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Contents

Stereochemistry of Electrochemical Reductions

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1. Introduction

Current interest in the electrochemical behavior of organic compounds is attested to by a number of recent books and reviews devoted to various aspects of the subject. The recent article in this series by Eberson and Schäfer [1] constitutes a good general introduction to the state of the art in synthetic and mechanistic organic electrochemistry, and also contains, in Chapter 1, many useful references for the interested reader who wishes to delve deeper into the extensive literature of organic electrochemistry.

A great deal of knowledge concerning the mechanisms of organic electrode reactions has been accumulated in recent years, principally through the application of sophisticated electrochemical techniques such as cyclic voltammetry, polarography, electron spin resonance (e.s.r.) spectroscopy, coulometry, and controlled-potential electrolysis [1]. Relatively less attention has been paid, on the other hand, to certain standard tools developed by physical organic chemists *for study of organic reaction mechanisms.* This review is devoted to a survey of progress in the applications of one of these tools, stereochemistry, to the understanding of mechanisms of organic electrode reactions. It should scarcely be necessary to point out the central role which stereochemical principles have played in our modern understanding of the mechanisms of organic reactions. Determination of the stereochemical course of a given organic reaction is usually one of the first steps taken in a study designed to unravel its mechanism. Despite the undoubted utility of stereochemical information in mechanistic studies, there are relatively few organic electrode reactions whose stereochemical course has been examined at all, and fewer yet which have been studied in much more than cursory fashion. This is unfortunate not only for the reasons outlined above but also because the synthetic organic chemist must know the stereochemical features of a reaction when he is considering using it in a synthetic scheme. It is hoped that by setting forth the current state of knowledge concerning the stereochemical course of electrochemical reductions, this review will not only provide that information (insofar as it is available) to those interested in synthetic applications and will afford a convenient summary for investigators carrying out or contemplating research in this field, but may also provide a stimulus for further work in this area by pointing out the many unsolved problems, intriguing results, and sometimes even outright contradictions which have been reported by those who have studied the stereochemistry of electrochemical reductions. It should be pointed out that a review article on this subject was recently published by Feoktistov [2]. The principal emphasis in that article was however upon data obtained from polarographic methods, rather than preparative-scale electrochemical reductions and it did not represent an exhaustive review of the Western literature on the subject. Certain areas, mainly polarographic, treated in detail there will not be discussed at length here.

1.1. Special Stereochemical Features of Electrochemical Reactions

It will be assumed in this review that the reader is familiar with the usual stereochemical concepts employed in organic chemistry. Reactions carried out at electrodes are sometimes complicated by special features, however, which are not commonly encountered in normal organic chemical practice and which one must therefore be aware of. These are all associated with the fact that electrochemical reactions at electrodes are heterogeneous processes.

1.1.1. Orientation

The orientation of the *electroactive substance* (the material undergoing electron-transfer at the electrode) with respect to the electrode surface can very substantially affect its electrochemical reactivity. This ought not be surprising: electron transfer is a heterogeneous process, and ought therefore to be substantially dependent upon the exact nature of the contact between the electroactive species and the electrode. Orientational effects ought to be particularly important when the electroactive species is adsorbed upon, and hence in intimate contact with, the electrode surface. What kinds of effects are associated with orientation of substances at an electrode surface? Generally what one observes is

a) efficient electron-transfer to a molecule or functional group when the electroactive material is oriented in a particular geometry with respect to the electrode surface, and

b) conversely, that reduction of the electroactive substance and other components of the medium may be slowed or even completely inhibited for other orientations of the electroactive material at the electrode.

The p.z.c., or *potential of zero charge*, is, as its name implies, the potential at which an electrode immersed in a given medium bears no net charge [3]. The charge on the electrode increases as its electrode potential is made increasingly positive or negative of the p.z.c. If a compound adsorbed on the electrode surface possesses a permanent dipole moment, it may change its orientation with respect to the electrode surface as the electrode potential is changed. For example, it has been found that at potentials positive and even slightly negative of the p.z.c., coumarin is adsorbed upon a mercury surface such that the plane of the coumarin ring is parallel to the electrode surface [4] (Fig. 1a). This behavior, which has also been observed with a number of other aromatic compounds [6-9], is not entirely unexpected; the flat geometry allows maximum overlap of the electrons in the π-cloud of the aromatic ring with the metal surface, an effect which ought to assume particular importance when the electrode bears a positive charge. If the electrode potential is swept toward increasingly negative potentials, it is found that at a point somewhat negative of p.z.c. the coumarin molecule reorients itself so that it is perpendicular to the electrode surface, with the positive end of the coumarin dipole closest to the neg-

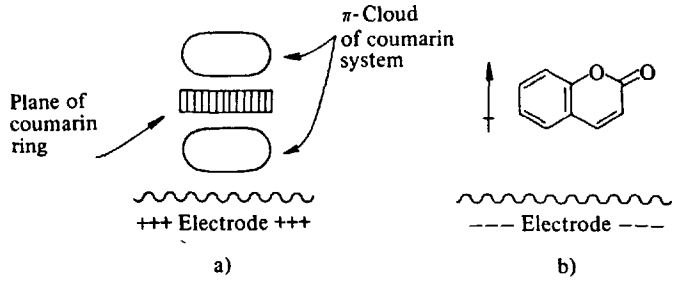
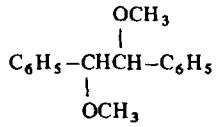


Fig. 1 a and b. a) Coumarin adsorbed parallel to electrode surface at potentials positive of p.z.c. b) Coumarin absorbed perpendicular to electrode surface at potentials negative of p.z.c.

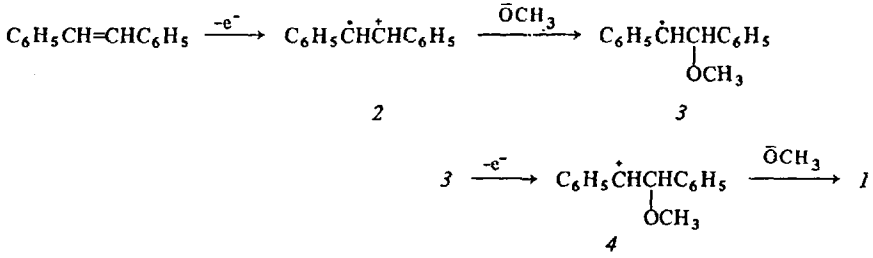
atively charged electrode (Fig. 1b). Reorientation occurs when the negative charge on the electrode surface has induced a large enough dipole in the adsorbed molecule to overcome the stabilization associated with the parallel surface orientation of coumarin [4]. In the parallel orientation electron-transfer to other components of the solution is not impeded by coumarin; presumably electron-transfer through the coumarin ring is mediated by the π-cloud of the coumarin system. Perpendicularly adsorbed coumarin, however, completely *inhibits* reduction of other components of the medium [5]. Differences in electrochemical behavior as a function of orientational differences have not been searched for, but studies in this area might provide useful information concerning the detailed geometrical requirements for electron-transfer to various functional groups. One area of possible synthetic interest is selective reduction of a molecule containing two identical functional groups, of which only one is oriented properly for reduction. This is very likely to be a rather general phenomenon. Such behavior has in fact already been observed with erythrosin and 2,2-dichloronorbornane (Section 2.1) and diketocholanic acid (Section 3.1).

1.1.2. Shielding

Intermediates generated at an electrode surface may react while still near the electrode. If so, one side of the intermediate may be wholly or partly shielded from attack by other reactants by the electrode itself. Such behavior is particularly common in the electrochemical oxidation of aromatic compounds since, as we have already seen with coumarin, aromatic compounds are generally tightly adsorbed parallel to the electrode surface at potentials positive of the p.z.c. For example, electrochemical oxidation of the stilbenes in alkaline methanol affords a mixture of *dl* and *meso*-1,2 dimethoxy-1,2-diphenylethane *(1)* [10]. It is found that *cis*-stilbene affords a mixture of isomers of *1* in which the



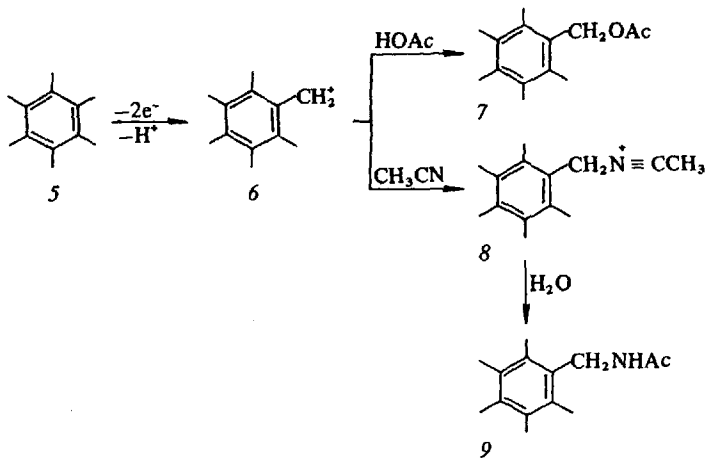
dl: meso ratio is 2:3, while the *dl:meso* ratio of *1* obtained from *trans*-stilbene is 2.2:1. The oxidation process amounts therefore to an overall preferred *cis* addition of the two methoxyl groups to the double bond. The probable mechanism of formation of *1* is as follows:



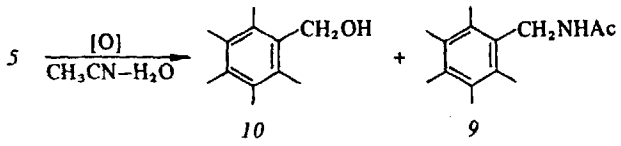
It is probable that adsorption of intermediates such as *2*, *3*, and *4* upon the electrode surface requires that both methoxyl groups be added from the same (unshielded) side of the planar system [11]. Shielding is probably less important in cathode processes than it is in oxidations. In order for shielding to dominate the stereochemistry of attack upon an electrochemically generated intermediate, the intermediate must either be tightly adsorbed upon the electrode or must be so reactive that it reacts before it can diffuse away from the electrode. The first of these two requirements is much more likely to be obeyed in oxidations of electron-rich species than in reductions generating carbanionic intermediates, which ought not to be absorbed on the electrode at negative potentials.

1.1.3. Medium Effects

The composition of the highly structured region of solution nearest to the electrode surface, the so-called *electrical double layer*, may differ very substantially from the composition of the bulk solution [12]. The chemistry of electrochemically generated intermediates may therefore differ greatly from what one would expect on the basis of the nominal solution composition if such intermediates react while still in the double layer. Anomalous behavior associated with a double layer composition which differs from the composition of the bulk solution may be termed a *medium effect*. Medium effects are more likely to be observed in electrochemical reactions involving highly reactive intermediates, since less reactive intermediates are more likely to survive long enough to permit diffusion out of the electrical double layer into bulk solution.



Medium effects can be quite striking. Nyberg [13] studied the electrochemical oxidation of hexamethylbenzene(*5*) in acetic acid-acetonitrile mixtures. Oxidation of *5* is known to generate the pentamethylbenzyl cation (*6*). The products obtained by Nyberg are pentamethylbenzyl acetate (*7*) and N-pentamethylbenzylacetamide (*9*). These are formed by nucleophilic attack upon *6* by acetic acid and acetonitrile, respectively. The latter process is the first step of a Ritter reaction, which is completed by reaction of nitrilium ion *8* with traces of water in the solvent. The ratio of *7* to *9* is highly dependent upon the *solvent composition* and the nature of the supporting electrolyte employed As expected, the relative proportion of amide *9* increases as the ratio of acetonitrile to acetic acid in the solvent is increased. In 99 : 1 acetonitrile-acetic acid, the ratio of *9* to *7* is 84:16 and 78:22 when the electrolyte is sodium perchlorate or tetra-*n*-butylammonium perchlorate (TEAP), respectively. When the electrolyte is tetra-*n*-butylammonium tetrafluoroborate, the ratio of *9* to *7* was however found to be 19:81. This dramatic dependence upon the nature of the supporting electrolyte was interpreted as a medium effect. Nyberg argued that the small tetrafluoroborate anion is solvated preferentially by acetic acid, the minor (1%) component of the mixed solvent system, and that this ion is solvated better by acetic acid than is the larger perchlorate anion[13]. It is known that the anion of the supporting electrolyte is present in the double layer in excess over its bulk concentration at the positive potentials at which oxidation of aromatic species takes place [12]. It was argued that the presence of tetrafluoroborate ion at the electrode surface, coupled with its preferential solvation by acetic acid, causes the acetic acid concentration at the electrode surface to be much higher than its bulk concentration.



Eberson and Olofsson [14] observed exactly the same effect, and advanced the same rationale, in their study of the electrochemical oxidation of *5* in acetonitrile-water mixtures, to afford mixtures of pentamethylbenzyl alcohol *(10)* and the amide *9*.

Besides being of obvious synthetic utility, these results remind us that changes in experimental parameters, particularly solvent, electrolyte, potential, and temperature, may cause substantial changes in the composition of the double layer and therefore may provoke changes in the course of electrochemical processes occurring at the electrode surface.

2. Electrochemical Cleavage of Single Bonds

2.1. Alkyl Halides

In 1949 von Stackelberg and Stracke proposed a general mechanistic scheme for the electrochemical reduction of alkyl halides (Scheme I) [15]. It was suggested that the reduction is stepwise, proceeding via initial one-electron

$$RX \xrightarrow[E_1]{e^-} R^{\cdot} + X^-$$

$$R^{\cdot} \xrightarrow[E_2]{e^-} R^-$$

$$R^- \xrightarrow{H^+} RH$$

Scheme I

reduction of the alkyl halide to generate a free radical, which is then reduced further to a carbanion, the latter then abstracting a proton from the solvent to afford the hydrocarbon. In spite of the fact that this mechanistic Scheme was initially formulated upon the basis of very little experimental evidence, it has withstood the test of time fairly well. It is now known, however, that the Scheme as originally formulated must be modified in several respects. For example, not stated explicitly by von Stackelberg and Stracke, but implicit in the statement that the only reaction open to the intermediate radical is reduction to a carbanion, is the assumption that potential E_1 necessary for cleavage of the carbon-halogen bond is quite negative of E_2, the reduction potential of

the radical, so that the radical is reduced as soon as it is formed. Sometimes, however, the intermediate radical is sufficiently long-lived to undergo rearrangement [16,17], dimerization [18] or combination with the electrode to form organometallic products [18-20] before reduction to a carbanion can occur.

A number of *stereochemical questions* may be raised in connection with Scheme I. These include the following:

a) is there a preferred orientation of the carbon-halogen bond with respect to the electrode surface?;

b) if so, can the electrode discriminate between identical groups in different stereochemical environments in a molecule?;

c) is reduction stereospecific, *i.e.*, do stereoisomeric halides afford stereoisomeric products?

Data bearing on all of these questions are now available, but since alkyl halides exist in great structural diversity, the answers to the questions sometimes depend upon the structure of the individual halide. Answers to all of these questions are not yet available for all structural types. Furthermore most stereochemical experiments have been conducted upon alkyl halides of rather special structures, so that it is often difficult to extrapolate the results to simpler systems.

Very little is known concerning the stereochemistry of electrochemical reduction of simple acyclic halides. Eberson studied the reduction of optically active α-methylbenzyl chloride *(11)* in dimethylformamide (DMF) containing deuterium oxide [21]. The α-deuterioethylbenzene *(12)* obtained from this

$$\underset{\text{Cl}}{\overset{|}{\text{C}_6\text{H}_5\text{CHCH}_3}} \xrightarrow[\text{D}_2\text{O}]{2e^-} \text{C}_6\text{H}_5\text{CDHCH}_3$$

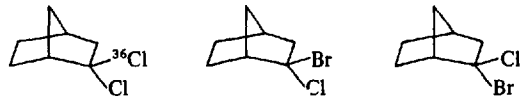
11 *12*

reaction is at least 98% racemic. This result is exactly what one would expect if the reduction proceeded via an intermediate α-methylbenzyl radical or carbanion. Optically active α-phenyl-α-chloropropionic acid *(13)* has on the other hand been reported to undergo reduction with predominant *inversion* of stereochemical configuration [22].

$$\underset{\text{Cl}}{\overset{|}{\overset{\text{CH}_3}{\overset{|}{\text{C}_6\text{H}_5\text{C}}}}}\text{CO}_2\text{R} \xrightarrow{2e^-} \overset{\text{CH}_3}{\overset{|}{\text{C}_6\text{H}_5\text{CH}}}\text{CO}_2\text{R}$$

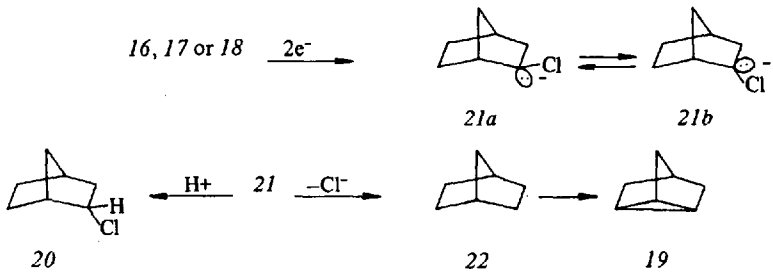
13, R = H
14, R = C_2H_5 *15*

Erickson and Fischer have not been able to reproduce this result, however [23]. They found that the products isolated from reduction of either *13* or its ethyl ester *(14)* are essentially completely racemic, thus making the stereochemistry of electrochemical reduction of *13* consistent with that of *11*.



16 *17* *18*

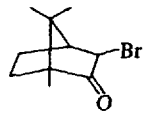
Recent experiments upon the electrochemical reduction of stereoisomeric geminal dihalides support the conclusion that configuration is lost during the reduction of most alkyl halides [24,25]. The compounds investigated were 2,2-dichloronorbornane-*exo*-^{36}Cl *(16)*, *exo*-2-bromo-*endo*-2-chloronorbornane *(17)*, and *exo*-2-chloro-*endo*-2-bromonorbornane *(18)*. Reduction of all three compounds consumes two Faraday/mole, and produces in each case a mixture of nortricyclene *(19)* and *endo*-norbornyl chloride *(20)*. A reduction scheme which accounts for the formation of these products is presented in Scheme II. Reduction of any of the three dihalides generates the rapidly interconverting pair of stereoisomeric carbanions *21a* and *21b*. Protonation of *21* from the less hindered *exo* direction will produce the *endo* chloride *20*, while ejection of chloride ion from *21* and intramolecular insertion [28] of the resulting carbene *22* affords *19*. The fact that both *17* and *18* lead to *20* demonstrates that equilibration between *21a* and *21b* is much faster than protonation of either.



Scheme II

The question [26,27] whether there is a preferred surface orientation for facile reduction of the carbon-halogen bond may also be answered by reference to the electrochemical behavior of *16–18*. This question was investigated in two ways. The first method involved determination of the amount of isotopically labelled chlorine remaining in *20* isolated from the electrochemical reduction of *16*. This proportion was found to be 7 ± 1% under a variety of experimental conditions [25,29]. This means that reduction of the *exo* chlorine

of *16* is faster than reduction of the *endo* chlorine by a factor of 13 ± 2. The second approach to this problem involved measurement of the relative rates of heterogeneous electron transfer to *17* and *18*, respectively. This analysis was based upon the fact that the polarographic current for a totally irreversible electrochemical process (reduction of alkyl halides is totally irreversible) increases exponentially with increasingly negative potential when measurements are restricted to potentials near the foot of the polarographic wave [30]. Logarithmic plots of polarographic current *vs.* potential in this region were constructed for *17* and *18;* the plots were parallel lines, hence the relative rates of reduction of *17* and *18* are independent of potential. The relative rates of reduction of *17* and *18*, under identical polarographic conditions, are simply equal to the ratio of the respective polarographic currents for reduction of the two halides. This ratio is 3, *i.e.*, electron transfer to *17* is faster than electron transfer to *18* by a factor of 3. Considering the approximations involved, this is in reasonable agreement with the value of 13 ± 2 determined from the measurements made upon *16*. Thus both approaches lead to the same conclusion, that reduction of an *exo* halogen in the norbornyl system is easier than reduction of an *endo* halogen. Other data support this conclusion. Butin, *et al.*, studied the polarographic behavior of seventeen 7-oxa-bicyclo-[2.2.1]-heptane derivatives [31]. They found that for any pair of stereoisomeric halides, the *exo* isomer is easier to reduce than the *endo* isomer. Lambert found that *exo*-norbornyl bromide is easier to reduce than the *endo* isomer [32]. Since stereochemical approach to the norbornyl ring system is less hindered from the *exo* direction, all of these data suggest that the halogen end of the carbon-halogen bond is nearest the electrode surface in the electron-transfer step. Sease and coworkers have reached this same conclusion on other grounds [33]. There is one set of data in the literature which is discordant with this conclusion, however. Limosin and Laviron determined the relative ease of polarographic reduction of several isomeric pairs of α-halocamphors, *e.g.*, *23* and *24* [34]. For any given pair of isomers, the *exo* isomer was always found to be easier to reduce than the *endo* isomer. Stereochemical approach upon the camphor system is generally easier from the *endo* direction, because of the steric bulk of the methyl group at C–7 which is *syn* with respect to the carbonyl group, hence one might expect that *endo* isomers would here be easier to reduce than *exo* isomers. The observed order of ease of reduction may, however, be due to the fact that the ground-state



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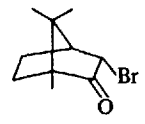

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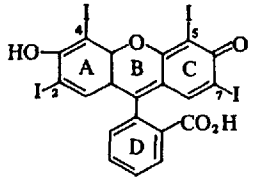
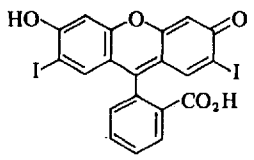


25

energies of *exo*-halocamphors are higher than their *endo* epimers because of steric compression of the *exo* halogen and the *syn*-7-methyl. A similar explanation could account for the well-known fact that in rigid α-halocyclohexanones, including those in the *t*-butyl-cyclohexane [35] and steroid [36] series, axial halogens are noticeably easier to reduce than equatorial halogens [37].

The selective removal of the *exo* chlorine of *16* demonstrates that it is indeed possible to remove one halogen from a dihalide in which the two halogens reside in different stereochemical invironments. Board and coworkers [38] discovered a very dramatic example of the same phenomenon in a study which also shows the value of adsorption studies in determining the nature of the *surface orientation of adsorbed molecules* and which incidentally, further supports the hypothesis that the halogen end of the carbon-halogen bond is nearest the electrode surface during electron transfer. These investigators examined the electrochemical behavior of erythrosin, *i.e.*, tetraiodofluoroscein *(25)*. This compound (as its disodium salt) exhibits three polarographic waves at −0.55, −0.80, and −1.2 V (*vs.* s.c.e.), of relative heights 1 : 2 : 2, respectively. Since the first wave is known to be associated with a two-electron reduction of the quinoid system of ring C to the corresponding phenol, the other two waves must each correspond to an uptake of four electrons. The only other reducible functionalities in *25* are the carbon-iodine bonds. It is known that electrochemical reduction of aryl iodides consumes two electrons per molecule. The existence of two discrete, well-separated reduction steps implies therefore that two of the iodine atoms in *25* are reduced more readily than the other two. Inspection of the structure of *25* does not reveal any obvious reason why this should be so. Adsorption studies, however, supplied an answer to this problem. Measurement of the amount of *25* necessary to coat a mercury surface of known area with a monomolecular film showed that each molecule of adsorbed *25* occupies an area of 117 Å^2 at the electrode surface. Examination of scale molecular models reveals that this can only be so if the molecule assumes a geometry in which the carbon-iodine bonds at C-4 and C-5 are perpendicular to the electrode surface, with these iodine atoms nearest to the electrode surface. If this is the orientation actually assumed by *25* at the electrode surface, one would expect that the iodine atoms at C-4 and C-5 ought to be more easily reduced than those at C-2 and C-7.



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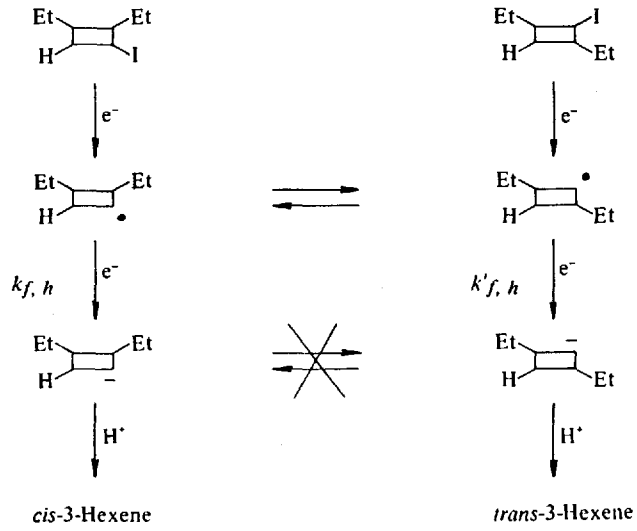
This was confirmed by experiment: controlled-potential electrolysis of *25* at −1.0 V, *i.e.*, on the plateau of the first four-electron wave, produced 2,7-diiodofluoroscein *(26)* as the major product.

At the beginning of this section the question was raised whether it might be possible to obtain stereoisomeric products from stereoisomeric starting materials. This amounts to asking the question whether or not it might be possible to intercept electrochemically generated stereoisomeric intermediates (radicals or carbanions) before they can completely equilibrate. As we have seen, this was not observed in the studies upon *17*[25], *18*[25], and optically active *11*[21], nor in the reinvestigation of optically active *13* by Erickson and Fischer [23]. In a few special structural cases, however, stereoisomeric intermediates have been intercepted. Fry and Mitnick [16] examined the electrochemical reduction of the stereoisomeric 3-iodo-3-hexenes. The *cis*-iodide affords a 3-hexene mixture which is richer in the *cis* isomer than is the 3-hexene mixture obtained from *trans*-3-iodo-3-hexene (Table 1).

Table 1. Stereochemistry of electrochemical reduction of the stereoisomeric 3-iodo-3-hexenes

Substrate	Phenol concentration M	Relative % products *trans*-3-Hexene	*cis*-3-Hexene
trans-3-Iodo-3-hexene	0	94	6
trans-3-Iodo-3-hexene	0.1	94	6
cis-3-Iodo-3-hexene	0	70	30
cis-3-Iodo-3-hexene	0.1	70	30

Since vinyl anions generally retain configuration [39] while isomeric vinyl radicals rapidly interconvert [40] these results constitute evidence that reductions of alkyl iodides do proceed via radical intermediates. Isomerization of stereoisomeric vinyl anions is ruled out by the lack of effect of phenol on the stereochemistry of the products (Scheme III). Since *cis* and *trans*-3-hexene are formed in differing proportions from the two halides, it may be concluded that the stereoisomeric vinyl radicals are being intercepted by electron trans-

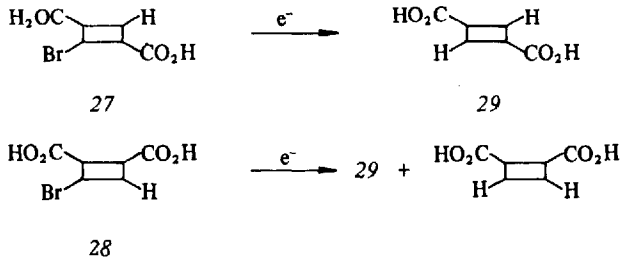


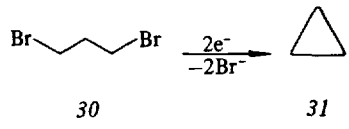
Scheme III

fer from the electrode ($k_{f,h}$ and $k'_{f,h}$) at rates comparable with rates (k_1 and k_{-1}) at which they are interconverting.

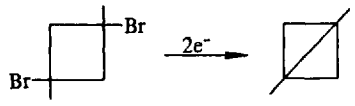
Elving, Rosenthal, Hayes and Martin [41] studied the electrochemical reduction of bromofumaric acid *(27)* and bromomaleic acid *(28)* in aqueous solution over a wide pH range. It was claimed that reduction of *27* proceeds stereospecifically to fumaric acid, and that reduction of *28* affords mixtures of maleic and fumaric acids. Because of the polar and hydrogen-bonding properties of the carboxyl groups in *27* and *28*, the relation of these results to those of Fry and Mitnick [16] is unclear.

The electrochemical reduction of 1,3-dibromides to cyclopropanes *(30 → 31)* appears to be fairly general [42-44] and has been applied to the synthesis of some rather strained ring systems, *e.g.*, *32*, *33*, and *34*. Rifi has sug-

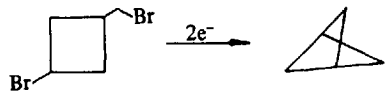




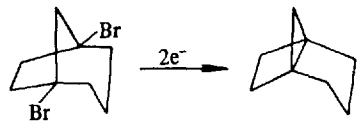
30 *31*



32[44]

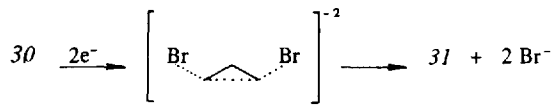


33[43]



34[42]

gested that ring closure is concerted with cleavage of the two carbon-bromine bonds, *i.e.*, that the transition state for electron transfer resembles *35* [42,43]. Stereochemical evidence has, however, recently been obtained which shows that this suggestion is incorrect. It was found by Fry and Britton [45] that *meso* and *dl*-2,4-dibromopentane *(36)* each afford a mixture of *cis* and *trans*-1,2-dimethylcyclopropane *(37)* upon electrochemical reduction in dimethylsulfoxide. Concerted ring closure ought to be stereospecific, *i.e.*, each isomer of *36* ought to be converted to a single (and different) isomer of *37*. Since the reduction of *meso* and *dl-36* is not stereospecific, reduction is not concerted. Formation of cyclopropanes from 1,3-dibromides must proceed in stepwise fashion, via an intermediate carbanion *(38)*. This conclusion is supported by the fact that reduction of *36* produces not only the *cis* and *trans* isomers of *37* but also products derived from base-promoted dehydrohalogenation of *36*, presumably by the intermediate carbanion [45].



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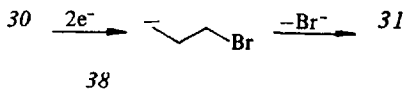
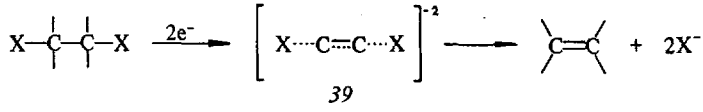


30 *31* *38*

There is on the other hand a great deal of evidence showing that the electrochemical reduction of 1,2-dihalides to olefins *can* occur via a concerted pathway, *i.e.*, via a transition state *(39)* in which both carbon-halogen bonds are partially broken and the carbon-carbon double bond is partially formed. An important, indeed critical, point of evidence supporting the conclusion that reduction is concerted lies in the remarkable ease with which vicinal dihalides are reduced. For example, the half-wave potentials of ethyl bromide and 1,2-dibromoethane are -2.08 V and -1.52 V (*vs.* s.c.e.), respectively; [15,46] those of ethyl iodide and β-chloroethyl iodide are -1.6 V and -0.9 V, respectively [47]. These very large differences must reflect the lower energy of delocalized transition state *39* relative to the transition state for reduction of an alkyl monohalide.

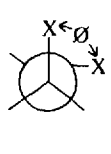


39

As one might expect, however, there are serious geometric constraints on this concerted reduction mechanism. Orbitals in the starting material are in the best position to be converted smoothly into the π-bond of the product olefin when ∅, the dihedral angle between the two halogen atoms when viewed along the axis of the carbon-carbon bond (see *40*) is either 180° or 0°, *i.e.*, when the two halogens are anti *(41)* or eclipsed *(42)*. This is exactly what has been observed experimentally: a plot of polarographic half-wave potential *vs* ∅ for a series of conformationally rigid dibromides showed that

a) reduction is easiest when ∅ = 180°,
b) is most difficult when ∅ = 90°, and
c) becomes very easy again when ∅ = 0° [46].

Reduction is probably stepwise when ∅ = 90°. (The plot in fact resembles very closely in form the familiar Karplus plot of the magnitude of vicinal proton-proton coupling constants as a function of ∅ [48]. The half-wave potentials of *43* and *44* are representative; the half-wave potential of a related monohalide *(45)* is given for reference [46]. These investigators did not examine the products of electrochemical reduction of the vicinal dihalides which they studied. If reduction is concerted, the products should



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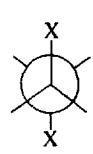

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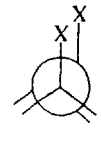

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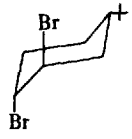
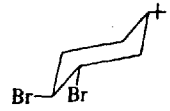


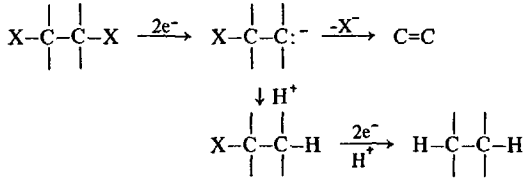
43, $E_{1/2}$ = −0.86 V



44, $E_{1/2}$ = −1.67 V

45, $E_{1/2}$ = −1.91 V

consist entirely of alkene, with no accompanying alkane formed by a stepwise removal of both halogens; if reduction is stepwise via an intermediate carbanion, some of the corresponding alkane should also be formed:

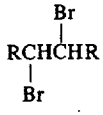


To test this point, McKeon and Koch examined the preparative scale electrochemical reduction of the diaxial bromides *43* and *46* in aqueous DMF [49]. They found only the corresponding olefins as products, under analytical conditions which could have detected as little as 3% of the alkanes, had they been formed. It is unfortunate that a dibromide for which $\varphi \approx 90°$ was not investigated by these workers. Reduction of such a compound ought to be stepwise, and alkane could be formed, especially in a proton-donating medium. Finally, Nelson and coworkers have suggested from their study of a series of rigid vi-

46

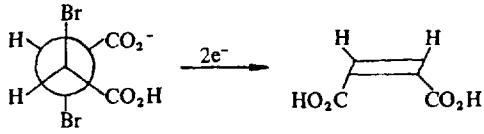
cinal dihalides that concerted reduction is observed when both halogens are eclipsed, *i.e.*, when $\emptyset = 0°$ [50].

Open-chain vicinal dihalides are apparently reduced concertedly via a conformation in which the two halogen atoms are anti to each other. This conclusion is based upon the fact that *meso* isomers of structures *47* are generally more easily reduced than the *dl* isomers, presumably because steric repulsions are less for *meso* than for *dl* in the transition state for electron transfer. The stereochemistry of the products of reduction of simple open chain vicinal di-

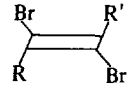


47, R = CH_2OH or CH_2ONO_2 [51]
R = n-C_4H_9 [46]
R = $CO_2C_2H_5$ [52]

halides has not been investigated. Presumably *meso* dihalides should afford *trans*-olefins, while *dl* dihalides should afford *cis* olefins. Elving, Rosenthal, and Martin found that electrochemical reduction of *meso*-2,3-dibromosuccinic acid (*meso-47*, R = CO_2H) does afford fumaric acid over a wide range of pH conditions [52]. *Dl*-2,3-dibromosuccinic acid (*dl-47*, R = CO_2H) exhibited more complicated behavior: fumaric acid was isolated from electrolyses carried out at pH less than 0.4 or greater than 6.9, while some maleic acid was obtained when the pH was between 0.4 and 6.9. The yield of maleic acid was highest (70%) at pH 4.0. It was suggested that because of steric and electronic repulsions the two carboxyl groups will assume an anti geometry with respect to each other at either very low or high pH. The bromine atoms will therefore not be anti to each other, hence reduction will be stepwise and thermodynamically controlled, leading to the more stable *trans* isomer, fumaric acid. At intermediate pH values, the dominant species in solution is the singly-ionized diacid, and it was argued that hydrogen bonding between the carboxylate ion and the remaining carboxyl group stabilizes the conformation leading to maleic acid:



These investigators also found that *dl* and *meso* diethyl 2,3-dibromosuccinate (*47*, R = CO_2Et) are both reduced to diethyl fumarate [52].



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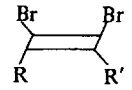
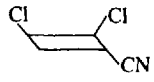


49

It appears that *vicinal dihalides* also undergo concerted reduction when the two halogen atoms are disposed *trans* about a double bond. Mairanovskii and Bergel'son found that for several pairs of isomers of general structure *48* and *49*, the *trans*-dibromide *(48)* is generally substantially easier to reduce than *cis;* for R = R′ = $(CH_3)_2COH$, the difference is 0.58V [53]. Jura and Gaul found that *50* and *51* exhibit very different polarographic behavior [54]. The



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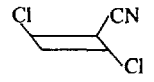

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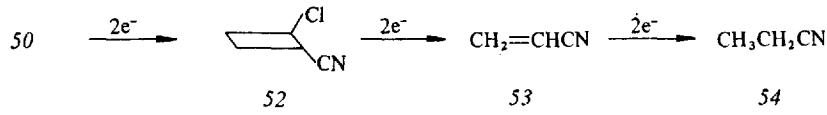
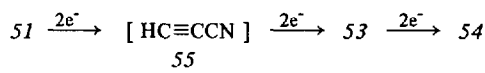


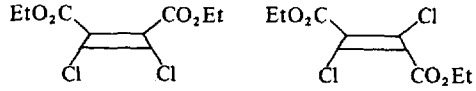
52 *53* *54*

cis isomer *(50)* exhibits three two-electron polarographic waves, associated with stepwise reduction of *50* to *52*, *53*, and finally propionitrile *(54)*. That *trans*-dichloride *(51)*, on the other hand, exhibits a four-electron wave was interpreted as arising from an initial concerted two-electron reduction of *51* to propiolonitrile *(55)*, which is reduced to acrylonitrile *(53)* in a second two-electron step as quickly as it is formed. The second polarographic wave of *51* is simply due to reduction of *53* to *54*. (A control experiment demonstrated



55

that *55* is easier to reduce than *51*, and hence that it should indeed be reduced to *53* as quickly as it is formed). These results parallel numerous reports in the chemical literature of stereospecific *trans*-elimination across the double bond [55]. The problem is apparently more complex, however. Elving and coworkers studied the electrochemical reduction of dibromomaleic and dibromofumaric acids and their diethyl esters (*49* and *48*, respectively, R = CO_2H and CO_2Et)

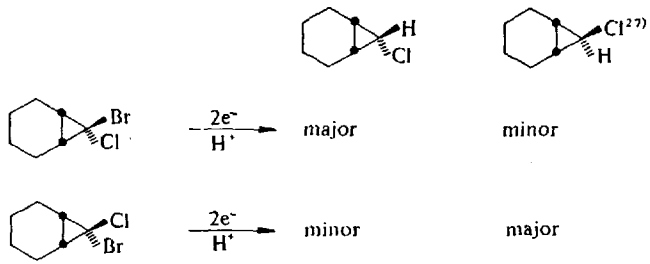


56 *57*

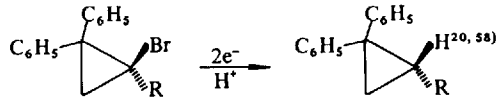
in protic media [56]. Unlike the preceding results, reductive elimination to produce the corresponding acetylene took place in *all* cases. Also surprisingly, the two dicarboxylic acids were equally easy to reduce, while the *cis* diester (*49*, R = CO_2Et) was actually *easier* to reduce than its *trans* isomer. These results constitute the only reported exception to the generalization that *trans*-1,2-dibromoolefins *(48)* are easier to reduce than their *cis* isomers *(49)*.

Feoktistov and coworkers [57] found a third mode of reactivity in a closely related system. The electrochemical reduction of the diethyl esters of dichloromaleic *(56)* and dichlorofumaric *(57)* acid was studied. Neither afforded the corresponding acetylene.

Several studies of the electrochemical reduction of *cyclopropyl halides* have been reported in the literature [20,26,27,58]. It appears that reduction occurs without complete loss of stereochemical integrity. The predominent stereochemical pattern involves reduction with overall (partial) retention of configuration. The following examples are representative:



The stereochemistry of the process appears to be influenced by a variety of experimental parameters [20], among which are the nature of the supporting electrolyte, solvent, electrode material, the identity of the halogen (bromine or iodine), and the nature of substituents at the reaction site, *e.g.*, while reduction of *58* (R = CH_3 or CO_2^-) occurs with partial retention of configuration, partial inversion is observed when R = CO_2H or CO_2CH_3 [58]. All of these factors suggest that the reaction involves adsorbed intermediates. Retention of configuration, which is most common (it is observed in all but the latter two cases) is probably associated with the fact that cyclopropyl carbanions retain their stereochemical integrity for much longer times than do other carbanions [59].



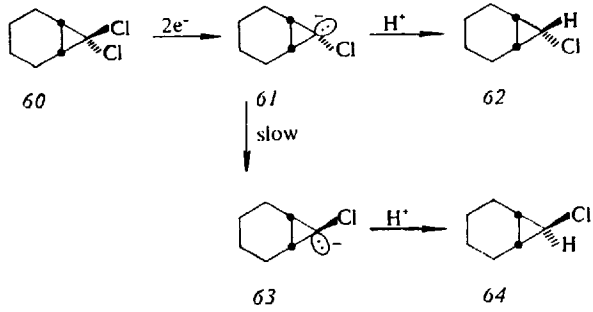
58 *59*

The partial loss of configuration often observed during reduction of cyclopropyl halides may actually occur via the corresponding cyclopropyl radicals, which lose configuration rapidly [60]. In that event, their behavior would resemble that of vinyl halides, as exemplified by the 3-iodo-3-hexenes [16]. Occasional cases of partial inversion could be associated with shielding of the cyclopropyl carbanion by the electrode surface, with concomitant protonation on the other face of the carbanion [58].

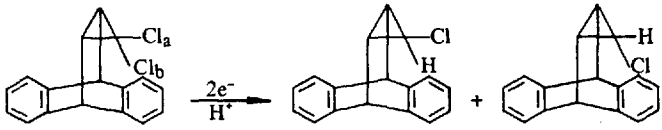
There is some evidence that carbanions generated by electrochemical reduction of cyclopropyl halides slowly lose their initial configuration. The electrochemical reduction of *60* probably occurs via preferential removal of the more sterically accessible halogen atom [26] to form carbanion *61* (although a dissenting viewpoint has been expressed by Erickson [27] and coworkers). In a good proton-donating medium (95% methanol-5% conc. hydrochloric acid), protonation of *61* is fast, and *62* is the exclusive product. In a poorer protonating solvent (DMF), isomerization of *61* to *63* can compete; the ratio of *62* to *64* in this solvent is 81 : 19 [26].

Steric acceleration of the isomerization can apparently occur: it is almost certainly Cl_a of *65* which is removed electrolytically, yet the major electrolysis product is *66* (*66*: *67* = 3.2:1) [27], probably because steric compression between Cl_b and the nearby aromatic ring in *65* can be relieved through isomerization (inversion).

As was indicated previously, several other parameters can affect the stereochemistry of reduction of cyclopropyl halides [20]. For example the optical purity of *59* produced from the electrochemical reduction of *58* in acetonitrile



60 *61* *62* *63* *64*



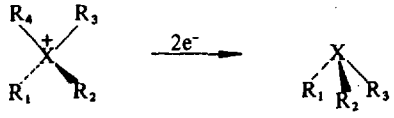
65 *66* *67*

containing tetraethylammonium bromide increases from 25% to 47% when the electrode is changed from mercury to glassy carbon [20]. Solvent and electrolyte play a role: the optical purity of *59* is 25% in DMF containing tetra-n-propylammonium perchlorate, 6–10% in acetonitrile containing tetra-n-butylammonium iodide, and 0% in 1,2-dimethoxyethane containing tetra-n-butylammonium perchlorate [20]. As indicated above, these effects suggest surface phenomena involving adsorbed intermediates.

2.2. Phosphonium and Arsonium Ions

Horner and his coworkers have made the important discovery that reduction of optically active phosphonium salts (*68*, X = P) proceeds with retention of configuration at phosphorus [61,62]. Cleavage of phosphonium salts can be carried out in aqueous solution at platinum, lead or mercury cathodes. The reaction is preferably carried out at a platinum electrode, upon a salt of which at least one substituent is a readily cleavable group, *e.g.*, allyl, benzyl, or phenacyl. At mercury or lead, saturated alkyl and aryl groups are also removed, hence there is relatively indiscriminate cleavage of the four substituents about P in *68* [61]. On the other hand, the potentials necessary for undesired cleavage of alkyl and aryl groups cannot be attained at platinum because of the low overvoltage of hydrogen upon platinum. Since *69* can be alkylated, new optically active phosphonium salts are accessible via this electrochemical reaction. The mechanism of electrochemical cleavage of phosphonium salts has not been studied. It probably involves a process in which the carbon-phosphorus bond is partially broken in the transition state for the initial electron transfer:

$$^{+}PR_4 \xrightarrow{e^-} [R \ldots . PR_3]^{\bullet} \longrightarrow PR_3 + R^{\bullet} \xrightarrow[H^+]{e^-} RH$$



68 *69*

This would explain the fact that groups which form stable radicals, *e.g.*, benzyl, allyl, or phenacyl, are easiest to remove electrolytically, and that tertiary alkyl groups are removed more readily than secondary or primary. This mechanism is more likely than one previously suggested, which involves a pentacoordinate phosphorus hydride *(70)* [61]:

$$R_4P^+ \xrightarrow[H^+]{2e^-} R_4PH \longrightarrow R_3P + RH$$

70

The reduction of optically active phosphonium salts by lithium aluminum hydride, which probably does involve *70* as an intermediate, affords *racemic* phosphines, presumably by pseudorotation in *70* before it decomposes [63].

Horner has extended this reaction to the electrochemical reduction of optically active arsonium salts (*68*, X = As), which also undergo cleavage with retention of stereochemical configuration at arsenic [64,65]. This is a convenient synthetic route of optically active arsines of known configuration.

2.3. Ammonium Ions

The electrochemical reduction of quarternary ammonium ions (*68*, X = N), to tertiary amines is possible if one of the four groups attached to nitrogen is one which forms a stable radical, *e.g.*, allyl, benzyl, or phenacyl. Because of the rapid inversion rates characteristic of tertiary amines, it is not possible to determine whether the initial cleavage occurs with retention of configuration at nitrogen as it does at phosporus and arsenic. One may however inquire into the stereochemistry at carbon in the cleavage reaction. Reduction of benzyltriethylammonium ion *(71)* in DMF affords a mixture of bibenzyl *(72)* and toluene [66,67]. The formation of *72* might occur by either a radical or an ionic path (Schemes IV and V). The problem was investigated stereochemically. Optically active α-methylbenzyltrimethylammonium ion *(73)* was reduced electrochemically, and the 2,3-diphenylbutane *(74)* formed in the elec-

$$\underset{71}{C_6H_5CH_2N^+(C_2H_5)_3} \xrightarrow{e^-} C_6H_5CH_2\cdot + N(C_2H_5)_3$$

$$2\,C_6H_5CH_2\cdot \longrightarrow \underset{72}{C_6H_5CH_2CH_2C_6H_5}$$

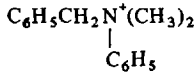
Scheme IV. Radical path

$$71 \xrightarrow{2e^-} C_6H_5CH_2^- + N(C_2H_5)_3$$

$$71 + C_6H_5CH_2^- \longrightarrow 72 + N(C_2H_5)_3$$

Scheme V. Ionic path

trolysis was isolated [66]. If the reaction follows the radical path a mixture of racemic and *meso* *74* will be formed, while the ionic path ought to afford a mixture of *meso* and optically active *74*. The *74* isolated from the electrolysis was completely optically inactive, supporting the radical machanism [66]. The latter was also supported by an analysis of the shape of the polarographic wave for the reduction of benzyldimethylanilinium ion *(75)*, which indicated the transfer of only one electron in the transition state for electron transfer [68].



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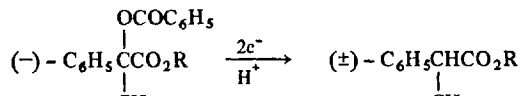
2.4. Other Single Bonds

While electrochemical cleavage of several other kinds of single bonds has been reported, stereochemical information is sparse. Erickson and Fischer examined the electrochemical reduction of 0-benzoylatrolactic acid (*76*, R = H) and its methyl ester (*76*, R = CH_3) [69]. The products (*77*, R = H and CH_3) of the



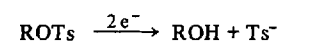
76 *77*

electrolysis are essentially completely racemic, which is what would be expected if the reduction involved radical or carbanion intermediates, and is, it will be recalled, exactly what was observed by Erickson and Fischer for the electrochemical cleavage of the corresponding chlorine compound *(13)* (Section 2.1) [23]. Erickson and Fischer also established the fact that reduction of

13 *78*

the sulfur derivative (*78*, R = H and C_2H_5) also affords racemic *77* [23].

Horner and Singer examined the electrochemical cleavage of alkyl *p*-toluenesulfonates *(79)* [70]. The reduction can be carried out under very mild



(Ts = *p*-toluenesulfonyl)

conditions (mercury cathode at 8–10 °C, in ethanol containing tetramethyl-

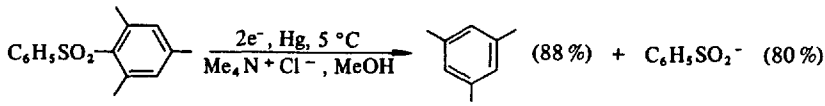
ammonium chloride). The alcohol corresponding to the starting *p*-toluenesulfonate is obtained in 75–95% yield, and, most important, the cleavage of the sulfur-oxygen bond proceeds with retention of configuration at carbon (less than 1% racemization). Electrolytic removal of the *p*-toluenesulfonyl group thus involves milder experimental conditions and is much cleaner stereochemically than are most other reagents, *e.g.*, lithium aluminum hydride, Raney nickel, or sodium in ammonia, commonly employed for the conversion of *p*-toluenesulfonates to alcohols. The reaction of sodium naphthalene with *p*-toluenesulfonates [70a] is comparable in ease and efficiency with the electrochemical method, but is probably not as safe for large scale operations.

Hoerner and Neumann found that electrochemical cleavage of the sulfur-nitrogen bond of alkyl *p*-toluenesulfonamides proceeds in high yield, under mild conditions, and with little or no racemization [71];

$$\text{Ts–L–(–)–Tyr} \xrightarrow[Me_4N^+Cl^-,\ EtOH]{2e^-,\ Hg,\ 10\,^\circ C} \text{L–(–)–Tyr} \quad (96\%)$$

(Ts = *p*-toluenesulfonyl; Tyr = tyrosine)

These investigators also observed that cleavage of the carbon-sulfur bond of aryl sulfones, a very difficult process to carry out by other means, proceeds smoothly at a mercury electrode even with sterically hindered sulfones, although the direction of cleavage of the latter appears to be governed by steric considerations, *e.g.* [72],



3. Reduction of Multiple Bonds

3.1. Carbonyl Compounds to Alcohols

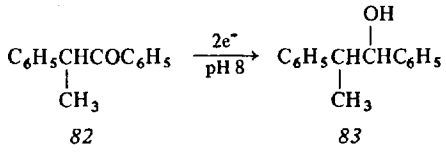
Arylalkyl ketones *(80)* are reduced to alcohols over the pH range of 7 to 12 (approx.) [73]. The reduction probably involves the following sequence of steps:

$$\text{ArCOR} \xrightarrow{e^-} \text{ArĊR}(-O^-) \xrightarrow{H^+} \text{ArĊR(OH)} \xrightarrow{e^-} \text{ArC̄R(OH)} \xrightarrow{H^+} \text{ArCHR(OH)}$$

80 *81*

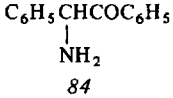
The reduction of ketones to alcohols by sodium in alcohol probably involves a very similar mechanism, but there appear to be some differences between

the stereochemistry of the two processes. For example, Mandell, Powers and Day found that electrochemical reduction of α-methyldesoxybenzoin *(82)*



82 *83*

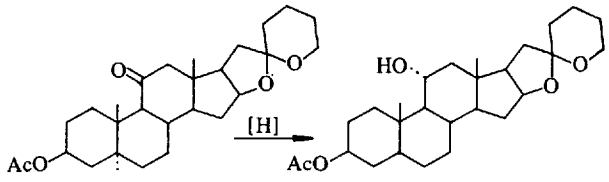
affords the *erythro* isomer of *83* in at least 92% stereochemical purity, while sodium-alcohol reduction of *82* affords a 64:36 mixture of the *erythro* and *threo* isomers of *83* [74]. Juday isolated the corresponding *erythro* alcohols from electrochemical reduction of *82* and of α-aminodesoxybenzoin *(84)* [75]. The stereochemical preference for *erythro* formation may implicate surface phenomena in the reaction. It is known, in fact, that radicals of structure *81*



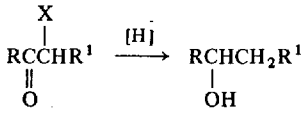
84

are strongly adsorbed upon the electrode surface during the electrochemical reduction of ketones [76]. There is evidence of a different sort which indicates that adsorption plays an important role in determining the stereochemistry of electrochemical reduction of ketones to alcohols. Horner and Degner effected an electrochemical *asymmetric synthesis* through use an optically active supporting electrolyte [77]. From the reduction of acetophenone in methanol containing (–)-ephedrine hydrochloride, they isolated R-(+)-α-methylbenzyl alcohol of 4.2% optical purity. When (+)-ephedrine hydrochloride was used as the supporting electrolyte, S-(–)-α-methylbenzyl alcohol (4.6% optical purity) was produced. These results suggest a high degree of orientation of both acetophenone and the chiral electrolyte at the electrode surface.

Dialkyl ketones undergo reduction to the corresponding alcohols over a wide pH range. Several groups have examined the stereochemical features of this reduction. Kabasakalian and McGlotten studied the polarographic behavior of a number of steroidal ketones [78]. They found that the relative ease of reduction of the carbonyl group depends upon its position on the steroid nucleus: the less steric hindrance there is to attack upon a carbonyl group, the easier it is to reduce electrochemically. These experiments suggest that it might be possible to reduce the less hindered carbonyl of a diketosteroid selectively. This is correct: Schenck and Kirchhof reduced the less hindered carbonyl (at C-3) of a 3,12-diketosteroid selectively on a preparative scale many years ago [79]. Kabasakalian and McGlotten found that steroidal ketones and α-hydroxy or acetoxy ketones are all reduced to the corresponding alcohol [80]. The hydroxyl group was found to be equatorial in all cases ex-



85 *86*

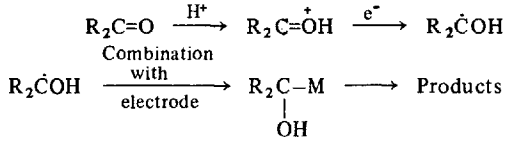


(X = H, OAc, or OH)

amined (cf. *85* → *86*), but small amounts of the axial alcohol would probably have escaped detection by these investigators.

Further evidence for surface effects upon the stereochemistry of electrochemical reduction of ketones comes from the discovery that the nature of the cathode material may effect stereochemistry. Reduction of 2-methylcyclohexanone affords pure *trans*-2-methylcyclohexanone at mercury or lead cathodes, a mixture of *cis* and *trans* alcohols (mostly *trans*) at nickel, and pure *cis* alcohol at copper [81]. Reduction could not be effected at platinum; presumably hydrogen evolution takes place before the potential necessary for reduction of the ketone can be reached.

Coleman, Kobylecki, and Utley studied the electrochemical reduction of the conformationally fixed ketones 4-*tert*-butylcyclohexanone and 3,3,5-trimethylcyclohexanone [82]. Stereochemically, the cleanest reductions took place at a platinum cathode in a mixture of hexamethylphosphoramide and ethanol containing lithium chloride. Under these conditions the equatorial alcohol predominated heavily (95% from 4-*tert*-butylcyclohexane and 91% from 3,3,5-trimethylcyclohexanone). In acidic media roughly equal quantities of axial and equatorial alcohol were produced. It was suggested that organolead intermediates are involved in the reductions in aqueous media. This is reasonable, based upon the probable mechanism of reduction in acid [83]. Reductions in acid at mercury cathodes in fact do result in the formation of



(M = a metal: Pb, Hg, etc.)

dialkylmercury compounds [84]; it is significant that the latter are known to be much more stable against protolysis than are organolead compounds [85].

3.2. Carbonyl Compounds to Pinacols

Arylalkyl and diaryl ketones are converted to the corresponding pinacols upon electrochemical reduction in acidic and very alkaline media:

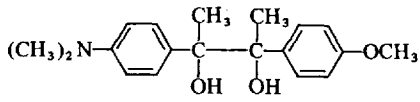
$$2\ \mathrm{ArCOR} \xrightarrow[2\mathrm{H^+}]{2\mathrm{e^-}} \mathrm{Ar}\underset{\mathrm{HO}}{\overset{\mathrm{R}}{\mathrm{C}}}\!-\!\underset{\mathrm{OH}}{\overset{\mathrm{R}}{\mathrm{C}}}\mathrm{Ar}$$

(R = aryl or alkyl)

The pinacols may of course exist in two diastereomeric modifications, *i.e.*, as *dl* or *meso* forms. Many carbonyl compounds have been reported to produce a mixture of diastereomeric pinacols; these include

furfural [86]
p-dimethylaminobenzaldehyde [87]
desoxybenzoin [75]
acetophenone [88]
piperonal [89]
anisaldehyde [89]
3,4-dimethoxybenzaldehyde [90]
p-tolualdehyde [89]
benzaldehyde [88,89]
3,5-dimethylbenzaldehyde [89]
p-chlorobenzaldehyde [89]
m-chlorobenzaldehyde [89].

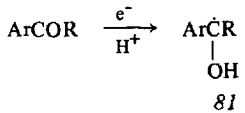
In a number of other cases it has been reported or implied that a single diastereomeric pinacol was formed, but the stereochemistry of the product was not determined; these reports come from studies upon the electrochemical reduction of 3-acetylpyridine [87], *p*-acetamidobenzaldehyde [91], β-dimethylaminopropiophenone [91], *p*-aminoacetophenone (which affords one pinacol in acid and the other in alkali)[92], and the reduction of a mixture of *p*-dimethylaminoacetophenone and *p*-methoxyacetophenone to afford a mixed pinacol *(87)* [93].



87

In view of the very considerable activity in this area it is surprising and somewhat dismaying to find that relatively little attention has been paid to the identity of the electrochemically generated pinacols. In none of the studies cited in the preceding paragraph was the stereochemistry of the products estab-

lished. Only in recent years, with the recognition that the stereochemistry of the pinacol can provide information concerning the nature of the dimerization step has attention been paid to this problem. Important contributions have been made in this area by Stocker and his coworkers, who have examined the effect of changes in a variety of experimental variables upon the stereochemistry of pinacols generated by electrochemical reduction of arylalkyl carbonyl compounds [94-96,98]. Stocker and Jenevein found that the *dl*:*meso* ratio in the mixture of pinacols produced by reduction of acetophenone ranges from *ca.* 1.0 to 1.4 in acid under a wide variety of experimental conditions, and from 2.5 to 3.2 in alkaline media [94]. In acid the pinacol arises by dimerization of neutral radicals:



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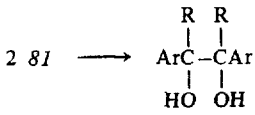
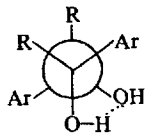


On simple steric grounds one would expect the *meso* pinacol to predominate; the fact that the *dl* isomer actually predominates was rationalized as due to favorable inter-radical hydrogen bonding in the *dl* transition state *(88)*. Support for this postulate was found in a study of the electrochemical reduction of 2-acetylpyridine [96]. Strong *intra*molecular hydrogen bonding in the radical *(89)* resulting from reduction of the latter does result in the predominance of *meso* isomer anticipated on steric grounds. Likewise, electrochemical reduction of *90* in acid affords the *meso* pinacol exclusively, presumably because of strong *intra*molecular hydrogen bonding in the intermediate radical [97].



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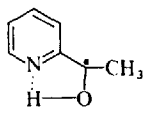
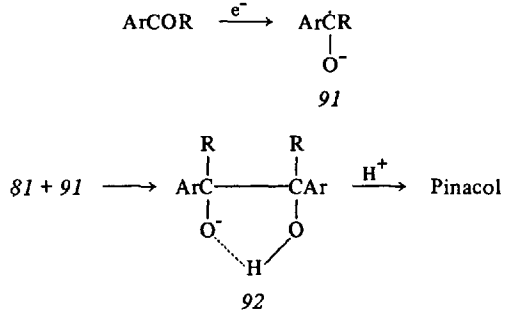


89

90

Stocker and Jenevein have suggested that in alkali the pinacols arise by combination of the neutral radical *81* with the radical anion *(91)* of the ketone:

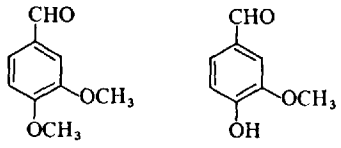


91

92

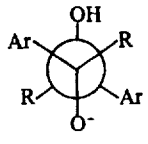
This suggestion appears reasonable, and accounts for the increased preference for formation of the *dl* pinacol in alkali, since the hydrogen bond in the transition state leading to *92* should be stronger than in *88.* Propiophenone and benzaldehyde exhibit behavior analogous to that observed with acetophenone [98].

There does exist one prominent exception to the generalization [95] that the *dl* pinacol heavily predominates in reductions carried out in alkali. When the aromatic ring bears a phenolic hydroxyl, reduction in alkali affords the *meso* pinacol exclusively. Thus reduction of *93* produces a mixture of *dl* and *meso* pinacols, but *94* affords the pure *meso* pinacol [90]. Other aromatic car-



93 *94*

bonyl compounds which have been found to produce a single pinacol cleanly in base are *p*-hydroxyacetophenone [87], 2-hydroxy-3-methoxybenzaldehyde [99], and *p*-hydroxybenzaldehyde [100]. Only in the last of these three examples was the stereochemistry of the pinacol established. Grimshaw and Ramsey [90] have argued that the *meso* isomer predominates in alkali in these cases because electrostatic repulsion between the two negatively charged aromatic rings forces these two groups to be as far apart as possible in the transition state for coupling to the *meso* isomer *(95).* However, this is unlikely to be the origin of this phenomenon, since the *dl* diastereomer can also be formed via a transition state *(96)* which also has the aromatic rings as far apart as possible,



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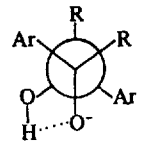
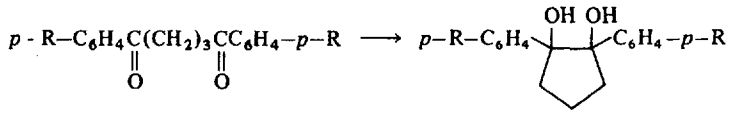


96

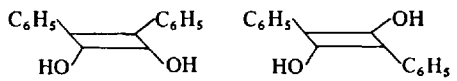
and has the added advantage of permitting hydrogen bonding between the two coupling radicals [95]. Gourley and Grimshaw [101] have advanced a rationalization similar to that of Grimshaw and Ramsey to account for the fact that, while reduction of ether *98* results in formation of *99* in which the *trans:cis* ratio is 3:79, the phenol *97* affords *99* in which the *trans:cis* ratio is 47:42. It is not clear why the *cis* pinacol should predominate to such a great extent in the reduction of *98* or the analogous compound with R = H [102].



97, R = OH
98, R = OCH_3

99

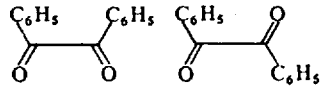
Vincenz-Chodkowska and Grabowski found that benzil *(100)* is reduced electrochemically to a mixture of *cis* and *trans* stilbenediols *(101* and *102)* [103].



101

102

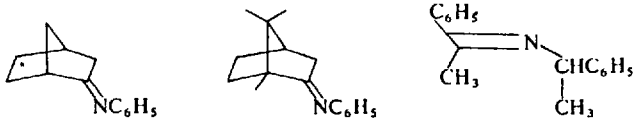
The relative proportions of these two compounds, which slowly rearrange to benzoin with a half-life of several hours, are strongly dependent upon changes in experimental parameters which are known to affect the strength of the electric field at the electrode surface, *i.e.*, electrode potential, ionic strength, temperature, and the nature of the supporting electrolyte. The ratio of *101* to *102* could be varied anywhere from 1:50 to 2:1, with *101* predominating at high field strengths. It is likely that field strength controls stereochemistry by affecting the relative population of rotamers *100a* and *100b* in the starting material.



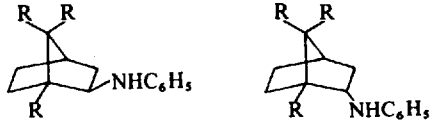
100a *100b*

3.3. Carbonyl Derivatives

Fry and Reed [29,104] examined the stereochemistry and mechanism of electrochemical reduction of several monoaryl imines *(103–105)* in DMF. Reduction of the bicyclic imines *103* and *104* may lead in each case to either *exo (106a* and *106b)* or *endo (107a* and *107b)* amine products. The stereochemical consequences of treatment of *103* and *104* with a variety of reducing agents are presented in Table 2, along with data pertaining to the reduction of (R)-(–)-*105* [29].



103 *104* *105*



106 (*a*, R=H; *b*, R=CH_3) *107*

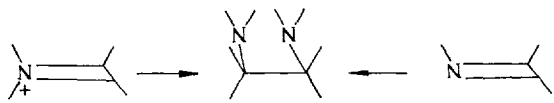
The data demonstrate that electrochemical reduction of these imines (at a mercury cathode) resembles a dissolving metal (sodium-ethanol) reduction much more closely than it resembles a reaction involving hydrogen addition from the less hindered side (catalytic hydrogenation). This is not particularly surprising. It is interesting to note, however, that while the electrolysis products are not kinetically controlled (as are those from catalytic hydrogenation), neither they nor the sodium-ethanol products are thermodynamically controlled, either, since thermodynamic control would afford *106a* as the major product from *103*. The predominance of *107a* disproves the frequent

Table 2. Stereochemistry of reduction of imines

Compound	Mode of reduction	Relative % products	
		Exo-amine	*Endo*-amine
103	H_2, platinum	0	100
103	Sodium-ethanol	33	67
103	Electrochemical	20	80
104	H_2, platinum	100	0
104	Sodium-ethanol	0	100
104	Electrochemical	0	100
		(R,R)-(-)-Amine	(R,S)-*Meso*-amine
(R)-(-)-*105*	H_2, platinum	90	10
(R)-(-)-*105*	Sodium-ethanol	42	58
(R)-(-)-*105*	Electrochemical	43	57

assertion in the literature [105] that sodium-alcohol reductions generally afford the more stable of a pair of epimeric products. Other data in the literature have also shown that this generalization is not valid [106,107]. The reason for predominance of the *endo* isomer from both *103* and *104* is not yet clear. Huffman and Charles found that sodium-alcohol reduction of norcamphor and camphor, the ketones corresponding to *103* and *104*, affords the *endo* alcohol as the predominant product from both ketones [108]. They accounted for this observation by the assumption that sterically hindered ketones are reduced by a different mechanism than that followed by unhindered ketones. The data of Fry and Reed render this assumption unlikely, however: it was found that *103* and *104* are reduced electrochemically by identical mechanisms, yet the product stereochemistry is still similar to that from sodium-ethanol reduction.

Diaryl imines [109] and immonium salts [110] have been found to undergo bimolecular reduction to *vic*-diamines under the appropriate experimental



conditions, but stereochemical information is not available in this area, save for the report by Law that benzal-*p*-toluidine affords a mixture of the *dl* and

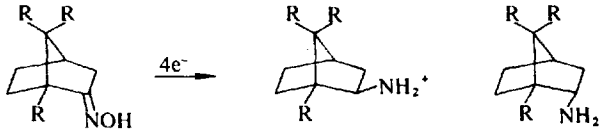
meso isomers of *108* upon reduction in neutral ethanol at a copper electrode [111].

$$2C_6H_5CH{=}NAr \xrightarrow{2e^-} \left(\begin{array}{c} C_6H_5CH \\ | \\ NHAr \end{array}\right)_2$$

108

(Ar=*p*-CH_3–C_6H_4)

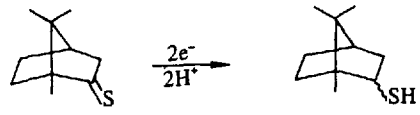
Fry and Newberg [112] examined the electrochemical reduction of norcamphor oxime *(109)* and camphor oxime *(110)* to the corresponding amines. The results of this study are shown in Table 3. It is clear from a comparison of these data with those in Table 2 that the electrochemical reduction of oximes *109* and *110* takes a very different stereochemical course from reduction of the corresponding anils *103* and *104*. Reduction of oximes apparently proceeds under kinetic control, affords products corresponding to protonation at carbon from the less hindered side of the carbon-nitrogen double bond, and affords the less stable epimeric amine in each case. It is not evident why the stereochemistry of reduction of anils and oximes should differ, however.



109, R=H
110, R=CH_3

Table 3. Stereochemistry of reduction of bicyclic oximes

Compounds	Mode of reduction	Relative % products	
		Exo-amine	*Endo*-amine
109	Sodium-ethanol	75	25
109	Lithium aluminum hydride	0	100
109	Electrochemical	0	100
110	Sodium-ethanol	4	96
110	Lithium aluminum hydride	99	1
110	Electrochemical	99	1



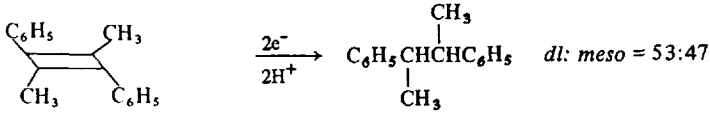
111

Electrochemical reduction of thioketones affords mercaptans under certain conditions. The stereochemistry of this process has not been examined. Reduction of thiocamphor *(111)* affords a single isomeric thioborneol [113] but although both isomeric mercaptans are known compounds [114,115], the stereochemistry of the product was not established [116].

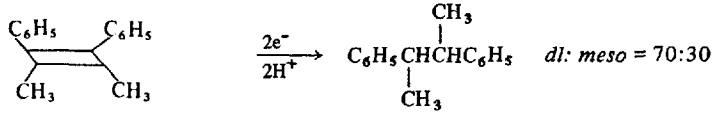
3.4. Olefins

Isolated double bonds are not electrochemically reducible. Conjugated double bonds are, however, reducible, and one may inquire into the stereochemistry of such processes. Because the area has not been investigated extensively, the stereochemical picture is confused and contradictory, however.

Horner and Röder examined the electrochemical reduction of the stereoisomeric α, α'-dimethylstilbenes *(112* and *113)* at a mercury cathode [117]. The reduction mechanism was not investigated, although the suggestion

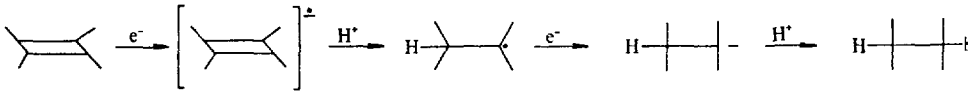


112

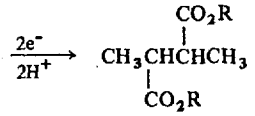


113

was made that adsorbed anions are involved. An ECE process of the following type is probably more likely:



Horner and Röder also found that dimethylmaleic acid (*112*, R = H) and its methyl ester (*112*, R = CH_3) are reduced stereospecifically to *meso*-2,3-dimethylsuccinic acid and its methyl ester, respectively (*113*, R = H and CH_3)[117].



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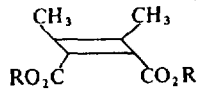
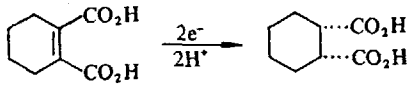
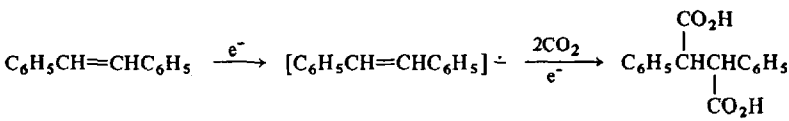


112

These results, which correspond to stereospecific *cis* addition of hydrogen to the double bond, stand in direct contradiction to the report [56] by Elving and coworkers that dimethylmaleic acid (*112*, R = H) affords *dl*-*113* (R = H) exclusively and that dimethylfumaric acid affords only *meso*-*113* (R = H). The latter results correspond of course to stereospecific *trans* addition of hydrogen across the double bond. Camilli, like Horner and Röder, however, observed *cis*-addition of hydrogen, albeit in a slightly different system [118]:



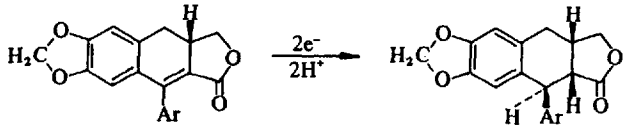
Dietz and Peover examined the electrochemical reduction of *cis* and *trans* stilbene *(114)* in DMF containing carbon dioxide [119]. The first electron transfer to *trans*-*114* affords a planar radical anion *(115)* which then undergoes rapid reaction with carbon dioxide to produce, ultimately, 2,3-diphenylsuccinic acid *(116)* in



114 *115* *116*

which the *dl*:*meso* ratio is 2.7:1. Reduction of *cis*-*114* affords a twisted radical anion whose half-life for conversion to *115* is at least 15 seconds. In the presence of carbon dioxide the twisted radical anion affords *116* in which the *dl*:*meso* ratio is 1.4:1 [119].

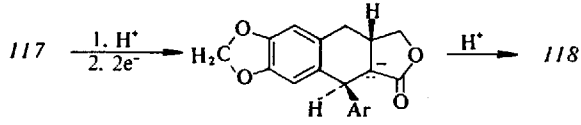
Klemm, Olson, and White discovered that the double bond in *117* may be reduced electrochemically with overall stereospecific *trans* addition of hydrogen to the double bond [120]. It was suggested that reduction proceeds by an initial two-electron reduction of the protonated starting material to carban-



117 *118*

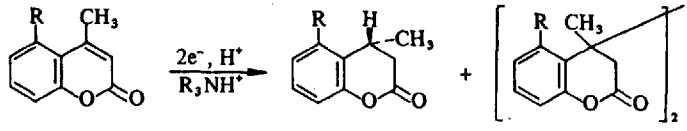
(Ar = 3,4,5-trimethoxyphenyl)

ion *119;* protonation of *119* to produce the thermodynamically more stable *cis* ring juncture would then afford *118.*



119

Gourley, Grimshaw, and Millar studied the electrochemical reduction of 4-methyl coumarins *(120)* in the presence of optically active supporting electrolytes [121]. The major products are the corresponding hydrodimers *(122),* but it is interesting to note that the saturated compounds *(121)* obtained as minor products are *optically active.* The results were rationalized as due to asymmetric hydrogen atom transfer from species *123* (with nine bonding

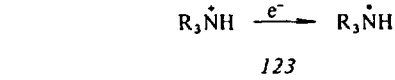


120 *121* *122*

(R = H or OCH_3)

electrons around nitrogen!), but other explanations seem possible, *e.g.,* asymmetric electron transfer to *120* from an optically active ammonium amalgam [122], or direct electron transfer to *120* adsorbed asymmetrically at the electrode surface in the presence of the optically active electrolyte, as suggested for the electrochemical asymmetric reduction of acetophenone in the presence of ephedrine salts (Section 3.1).

The electrochemical reduction of activated double bonds may also be used in the characterization of olefins. In all cases in which pairs *(124* and *125)* of 1,2-disubstituted olefins in which both X and Y are electron-withdrawing



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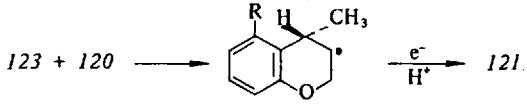
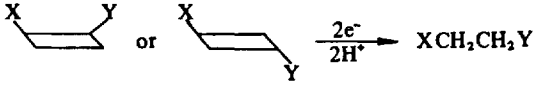


substituents have been studied polarographically, it has been found that the *trans* olefin is easier to reduce than the *cis* isomer [123-125]. It apperars that this generalization is good enough to be used as a criterion for assignment of configuration to an unknown isomeric pair of olefins.



124 *125*

(X, Y = COR, CO_2R, CN, etc.)

3.5. Acetylenes

The stereochemistry of electrochemical reduction of acetylenes is highly dependent upon the experimental conditions under which the electrolysis is carried out. Campbell and Young found many years ago that reduction of acetylenes in alcoholic sulfuric acid at a spongy nickel cathode produces *cis*-olefins in good yields [126]. It is very likely that this reduction involves a mechanism akin to catalytic hydrogenation, since the reduction does not take place at all at cathode substances, such as mercury, which are known to be poor hydrogenation catalysts. The reduction also probably involves the adsorbed acetylene as an intermediate, since olefins are not reduced at all under these conditions and since hydrogen evolution does not occur at the cathode until reduction of the acetylene is complete. Acetylenes may also be reduced to *cis* olefins in acidic media at a silver-palladium alloy cathode [127].

Benkeser and Tincher [128], on the other hand, reduced acetylenes preferentially to *trans* olefins using solvated electrons generated at a platinum cathode by electrolytic reduction of lithium chloride in methylamine [lithium metal is formed from lithium ion at the cathode in this electrolysis; its dissolution in methylamine generates the solvated electron and regenerates lithium

ion]. *Trans: cis* ratios in the resulting olefin mixtures ranged from 92:8 to 98:2. This method is experimentally simple and requires only a catalytic amount of lithium chloride and hence ought to be safer for large scale preparations than the use of sodium in ammonia, which is the usual method of effecting this transformation. The diethyl ester of acetylenedicarboxylic acid is reduced to diethyl fumarate [56]. Horner and Röder have found that acetylenes are slowly reduced to (predominantly *trans*) olefins upon electrochemical reduction at a mercury cathode in a protic solvent containing a quarternary ammonium salt as supporting electrolyte [117]. It is not clear whether this reduction involves direct electron transfer to the acetylene or whether the first step involves formation of a tetraalkylammonium amalgam [122], which is the actual reducing agent. Reduction of the diethyl ester of acetylenedicarboxylic acid to diethyl fumarate [56] occurs at rather positive potential and hence must involve direct electron transfer from the electrode to the acetylene.

4. Coupling of Activated Olefins

A wide variety of activated olefins *(126)* undergo reductive electrochemical dimerization to compounds of structure *127* (electrolytic hydrodimerization) [129]. While the product *127* is capable of existing in either *dl* or *meso* modifications, relatively little attention has been paid to the stereochemistry of hydrodimers

$$2\ \mathrm{RCH{=}CHX} \xrightarrow[2\mathrm{H^+}]{2\mathrm{e^-}} \mathrm{XCH_2CH(R)CHCH_2X}\ (\mathrm{R})$$

126 *127*

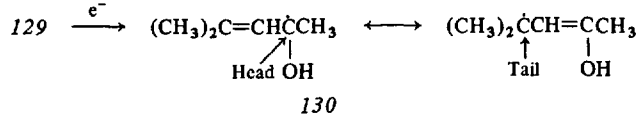
(X = CN, CO_2R', COR', etc.)

or to factors affecting the stereochemical course of the hydrodimerization process.

Before discussing what is known concerning the stereochemistry of hydrodimerization, it will be useful to discuss probable mechansims of hydrodimerization. It appears that a different mechanism is followed in acid than in neutral or alkaline media. In acid the first step is a one-electron reduction of the protonated starting material to form a neutral radical, which then dimerizes. Thus for mesityl oxide *(128)*:

$$(\mathrm{CH_3})_2\mathrm{C{=}CHCOCH_3} \xrightarrow{\mathrm{H^+}} (\mathrm{CH_3})_2\mathrm{C{=}CHC(OH^+)CH_3}$$

128 *129*



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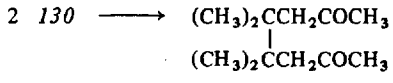
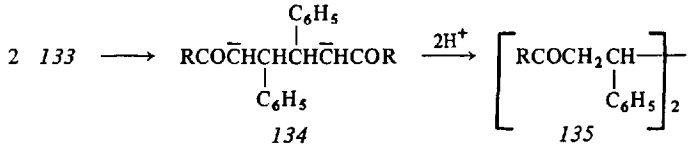


131

In neutral, alkaline, and aprotic media the first step is a one-electron reduction of the neutral ketone to a radical anion. There is some disagreement [130,131,144] concerning whether the next step is dimerization of the radical anion or reaction between it and the starting material. These two possibilities may be illustrated for benzylidene ketones *(132)* as follows:

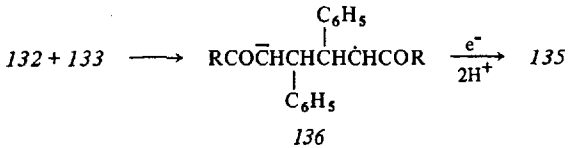
132 *133*

then



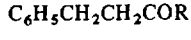
134 *135*

or



136

The reaction is complicated in aprotic media by polymerization of the olefin at the electrode [132] apparently because anions such as *134* or *136* can initiate anionic polymerization of the activated olefin. Steric hindrance about the double bond can retard polymerization; yields of hydrodimer from *132* in dimethylformamide as a function of the size of R are: R = hydrogen or methyl, 0%, R % *n*-propyl, 25%; R % *i*-propyl, 65%; R % *t*-butyl, 95% [132]. Saturation of the double bond to produce, *e.g.*, *136* from *132*, is a side reaction in neutral



136

media. This process is particularly important when dimerization is sterically inhibited; note the contrast between the behavior of *137* and *138* [133]:

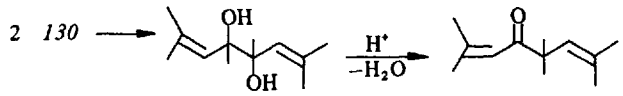
$$CH_3COCH{=}CH_2 \xrightarrow{e^-} CH_3CO(CH_2)_4COCH_3$$

137

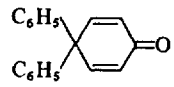
$$t\text{-}BuCOCH{=}CH_2 \xrightarrow{e^-} t\text{-}BuCOCH_2CH_3$$

138

Steric factors are also important in hydrodimerizations carried out in acidic media. Excessive steric hindrance about the β-carbon in an α, β-unsaturated carbonyl compound can retard tail-to-tail coupling, *e.g.*, 2 *130* → *131*, and lead to products of head-to-head (and occasionally head-to-tail) coupling. Thus in the reduction of mesityl oxide at pH ≳4 there is also formed a small amount of ketone *140*, apparently formed *via* head-to-head coupling of *130* and subsequent pinacol rearrangement of *139* [134]:



139 *140*



141

Head-to-head coupling to produce pinacols is the exclusive pathway when the β-carbon is badly sterically hindered, as in steroidal enones and dienones [135-137], or the dienone *141* [138].

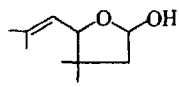
Head-to-head coupling ought to be more important in α, β-unsaturated aldehydes, in which steric hindrance about the carbonyl carbon (head) is less than in the corresponding ketones. Indeed, β, β-dimethylacrolein *(142)* affords *143* (a *dl*:*meso* mixture), *144*, and *145* in 24, 67, and 9% yield, respectively, upon electrochemical reduction at pH 5.0 [139]. Tail-to-tail coupling does not occur;

$(CH)_2C{=}CHCHO$

142

$(CH_3)_2C{=}CHCHCHCH{=}C(CH_3)_2$ (OH at the CHCHCH carbon; OH)

143



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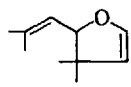
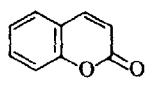


145

143 is the head-to-head product, while *144* and *145* arise via head-to-tail coupling, followed by intramolecular cyclization. This behavior contrasts markedly with the almost complete tail-to-tail coupling observed with the related ketone *128* [134].

There is little data in the literature concerning the stereochemical identity of pinacols and hydrodimers produced in the electrochemical reduction of α,β-unsaturated ketones. Lund found that the pinacols produced from electrochemical reduction of steroidal ring A ketones in alkali are generally different from those obtained from electrolysis in acid [137]. Three isomers are possible in principle: referring to the respective configurations of the two hydroxyl groups, the isomeric pinacols may be diequatorial, diaxial, or axial-equatorial. Lund assigned the stereochemistry of the pinacols arising from reduction of androsta-1, 4-diene-17-β-ol-3-one in acid and base, respectively, on semi-intuitive grounds. These assignments have been criticized by Bladon, Cornforth, and Jaeger, however [136]. It appears that the only steroidal pinacol whose stereochemistry has been definitely established is that from reduction of cholestenone in ethanol containing sodium acetate. This was shown to be the diequatorial pinacol by chemical degradation [136].

Harle and Lyons isolated a mixture of *dl* and *meso* hydrodimers from the electrochemical reduction of coumarin *(146)* at pH 6.8 [140] Archer and Grimshaw, on the other hand, isolated a single diastereomer in high yield upon electrolytic reduction of 3-phenylcoumarin *(147)* in methanolic hydrogen chloride [141]. Interpretation of the difference between the behavior of *146* and *147*



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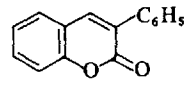
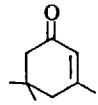


147

is difficult because the two pairs of investigators employed different experimental conditions and because the stereochemistry of the hydrodimer from *147* was not established. Isophorone *(148)* likewise affords only a single diastereomeric hydrodimer also of unknown configuration [142].

Useful information concerning the stereochemistry of hydrodimerization comes from a study of the electrochemical behavior of *trans*-2,2,6,6-tetra-



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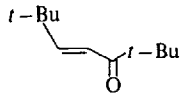

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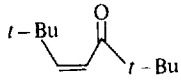

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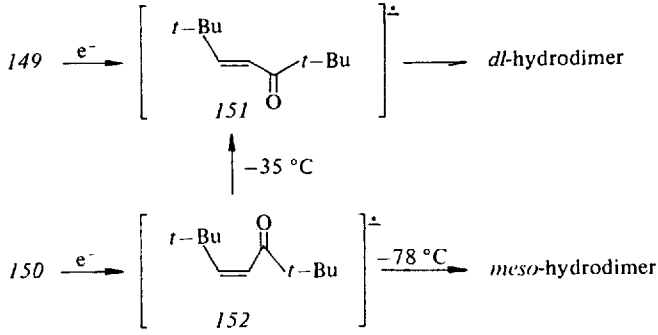

methyl-4-hepten-3-one *(149)* and its *cis* isomer *(150)* in dimethylformamide [143]. The *trans* isomer *149* undergoes one-electron reduction to a radical anion *(151)*, detectable by e.s.r. spectroscopy, which then reacts further to afford the *dl* hydrodimer. Reduction of *150* affords the *meso* hydrodimer when electrolysis is carried out at −78 °C, and the *dl* hydrodimer at −35 °C. Apparently at the higher temperature the *cis* radical anion *(152)* can isomerize to *151* before coupling. It appears that experimental conditions exert a very considerable influence upon the stereochemistry of hydrodimerization: controlled-potential reduction of *132* (R = *t*-Bu) in an acetate buffer in 50% ethanol-water afforded a mixture of *meso* and *dl* hydrodimers in which the major isomer was the *meso* diastereomer (*meso*:*dl* ≈ 10 : 1) [144]. By way of contrast, reduction in dimethylsulfoxide containing 0.1 M lithium perchlorate and 7% water or 0.1 M tetra-*n*-butylammonium perchlorate and 0.075M lithium perchlorate afforded a mixture of dimers in which the *dl* diastereomer predominates (*meso*/*dl* ≤ 0.25) [111]. Electrochemical reduction of *132* (R = C_6H_5) (pre-

sumably *trans*) in DMF affords the *meso* hydrodimer [145]. The situation is obviously unsatisfactory with respect to an understanding of the factors controlling the stereochemistry of hydrodimerization.

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Electrochemical Oxidation of Biologically-Important Purines

at the Pyrolytic Graphite Electrode

Relationship to the Biological Oxidation of Purines

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Contents

1. Introduction

Most studies on the electrochemistry of biologically-important molecules have been concerned with reduction reactions, principally at the dropping mercury electrode. Such processes are relatively simple and involve primarily hydrogenation of one or more double bonds within the substrate molecules. Although these processes are of electrochemical interest, in fact they generally reveal little information of direct relevance to biological, particularly metabolic, processes. This is so because metabolism (or better catabolism) of most organic molecules in biological systems proceeds by oxidation or other non-reductive reactions. The major exceptions to this are primarily the coenzymes involved in certain metabolic processes which act as the electron (or hydride ion-) acceptors for electrons removed from substrate molecules. Most of the coenzymes are themselves reoxidized ultimately by oxygen by way of the respiratory or electron-transport chain. Oxidation of purines is very important in the catabolism of these compounds in all organisms. Purines themselves are found in every living cell in every living organism principally as components of the nucleic acids. The particular sequence of purines and pyrimidines in the nucleic acids contain the information for direction of protein synthesis and for transfer of genetic information. In addition, certain purines, particularly adenine and to a lesser extent guanine, are important in intermediary metabolism in the form of certain of their nucleotides. Other purines are implicated in various diseases, or ailments, for example excessive formation of uric acid leads to precipitation of this compound in bone joints and results in the common and painful affliction called gout. Other purines are used extensively as drugs, for example 6-thiopurine is one of the three or four most effective drugs for treatment of certain leukemias. Theophylline, theobromine and caffeine are effective diuretics, particularly the latter compound and are imbibed in very large quantities from tea, coffee and many soft drinks. Theophylline is extensively utilized as a mild cardiovascular stimulant.

It is accepted that all of these compounds are metabolically oxidized in man or other organisms, but without exception the mechanism of the biological oxidation is at best only understood to a fragmentary and incomplete extent, and often even the products of biological oxidation, *i.e.* the metabolites have not been totally identified. Electrochemistry offers one of the possible ways to investigate potential mechanisms of biological oxidations of the purines and presumably other suitable molecular systems. At first sight it might appear that the possibility of any relationship between biological (*i.e.* enzymatic) and electrochemical reactions is remote. However, there are several marked similarities between the two types of processes. First, the electron-transfer reaction at both an electrode surface and at the active site of an enzyme is essentially a heterogeneous process. It is fairly certain that at a charged electrode surface a purine molecule is oriented in a very specific manner

when the electron-transfer reaction occurs. Enzymes are also known to demand a very specific orientation of the substrate at the active site for reaction, as well as restricting their activity to particular types of molecular substrates. Both enzymatic and electrochemical processes occur in aqueous solution, although the presence of *free* water in enzymatic reactions may not be necessary and hence it is reasonable to propose that electrochemical reactions in non-aqueous media could yield information of relevance to biological reactions. Many biological reactions occur at charged membrane or ribosomal surfaces just as an electrode reaction occurs at a charged electrode surface. Both biological and electrochemical reactions normally occur in solutions containing large excesses of inert electrolytes at comparable concentrations, although these electrolytes are present for somewhat different reasons. Finally, both types of reactions take place or are studied at very similar temperatures. *A priori* therefore, there are a number of at least superficial similarities between the conditions of biological and electrochemical redox processes and accordingly it would appear that useful information regarding biological redox mechanisms can be obtained by way of *in vitro* electrochemical studies. In this paper a detailed review of the mechanism of the electrochemical oxidation of a number of biologically-important purines will be presented. These mechanisms will be compared to known biochemical-biological data, and it will be demonstrated that in some instances the electrochemical data is of value in interpretation of biological observations.

2. Uric Acid

2.1. Electrochemical Oxidation

Fichter and Kern [1] first reported that uric acid could be electrochemically oxidized. The reaction was studied at a lead oxide electrode but without control of the anode potential. Under such uncontrolled conditions these workers found that in lithium carbonate solution at 40–60 °C a yield of approximately 70% of allantoin was obtained. In sulfuric acid solution a 63% yield of urea was obtained. A complete material balance was not obtained nor were any mechanistic details developed. In 1962 Smith and Elving [2] reported that uric acid gave a voltammetric oxidation peak at a wax-impregnated spectroscopic graphite electrode. Subsequently, Struck and Elving [3] examined the products of this oxidation and reported that in 1 *M* HOAc complete electrochemical oxidation required about 2.2 electrons per molecule of uric acid. The products formed were 0.25 mole CO_2, 0.25 mole of allantoin or an allantoin precursor, 0.75 mole of urea, 0.3 mole of parabanic acid and 0.30 mole of alloxan per mole of uric acid oxidized. On the basis of these products a scheme was developed whereby uric acid (I, Fig. 1) is oxidized in a primary 2*e* process to a shortlived dicarbonium ion (IIa, IIb, Fig. 1) which, being unstable, under-

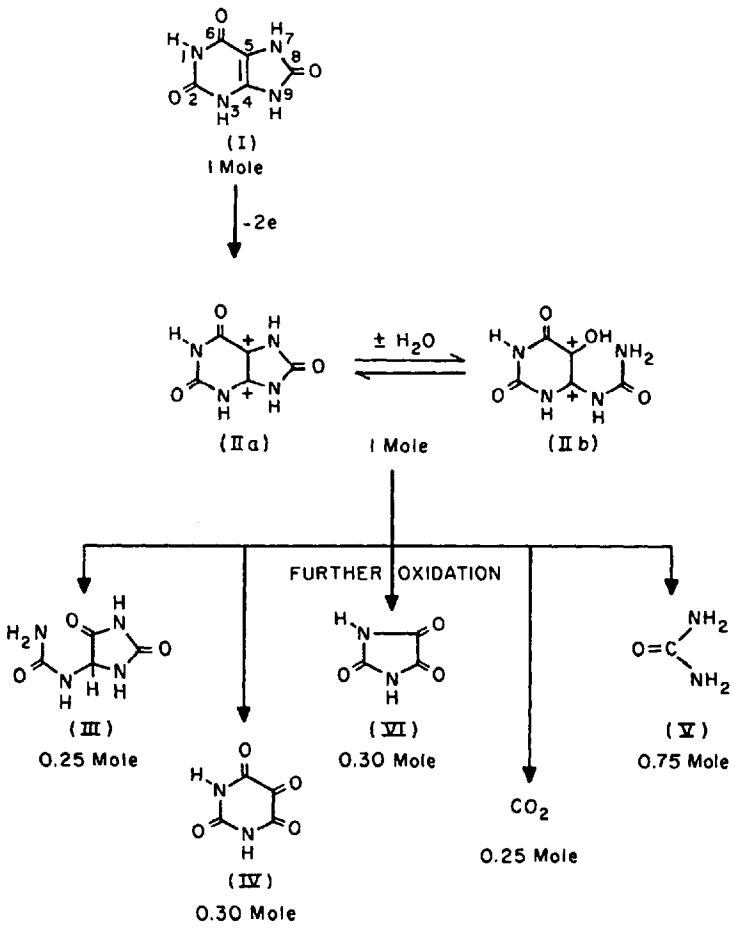


Fig. 1. Proposed mechanism of electrochemical oxidation of uric acid at a wax-impregnated spectroscopic graphite electrode in 1 *M* HOAc according to Struck and Elving [3]

went hydrolysis to allantoin (III, Fig. 1) (or its precursor), hydrolysis to alloxan (IV, Fig. 1) and urea (V, Fig. 1) and further oxidation to parabanic acid (VI, Fig. 1) and urea. That the $C_4{=}C_5$ double bond was the site of the oxidation was evidenced by the fact that upon initiation of the electrochemical oxidation the characteristic UV absorption spectrum of uric acid began to decrease and ultimately disappeared. The UV spectrum of uric acid is associated with a $\pi \rightarrow \pi^*$ transition associated with the $-C_4{=}C_5-$ chromophore. There are several major objections to this mechanism. First there is no real evidence in favor of the dicarbonium ion, indeed it is more plausible if any positive

Table 1. Linear *Ep* vs. pH relationships for oxidation of some purines at the stationary pyrolytic graphite electrode [a]

Compound	Peak	pH Range	*Ep* Volt *vs.* SCE	Reference
Purine		0–14	NO[b]	2)
Theobromine (3,7-dimethyl-xanthine)	I	2.3–5.5	1.67–0.064 pH	35,37)
Caffeine (1,3,7-trimethyl-xanthine)	I	2.3–5.5	1.59–0.042 pH	35,37)
Adenine	I	3.6–10	1.39–0.051 pH[c]	49)
Theophylline (1,3-dimethyl-xanthine)	I II	4–9 2.3–8.5	1.35–0.069 pH[d] 1.45–0.056 pH	35,39)
1,7-Dimethyl-xanthine	I	0–12.5	1.31–0.059 pH	35)
Hypoxanthine	I	0–5.7	1.27–0.067 pH[e]	2)
3-Methylxanthine	I II	5.5–12.5 0–11.9	1.20–0.056 pH[d] 1.27–0.050 pH	35)
7-Methylxanthine	I II	7–12.5 0–12.5	1.19–0.049 pH[d] 1.22–0.042 pH	35)
Guanine	I	0–12.5	1.12–0.065 pH	6)
Xanthine	I	0–12.5	1.07–0.060 pH	35)
Isoguanine	I	2*M* H_2SO_4	1.05[e,f]	2)
1-Methylxanthine	I	0–12.5	1.05–0.049 pH	35)
6-Thiopurine	I II III	2–8 0–12 2–10	0.51–0.047 pH[d] 0.81–0.052 pH 1.88–0.136 pH	65)
2,6-Dithiopurine	I II III	1–8 4.7–9 4.7–12.5	0.61–0.057 pH[d] 1.26–0.062 pH 1.86–0.100 pH	75)
Uric acid	I	2.3–5.7	0.76–0.069 pH[e]	2)
2-Thiopurine	I II	0–9 4–13	0.36–0.049 pH 1.83–0.082 pH	74)

[a] Except where otherwise stated the scan rate was 3.3 mV. sec^{-1}.
[b] Not oxidized.
[c] Scan rate 60 mV. sec^{-1}.
[d] Adsorption peak.
[e] Equation for the half-peak potential, $E_{p/2}$, at wax-impregnated spectroscopic graphite electrode.
[f] Only one data point available.

charge is localized on surrounding nitrogen atoms. Second, the peak for oxidation of uric acid is strongly pH dependent [2] (Table 1) and hence protons must be involved in the electrode reaction, which the mechanism does not indicate. Third, uric acid is very readily oxidizable and it was claimed that the primary electrode product, the dicarbonium ion, is further oxidized at potentials where uric acid is oxidized. The probability of a doubly positively charged species readily losing two further electrons to give parabanic acid is remote. Finally, oxidation of the dicarbonium ion to parabanic acid is, as just stated, a two-electron process. Formation of 0.3 mole of parabanic acid per mole of uric acid would therefore require a total transfer of 2.6*e* not 2.2*e* as claimed. A subsequent study of the electrochemical oxidation of uric acid at a pyrolytic graphic electrode (PGE) [4] revealed that in 1 *M* HOAc very close to 2*e* were required to oxidize uric acid. The same products were obtained as were reported by Struck and Elving [3], although only traces of parabanic acid were found. The major products were alloxan and urea along with a smaller amounts of allantoin. [The actual molar amounts of products are shown in Fig. 3] The peak potential for the anodic peak of uric acid at the PGE was strongly pH dependent (Table 1). It has also been shown by use of fast sweep cyclic voltammetry that oxidation of uric acid gives rise to a very unstable but very easily reducible product [5]. Thus, for example, in the case of uric acid in acetate buffer pH 4.7, on the first potential cycle at a clean electrode a single voltammetric oxidation peak is observed at $E_p = ca$ 0.6 V *vs* SCE (Peak I_a, Fig. 2). If the positive-going potential sweep is reversed after having scanned the single oxidation peak, then on the negative-going sweep two well-formed cathodic peaks are observed, the first at $E_p \approx +0.4$ V (Peak I_c, Fig. 2) and the second at $E_p \approx -0.9$ V (Peak II_c, Fig. 2). The former peak could not be observed unless the anodic peak was first scanned and unless the scan rate exceeded about 0.5 volt sec^{-1}. In fact as the scan rate was increased so the height of this cathodic peak approached that of the primary oxidation peak. The cathodic peak II_c was originally proposed to be due to reduction of parabanic acid [5] a reaction which occurs at about this potential. This, may in part be true, but the very small quantity of parabanic acid formed at the PGE precludes this compound being the major contributor to the process occurring; further discussion of this peak will be presented later. In summary, the primary product of the 2*e* oxidation of uric acid is a very readily reducible species which is also very unstable as evidenced by cyclic voltammetry. The oxidation peak of uric acid and the reduction peak of the unstable product form an almost reversible couple, and since the potentials for both peaks shift close to 60mV more negative for each unit increase of pH [2,6] it is possible to conclude that the number of protons and electrons involved in the electrode process are equal *i.e.* 2. It was proposed in several early papers by Dryhurst [5,6] that the primary product of this $2e-2H^+$ reaction was a 4,5-diol. However, this compound would not be expected to be electrochemically reducible, although it can be expected to readily decompose to the

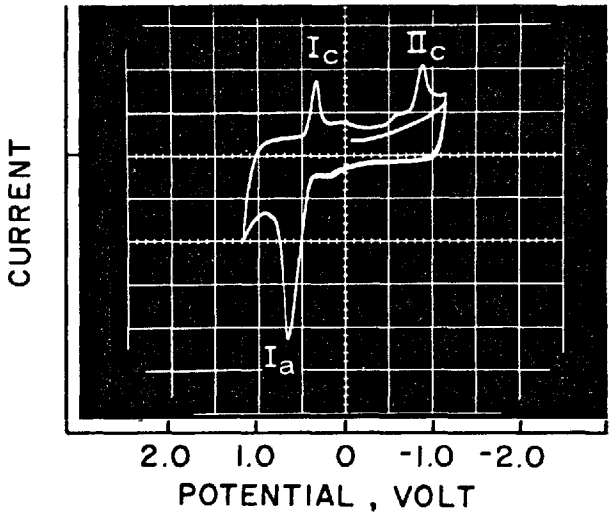


Fig. 2. Cyclic voltammogram of saturated uric acid in acetate buffer pH 4.7 at a clean stationary PGE

Scan pattern: 0.0V → −1.10V → 1.20V → −1.10V → 0.0V
Scan rate: 4.6V sec^{-1}; current sensitivity: 200 μa per division
Current above axis marker is cathodic

observed products. Accordingly, it was subsequently proposed that the primary $2e-2H^+$ product of the electrooxidation of uric acid (I, Fig. 3) is a bis-imine which can exist in two tautomeric forms (IIa, IIb, Fig. 3). Such a system of conjugated double bonds would be expected to be very readily electrochemically reducible. Although no electrochemical data are available on the reducibility of bis-imines, in fact it is well known that aldimines and ketimines are very readily reduced electrochemically [7-9]. The expected ease of reduction of structures IIa and/or IIb along with their expected facile hydration across the imine N=C double bonds accounted nicely for part of the observed cyclic voltammetry of uric acid. Thus, provided the sweep rate is fast enough, IIa or IIb formed as the primary products of oxidation of uric acid can be detected as very readily reducible species. At slow scan rates IIa or IIb cannot be detected because they are hydrated too rapidly. Hydration of IIa or IIb would result in the formation of uric acid-4,5-diol (III, Fig. 3) which, being a typical intermediate of an imine-like hydrolysis, would be expected to readily fragment to the observed products, allantoin, alloxan, parabanic acid and urea (Fig. 3). There is definite evidence for the formation of an electrochemical product having the 4,5-diol type of structure [10]. Thus, electrochemical oxidation of uric acid in aqueous acetate buffers pH 3.7 and 4.7 at the PGE

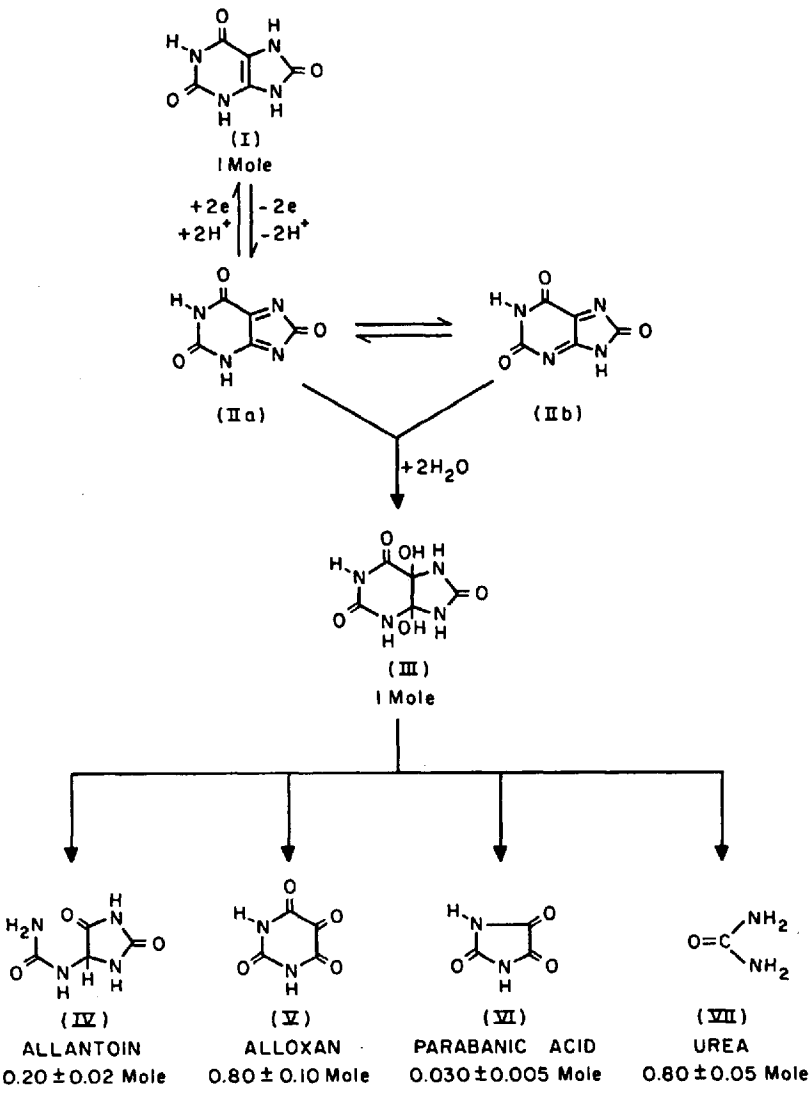


Fig. 3. Primary electrochemical mechanism and products formed on oxidation of uric acid at the PGE in 1 *M* HOAc

results in transfer of 2*e* and $2H^+$ and 90% or more of the initial uric acid oxidized gives rise to allantoin, along with small amounts of parabanic acid and alloxan. Addition of increasing amounts of methanol to solutions of uric acid

in the same buffers results in a systematic decrease in the cathodic bis-imine peak observed cyclic voltammetrically until in 50% methanolic solutions the latter peak cannot be observed at all under sweep rate conditions where it is very prominent in totally aqueous solutions. In a 50% methanolic solution the oxidation still involves only 2*e* but only about 20% of the initial uric acid gave rise to allantoin and no parabanic acid or alloxan were formed. Since the UV spectrum of uric acid in methanolic solutions was also destroyed upon electrolysis attack must have occurred at the C_4=C_5 bond. The mechanism of the process in methanol has been rationalized [10] by assuming that under voltametric conditions the greater nucleophilicity of methanol (or methoxide ion) results in preferential and very rapid formation of 4,5-dimethoxy uric acid rather than the uric acid–4,5-diol. The former compound would not be expected to fragment in the same way as the diol, and hence does not give appreciable quantities of alloxan, allantoin or parabanic acid.

The evidence therefore strongly supports the primary formation of a bis-imine (IIa, IIb, Fig. 3) which can be detected cyclic voltammetrically. There is also substantial, although indirect, evidence that the bis-imine hydrates in aqueous solutions to give uric acid–4,5-diol. A plausible mechanism for formation of allantoin from uric acid-4,5-diol has been written [4]. Protonation of uric acid-4,5-diol (I, Fig. 4) should lead to cleavage of the C_5–C_6 bond of the diol forming an imidazole isocyanate (II, Fig. 4), which following the expected proton shift giving III would be very readily hydrolyzed to allantoin (V) and CO_2. A simple fragmentation of the 4,5-diol to alloxan (VI, Fig. 4) and urea (VII) has also been written [4]. Although only minor amounts of parabanic acid are formed on oxidation of uric acid at the PGE, large amounts are claimed to be formed at spectroscopie graphite electrodes [3]. Formation of parabanic acid necessarily involves some secondary electrochemical oxidation. A mechanism is shown in Fig. 4 where uric acid–4,5-diol undergoes a ring opening reaction to give the structures VIIIa and VIIIb. In acid solution VIIIa should readily cleave across the original C_5–C_6 bond to give an isocyanate (IX) and 2-oxy-4,5-dihydroxyimidazole (X). Alternatively the structure VIIIb could undergo a ring closure to give XI which in turn should also cleave to give X and XI. Simple hydrolysis of the isocyanate (IX) would give rise to urea and CO_2. 2-Oxy-4,5-dihydroxyimidazole (X) is of course an enediol. Enediols are known to be very readily oxidizable to α-diketones [11] even by such weak oxidizing agents as cupric ion or, on occasion, oxygen. Accordingly, it has been proposed that the parabanic acid (XIV, Fig. 4) which appears during the electrooxidation of uric acid is formed as a result of the electrochemical oxidation of the enediol (X, Fig. 4) in a $2e$-$2H^+$ process [4]. It is important to realize that the parabanic acid could, according to the mechanism proposed in Fig. 4, arise from either the original pyrimidine or imidazole rings of uric acid *via* intermediates (VIIIa) or (XI). The larger amount of parabanic formed on electrooxidation of uric acid at spectroscopic graphite [3] than at pyroly-

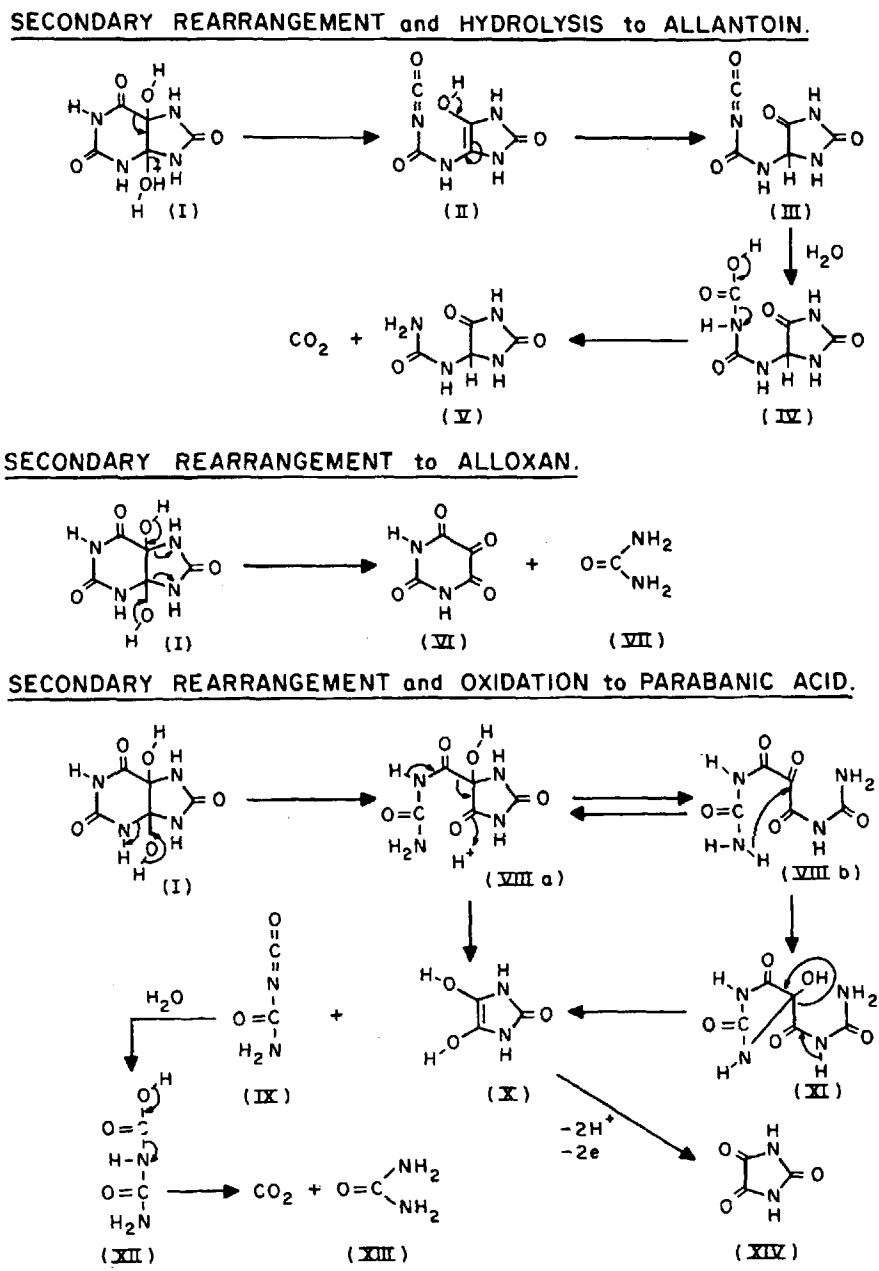
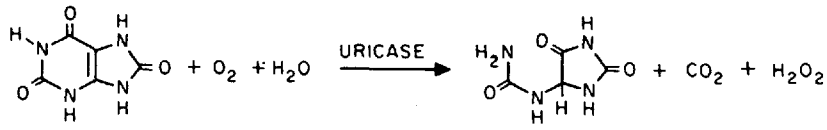


Fig. 4. Mechanisms for decomposition of uric acid-4,5-diol to allantoin (V), alloxan (VI), urea (VII) parabanic acid (XIV) and CO_2

tic graphite [4] can only be rationalized by proposing some specific effect brought about by the nature of electrode material.

2.2. Biological Oxidation

Uric acid is one of the principal products of purine metabolism in man [12,13]. However, in many other organisms further oxidative degradation of the purine molecule occurs. One of the most important enzymes involved in uric acid oxidation is uricase, which has been studied to some extent *in vitro*. It will act quite well, for example, when injected intravenously [14], although it does not occur naturally in man. Early workers proposed that in the presence of uricase, uric acid was quantitatively oxidized to allantoin, CO_2 and H_2O_2 according to Eq. 1 [15-19]. Other workers [20,21] showed that under certain conditions the uptake of oxygen was somewhat more rapid than the evolution of CO_2. This was interpreted to mean that an intermediate was formed in the reaction. By using water, and gaseous oxygen labelled with ^{18}O, Bentley and Neuberger [22] were able to show that the oxygen atoms of the hydrogen peroxide formed in the oxidation of uric acid in the presence of uricase were derived from molecular oxygen. Hence, uricase can be regarded as act-



(1)

ing as a catalytic site for transfer of two electrons from uric acid to (presumably) dissolved oxygen. By labelling the C_6 position of uric acid with ^{14}C it was found that the CO_2 formed in the enzymatic oxidation was derived from that carbon atom. On the basis of their own and other experiments [17], Bentley and Neuberger [22] concluded that the mechanism of uricase oxidation of uric acid involved transfer of 2*e* from the monoanion[a] of uric acid (I, Fig. 5) to give a carbonium ion (II, Fig. 5). It was proposed that the electron-transfer process was the only step that involved the enzyme, and that further changes were purely chemical reactions arising from the reactivity of the intermediates. The carbon atom at position 5 of the carbonium ion (II, Fig. 5), being strongly electrophilic, was proposed to interact with the N_1 nitrogen to give III, which being unstable, interacts with hydroxyl ion to give IV. This in turn

[a] The optimum reactivity of uricase is at *ca.* pH 9.25 where uric acid exists predominantly as its monoanion.

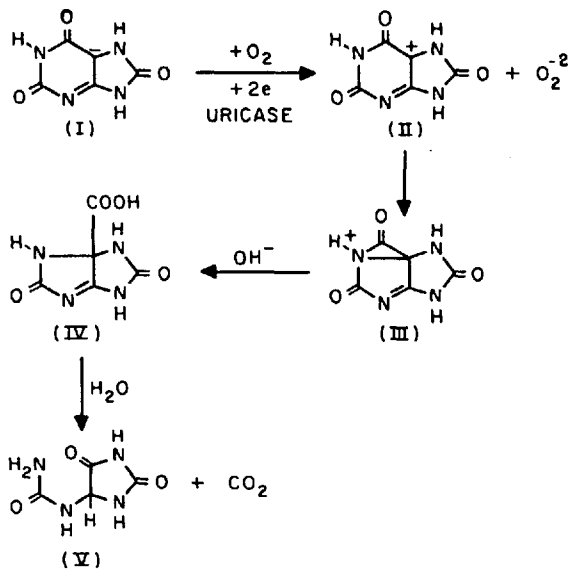


Fig. 5. Mechanism of uricase catalyzed oxidation of uric acid according to Bentley and Neuberger [22]

hydrolyzes and decarboxylates giving allantoin and CO_2 which are the observed major products in moderately alkaline solution.

Agner [23,24] Paul and Avi-Dor [25] and Canellakis *et al.* [26] have studied the oxidation of uric acid in the presence of various peroxidase enzyme systems. The identity and yield of various products was found to depend on the pH and buffer system employed. A combination of the findings of these workers suggests that in the presence of a peroxidase enzyme (*e.g.*, lactoperoxidase, verdoperoxidase, horseradish peroxidase) and H_2O_2, uric acid (I, Fig. 6) is oxidized *via* a very unstable intermediate to uric acid -4,5-diol (II, Fig. 6). At moderate pH the diol was then proposed to decompose, *via* an unknown species D, to allantoin (VI). At low pH decomposition primarily to alloxan (V) occurred, *via* an unspecified intermediate B. At moderately high pH it was proposed that the ultimate product observed, alloxanic acid (IV) was formed by breakdown of 5-ureido-2-imidazolidone-4,5-diol-4-carboxylic acid (III). Paul and Avi-Dor [25] suggested that in the case of 1-methyl uric acid (I, Eq. 2) the primary product of the enzymatic reaction was a bis-imine (II, Eq. 2) which then hydrated to give the appropriate 4,5-diol (III, Eq. 2). There was no direct evidence to support the existence of intermediates II or III, although many years earlier Blitz and Max [27] had suggested that the 4,5-diol was an intermediate in the nitric acid oxidation of uric acid to alloxan or the oxidation with alkaline per-

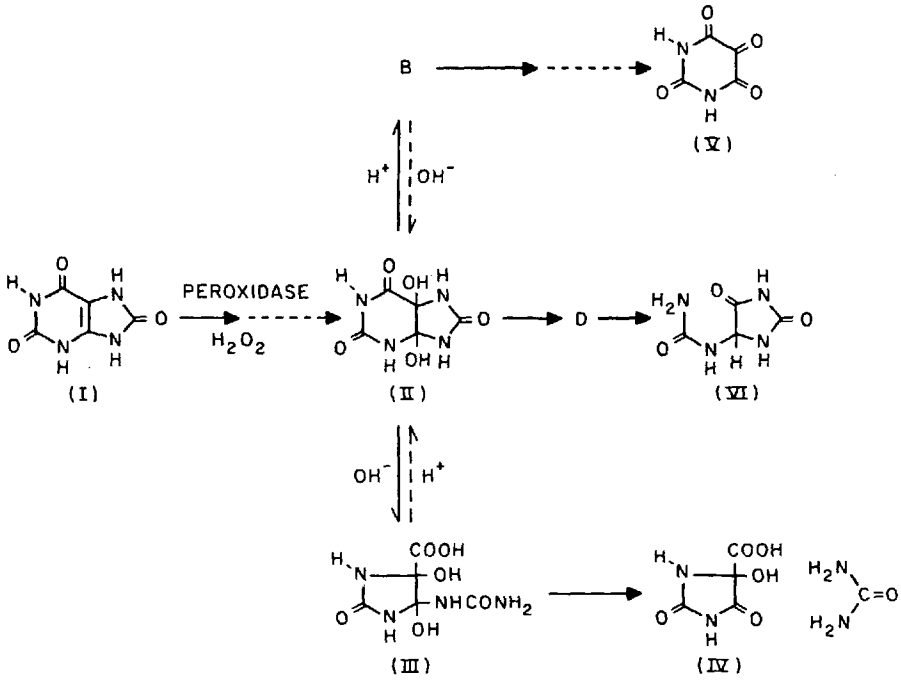
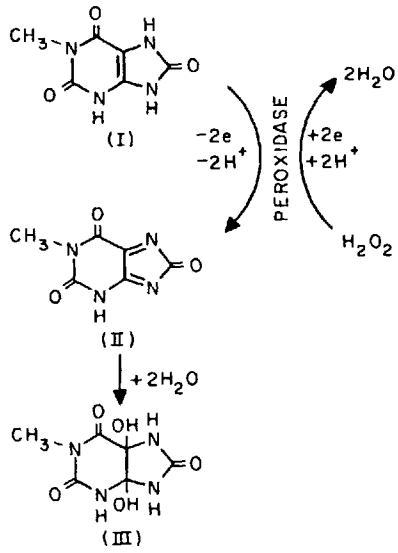


Fig. 6. Generalized mechanism of oxidation of uric acid by peroxidase enzymes [25,26]



(2)

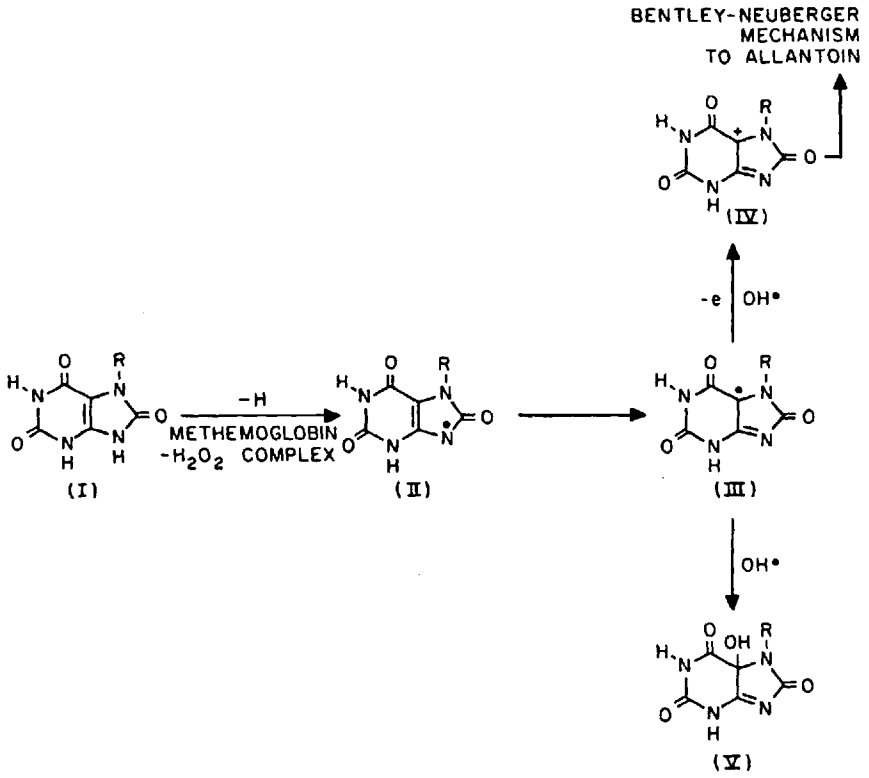


Fig. 7. Mechanism of oxidation of uric acid by methemoglobin and H_2O_2 according to Howell and Wyngaarden [29]

manganate to allantoin. Indeed, Blitz [28] reported that the dimethyl ether of III (Eq. 2) could be isolated after oxidation of uric acid with chlorine in methanol.

More recently Howell and Wyngaarden [29] studied the oxidation of uric acid with methemoglobin (a hemeprotein) and H_2O_2. At pH 5 the rate of oxidation is optimum and allantoin is one of the major oxidation products. In view of the fact that hydroxyl radicals were thought to be liberated in the reaction and that methemoglobin formed a peroxide complex, a mechanism was proposed where the hydrogen atom at N_9 of the uric acid (Fig. 7) was removed by the methemoglobin-peroxide complex giving a radical (II, Fig. 7). This could exist in at least two structural forms (II or III, Fig. 7), one of which (III) might react in two ways:

(a) loss of an electron to a hydroxyl radical to give the carbonium ion (IV) which could then undergo the Bentley-Neuberger [22] transformation to allantoin (Fig. 5), or

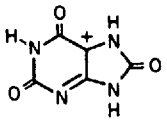
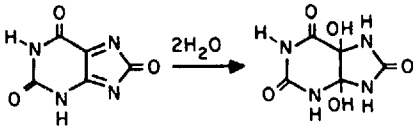
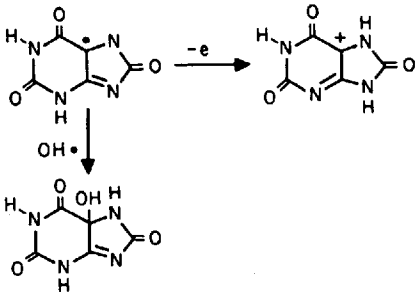
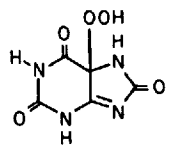
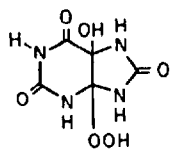
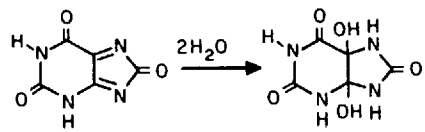
(b) addition of a hydroxyl radical to (III) would give V. A complex mechanism for decomposition of V to allantoin was propsed [29]. However, it is interesting to note that hydration of the structures IV or V would lead to uric acid-4,5-diol which could decompose as outlined earlier. Soberon and Cohen [30] have shown that uric acid is oxidized in the presence of myelo-peroxidase and peroxide to alloxan and other products by mechanisms which are similar to those previously discussed.

2.3. Correlations between Electrochemical and Biological Oxidations of Uric Acid

A tabulation of proposed intermediates and major products observed in the biological and electrochemical oxidations of uric acid are presented in Table 2. Also included in this table are similar data for the photodynamic oxidation of uric acid and for oxidation under the influence of ionizing radiation. Clearly, the preponderence of evidence favors an intermediate or primary product formed by attack of the $C_4{=}C_5$ bond. Comparison of the ultimate products obtained by the various oxidative processes reveals that the products formed upon enzymatic (particularly with peroxidase enzymes) and electrochemical oxidation (at the PGE) are essentially identical with respect to the nature, yields and effects of pH on these in as far as they have been investigated. Accordingly, it is pertinent to consider what the electrochemical information reveals about the biological processes that is not available from the enzymatic and other related studies. First, not only can some type of unstable primary product or intermediate be inferred from the nature of the products formed electrochemically, but the cyclic voltammetric evidence allows one to physically detect it and confirm that it is extremely unstable and that it is very easily reducible, *i.e.*, the bis-imine. The electrochemical data also positively indicate that $2e$ and $2H^+$ are involved in the primary reaction. In addition, the electrochemical data also support the view that uric acid-4,5-diol is almost certainly formed at some stage of the reaction by hydration of the bis-imine because of the formation of the 4,5-dimethyl derivative when methanol is present. Finally, the ultimate products observed electrochemically can all be explained by secondary reactions of the uric acid-4,5-diol using currently acceptable organic reaction mechanisms. The enzymatic reactions which give essentially the same products are based on the existence of a reactive unstable intermediate. It seems reasonable therefore in view of these findings to propose that the enzymatic and electrochemical reaction mechanisms are very similar if not identical.

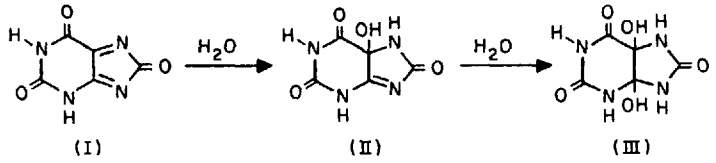
The nature of the species that gives rise to the more negative cathodic peak observed on cyclic voltammetry of uric acid (Fig. 2) is not clear. It must be due to some relatively transient species since the peak is not pronounced at the completation of the electrolysis nor is a large amount of parabanic acid formed,

Table 2. Structures of proposed primary products or intermediates formed on oxidation of uric acid along with the major products of the oxidations

Oxidizing system	Structure of primary product or intermediate	Major final products	Reference
Uricase/O_2 at pH *ca.* 9		Allantoin	22)
Peroxidases/H_2O_2		Low pH-Alloxan Intermediate pH-Allantoin High pH-Alloxanic acid	25,26)
Hemeproteins/H_2O_2		Allantoin	29)
Rose bengal $h\nu/O_2/H_2O$ in alkaline solution		Triuret, sodium oxonate, allantoxaidin	31,32)
γ-Radiation		Not known	33,34)
Pyrolytic graphite electrode		Low pH-Alloxan Intermediate pH-Allantoin	3,4,5)

which is reduced at very similar potentials. Many intermediate species shown in Fig. 4 could be proposed as being responsible for the peak. Another possible explanation is that the bis-imine (I, Eq. 3) could be hydrated in a stepwise fashion, first to the tertiary alcohol (II, Eq. 3) then to the uric acid-4,5-diol (III,

Eq. 3). Species II might be the species responsible for the major contribution to the more negative cathodic peak observed cyclic voltammetrically.



(I) (II) (III) (3)

3. Xanthines

3.1. Electrochemical Oxidation

Xanthine is electrochemically oxidized at the PGE by way of a single pH-dependent voltammetric peak (Table 1) that involves over-all $4e$ and $4H^{+}$ [4]. Evidence favors the view that the reaction proceeds by two $2e - 2H^{+}$ oxidations [35]. The first, potential-controlling reaction is a $2e - 2H^{+}$ oxidation of the $N_7{=}C_8$ (or $N_9{=}C_8$) bond of xanthine (I, Fig. 8) to give uric acid (II, Fig. 8). Since uric acid is more readily electrochemically oxidized than is xanthine (Table 1) the former compound is immediately oxidized in a further $2e - 2H^{+}$ process to the uric acid bis-imine (IIIa or IIIb, Fig. 8). Hydration of this species would give rise to uric acid-4,5-diol which then undergoes exactly the same secondary reactions as described previously (Fig. 4) so that the same products are observed from xanthine as are observed for uric acid [4]. The involvement of uric acid and its bis-imine in the electrochemical oxidation of xanthine is very evident from fast sweep cyclic voltammetry. The first potential sweep for xanthine at a clean PGE is shown in Fig. 9 which exhibits only a single oxidation peak (peak Ia, Fig. 9) which corresponds to the $4e - 4H^{+}$ oxidation of xanthine to the uric acid bis-imine (I → IIIa,b Fig. 8). Provided the sweep rate is fast enough the bis-imine can be detected as a reduction peak (peak Ic, Fig. 9) once having scanned peak Ia. Peak Ic of course correponds to the reduction of the bis-imine to uric acid (IIIa, IIIb → II, Fig. 8). On the second positive-going sweep the uric acid formed in the latter process is reoxidized to the bis-imine and gives rise to peak IIa. The very negative reduction peak in Fig. 9 was thought [35] originally to be due to reduction of parabanic acid to 5-hydroxyhydantoin [36]. However, in view of the very small amount of parabanic acid actually formed in the oxidation of xanthine it must be assumed that the peak is due to some other reducible intermediate species (*vide supra*).

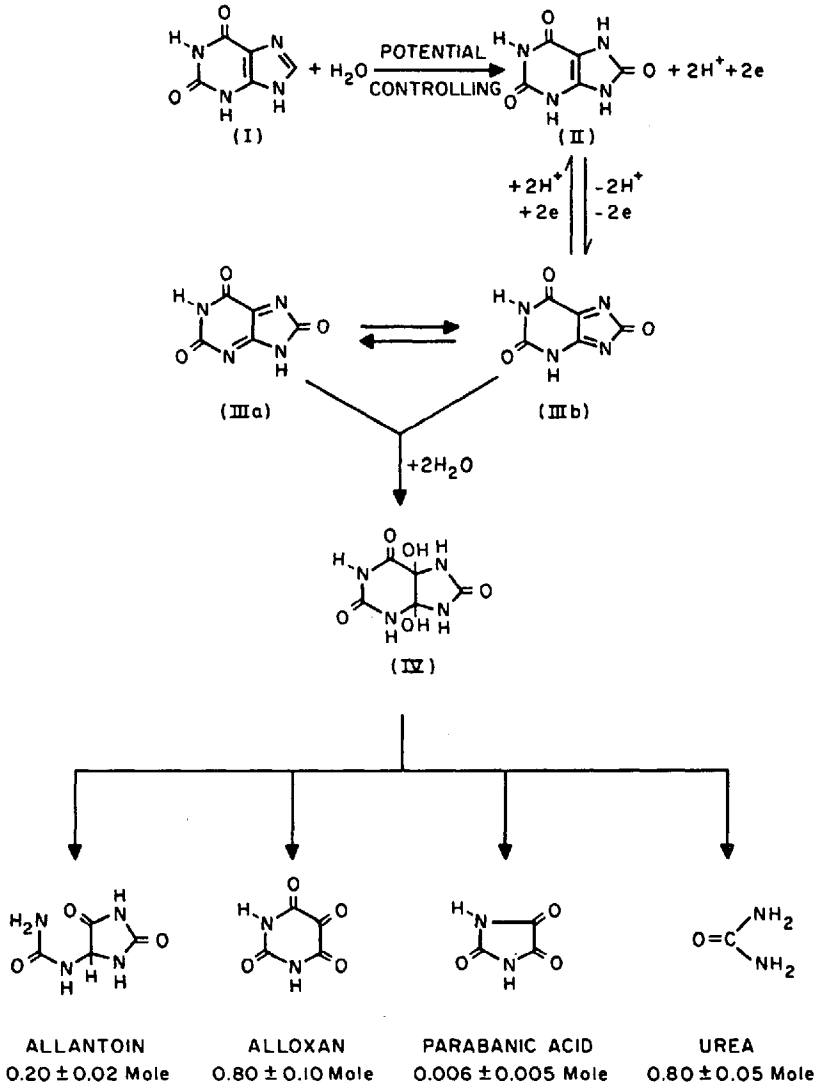


Fig. 8. Primary electrochemical mechanism and products formed on oxidation of xanthine at the PGE in 1 *M* HOAc

Studies of the linear sweep and cyclic voltammetric behavior of N-methylated xanthines [35,37] reveals that they undergo electrochemical oxidation over a fairly wide pH range at the PGE (Table 1). All but three of the xanthines studied show just a single voltammetric oxidation peak, although it is prob-

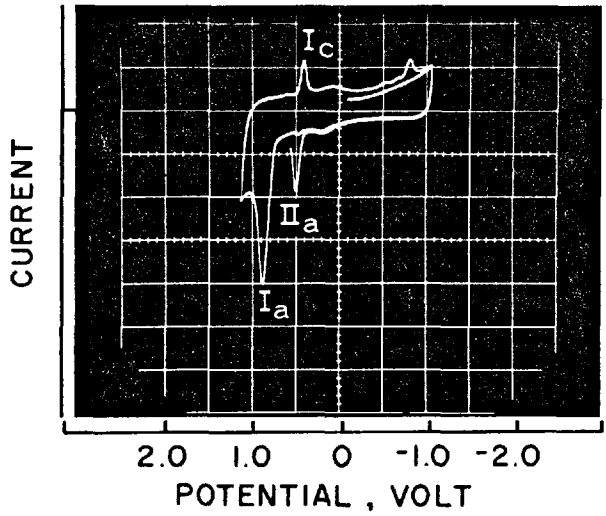


Fig. 9. Cyclic voltammogram of saturated xanthine at a clean, stationary PGE in acetate buffer pH 4.7

Scan rate: 4.6 V sec^{-1}
Current sensitivity: 200 μa per division
Current above axis marker is cathodic

able that the additional peaks observed at most negative potentials (peak I, Table 1) for some xanthines are due to adsorption processes [35]. Fast sweep cyclic voltammetry of many methylated xanthines revealed that methylation of the xanthine at N_7 caused a very pronounced decrease, even perhaps elimination, of the cathodic peak corresponding to reduction of the bis-imine primary oxidation product to the appropriate uric acid (*i.e.*, equivalent to peak Ic, Fig. 9). In the case of 3,7-dimethylxanthine (theobromine) and 1,3,7-trimethylxanthine (caffeine) there was no evidence at all from fast sweep cyclic voltammetry that peaks equivalent to peaks Ic and IIa of xanthine were present. In order to demonstrate that the effect of N_3 and N_7 dimethylation of xanthines was principally to alter the stability or lifetime of a bis-imine-type intermediate such that it could not be detected cyclic voltammetrically, rather than to completely alter the overall mechanism, Hansen and Dryhurst [37] examined the nature and amounts of the products of electrochemical oxidation of theobromine and caffeine. Both of these compounds are oxidized by way of a single voltammetric oxidation peak at the PGE in a process that involves overall 4*e*. In view of the pH dependence of the peak observed for both compounds (Table 1) and the nature of the products formed (Figs. 11–13) a mechanism can be proposed where, as with xanthine, the in-

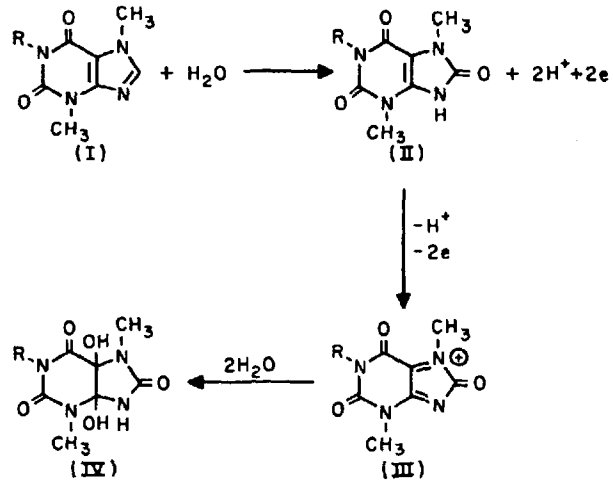


Fig. 10. Mechanism of primary electrochemical oxidation of theobromine (I, R=H) and caffeine (I, R=CH_3) at the PGE

itial $2e - 2H^+$ reaction involves oxidation of the $N_9=C_8$ double bond of the methylated xanthine (I, Fig. 10) to give the corresponding methylated uric acid (II, Fig. 10). The follow up electrochemical reactions can be described as removal of a proton and $2e$ from the $C_4=C_5$ double bond of the uric acid to give not a bis-imine but rather a bis-iminum ion (II → III, Fig. 10). This iminium ion would be expected to be extraordinarily susceptible to hydration [38] so that the corresponding methylated uric acid-4,5-diol (IV) would be formed very rapidly. It is logical therefore that the failure to detect an unstable, reducible product by fast sweep voltammetry of theobromine and caffeine is not due to the fact that a reducible species is not produced, but rather to the fact that the reducible iminium ion (III) would be extremely rapidly hydrated to give the electrochemically inactive substituted uric acid-4,5-diol (IV). The observed electron number and amount of substituted parabanic acids formed upon oxidation of theobromine and caffeine indicate that the primary route of decomposition of a 3,7-dimethyluric acid-4,5-diol is to allantoin and alloxan derivatives. Nevertheless, small amounts of parabanic acid are produced. In the case of theobromine the appropriate uric acid-4,5-diol (IV, Fig. 11) could undergo ring opening across the $-N_3-C_4-$ bond to give Va which upon protonation and fragmentation would give 2-oxy-3-methyl-4,5-dihydroxyimidazole (VII) and the isocyanate (VIII). Electrochemical oxidation of the former would lead to methyl parabanic acid (IX) derived from the imidazole moiety of the original compound, while hydrolysis of VIII would yield CO_2 and N-methylurea. The same products could be ob-

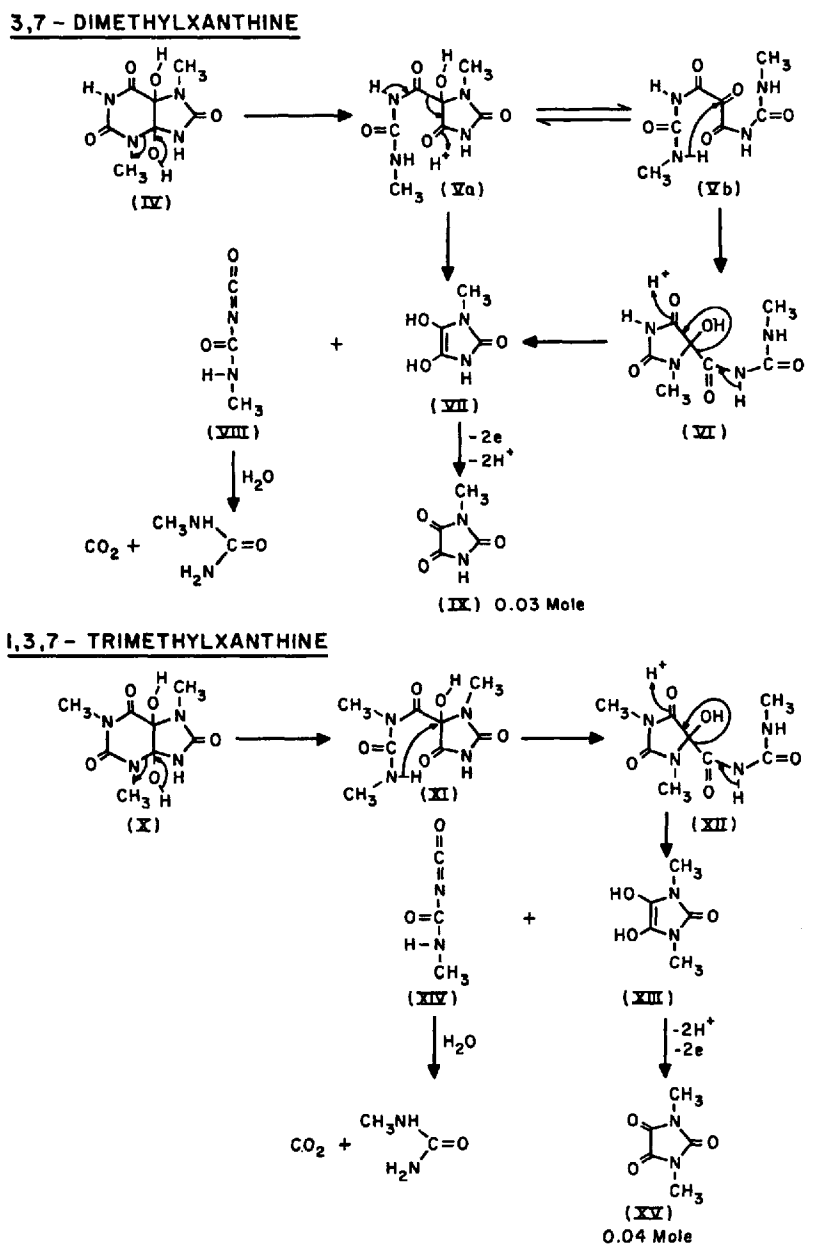


Fig. 11. Mechanism for formation of parabanic acids from the methylated uric acid-4,5-diol derived from theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine). Molar amounts of products are those formed in 1 *M* HOAc

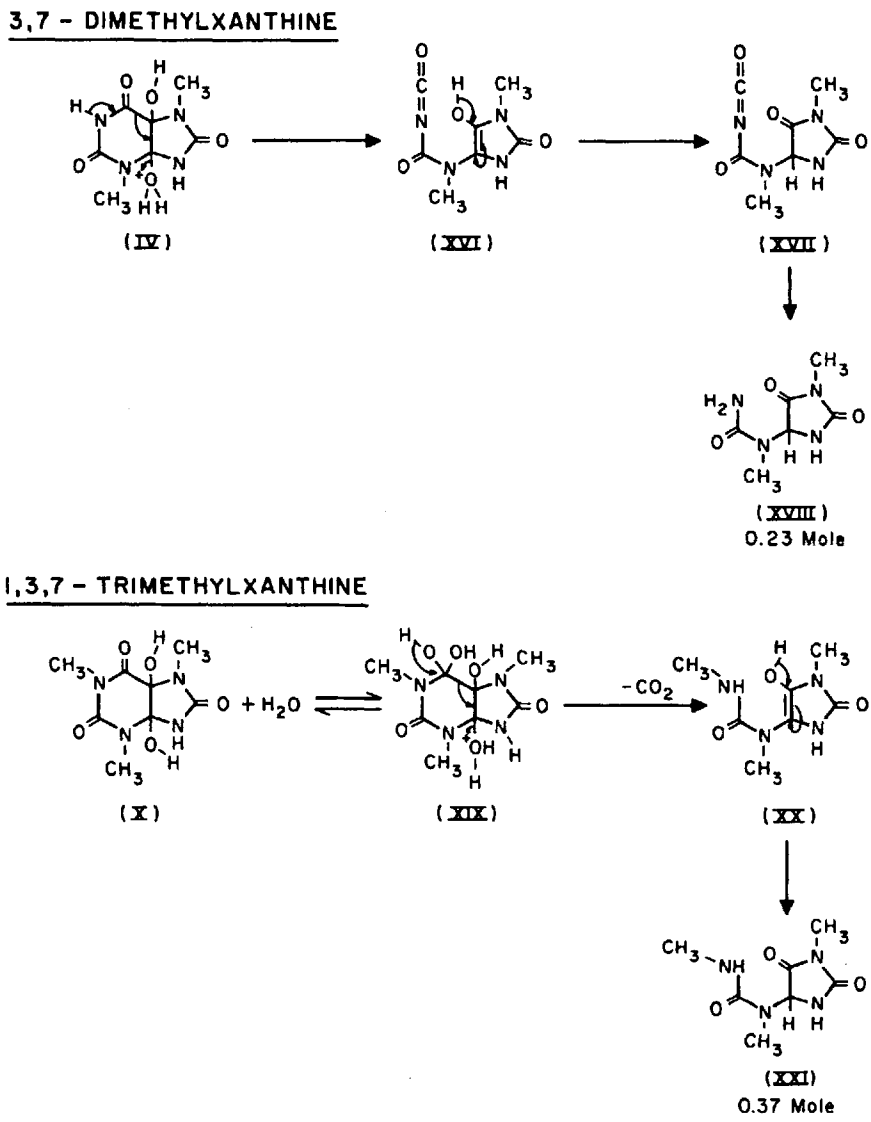


Fig. 12. Mechanism for formation of allantoins from the methylated uric acid-4,5-diol derived from theobromine and caffeine. Molar amounts of products are those formed in 1 *M* HOAc

tained *via* intermediates Vb and V1, although in this case the N-methyl-parabanic acid that results would originate from the pyrimidine ring moiety

of the original compound. In the case of caffeine only dimethylparabanic acid is produced [37]. A mechanism analogous to IV → Va (Fig. 11) is not possible with caffeine which is methylated at N_1, the only route to parabanic acid is first by opening of the N_3–C_4 bond of X (Fig. 11) to give XI followed by ring closure to XII. This, upon protonation and fragmentation, would give 2-oxy-1,3-dimethyl-4,5-dihydroxyimidazole (XIII) which should be readily oxidized to dimethyl parabanic acid (XV). The isocyanate (XIV) would hydrolyze to CO_2 and methylurea.

Formation of methylated allantoins from the uric acid-4,5-diols (IV, X, Fig. 11) would likely proceed by different mechanisms. Protonation of the diol (IV, Fig. 12) derived from theobromine would lead to ring opening at the C_6–C_5 position giving an imidazole isocyanate (XVI, Fig. 12). This could readily form XVII which after hydrolysis and loss of CO_2 would give dimethylallantoin (XVIII). On the other hand, the uric acid diol derived from caffeine (X, Fig. 12) cannot fragment by this mechanism. Accordingly, either or both of the processes could occur *via* the form of the diol hydrated at the C_6 carbonyl group (XIX, Fig. 12) which could readily lose CO_2 to give XX followed by rearrangement to trimethylallantoin (XXI).

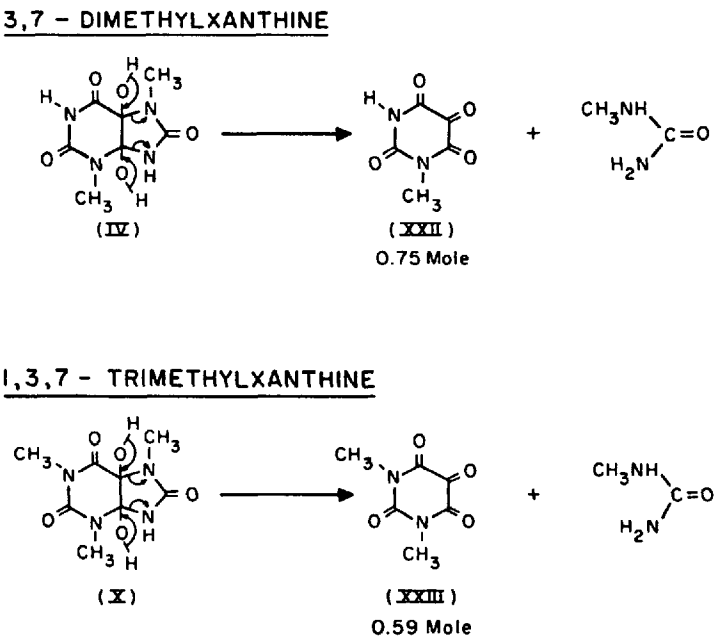


Fig. 13. Proposed mechanism for formation of alloxans from the methylated uric acid-4,5-diol derived from theobromine and caffeine. Molar amounts of products are those formed in 1 *M* HOAc

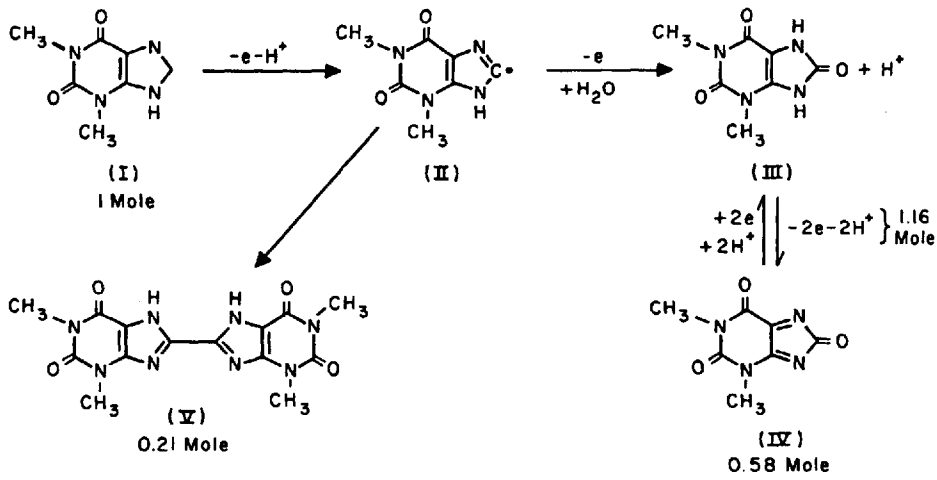
Secondary rearrangement of the uric acid-4,5-diol derived from theobromine or caffeine to give a methylated alloxan is shown in IV → XXII and X → XXIII, Fig. 13 respectively.

Hansen and Dryhurst [39] found that theophylline (1,3-dimethylxanthine) in 1 *M* HOAc is electrochemically oxidized by way of a single, pH-dependent (Table 1) voltammetric peak at the PGE in a process that involves about 3*e* per molecule of theophylline oxidized. The products and their quantitative yields are shown in Fig. 14. These products differ from those obtained from other xanthines in that a theophylline dimer, 8-(1,3-dimethylxanthyl)-1,3-dimethylxanthine V, Fig. 14 is formed. All of the remaining products are similar to those obtained from other xanthines with the obvious methylation differences. In view of dimer formation it is obvious that the first two electrons must be removed from theophylline (I, Fig. 14) in a stepwise manner, resulting first in formation of a free radical (II, Fig. 14). About 40 per cent of the free radical dimerizes to give V, while the remainder is further oxidized to 1,3-dimethyl uric acid (III). This is then further oxidized to the bis-imine (IV). The bis-imine is susceptible to hydrolysis, but is sufficiently stable to be detected by fast sweep cyclic voltammetry as a cathodic peak corresponding to reaction IV → III. The 1,3-dimethyl uric acid (III) so formed can be detected cyclic voltammetrically since it is more readily oxidizable than theophylline [35,39]. Hydration of IV results in formation of 1,3-dimethyl uric acid-4,5-diol (VI) which can undergo a ring opening at the N_3–C_4 bond to give the substituted imidazole (VIIa) or its hydrated derivative (VIIIb). Protonation and fragmentation of the latter would yield dimethyl urea and CO_2 and the 4,5-dihydroxy-imidazole (VIII) which is readily electrochemically oxidized to parabanic acid (IX). Again, unsubstituted parabanic acid can only result from the original imidazole moiety of the purine ring. The hydrated form of 1,3-dimethyl uric acid (VIa) upon protonation can give rise to X which should readily form dimethylallantoin (XI). Simple cleavage of VI would yield dimethyl alloxan (XII) and urea.

3.2. Biological Oxidations

Most *in vitro* studies of xanthines have centered around the enzyme xanthine oxidase. Bergmann and co-workers [40,41] have examined the main oxidative pathways in the xanthine oxidase catalyzed oxidation of purines. The mechanism proposed by these workers [41] is that the enzyme binds a specific tautomeric form of the substrate, regardless of whether or not that form represents the major structure present in solution. It is then proposed that the purine, *e.g.*, xanthine, undergoes hydration at the N_7=C_8 double bond either prior to or simultaneously with dehydrogenation of the same position. Accordingly, the process would involve either pathway *a* or *b*, Fig. 15. Route *a* would give a lactim form of the oxidized purine, while *b* would give the cor-

A. PRIMARY ELECTRODE PROCESSES



B. SECONDARY HYDRATION, REARRANGEMENT AND OXIDATION TO PARABANIC ACID

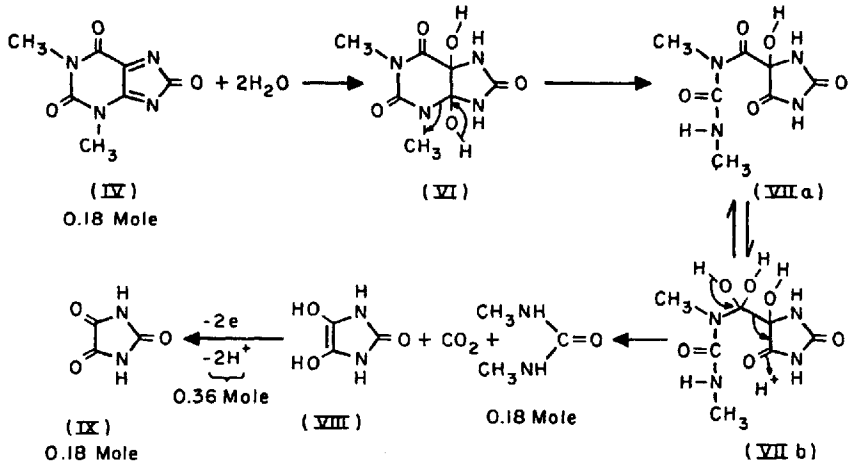
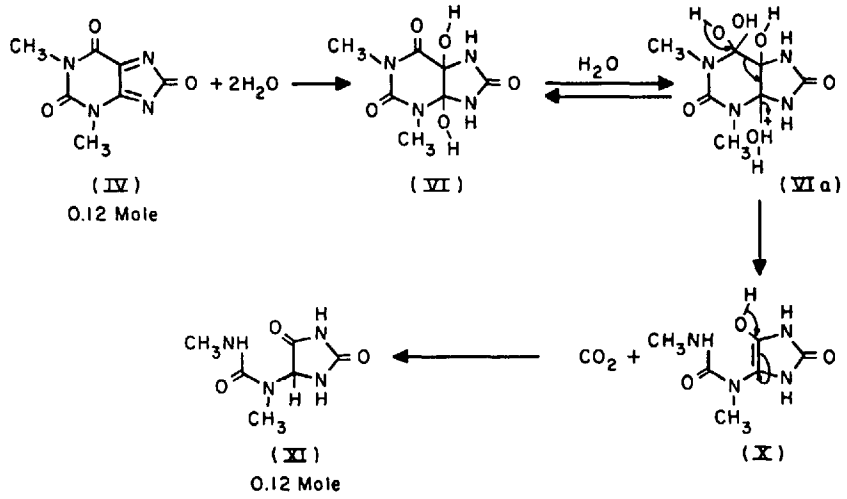
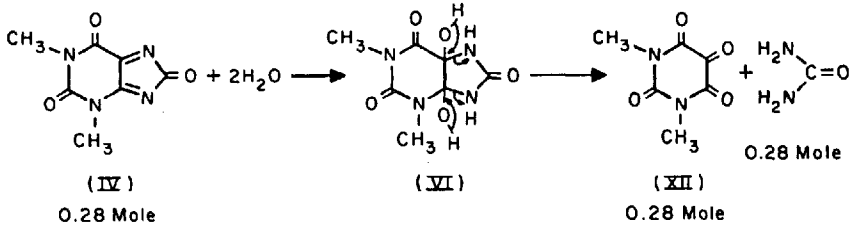


Fig. 14. Products and mechanism of the electrochemical oxidation of theo-

C. SECONDARY HYDRATION AND FRAGMENTATION TO DIMETHYL ALLANTOIN



D. SECONDARY HYDRATION AND FRAGMENTATION TO DIMETHYL ALLOXAN



phylline at the PGE. Molar amounts of products are those formed in 1 *M* HOAc

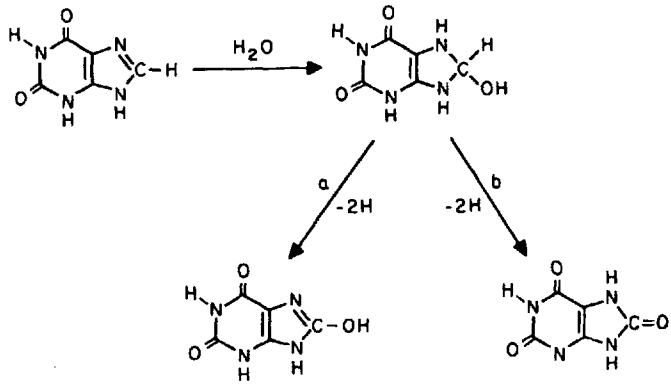


Fig. 15. Proposed generalized route for oxidation of xanthine (and other purines) by xanthine oxidase [41]

responding lactam. Many of the early studies on the metabolism of the methylated xanthines, caffeine, theophylline and theobromine in man suggested that all three compounds were oxidized to uric acid [42,43]. However, Buchanan *et al.* [44] subsequently reported that uric acid is not excreted as a result of metabolism of caffeine and theophylline, but rather that 1-methyl, 3-methyl- and 1,3-dimethyl uric acids are produced. Indeed, Brodie *et al.* [45] have shown that the primary metabolic oxidation product of theophylline is 1,3-dimethyl uruc acid. Weingeld and Christman [46] succeeded in isolating 1-methyl uric acid from human urine after ingestion of caffeine and both 1-methyl and 1,3-dimethyl uric acid after ingestion of theophylline. A report by Cornish and Christman [47] outlines the possible metabolic pathways for caffeine, theophylline and theobromine. In summary, 62 per cent of theobromine, 77 per cent of theophylline and 66 per cent of caffeine is claimed to be excreted in the form of methylxanthines and methyl uric acids within 48 h. There is a considerable amount of demethylation in man, the order of demethylation being N_3, N_7, N_1 although complete demethylation does not appear to occur.

3.3. Comparison of Electrochemistry and Biochemistry of Xanthines

There is no doubt that electrochemically xanthine is initially oxidized to uric acid, which is then further oxidized to a bis-imine that undergoes hydrolysis giving ultimately alloxan, allantoin and urea. There is no single enzyme in man that will bring about such a fragmentation of xanthine. However, there are organisms that possess a combination of enzymes, *e.g.*, xanthine oxidase and certain peroxidases, that under conditions comparable to those employed in the

electrochemical studies, would give rise to identical products, and identical mechanisms could be utilized to explain the processes.

In the case of the methylated xanthines, particularly theophylline, theobromine and caffeine, the preponderance of data on the metabolism of these compounds in man suggests that a methylated uric acid is the principal product. However, the data presented earlier proposes at best a 77 per cent accounting of the methylated xanthine administered. The question can be raised as to whether the final products observed upon electrochemical oxidation of these compounds aids these studies. Very recently studies of metabolism of caffeine have revealed that 3,6,8-trimethylallantoin is a metabolite of caffeine [48]. This methylated allantoin is, of course, a major product observed electrochemically. The mechanism developed for the electrochemical oxidation seems to nicely rationalize the observed products and electrochemical behavior. The mechanism of biological oxidation could well be very similar, although insufficient work has yet been performed to come to any definite conclusions. There is however, one major difference between the electrochemical and biological reactions which is concerned with the fact that in the former situation no demethylation occurs whereas in the latter systems considerable demethylation appears to take place.

4. Adenine

4.1. Electrochemical Oxidation

Adenine is oxidized by way of a single, pH-dependent voltammetric peak at the PGE (Table 1) [49] that involves a total of 6*e*. The probable mechanism involves three $2e - 2H^+$ oxidations of adenine (I, Fig. 16) to give 2-oxyadenine (II, Fig. 16), then 2,8-dioxyadenine and then the bis-imine (IV). The latter can be detected by fast-sweep cyclic voltammetry as a cathodic peak due to reduction of the bis-imine (IV) to 2,8-dioxyadenine (III) which in turn can be detected on the subsequent positive positive going sweep as an anodic peak [5]. Hydration of the bis-imine gives the corresponding 4,5-diol (V) which upon hydrolysis and fragmentation gives the 2-oxy-4,5-dihydroxy imidazole (V → VII) and an isocyanate (VIII). The enediol (VII) is readily oxidized to parabanic acid (X) (a major product from adenine), part of which hydrolyzes to oxaluric acid (X_a). The isocyanate (VIII) is hydrolyzed to urea, ammonia and CO_2. Similarly, fragmentation of V would lead to allantoin (V → XI → XIV). Additional products were observed in the study of Dryhurst and Elving [49] but were due to reactions occuring at the counter electrode that was necessarily present in the working electrode compartment and hence are of no interest in the present context.

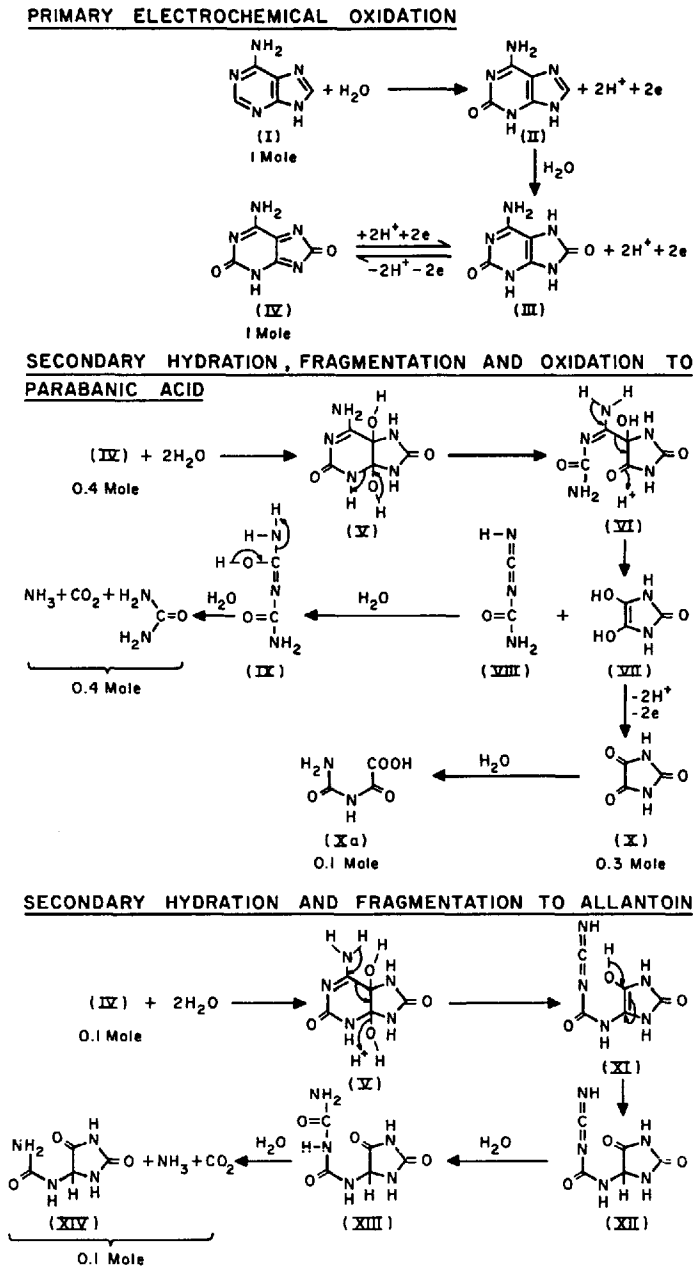


Fig. 16. Mechanism for the electrochemical oxidation of adenine at the PGE. Molar amounts of products are those formed in 1 *M* HOAc

4.2. Biological Oxidations

The oxidation of adenine in animal tissues occurs as a result of the enzyme xanthine oxidase [50-52] and the product is 2,8-dioxyadenine [52,53]. Wyngaarden and Dunn [54] consider that 8-oxyadenine is the principle intermediate in the oxidation of adenine. Bergmann and co-workers [55] came to the same conclusions. However, by examining various adenine derivatives they concluded that adenine-like compounds are oxidized in the presence of xanthine oxidase only if at least one hydrogen atom is present on the 6-amino group and provided that the imidazole ring contains a free N–H group. The biochemistry of adenine is extensively covered in the reviews of Lister [56], Robins [57], and Balis [58].

4.3. Correlations between Electrochemical and Biological Oxidation of Adenine

The initial electrochemical and biological oxidation with xanthine oxidase are essentially identical. However, electrochemically 2,8-dioxyadenine the final product in the presence of xanthine oxidase is much more readily oxidizable than adenine [59] so that considerable further oxidation occurs. To the authors knowledge, 2,8-dioxyadenine is not a major metabolite of adenine in man or other higher organisms. Accordingly, it is likely that other enzymes accomplish further degradation of 2,8-dioxyadenine. The relationship between the products so formed and the mechanism of the reaction to the related electrochemical processes has yet to be studied.

5. Guanine

5.1. Electrochemical Oxidation

Guanine shows a single, pH-dependent voltammetric oxidation peak at the PGE (Table 1) [6]. Examination of the reaction by linear and cyclic sweep voltammetry and by controlled potential electrolysis reveals that guanine (I, Fig. 17) is oxidized by an initial $2e - 2H^+$ oxidation at the $-N_9{=}C_8$-bond to give 8-oxyguanine (II, Fig. 17) which is immediately further oxidized in a further $2e - 2H^+$ process to a bis-imine (III). Hydration of III gives a 4,5-diol (IV) which can rearrange to the enediol (VI) and isocyanate (VII) as shown in Fig. 17. Secondary electrochemical oxidation of VI gives parabanic acid. Hydrolysis of the isocyanate VII gives guanidine and CO_2. Hydrolysis and fragmentation of IV results in the formation of oxalyl guanidine (XII) and CO_2. Close to $4.7e$ are transferred during the oxidation of guanine, which is accounted for very nicely by $4e$ involved in the primary electron transfer processes (I → II → III, Fig. 17) plus the extra electrons required to oxidize 2-oxy-4,5-dihydroxy-

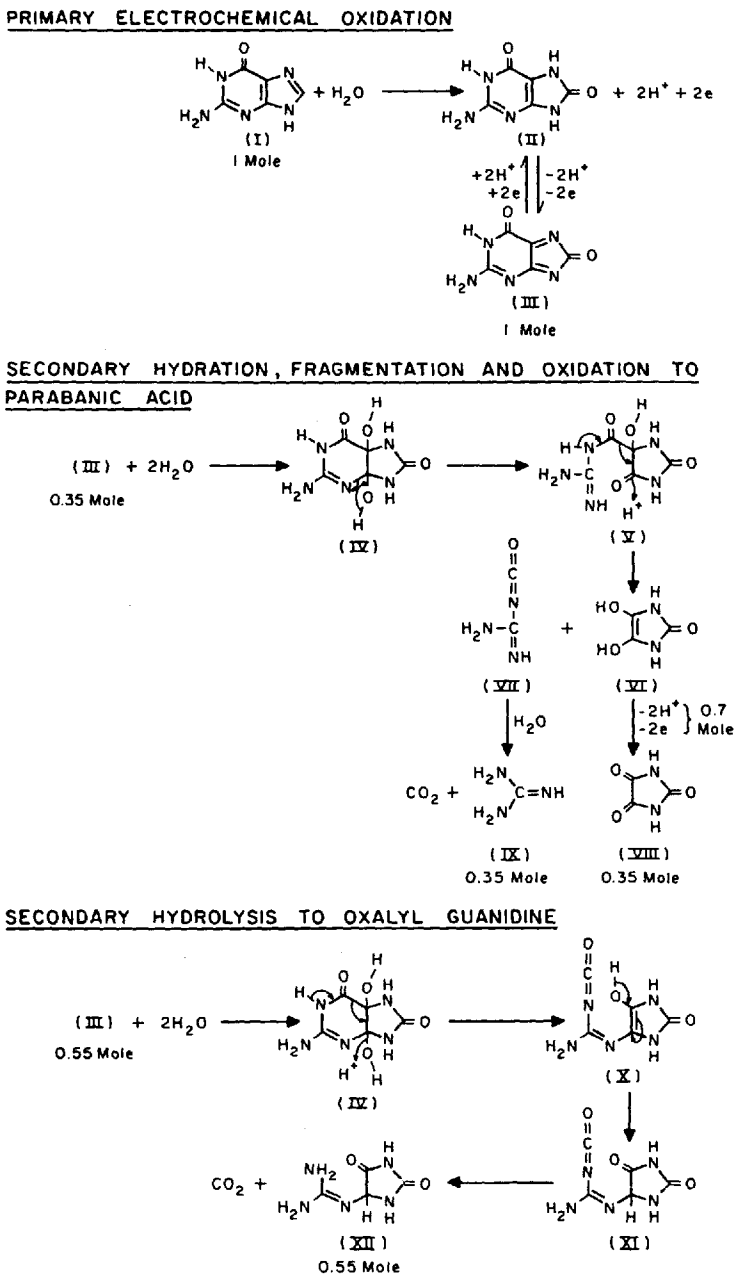


Fig. 17. Mechanism for the electrochemical oxidation of guanine at the PGE. Molar amounts of products are those formed in 1 *M* HOAc

imidazole (VI) to parabanic acid (VIII). The bis-imine (III) can be readily detected by cyclic volammetry [6].

5.2. Biological Oxidations

Very little work has been reported on the mode of biological oxidation of guanine. In many animals guanine appears to be converted in part to allantion [60], although in xanthinuric man it is primarily oxidized to xanthine [61]. Since 8-oxyguanine is known to exist in nature [62,62], Wyngaarden [64] examined the possibility of xanthine oxidase being the agent responsible for such oxidation of guanine. In the presence of very large amounts of xanthine oxidase guanine was slowly oxidized but not to 8-oxyguanine rather to uric acid. It was therefore concluded that the xanthine oxidase was contaminated with guanase which is responsible for the deamination process.

5.3. Correlation between Electrochemical and Biological Oxidation Guanine

There is really too little information on the biological oxidation of guanine to attempt to compare it with the electrochemical oxidation. It might be useful to bear in mind the electrochemical mechanism when further studies of the biological oxidation of this compound are carried out.

6. 6-Thiopurine

6.1. Electrochemical Oxidation

6-Thiopurine (6-mercaptopurine) gives rise to three pH-dependent voltammetric oxidation peaks at the PGE (Table 1) [65]. The first, least positive peak is an adsorption pre-peak due to the one-electron oxidation of 6-thiopurine (I, Fig. 18) to an adsorbed layer of product, bis (6-purinyl) disulfide (III, Fig. 18) presumably *via* a free radical (II). The second peak is the same reaction except the product is the dissolved form of III. At low pH a further slow chemical oxidation of the disulfide occurs to give, probably, either a sulfone (IVa) or a sulfoxide (IVb). At higher pH, *e.g.*, at pH 9, in an ammonia buffer, the disulfide (III) decomposes rapidly under conditions of prolonged electrolysis to give 75 per cent of the original 6-thiopurine and 25 per cent of a mixture of purine-6-sulfinic acid (V) and purine-6-sulfonamide (VI), so that a type of cyclic process occurs and for complete oxidation of 6-thiopurine close to 4*e* are transferred. The third, most positive peak is observed only at high pH. In a non ammonia-containing buffer such as carbonate pH 9 a straightforward $6e - 6H^+$ oxidation to purine-6-sulfonic acid (VII) occurs, while in an ammonia buffer of the same pH a mixture of purine-6-sulfinic acid

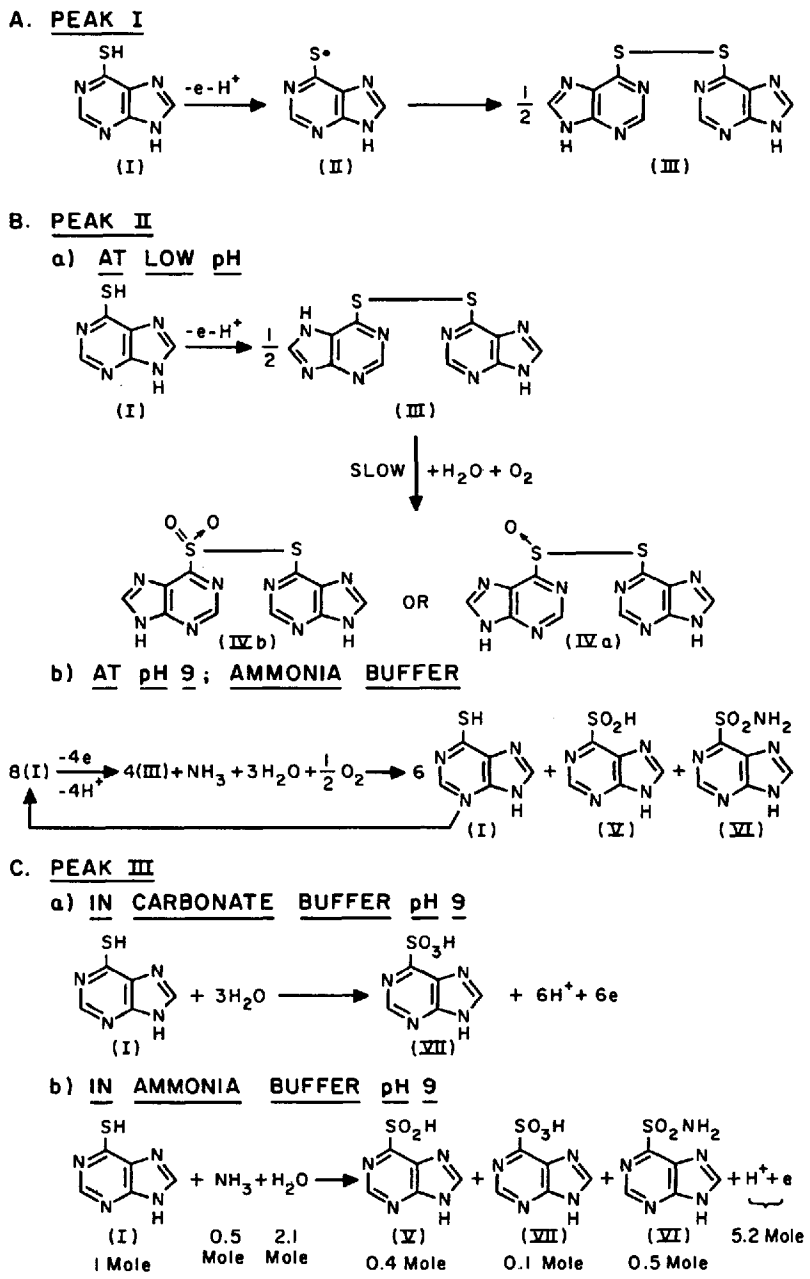


Fig. 18. Mechanism for the electrochemical oxidation of 6-thiopurine at the PGE

(V), purine-6-sulfonic acid (VII) and purine-6-sulfonamide are produced with around 5.2*e* being transferred.

6.2. Biological Oxidation

6-Thiopurine does not occur naturally, but is one of the most effective drugs available for the treatment of acute leukemia [66]. A discussion of the therapeutic action of 6-thiopurine is presented elsewhere [67]. The mechanism of metabolic breakdown of 6-thiopurine even in terms of a complete picture of its metabolites in man and other organisms is apparently not known. However various studies in man have revealed that 6-thiopurine (I, Fig. 19) is at least partially oxidized to 6-thiouric acid (II, Fig. 19) although inorganic sulfate anf other unidentified products are obtained [68,69]. Xanthine oxidase catalyzes formation of 6-thiouric acid as a major metabolite of 6-thiopurine in bacteria [70], mice [71] and man [69]. Bergmann and Ungar [72] have shown that 6-thiopurine is attacked, in the presence of xanthine oxidase, first at C_8 then at C_2. However, in the purine oxidizing system *pseudomonas aeroginosa* 6-thiopurine is attacked first at C_2 then at C_8, but further oxidation to unidentified products occurs [73].

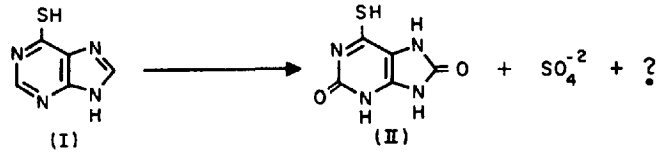


Fig. 19. Partial description of metabolism of 6-thiopurine in mammals

6.3. Correlations between Electrochemical and Biological Oxidation of 6-Thiopurine

Electrochemically all thiopurines that have been studied (6-thiopurine, 2-thiopurine [74], 2,6-dithiopurine [75] (Table 1) are oxidized *only* at the exocyclic sulfur group. The reported biological oxidations of these compounds all indicate that oxidation occurs at the ring C=N double bonds. However, electrochemical studies have been limited to three compounds so far, it is possible that other thiopurines might behave differently. The small amount of data and incomplete metabolic information, on 6-thiopurine in particular, certainly does not rule out the possibility of oxidations of the exocyclic thiol grouping occurring. Again, therefore, it is interesting to speculate that some of the electrooxidation products might well be the missing biological products. Elec-

trochemical methods have been developed to analyze for mixtures of these potential metabolites [76].

7. Adsorption of Purines at Charged Electrode Surfaces

A number of reports have appeared concerned with the adsorption of purines at a dropping mercury electrode [77-80] but these are confined to studies at potentials far removed from those where electrochemical oxidation occurs. More recently some qualitative studies on the adsorption of certain purines at the PGE have appeared with a view to understanding the adsorption of these compounds at positively charged electrodes. Since many biological reactions occur at charged membrane or ribosomal surfaces it is of considerable interest to investigate these phenomena.

By use of linear sweep volammetry at various scan rates, concentration studies and AC voltammetry, Dryhurst [81] has shown that guanine is quite strongly adsorbed at the PGE before it is electrochemically oxidized and that possibly at least one product of the oxidation is adsorbed to some extent. Significantly however, in the presence of guanosine (the nucleoside of guanine) adsorbed guanine is displaced from the electrode surface, *i.e.*, guanosine is apparently more strongly adsorbed than guanine. In a very similar manner adenine is also adsorbed at the PGE and is also displaced from the electrode by adenosine [82]. The actual biological significance of these findings is not yet clear. It is interesting to speculate whether nucleotides will displace adsorbed nucleosides from a positively charged electrode surface and whether these findings have any implications on the relative reactivity or susceptibility to reaction at charged biological membrane surfaces.

An interesting study related to these findings has been reported by Dryhurst and De [83] who examined the adsorption of uric acid an the PGE and the effect of allopurinol (4-oxypyrazolo [3,4-d] pyrimidine) on this adsorption. Allopurinol is a drug that is widely used for the treatment of gout [84] (*vide supra*) and functions by inhibiting xanthine oxidase which is the enzyme responsible for oxidizing many purines to uric acid. The investigation was carried out in order to develop an analytical method for determination of allopurinol and uric acid. It is found that uric acid, which is very easily oxidized (Table 1) is strongly adsorbed at the PGE. However, addition of allopurinal to a solution of uric acid results in a displacement of adsorbed uric acid from the electrode surface, presumably because allopurinal is more strongly adsorbed. The same effect is not apparent at negatively charged mercury surfaces [83]. These findings could be significant in retrospect since it may indicate that the facility of a purine to be oxidized or otherwise chemically altered at an enzyme or other interface may be dependent not only upon stereochemical factors but also on the charged nature of the catalytic or reactive surface.

8. Conclusions

In the case of uric acid there is an impressive degree of parallelism between the enzymatic and electrochemical products. The electrochemical information has clearly demonstrated the existance of unstable products and the nature of at least some of these products, and has allowed a much more detailed mechanism to be written. In the case of less oxygenated purines the initial positions of electrochemical and enzymatic (xanthine oxidase) attack appear to be the same. However, on reaching the point where the purine ring is oxidized at all unsubstituted C=N bonds xanthine oxidase for example ceases to promote any further reaction, while electrochemically the C_4=C_5 double bond is further oxidized. This electrochemical behavior parallels closely that expected with dual enzyme systems, *e.g.*, xanthine in the presence of xanthine oxidase and uricase or a suitable peroxidase. In the case of methylated xanthines it is felt that the electrochemical data should aid in unravelling the total metabolite picture, and suggest possible mechanisms. Indeed, as indicated earlier, it is possible that the presently incomplete knowledge of metabolsm of caffeine might be completed using information gathered electrochemically.

In the case of the thiopurines the electrochemical processes do not appear to agree at all with the known biological oxidations. However, again in the case of 6-thiopurine not even a complete picture of the metabolites is available. The electrochemical data indicates that thiopurines are very readily oxidized to disulfides and hence to sulfinic or sulfonic acids. In view of well-known sulfide-disulfide transformations in biological situations (*e.g.*, L-cysteine to L-cystine), it is not unlikely that part of the metabolic degradation pathway for thiopurines might proceed *via* reactions of the sulfide moiety.

In conclusion therefore it is felt that electrochemistry does offer a valuable technique to study the electron-transfer reactions of biologically-important molecules. The mechanisms and products observed electrochemically do appear to be similar in many instances to those of the biological reactions. In cases where the biological products or mechanisms are not known electrochemical studies should prove useful in suggesting potential reaction routes and products.

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