of organic chemistry, who eventually solved the problem and in the process made a series of astounding discoveries. At the age of 26 Pasteur, while studying crystals of potassium acid tartrate, the precipitate found in a very acidic wine, discovered optical isomerism. He perceived the relationship between optical activity and molecular asymmetry—a discovery that led van't Hoff and LeBel to propose the tetrahedral carbon atom a quarter of a century later. In the course of his studies, Pasteur discovered most of the methods used for resolving racemic mixtures into their enantiomers: by crystallization and hand picking, by fractional crystallization of diastereomers, and by the use of enzymes generated by bacteria.

As an industrial consultant to the wine industry, Pasteur showed conclusively for the first time that fermentation is the result of the activity of minute living organisms and that when fermentation fails, either the proper organism is not present or the conditions for proper growth are lacking. He invented the process of pasteurization, a brief heating to kill microorganisms, so that wine and vinegar could be shipped without spoiling. A grant from the British brewing industry allowed him to perfect the pasteurization of beer so it could be shipped around Africa to India without deterioration. Later the process was applied to milk.

In the century since Pasteur carried out his pioneering work many of the details of the biochemistry of the fermentation process have been elucidated, but even now the exact details of interaction of enzymes (large globular proteins of MW 60,000 to 360,000) with the substrate (the small molecule such as pyruvic acid or glucose) are still not entirely understood.

Biosynthesis of Ethanol



Sucrose

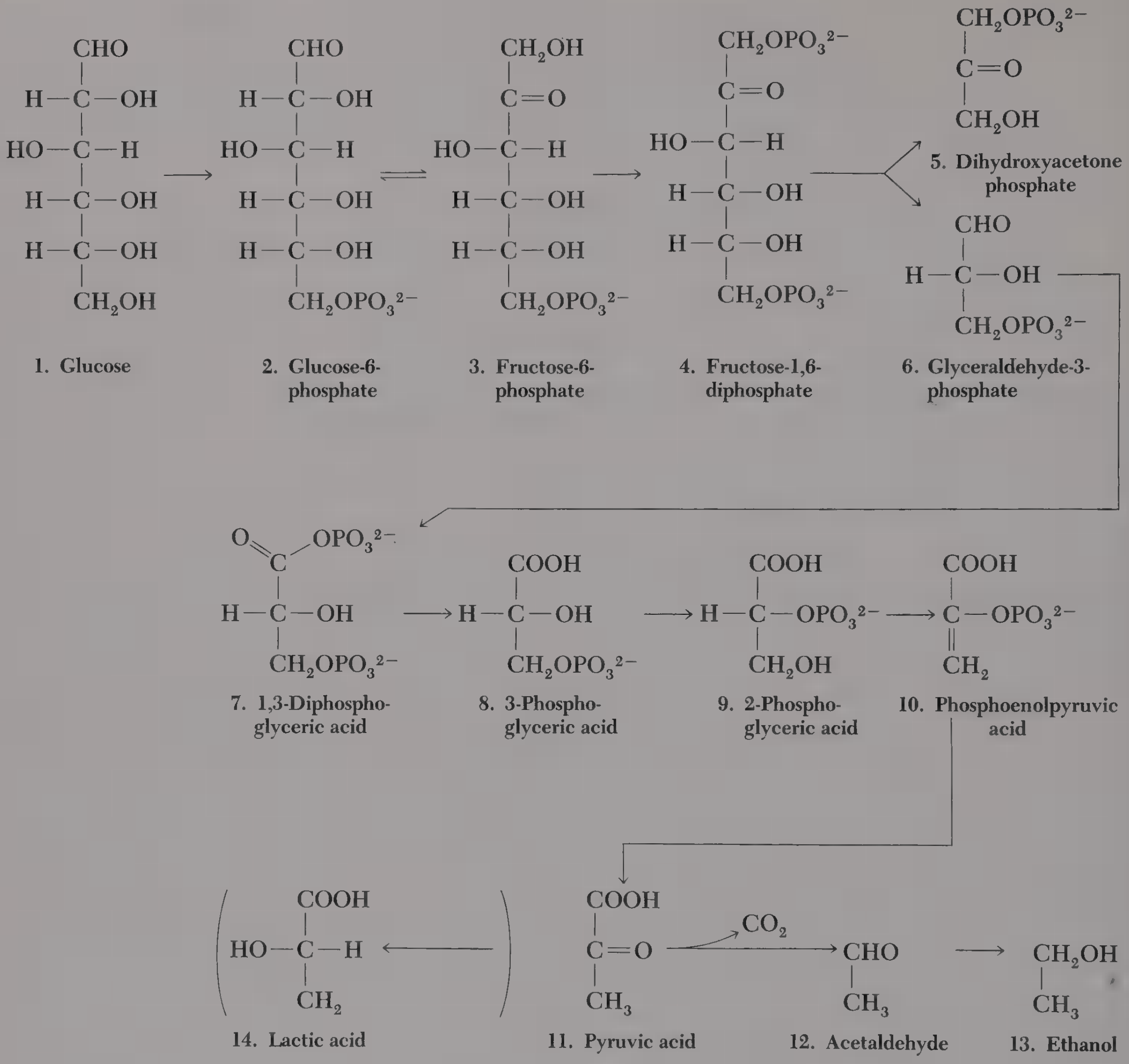
Sucrose, ordinary table sugar, is converted into ethanol and carbon dioxide with the aid of some fourteen enzymes as catalysts, in addition to adenosine triphosphate (ATP), phosphate ion, thiamine pyrophosphate, magnesium ion, and reduced nicotinamide adenine dinucleotide (NADH), all present in yeast.

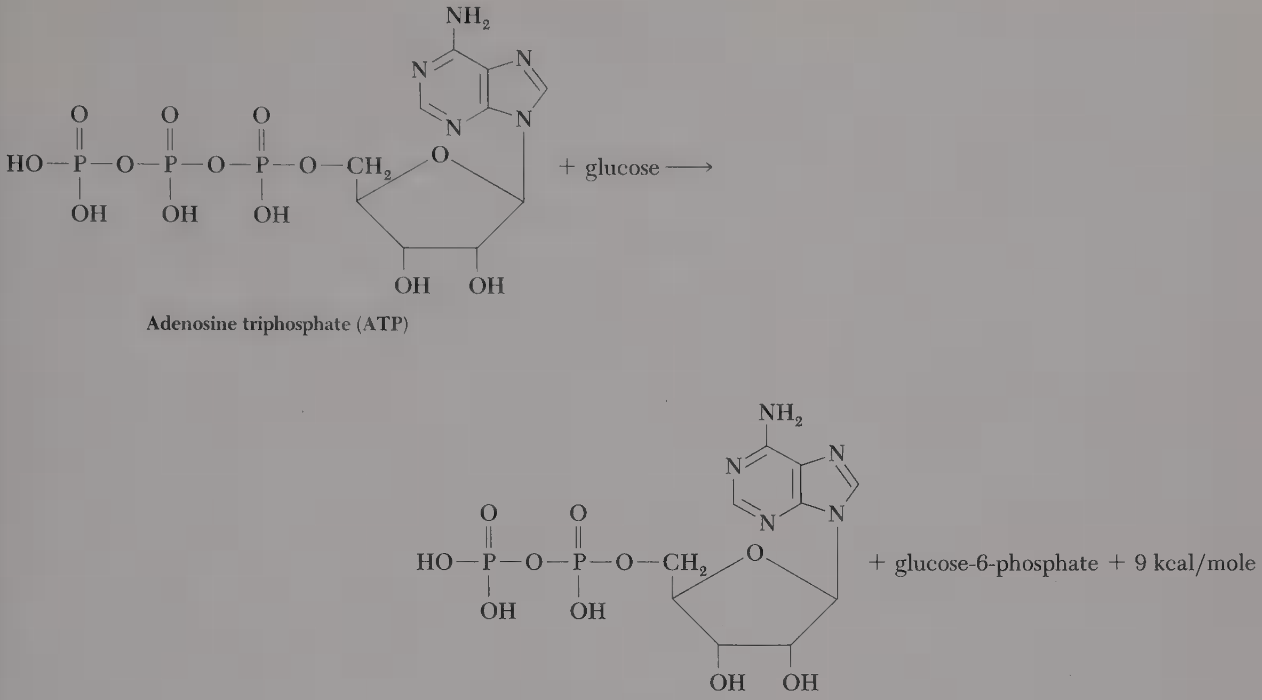
The fermentation process—known as the Emden-Meyerhof-Parnas scheme—involves the hydrolysis of sucrose to glucose and fructose which, as their phosphates, are cleaved to two three-carbon fragments. These fragments, as their phosphates, eventually are converted to pyruvic acid, which is decarboxylated to give acetaldehyde. Acetaldehyde, in turn, is reduced to ethanol in the final step. Each step requires a specific enzyme as a catalyst and often inorganic ions, such as magnesium and, of course, phosphate. Thirty-one kilocalories of heat are released per mole of glucose consumed in this sequence of anerobic reactions.

This same sequence of reactions, up to the formation of pyruvic acid, occurs in the human body in times of stress when energy is needed, but not enough oxygen is available for normal aerobic oxidation. The pyruvic acid under these conditions is converted to lactic acid. It is the buildup of lactic acid in the muscles which is partly responsible for the feeling of fatigue.

The first step in the sequence is the formation of glucose-6-phosphate (**2**) from glucose (**1**). The reaction requires adenosine triphosphate (ATP), which is converted to the diphosphate (ADP) by catalysis with the enzyme glucokinase, which requires magnesium ion to function. This conversion is one of the reactions in which energy is released. In the living organism this energy can be used to do work; in fermentation it simply creates heat.

In the next step glucose-6-phosphate (**2**) is converted through the enol of the aldehyde to fructose-6-phosphate (**3**) by the enzyme phosphoglucoisomerase. The fructose monophosphate **3** is converted to the diphosphate **4** by the action of ATP under the influence of phosphofructokinase with the release of

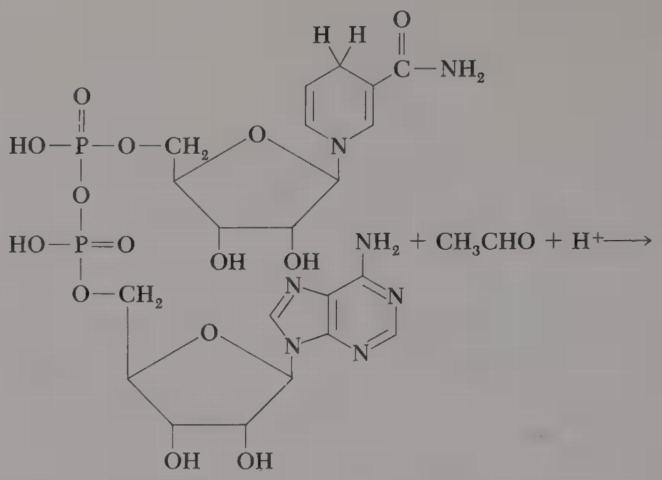

1. Glucose
2. Glucose-6-phosphate
3. Fructose-6-phosphate
4. Fructose-1,6-diphosphate
5. Dihydroxyacetone phosphate
6. Glyceraldehyde-3-phosphate
7. 1,3-Diphosphoglyceric acid
8. 3-Phosphoglyceric acid
9. 2-Phosphoglyceric acid
10. Phosphoenolpyruvic acid
11. Pyruvic acid
12. Acetaldehyde
13. Ethanol
14. Lactic acid
CO2



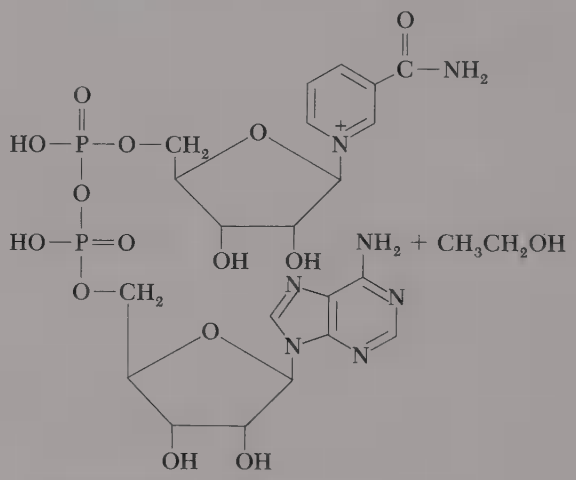
Adenosine triphosphate (ATP)

Adenosine diphosphate (ADP)

more energy. This diphosphate, **4**, undergoes a reverse aldol reaction catalyzed by aldolase to give dihydroxyacetone phosphate (**5**) and glyceraldehyde-3-phosphate (**6**). The latter two are interconverted by means of triosphosphate isomerase. The aldehyde of glyceraldehyde phosphate (**6**) is oxidized by nicotinamide adenine dinucleotide (NAD^+) in the presence of another enzyme to a carboxyl group that is phosphorylated with inorganic phosphate. In the next reaction ADP is converted to ATP as **7** loses phosphate to give **8**. A mutase converts the 3-phosphate, **8**, to the 2-phosphate, **9**. An enolase converts **9** to **10** and a kinase converts **10** to pyruvic acid (**11**). A decarboxylase converts pyruvic acid (**11**) to acetaldehyde (**12**) in the fermentation process. Yeast alcohol dehydrogenase (YAD), a well studied enzyme, catalyzes the reduction of acetaldehyde to ethanol. The reducing agent is reduced nicotinamide adenine dinucleotide, NADH.



Reduced nicotinamide adenine dinucleotide, NADH



Nicotinamide adenine dinucleotide, NAD^+

Enzymes are remarkably efficient catalysts, but they are also labile (sensitive) to such factors as heat and cold, changes in pH, and various specific inhibitors. In the first experiment of this chapter you will have an opportunity to observe the biosynthesis of ethanol and to test the effects of various agents on the enzyme system.

This experiment involves the fermentation of 50 g of ordinary cane sugar using baker's yeast. The resulting dilute solution of ethanol, after removal of the yeast by filtration, can be distilled according to the procedures of Chapter 2.

EXPERIMENTS

1. Fermentation of Sucrose

Macerate (grind) one-half cake of yeast or half an envelope of dry yeast in 50 ml of water in a beaker, add 0.35 g of disodium hydrogen phosphate, and transfer this slurry to a 500-ml round-bottomed flask. Add a solution of 51.5 g of sucrose in 150 ml of water, and shake to ensure complete mixing. Fit the flask with a one-hole rubber stopper containing a bent glass tube that dips below the surface of a saturated aqueous solution of calcium hydroxide (limewater) in a 6-in. test tube (Fig. 61.1). The tube in limewater will act as a seal to prevent air and unwanted enzymes from entering the flask but will allow gas to escape. Place the assembly in a warm spot in your desk (the optimum temperature for the reaction is 35°) for a week, at which time the evolution of carbon dioxide will have ceased. What is the precipitate in the limewater?

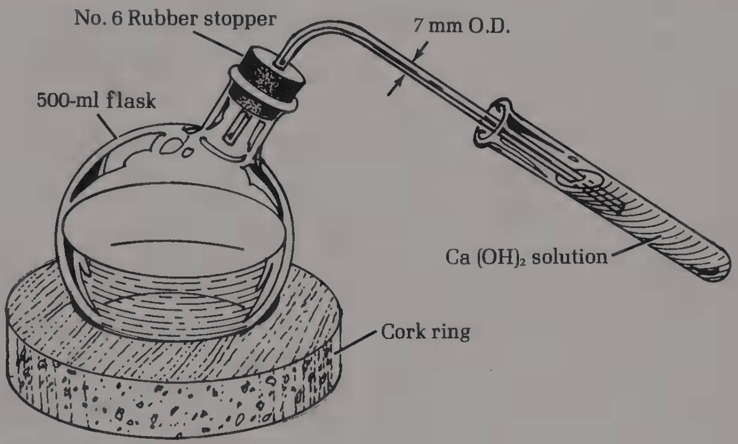


FIGURE 61.1 Apparatus used in the fermentation of sucrose experiment.

▼
Reaction time: 1 week

▼
Filter aid

Upon completion of fermentation add 10 g of filter aid (diatomaceous earth or face powder) to the flask, shake vigorously, and filter. Use a 5.5-cm Büchner funnel placed on a neoprene adapter or Filtervac atop a 500-ml filter flask that is attached to the water aspirator through a trap by vacuum tubing Fig. 61.2). Since the apparatus is top-heavy, clamp the flask to a ring stand. Moisten the filter paper with water and apply gentle suction (water supply to aspirator turned on full force, clothespin on trap partially closed), and slowly pour the reaction mixture onto the filter. Wash out the flask with a few milliliters of water from your wash bottle and rinse the filter cake with this water. The filter aid is used to prevent the pores of the filter paper from becoming clogged with cellular debris from the yeast.

The filtrate, which is a dilute solution of ethanol contaminated with bits of cellular material and other organic compounds (acetic acid if you are not careful), is saved in a stoppered flask until being distilled following the procedure of Chapter 2.

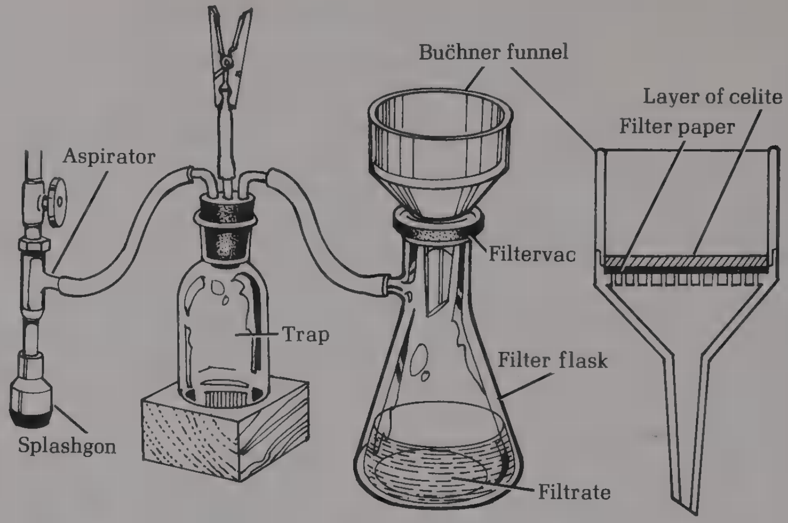


FIGURE 61.2 Vacuum filtration apparatus.

2. Effect of Various Reagents and Conditions on Enzymatic Reactions

About 15 min after mixing the yeast, sucrose, and phosphate, remove 20 ml of the mixture and place 4 ml in each of five test tubes. To one tube add 1.0 ml of water, to the next add 1.0 ml of 95% ethanol, to the next add 1.0 ml of 0.5 *M* sodium fluoride. Heat the next tube for 5 min in a steam bath and cool the next tube for 5 min in ice. Add 10 to 15 drops of mineral oil on top of the reaction mixture in each tube (to exclude air, since the process is anaerobic). Place tubes in a beaker of water at room temperature for 15 min, then take them one at a time and connect each to the manometer as shown in Fig. 61.3. Allow about 30 sec for temperature equilibration, then clamp the vent tube

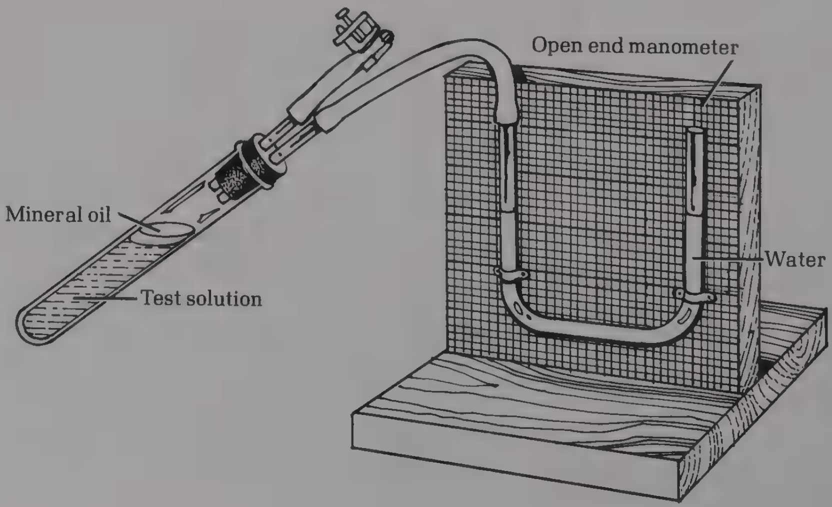


FIGURE 61.3 Manometric gasometer.

and read the manometer. Record the height of the manometer fluid in the open arm of the U-tube every minute for 5 minutes or until the fluid reaches the top of the manometer. Plot a graph of the height of the manometer fluid against time for each of the four reactions. What conclusions can you draw from the results of these five reactions?

WINEMAKING

The sugar in almost every naturally sweet substance has been fermented to alcohol. Mead from honey, hard cider from apples, and birch beer from birch sap are examples. But the juice of the wine grape fermented to wine has won enduring favor. Only the most ardent amateur winemaker, however, has the patience to contend in late August and early September with the pressing of freshly harvested grapes. This step is no longer necessary. Recently grape concentrates have become widely available to the general public. These concentrates bear no relation to the grape concentrates on the grocer's shelves used for making grape juice. They are instead the pure juices of specific species of *Vinis vinifera* and have been used for years by major wineries for blending purposes and providing uniformity of taste from year to year. With some fairly simple analytical chemistry and the judicious application of a knowledge of organic chemistry, these concentrates can be made into wines that, if not great, rise well above the mediocre.

The process is fairly simple. The concentrate is diluted, analyzed for its sugar content and its organic acid content and adjusted accordingly, then in sterilized apparatus inoculated with wine yeast, allowed to ferment vigorously for a few days in a large bucket, siphoned from a precipitate of yeast cells into a large glass bottle and set aside for completion of the fermentation process, which is followed by analysis. The siphoning is repeated two or three times at intervals to leave behind spent yeast cells. After storage for a period of time in the large container the wine is ready for transfer to sterilized wine bottles which are filled, corked, and labelled.

The experiments that follow give a procedure for preparing wine from commercially available grape concentrate on the scale usually used for this process by amateurs: 5 gal. In addition a procedure is given for preparing a wine from cranberry concentrate on a 1-gal. scale.

Sterilization.

Containers and apparatus that come into contact with wine at all stages in its manufacture must be sterile, that is, free of unwanted microorganisms. This sterilization, fortunately, is not difficult to carry out. Almost all unwanted microorganisms will not reproduce in very dilute solutions of sulfurous acid, whereas the principal strains of wine yeast are not very sensitive to this substance. Early vintners burned sulfur in their casks. Now it is much more convenient to use sodium metabisulfite:

$$Na_2S_2O_5 + H_2O \longrightarrow 2NaHSO_3$$

$$NaHSO_3 + H^+ \longrightarrow Na^+ + H_2SO_3$$

Wine, as we shall see, is naturally acidic and furnishes the hydrogen ion to produce sulfurous acid from bisulfite. In the aging process sulfurous acid is removed as the bisulfite addition products of aldehydic substances in the wine.

Sugar Analysis.

The alcoholic content of the wine is governed by the amount of sugar in the original grape juice solution which is called *must.* If the alcoholic content is below about 9% the wine will be unstable and will not keep. When the alcoholic content of the fermenting solution reaches about 12% the alcohol produced will kill the yeast cells, so no more alcohol can be produced. If excess sugar is present when the alcohol content reaches 12–13% it will remain unfermented and the resulting wine will be *sweet.* If the sugar content of the starting must is adjusted so that virtually all the sugar ferments and the alcohol concentration is about 10–12%, the result is a *dry* wine. The sugar content of the must is easily measured with a hydrometer, a device that measures the density of a liquid (Fig. 61.4). As the density of the liquid increases, the hydrometer floats higher in the liquid. A hydrometer measuring in the range 0.990 to 1.170 will cover the necessary range.

To determine the density, transfer the liquid to a graduated cylinder or other container of adequate depth and side clearance, spin the hydrometer to free it of clinging air bubbles, and read the density at the meniscus. The percent alcohol in the resulting wine is related to the density according to

$$\%\ \text{alcohol} = (140)(\text{density}) - 140.5$$

So if a wine of 11.4% alcohol is desired the density of the solution before fermentation begins must be 1.085.

If the density is too low, add sugar to bring it to the desired level. Addition of 64 g of sucrose per gallon will raise the density by 0.005.

The concentration of sugar in the finished wine is most easily measured using a Clinitest or similar kit used by diabetics and available in most pharmacies.

Acid Analysis.

Much of the characteristic tart taste of wine can be attributed to the presence of a mixture of three organic acids—tartaric, malic, and citric—half or more of which is tartaric and most of the remainder malic.

To carry out the analysis boil 200 ml of water in a 500-ml Erlenmeyer flask, add 1 ml of a 1% phenolphthalein solution, and titrate with 0.1 *N* sodium hydroxide solution to a faint but definite pink color. Pipette 5.00 ml of must into the hot solution and titrate to the same color. The end point is most easily seen in yellow light. Alternatively, cool the solution to room temperature after the must has been added and titrate to pH 8.2 using a meter to measure pH. Even though a mixture of acids is being titrated, wine acidity is usually expressed as percent tartaric acid.

$$\text{Tartaric acid, g/100 ml} = \frac{(\text{vol.NaOH})(\text{normality NaOH})(75.04)(100)}{(1000)(\text{vol.sample})}$$

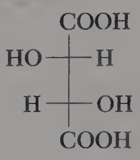
Red wines have an acidity of 0.5–0.62% and white and rosé wines are in the range 0.55–0.68%.

If the acidity is too low (the usual case) it can be increased 0.1% by the addition of 3.5 g of blended acid per gallon of must. The blended acid is prepared from 50% tartaric acid, 30% malic acid, and 20% citric acid.

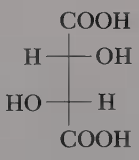
EXPERIMENT

Preparation of 5 Gallons (18.9 Liters) of Dry White Wine.

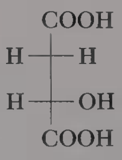
White wine matures more rapidly and is easier to titrate than red wine and is therefore a good choice for a first experiment. The primary fermenter should be an 8-gal bucket made of white food-grade polyethylene. Plasticizers leached from polyvinyl chloride containers can spoil the taste of wine. Pour 5.0 gal of water into the fermenter, mark the meniscus, then empty the fermenter and rinse it with a small quantity of 5% sodium metabisulfite ($Na_2S_2O_5$) sterilizing solution (which can be reused). Add 1 gal of grape concentrate, taking care to transfer all solids to the fermenter. Add distilled water to the 5.0-gal mark. If distilled water is not available use bottled water or ordinary tap water that has been boiled to expel chlorine and then shaken vigorously to reaerate it. Stir the mixture well and then analyze for sugar by measuring the density as described above. By the addition of sugar adjust the density to 1.085, a density that will give a 12% alcohol wine. Preferably dissolve the sugar to be added in a portion of the must and then mix the two resulting solutions. Check the density. Analyze for organic acids as described above. Add blended acid to bring the level to 0.6%.



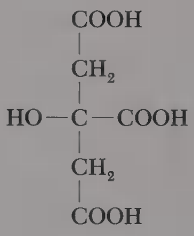
D-(−)-Tartaric acid



L-(+)-Tartaric acid



D-(−)-Malic acid



Citric acid

The must is often short of nitrogenous substances and phosphorus, both needed for the initial vigorous aerobic yeast growth. Add 10 g per gallon of ammonium phosphate ($(NH_4)_2HPO_4$) or a 50:50 mixture of urea (NH_2CONH_2) and sodium phosphate (Na_3PO_4). It is also advisable to add 3 mg per gallon of thiamine hydrochloride (vitamin B_1), an important growth factor for yeast.

Remove 200 ml of the must with a sterilized (bisulfite) pipette and place it in a sterile 500-ml Erlenmeyer flask. Warm the solution to 37°, add about 20 g of sucrose, and then add one package of dry Montrachet wine yeast (a variety of *Saccharomyces ellipsoideus*). Do not use baker's yeast (*S. cerevisiae*) unless you like wine that smells like bread. Place the flask in a warm place. After two or three hours the fermentation should be proceeding vigorously. Add this growing culture to the fermenter, stir the solution, and cover the fermenter with a piece of clear plastic food wrap or polyethylene tied on with string or a length of rubber tubing. The primary fermentation should take place in a room at a temperature not much above 21° (70°F). After about a day the fermentation should be proceeding vigorously and the plastic cover should be billowed up. Stir the must daily. In four to six days the vigorous fermentation will moderate and a slower and more sedate fermentation will begin. At this point the must should be siphoned (*racked* in winemaker's terminology) into a sterile 5-gal bottle, leaving behind the layer of dead yeast cells that has settled to the bottom of the primary fermenter. The density of the must should have decreased markedly.

About three weeks after the first racking, when most of the fermentation has been completed, the wine is racked for the second time. It is then racked twice more at intervals of three months. Each racking will leave behind a layer of dead yeast cells, which if allowed to autolyse would impart a musty taste to the wine. During this period of bulk storage the once cloudy must will become brilliantly clear and a number of complex and subtle chemical reactions will occur to transform the raw must to a mellow and palatable wine.

It is good practice to add 0.5 g of sodium metabisulfite per gallon (50 ppm) to white wine on the second and successive rackings. Dissolve the bisulfite in a small quantity of water prior to addition. The sulfur dioxide serves as an antioxidant:

$$2SO_2 + O_2 \longrightarrow 2SO_3$$

$$SO_3 + H_2O \longrightarrow H_2SO_4$$

The five-gallon glass bottle (carboy) should be topped with water to minimize contact of the must with oxygen and the carboy should be fitted with an air lock (Fig. 61.6a and b). An air lock allows the escape of carbon dioxide from the slowly fermenting solution, but prevents oxygen and unwanted microorganisms from entering the secondary fermenter.

After some six to nine months of bulk storage the wine is ready to bottle. Use wine bottles of the appropriate shape, wash them thoroughly, and sterilize them by rinsing with 10% sodium metabisulfite solution which is allowed to drain from the bottles. Siphon the wine into each bottle to within 1.5 cm of the closure and close the bottle with a sterilized screw cap or a new, sterilized, cylindrical wine-bottle cork. These corks, sterilized with bisulfite solution, require a corking device to compress them sufficiently to be driven into the bottle neck. Do not use tapered laboratory corks, which are not sufficiently impervious to oxygen or wine for long-term storage. A foil cap can be applied over the top of the bottle and a neat label should be attached. Store the bottles on their sides in a cool, dark place. The wine will continue to improve on storage. White wine reaches its peak in perhaps a year and red wine in two or more years.

Grape-Cranberry Wine.[2]

To a 2-gal polyethylene bucket add 6 oz of frozen grape juice concentrate, 12 oz of frozen cranberry cocktail concentrate, a pectic enzyme tablet (to break down pectin in the cranberries), and 0.5 g of sodium metabisulfite (added to kill unwanted bacteria). Dilute this mixture with distilled water to a volume of slightly less than 1 gal (3.78 liters) and measure the density of the solution with a hydrometer. Add sugar (1 lb or more may be required) to bring the density up to 1.079, which will give a wine containing 10.5% alcohol. (The density increases 0.005 units for each 64 g of sugar added.) Check the density once more, then titrate the mixture with 0.1 *N* NaOH to determine the acidity. Add a blend of tartaric, malic, and citric acids to bring the acidity to 0.61g/100 ml (expressed as tartaric acid). Each 3.5 g of blended acid per gallon will raise the acidity by 0.1 g/100 ml (12 to 17 g may be required). Allow the must to stand, covered with a sheet of plastic for 24 hrs, during which time the sulfur dioxide content will decrease. Then inoculate the mixture with a package of dried Montrachet yeast and allow fermentation to proceed at about 21° (70° F) for three days, stirring the mixture each day. Siphon the fermenting must into a gallon bottle and attach a fermentation lock (Fig. 61.6a and b). Rack (siphon) the wine into another sterilized 1-gal bottle after three weeks and again after three months, leaving a residue of dead yeast cells behind each time. After six months the wine should be clear. If the taste is too acidic, sweeten with sugar to taste just before drinking or add 1g potassium sorbate to prevent further fermentation, add sugar to taste and bottle. The resulting wine will be a fairly dry, fruity rosé type.

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[2] Procedure of J. M. Bobbitt of the University of Connecticut.

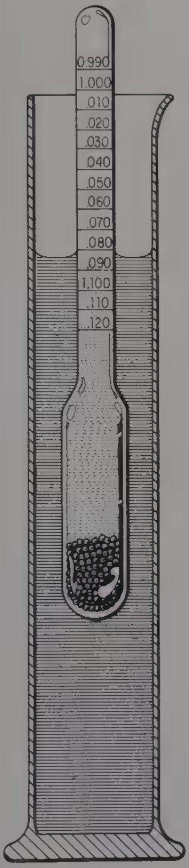


FIGURE 61.4 Hydrometer reading 1.085.

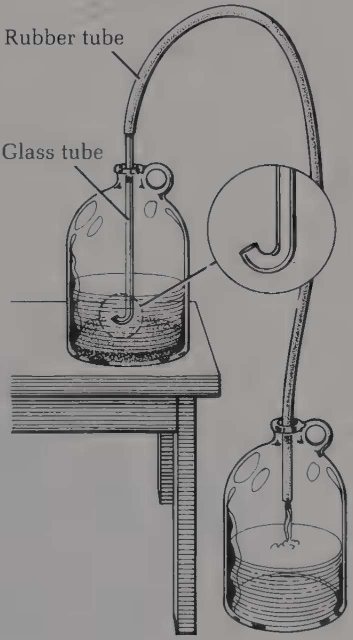


FIGURE 61.5 Siphon.

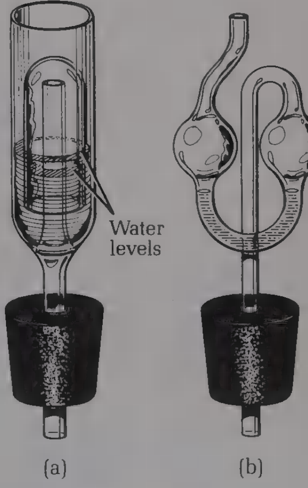


FIGURE 61.6 Air locks.

BIBLIOGRAPHY

1. P. Duncan and B. Acton, *Progressive Winemaking*, Standard Press, Andover, Hants, England, 1976.
2. C. J. J. Berry, *First Steps in Winemaking*, Standard Press, Andover, Hants, England, 1975.
3. M. A. Amerine and C. S. Ough, *Wine and Must Analysis*, John Wiley and Sons, New York, 1974.

WINEMAKING SUPPLIES: (Grape concentrates, yeasts, hydrometers, carboys, books, etc.) are available in most large cities. Catalogs for mail order purchase can be obtained from Wine Art which has 80 stores throughout the country, e.g. P.O. Box 296 Lexington, MA 02173; 254 Shippan Avenue, Stamford, CN 06902; 705 East 6th St., Denver, CO 80203; 311 Main Street, Ames, IA 50010; 4508 North Keystone, Indianapolis, IN 46205; and 460 Fletcher Parkway, San Diego (El Cajon), CA 92020. Also Simplex, Box 12276, 4805 Lyndale Avenue North, Minneapolis, MN 55412; Presque Isle Wine Cellars, 9440 Buffalo Road, North East, PA 16428; Vynox Industries, 400 Avis Street, Rochester, NY 14615; and New England Winemaking Supply, 501 Worcester Road, Rt. 9, Framingham, MA 01701.

SPECTRA INDEX

^{1}H nmr Spectra

Infrared Spectra

^{13}C nmr Spectra

GENERAL INDEX

2 3 4 5 6 7 8 9 0

MULTIPLES OF ELEMENTS' WEIGHTS AND THEIR LOGARITHMS

C	12.0112	1.07958
C_2	24.0223	1.38061
C_3	36.0335	1.55671
C_4	48.0446	1.68164
C_5	60.0557	1.77855
C_6	72.0669	1.85774
C_7	84.0780	1.92468
C_8	96.0892	1.98267
C_9	108.100	2.03383
C_{10}	120.111	2.07958
C_{11}	132.123	2.12098
C_{12}	144.134	2.15877
C_{13}	156.145	2.19353
C_{14}	168.156	2.22571
C_{15}	180.167	2.25568
C_{16}	192.178	2.28370
C_{17}	204.190	2.31003
C_{18}	216.201	2.33486
C_{19}	228.212	2.35834
C_{20}	240.223	2.38061
C_{21}	252.234	2.40180
C_{22}	264.245	2.42201
C_{23}	276.256	2.44131
C_{24}	288.268	2.45980
C_{25}	300.279	2.47752
C_{26}	312.290	2.49456
C_{27}	324.301	2.51095
C_{28}	336.312	2.52674
C_{29}	348.323	2.54198
C_{30}	360.334	2.55671
C_{31}	372.346	2.57095
C_{32}	384.357	2.58473
C_{33}	396.368	2.59810
C_{34}	408.379	2.61106
C_{35}	420.390	2.62365
C_{36}	432.401	2.63589
C_{37}	444.413	2.64779
C_{38}	456.424	2.65937
C_{39}	468.435	2.67065
C_{40}	480.446	2.68164
C_{41}	492.457	2.69237
C_{42}	504.468	2.70283
C_{43}	516.479	2.71305
C_{44}	528.491	2.72304
C_{45}	540.502	2.73280
C_{46}	552.513	2.74234
C_{47}	564.524	2.75168
C_{48}	576.535	2.76083
C_{49}	588.546	2.76978
C_{50}	600.558	2.77855
H	1.00797	0.003448
H_2	2.01594	0.304478
H_3	3.02391	0.480569
H_4	4.03188	0.605508
H_5	5.03985	0.702418
H_6	6.04782	0.781599
H_7	7.05579	0.848546
H_8	8.06376	0.906538
H_9	9.07173	0.95769
H_{10}	10.0797	1.00345
H_{11}	11.0877	1.04484
H_{12}	12.0956	1.08263
H_{13}	13.1036	1.11739
H_{14}	14.1116	1.14958
H_{15}	15.1196	1.17954
H_{16}	16.1275	1.20757
H_{17}	17.1355	1.23390
H_{18}	18.1435	1.25872
H_{19}	19.1514	1.28220
H_{20}	20.1594	1.30448
H_{21}	21.1674	1.32567
H_{22}	22.1753	1.34587
H_{23}	23.1833	1.36518
H_{24}	24.1913	1.38366
H_{25}	25.1993	1.40139
H_{26}	26.2072	1.41842
H_{27}	27.2152	1.43481
H_{28}	28.2232	1.45061
H_{29}	29.2311	1.46585
H_{30}	30.2391	1.48057
H_{31}	31.2471	1.49481
H_{32}	32.2550	1.50860
H_{33}	33.2630	1.52196
H_{34}	34.2710	1.53493
H_{35}	35.2790	1.54752
H_{36}	36.2869	1.55975
H_{37}	37.2949	1.57165
H_{38}	38.3029	1.58323
H_{39}	39.3108	1.59451
H_{40}	40.3188	1.60551
H_{41}	41.3268	1.61623
H_{42}	42.3347	1.62670
H_{43}	43.3427	1.63692
H_{44}	44.3507	1.64690
H_{45}	45.3587	1.65666
H_{46}	46.3666	1.66621
H_{47}	47.3746	1.67555
H_{48}	48.3826	1.68469
H_{49}	49.3905	1.69364
H_{50}	50.3985	1.70242
H_{51}	51.4065	1.71102
H_{52}	52.4144	1.71945
H_{53}	53.4224	1.72772
H_{54}	54.4304	1.73584
H_{55}	55.4384	1.74381
H_{56}	56.4463	1.75164
H_{57}	57.4543	1.75932
H_{58}	58.4623	1.76688
H_{59}	59.4702	1.77430
H_{60}	60.4782	1.78160
H_{61}	61.4862	1.78878
H_{62}	62.4941	1.79584
H_{63}	63.5021	1.80279
H_{64}	64.5101	1.80963
H_{65}	65.5181	1.81636
O	15.9994	1.20410
O_2	31.9988	1.50513
O_3	47.9982	1.68122
O_4	63.9976	1.80616
O_5	79.9970	1.90307
O_6	95.9964	1.98225
O_7	111.996	2.04920
O_8	127.995	2.10719
O_9	143.995	2.15835
O_{10}	159.994	2.20410
N	14.0067	1.14634
N_2	28.0134	1.44737
N_3	42.0201	1.62346
N_4	56.0268	1.74840
N_5	70.0335	1.84531
N_6	84.0402	1.92449
S	32.064	1.50602
S_2	64.128	1.80705
S_3	96.192	1.98314
S_4	128.256	2.10808
S_5	160.320	2.20499
S_6	192.384	2.28417
F	18.9984	1.27872
F_2	37.9968	1.57975
F_3	56.9952	1.75584
F_4	75.9936	1.88078
F_5	94.992	1.97769
F_6	113.99	2.05687
F_7	132.989	2.12382
F_8	151.987	2.18181
F_9	170.986	2.23296
F_{10}	189.984	2.27872
Cl	35.453	1.54966
Cl_2	70.906	1.85069
Cl_3	106.359	2.02678
Cl_4	141.812	2.15172
Cl_5	177.265	2.24863
Br	79.904	1.90257
Br_2	159.808	2.2036
Br_3	239.712	2.37969
Br_4	319.616	2.50463
Br_5	399.52	2.60154
I	126.904	2.10348
I_2	253.809	2.40451
I_3	380.713	2.5806
OCH_3	31.0345	1.49185
$(OCH_3)_2$	62.0689	1.79288
$(OCH_3)_3$	93.1034	1.96897
$(OCH_3)_4$	124.138	2.09391
$(OCH_3)_5$	155.172	2.19082
$(OCH_3)_6$	186.207	2.27
$(OCH_3)_7$	217.241	2.33695
$(OCH_3)_8$	248.276	2.39494
$(OCH_3)_9$	279.31	2.44609
$(OCH_3)_{10}$	310.345	2.49185
OC_2H_5	45.0616	1.65381
$(OC_2H_5)_2$	90.1231	1.95484
$(OC_2H_5)_3$	135.185	2.13093
$(OC_2H_5)_4$	180.246	2.25587
$(OC_2H_5)_5$	225.308	2.35278
$(OC_2H_5)_6$	270.369	2.43196
$(OC_2H_5)_7$	315.431	2.49891
$(OC_2H_5)_8$	360.492	2.5569
$OCOCH_3$	59.045	1.77119
$(OCOCH_3)_2$	118.090	2.07222
$(OCOCH_3)_3$	177.135	2.24831
$(OCOCH_3)_4$	236.180	2.37325
$(OCOCH_3)_5$	295.225	2.47016
$(OCOCH_3)_6$	354.270	2.54934
$(OCOCH_3)_7$	413.315	2.61629
$(OCOCH_3)_8$	472.360	2.67428
$(OCOCH_3)_9$	531.405	2.72543
$(OCOCH_3)_{10}$	590.450	2.77119
$(H_2O)_{1/2}$	9.00767	0.95461
H_2O	18.0153	1.25564
$(H_2O)_{1\frac{1}{2}}$	27.0230	1.43174
$(H_2O)_2$	36.0307	1.55668
$(H_2O)_3$	54.0460	1.73277
$(H_2O)_4$	72.0614	1.85771
$(H_2O)_5$	90.0767	1.95462
$(H_2O)_6$	108.092	2.0338
P	30.9738	1.491
P_2	61.9476	1.79203
P_3	92.9214	1.96812
P_4	123.895	2.09306
P_5	154.869	2.18997
P_6	185.843	2.26915
Na	22.9898	1.36154
Na_2	45.9796	1.66257
Na_3	68.9694	1.83866
K	39.102	1.5922
K_2	78.204	1.89323
K_3	117.306	2.06932
Ag	107.868	2.0329
Ag_2	215.736	2.33393
Cu	63.546	1.80309
Cu_2	127.092	2.10412
Cr	51.996	1.71597
Hg	200.59	2.30231
Pb	207.19	2.31637
Pt	195.09	2.29024
Pt_2	390.18	2.59127
Se	78.96	1.89741
Th	204.37	2.31042

Log $C/CO_2 = 0.436029$ Log $H_2/H_2O = 0.048836$

ATOMIC WEIGHTS (1966)

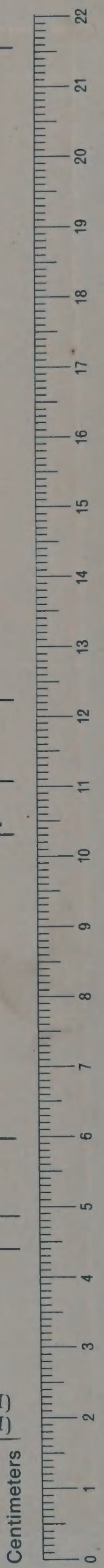
Aluminum	Al	26.9815	Molybdenum	Mo	95.94
Antimony	Sb	121.75	Nickel	Ni	58.71
Arsenic	As	74.9216	Nitrogen	N	14.0067
Barium	Ba	137.34	Osmium	Os	190.2
Beryllium	Be	9.0122	Oxygen	O	15.9994
Bismuth	Bi	208.980	Palladium	Pd	106.4
Boron	B	10.811	Phosphorus	P	30.9738
Bromine	Br	79.904	Platinum	Pt	195.09
Cadmium	Cd	112.40	Potassium	K	39.102
Calcium	Ca	40.08	Praseodymium	Pr	140.907
Carbon	C	12.01115	Rhodium	Rh	102.9055
Cerium	Ce	140.12	Ruthenium	Ru	101.07
Cesium	Cs	132.905	Selenium	Se	78.96
Chlorine	Cl	35.453	Silicon	Si	28.086
Chromium	Cr	51.996	Silver	Ag	107.868
Cobalt	Co	58.9332	Sodium	Na	22.9898
Copper	Cu	63.546	Strontium	Sr	87.62
Europium	Eu	151.96	Sulfur	S	32.064
Fluorine	F	18.9984	Tantalum	Ta	180.948
Gold	Au	196.967	Tellurium	Te	127.60
Hydrogen	H	1.00797	Thallium	Tl	204.37
Iodine	I	126.9044	Thorium	Th	232.038
Iridium	Ir	192.22	Tin	Sn	118.69
Iron	Fe	55.847	Titanium	Ti	47.90
Lead	Pb	207.19	Tungsten	W	183.85
Lithium	Li	6.939	Vanadium	V	50.942
Magnesium	Mg	24.312	Ytterbium	Yb	173.04
Manganese	Mn	54.9380	Zinc	Zn	65.37
Mercury	Hg	200.59	Zirconium	Zr	91.22

ACIDS AND BASES

	SP. GR.	% BY WT	MOLES PER L	GRAMS PER 100 ML
Hydrochloric acid, concd.	1.19	37	12.0	44.0
Constant boiling (252 ml concd. acid + 200 ml water, b.p. 110°)	1.10	22.2	6.1	22.2
10% (100 ml concd. acid + 321 ml water)	1.05	10	2.9	10.5
5% (50 ml concd. acid + 380.5 ml water)	1.03	5	1.4	5.2
1 N (41.5 ml concd. acid diluted to 500 ml)	1.02	3.6	1	3.6
Hydrobromic acid, constant-boiling (b.p. 126°)	1.49	47.5	8.8	70.7
Hydriodic acid, constant-boiling (b.p. 126°)	1.7	57	7.6	97
Sulfuric acid, concd.	1.84	96	18	177
10% (25 ml concd. acid + 398 ml water)	1.07	10	1.1	10.7
1 N (13.9 ml concd. acid diluted to 500 ml)	1.03	4.7	0.5	4.9
Nitric acid, concd.	1.42	71	16	101
Sodium hydroxide, 10% solution	1.11	10	2.8	11.1
Ammonia solution, concd.	0.90	28.4	15	25.6

BUFFER SOLUTIONS (0.2 *M*, except as indicated)

pH	COMPONENTS	pH	COMPONENTS
0.1	1 *N* Hydrochloric acid	8.0	11.8 g Boric acid + 9.1 g Borax ($Na_2B_4O_7 \cdot 10H_2O$) per l
1.1	0.1 *N* Hydrochloric acid	9.0	6.2 g Boric acid + 38.1 g Borax per l
2.2	15.0 g ᴰ-Tartaric acid per l (0.1 *M* solution)	10.0	6.5 g $NaHCO_3$ + 13.2 g Na_2CO_3 per l
3.9	40.8 g Potassium acid phthalate per l	11.0	11.4 g Na_2HPO_4 + 19.7 g Na_3PO_4 per l
5.0	14.0 g KH-Phthalate + 2.7 g $NaHCO_3$ per l (heat to expel carbon dioxide, then cool)	12.0	24.6 g Na_3PO_4 per l (0.15 *M* solution)
6.0	23.2 g KH_2PO_4 + 4.3 g Na_2HPO_4 (anhyd., Merck) per l	13.0	4.1 g Sodium hydroxide pellets per l (0.1 M)
7.0	9.1 g KH_2PO_4 + 18.9 g Na_2HPO_4 per l	14.0	41.3 g Sodium hydroxide pellets per l (1 M)



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