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Marine Natural Products – Diversity and Biosynthesis

Editor: Paul J. Scheuer

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Preface

Marine Natural Products – Diversity and Biosynthesis contains four chapters, which review some of the most lively research topics in the field, all written by active contributors.

Steroid chemistry, the glamor area of natural product research in the years following World War II, was replaced by macrolides, alkaloids, and prostanoids. Sterol chemistry again became exciting when an unanticipated rich diversity of marine invertebrate sterols were isolated, notably from sponges. After pioneering research in Italy, the field was reborn in Carl Djerassi's laboratory at Stanford. As a result, we now have a better and more detailed knowledge of sterol biosynthesis than of any other class of marine natural products. The authors of Chapter 1 are Stanford alumni.

Naturally occurring isocyano compounds are no longer an oddity since their frequent isolation from members of the class of Demospongiae. Their biogenesis has intrigued chemists ever since the first terrestrial isocyano compound (an amino-acid derivative) was described from a micro-organism. All known marine isocyano compounds are terpenoid and their biosynthesis continues to pose an unsolved problem, which is discussed in Chapter 2.

Early natural product research with marine mollusks supported a conclusion that all secondary metabolites of mollusks are diet-derived, which proved to be premature. In fact, the biosynthetic capability of some marine mollusks covers a wide spectrum of chemical classes and substances. Much of this recent insight has resulted from work in the laboratories of the authors of Chapter 3.

Oxylipin is a term that has not yet fully entered the scientific vocabulary. Indeed, a new word for oxidized fatty acid-derived compounds was needed, as the older terms – prostanoids, eicosanoids – proved to be too narrow for an ever-widening spectrum of discoveries. Much of this research has come from the laboratory of the authors of Chapter 4. Their account demonstrates the

diversity and highlights some of the unique biosynthetic pathways of oxylipins.

I should like to thank all authors for their contributions and Springer-Verlag for including the volume in their Topics in Current Chemistry series.

Honolulu, April 1993 Paul J. Scheuer

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Biosynthesis of Marine Sterols

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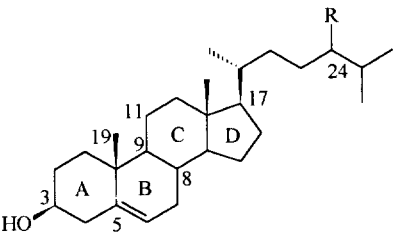
Bill J. Baker and Russell G. Kerr

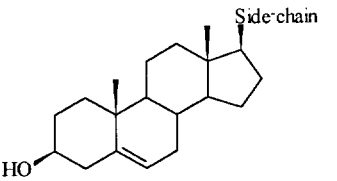
Marine sterols have been the subject of intense research since Bergmann first discovered that marine organisms elaborate unique sterols. Much of this research has been directed at the isolation and characterization of novel marine sterols, but recently studies have begun to investigate their biosynthetic origin. The study of an organism and its ability to perform de novo sterol biosynthesis, as well as its metabolic processes such as alkylation, dealkylation, and rearrangement, has provided insight into sterol sequestration and utilization. These efforts to understand the biosynthetic origin of marine sterols are reviewed.

1 Introduction

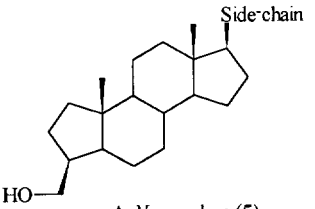
The surge of interest in marine sterols, stimulated by some several hundred new and unique sterol structures found in marine invertebrates over the last two



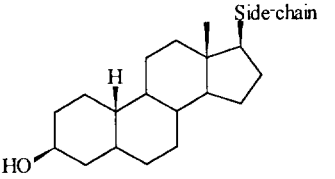
R = H, Cholesterol (**1**)
R = Me, 24-Methylcholesterol (**2**)
R = Et, 24-Ethylcholesterol (**3**)



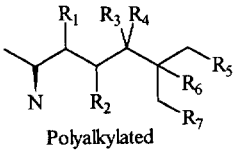
Conventional, Δ^5, sterol nucleus (**4**)



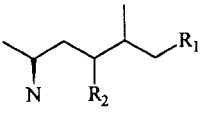
A-*Nor* nucleus (**5**)



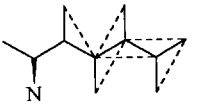
19-*Nor* nucleus (**6**)



Polyalkylated side-chains (**7**)



26-*Nor* side-chains (**8**)



Cyclopropa(e)ne side-chains (**9**)

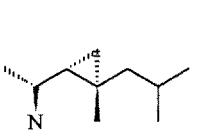
Fig. 1. Terrestrial (**1–3**) and marine (**4–9**) sterols

decades, has brought with it an interest in their biosynthetic origin. Sterols play a critical role in both the physiology and biochemistry of all eukaryotic organisms [1]; in higher animals cholesterol (**1**) is required as a cell membrane component, as are 24-alkylated sterols (**2, 3**) in plants (Fig. 1). Steroid hormones regulate physiological processes involved in reproduction, maturation, and related phenomena. There is evidence for ecological roles of sterols in the marine environment [2]. Efforts to explain the origin and function of unique marine sterols have led to the development of new biosynthetic techniques that address the difficulties found in marine systems; many of these techniques will have applicability in studying the biosynthesis of other marine-derived structures. In our report below we will attempt to summarize the wealth of information that has been gained about marine sterol biosynthesis and the concomitant understanding about the interaction of marine organisms that results from these studies. The field has been reviewed periodically [3, 4, 5] and several recent reviews address some specific aspects of marine sterol biosynthesis, such as sponge sterol biosynthesis [6, 7] and sponge phospholipid biosynthesis [8]; a report on sterols in the marine environment has appeared [2].

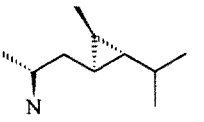
Marine organisms frequently contain a complex mixture of sterols. The biosynthetic origin of these compounds is complicated by the fact that there are four possible sources. The sources by which marine invertebrates may obtain sterols, first described by Goad [3], are: (1) de novo biosynthesis, (2) assimilation from diet, (3) modification of dietary sterols and (4) assimilation of symbiont-produced sterols or sterol precursors. In any examination of the biosynthetic origin(s) of sterols one must consider all of these factors; needless to say, a complex picture is emerging.

2 On the Uniqueness of Marine Sterols

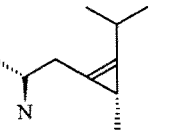
While terrestrial plant and animal monohydroxy sterols are relatively simple, sterols from marine organisms can take on an enormous variety of substitutions and rearrangements. A single general formula (Fig. 1) can describe most terrestrial sterols (**1–3**). No single template will suffice for marine sterols; three sub-structures (**4–6**) for sterol nuclei have been proposed by Djerassi [7] to describe the marine monohydroxy sterols, and these nuclei are accompanied by a wide spectrum of side-chains (**7–9**) (Fig. 1); perhaps the most intriguing marine sterols include cyclopropane (**10, 11**) – and cyclopropene (**12, 13**) – containing sterols (Fig. 2) and the biosynthetically related acetylenes (**14, 15**). Additional variation of the sterol nucleus can be found in the various *seco*-sterols such as the 9,11-*seco*-sterols (**16**) [2] and the recently described 8,9-seco sterols **17** [9] (Fig. 3). Other unique marine-derived sterols [2] are the highly degraded sterols **18** and **19** (Fig. 3) isolated from the sponge *Dictyonella incisa*; sterols from echinoderms with up to nine hydroxy (or acetoxy) groups (**20**); and sterols with



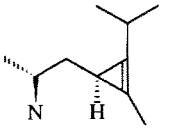
23-methyl-22,23-methylene-cholesterol (**10**)



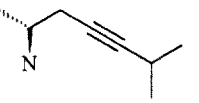
Dihydrocalysterol (**11**)



Calysterol (**12**)



23H-Isocalysterol (**13**)



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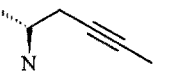

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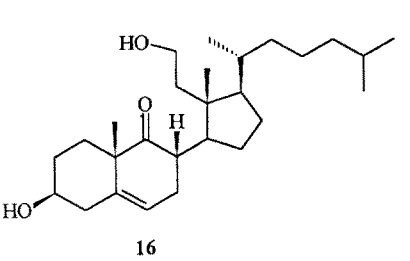
Fig. 2. Marine sterol side-chains (*N* = nucleus **4**)



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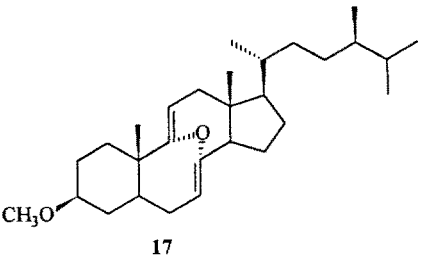

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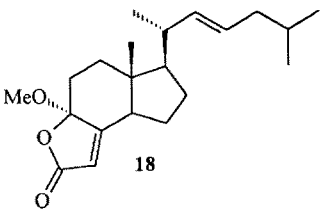

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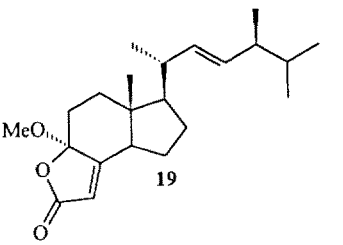

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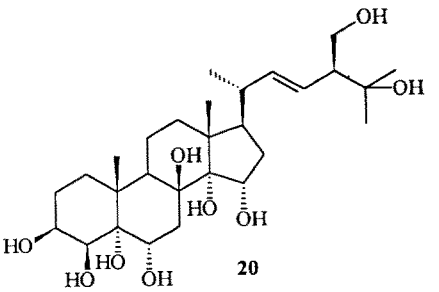

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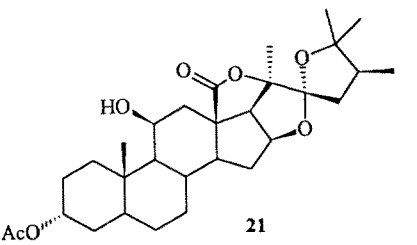

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Fig. 3. Unusual marine steroids

highly oxygenated side-chains, such as **21**, isolated from the gorgonian *Isis hippuris*. These unique sterol structures (Figs. 1–3) illustrate the far more complex sterol biosynthetic capability found in the marine realm. Given the complex and varied sterol mixtures of marine organisms, biosynthetic relationships and metabolic pathways are exciting targets for study.

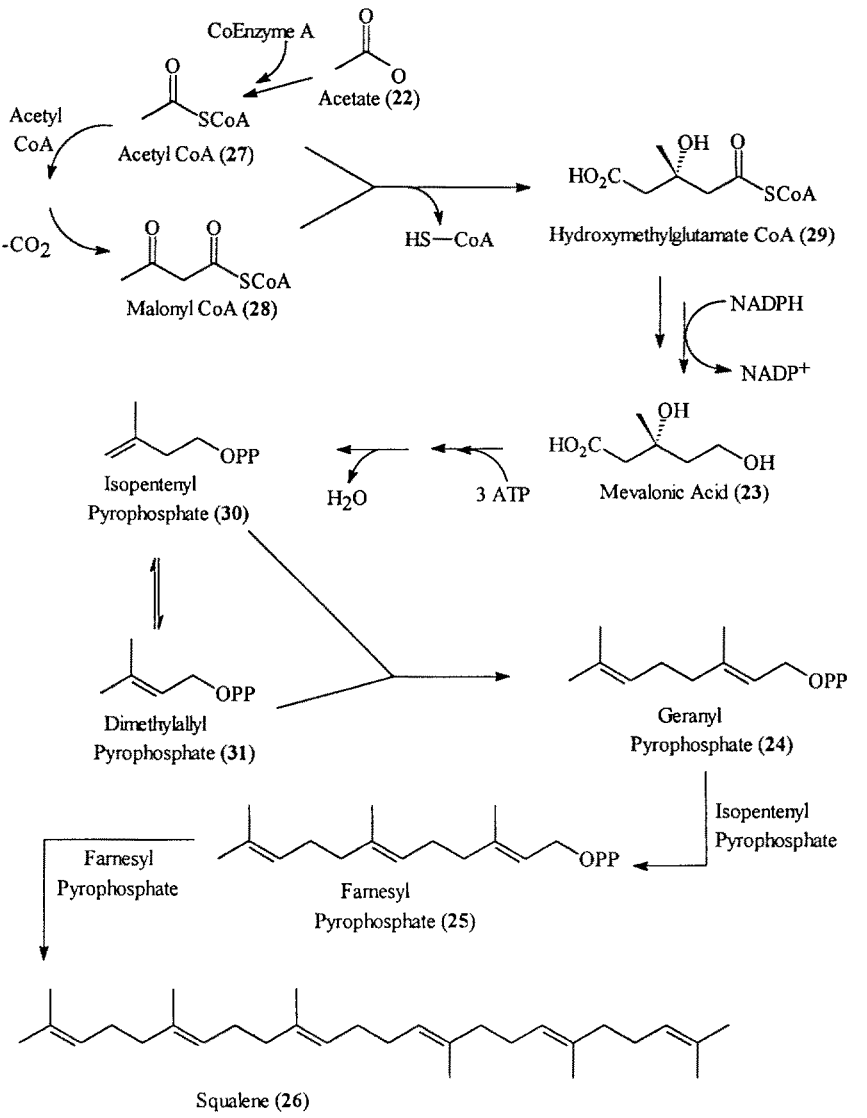
It has been suggested [6] that these unusual sterols, especially in those cases where these unusual sterols comprise the entire sterol content of the organisms, likely replace "conventional" sterols as cell-membrane components. Evidence for this comes from subcellular fractionation and subsequent analysis of two marine sponges [10]. The sterol composition of the membrane isolates was found to be identical to that of the intact sponge. Most common variation of the marine sterol is in the side-chain, situated deep in the lipophylic environment of the phospholipid bilayer. This suggests that unusual fatty acids might accompany the sterols, and indeed this is often the case [8].

3 Biosynthesis of Sterol-Related Triterpenes – De Novo Sterol Biosynthesis

One of the fundamental questions in sterol metabolism is whether an organism is capable of de novo sterol biosynthesis. This metabolic capability has been addressed in most of the common marine phyla using a number of different techniques. De novo sterol biosynthesis in higher organisms has been studied in great detail. In brief, this process involves the conversion of acetate (**22**) through mevalonate (**23**), geranyl pyrophosphate (**24**), farnesyl pyrophosphate (**25**) to the acyclic triterpene squalene (**26**) (Scheme 1) [11]. Photosynthetic and non-photosynthetic organisms share a common pathway to this point. A bifurcation in sterol biosynthesis occurs at the step of squalene cyclization. In photosynthetic organisms, cycloartenol (**32**) is the primary cyclization product, while in non-photosynthetic organisms, lanosterol (**33**) is formed. The metabolism of these cyclic triterpenes to sterols (Scheme 2) involves the loss of the 14α-, 4α- and 4β-methyl groups and modification of the double bond [1].

3.1 Biosynthesis from Acetate and Mevalonate

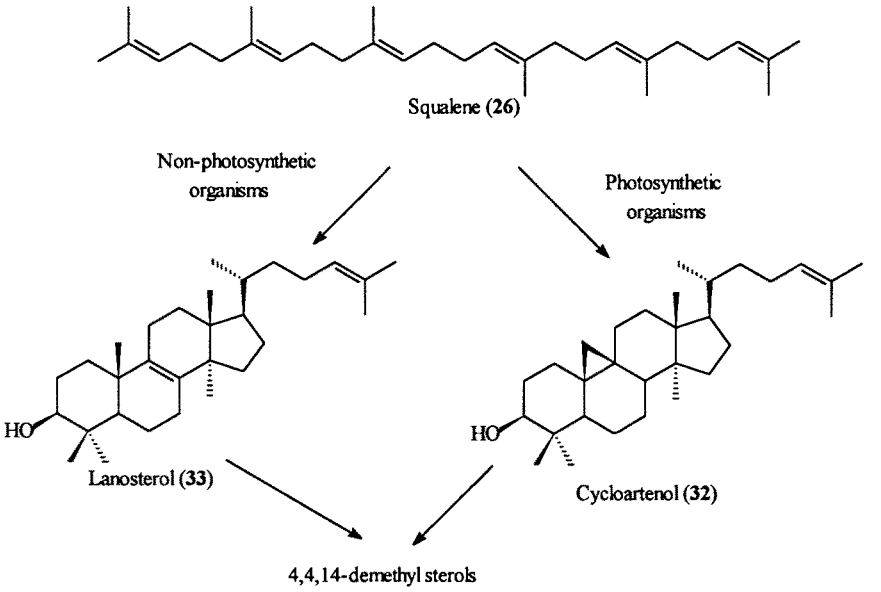
The number of organisms which have been shown to be capable of de novo biosynthesis is quite large; in fact it has recently been suggested that all sponges are capable of de novo biosynthesis of sterols [12, 13]. The early biosynthetic studies utilizing acetate (**22**) and mevalonate (**23**) [3, 12, 14] resulted in ambiguous results; these ambiguities turn out to be a result of the nature of working with these water-soluble precursors. Djerassi has suggested [7, 13] that poor uptake or alternate metabolism of these water-soluble precursors, and not their



Scheme 1. Biosynthesis of Squalene (**26**)

inability to perform de novo sterol biosynthesis has accounted for many of the inconclusive or negative results and that lipid precursors, such as squalene (**26**), are better suited for investigation of de novo sterol biosynthesis.

Utilizing such lipid precursors, several holothuroids were shown to be capable of de novo production of 4- and 4,4-demethyl sterols, but incapable of further steroid elaboration (Scheme 3) [15]. The incorporation of sterol precur-

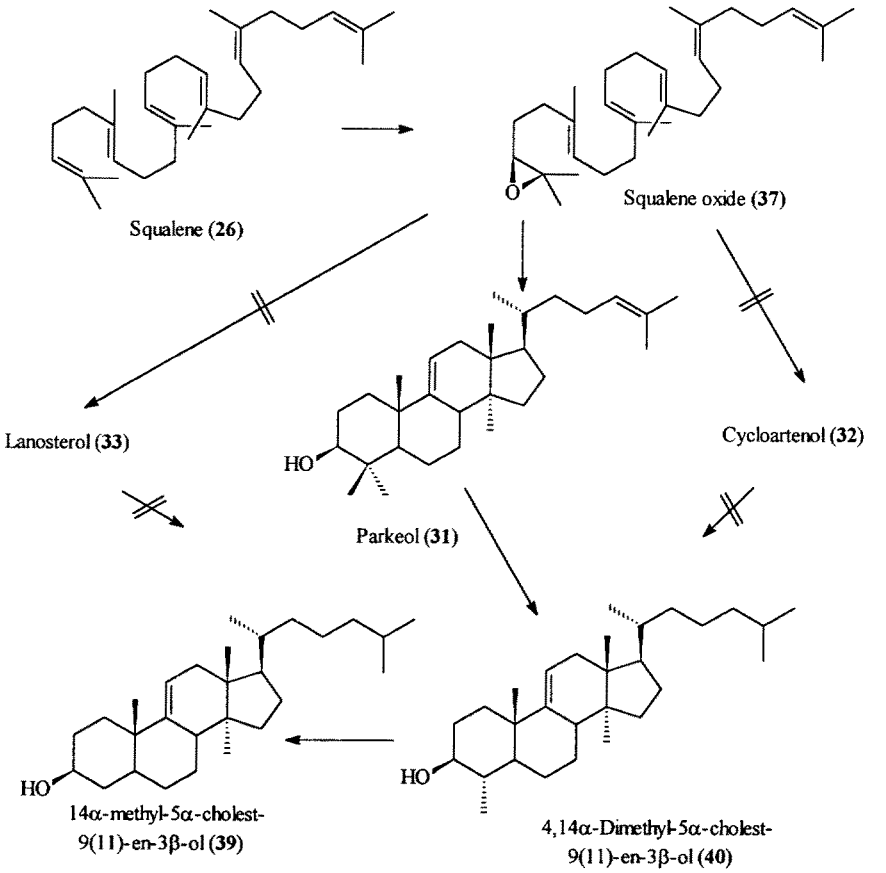


Scheme 2. Metabolism of Squalene (**26**) in photosynthetic and non-photosynthetic organisms

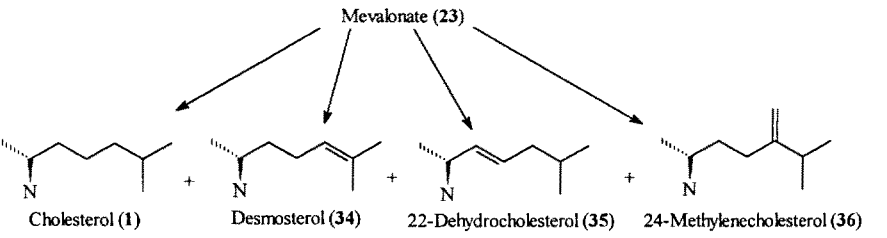
sors into crustaceans has yet to be demonstrated [5, 16]. The red alga *Rhodymenia palmata* [17] can incorporate acetate (**22**) and mevalonate (**23**). This result was also obtained with several bivalve molluscs [18]; however, lack of conversion of acetate was observed with *Mytilus edulis*, *Atrina fragilis* and *Ostrea edulis* [18]. An independent study of *Mytilus edulis* [19] however described the transformation of mevalonate to cholesterol (**1**), desmosterol (**34**), 22-dehydrocholesterol (**35**) and 24-methylenecholesterol (**36**) (Scheme 4); this was the first investigation of the biosynthetic origin of 22-dehydrocholesterol. As is evident from the two sets of experiments with *M. edulis*, it is often risky to make conclusions regarding lack of metabolic activity when an organism fails to transform a precursor. The conversion of acetate to cholesterol has also been demonstrated in the tunicates *Styela plicata*, *Microcosmus sulcatus*, *Ciona intestinallis* and *Halocynthia papillosa* [3].

3.2 Biosynthesis and Cyclization of Squalene (26)

As described earlier, the dichotomy at the point of squalene (**26**) cyclization has been used as a distinguishing feature of photosynthetic and non-photosynthetic organisms [1]. Cycloartenol (**32**) is the primary cyclization product in the former group, while in the latter, lanosterol (**33**) is initially produced (Scheme 2). Recent investigations into the cyclization of squalene in marine organisms have provided interesting results.



Scheme 3. Origin of sterols in Holothuroidea



Scheme 4. Metabolism of Mevalonate (**23**) in the clam *Mytilus edulis*

Until recently, the only marine example of cycloartenol (**32**) production was in the chrysophyte *Ochromonas* sp. [20]. A survey, documenting the products of squalene oxide (**37**) cyclization (see Scheme 3) using crude enzyme preparations of various algal phyla has recently been reported [21]. Interestingly, while all

examples from the bacillariophytes, chlorophytes, chrysophytes, cryptophytes, prymnesiophytes, raphidophytes and rhodophytes converted squalene oxide to cycloartenol, as one might expect for photosynthetic organisms, all representatives of the dinoflagellates produced lanosterol (**33**). This clear difference in metabolism is of obvious taxonomic significance; however, these results are in contrast to the idea that the mode of squalene (**26**) cyclization is linked to a distinction between photosynthetic and non-photosynthetic organisms.

Sea cucumbers (Holothuroidea, Echinodermata) appear to be unique in their mode of squalene oxide (**37**) cyclization. Tritium-labeled lanosterol (**33**), cycloartenol (**32**) and parkeol (**38**) were individually administered to the sea cucumber *Holothuria arenicola*. While the former two triterpenes were not metabolized [22], parkeol was efficiently transformed into 14α-methyl-5α-cholest-9(11)-en-3β-ol (**39**) (Scheme 3). Other Δ^7 sterols present in *H. arenicola* were not found to be radioactive and were thus assumed to be of dietary origin. The intermediacy of parkeol was confirmed by the feeding of labeled mevalonate (**23**) and squalene (**26**) to the sea cucumber *Stichopus californicus* [15]. Both precursors were transformed into parkeol, but not lanosterol nor cycloartenol, and to 4,14α-dimethyl-5α-cholest-9(11)-en-3β-ol (**40**) and 14α-methyl-5α-cholest-9(11)-en-3β-ol. Thus, while all other eukaryotes produce either cycloartenol or lanosterol, parkeol is the intermediate between triterpenes and the 14-methyl sterols in sea cucumbers.

The existence of de novo sterol biosynthesis in sponges (Porifera) was in question for a number of years, as early experiments using radiolabeled acetate (**22**) and mevalonate (**23**) yielded either no or very little radioactivity in the sterols [14, 23, 24]. Sponges were subsequently shown to be capable of this process by feeding of the cyclic triterpene lanosterol (**33**) [25]. This precursor was chosen because, unlike acetate and mevalonate, it is not water-soluble and thus the filter-feeding sponge should be better able to "extract" the precursor from the sea water. Also, acetate is a poor choice of precursor as it is efficiently used for numerous other pathways. In addition to metabolizing lanosterol, many sponges also transformed cycloartenol (**32**) to their 4,4,14-demethyl sterols [25]. Sponges are the only organisms thus far shown to be capable of transforming both lanosterol and cycloartenol. Perhaps not surprisingly, subsequent feeding experiments [26] with radiolabeled squalene (**26**) demonstrated that squalene oxide (**37**) is only cyclized to lanosterol and that the observed metabolism of cycloartenol merely reflects a lack of specificity. Evidently, cycloartenol is transformed to the 4,4,14-demethylsterols via lanosterol.

4 Metabolism of 4,4,14-Tridemethyl Sterols

4.1 Nuclear Modifications

Most common among the nuclear modifications is the saturation and desaturation that results in the many olefinic isomers, some of which are exceedingly rare

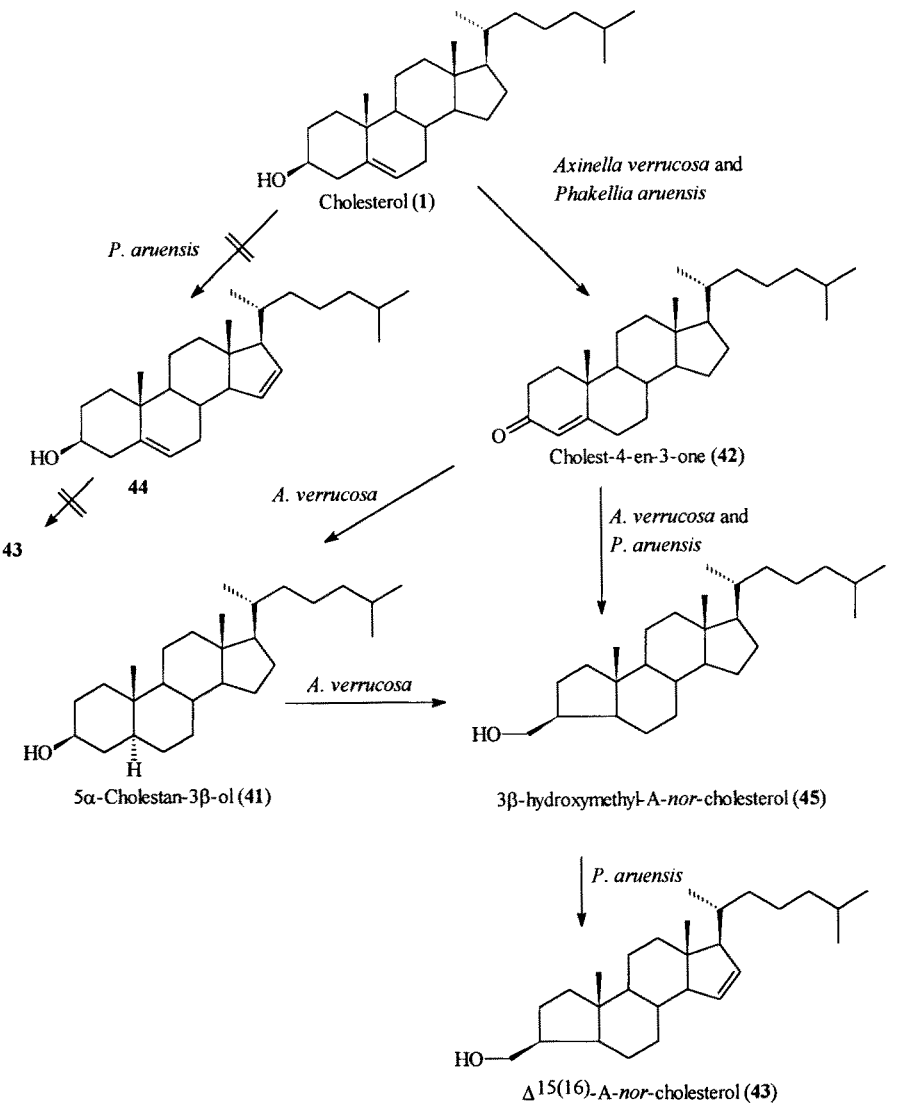
even in marine organisms [2]: isomers Δ^5, Δ^7, Δ^8, $\Delta^{9(11)}$, $\Delta^{5,7}$, $\Delta^{7,9(11)}$, $\Delta^{8,14}$, $\Delta^{14(15)}$, $\Delta^{15(16)}$, $\Delta^{5,17(20)}$, $\Delta^{8(9),14}$, $\Delta^{5,7,9(11)}$ have been reported. The ability of sponges to convert Δ^5 or Δ^7 nuclei into the corresponding $\Delta^{5,7}$ nucleus is interesting. Eukaryotic organisms produce Δ^7 sterols via isomerization of $\Delta^{8(9)}$ nuclei; however, some protozoans, echinoderms and insects convert the $\Delta^{5,7}$ nucleus, derived from the corresponding Δ^5 analog, into the Δ^7 nucleus. Regarding the ability of sponges to perform both of these operations [28], questions arise about these primitive animals; Garson [30] has suggested the assistance of symbionts. The conversions appear to be facile and mediated by specific enzymes [5]. Little is known about the biosynthesis of some interesting nuclear modifications, such as the origin of the *seco* sterols, but an understanding of A-*nor* and 19-*nor* sterol biosynthesis is at hand.

4.1.1 Ring Contractions

In a series of seminal papers on biosynthetic studies with marine organisms, Minale, Sodano and coworkers have documented the origin of the A-*nor* ring system (**5**), using radiotracer techniques [29–32]. They have shown (Scheme 5) that the ring contraction originates from cholesterol (**1**) rather than 5α-cholestan-3β-ol (**41**), which appears to be a side-product. Using the sponge *Axinella verrucosa*, which contains predominantly the A-*nor* sterols, dietary cholesterol is efficiently converted to the corresponding A-*nor* cholesterol via the intermediacy of Δ^4-cholesten-3-one (**42**). It was subsequently shown by the Djerassi group that the A-*nor* sterol bearing an unusual 15,16 double bond (**43**) derives from the corresponding A-*nor* cholesterol rather than Δ^{15} cholesterol (**44**) in the Australian sponge *Phakellia aruensis* [33]. The exact mechanism, whereby the C-2/C-3 bond is broken and a new bond formed between C-2 and C-4 is yet to be understood. Most sponges which contain A-*nor* sterols lack sterols with conventional nuclei, suggesting a highly efficient enzymatic system for the ring contraction.

4.1.2 Dealkylation

Loss of the 19-methyl group from the cholesterol nucleus (**4**) (Scheme 6) has been shown in sponges to proceed via the corresponding carboxylic acid (**46**) [34]. Early studies showed that dietary cholesterol (**1**) was converted by *Axinella polypoides* into the 19-*nor* stanol **47** [14]. Subsequent feeding experiments with a variety of putative intermediates demonstrated the stepwise conversion of 19-hydroxy (**48**) and 19-carboxylic acid (**46**), both at the 3-oxo level of oxidation, into **47**. The intermediacy of a dienolate (**46a**) is suggested to assist the decarboxylation [34]. 19-*Nor* sterols are also found in the gorgonian coral *Pseudoplexaura porosa* [35], for which no biosynthetic work has been performed. However, occurrence of 19-oxygenated sterols in two corals of the genus *Lobophytum* [36] suggests a similar mechanism.

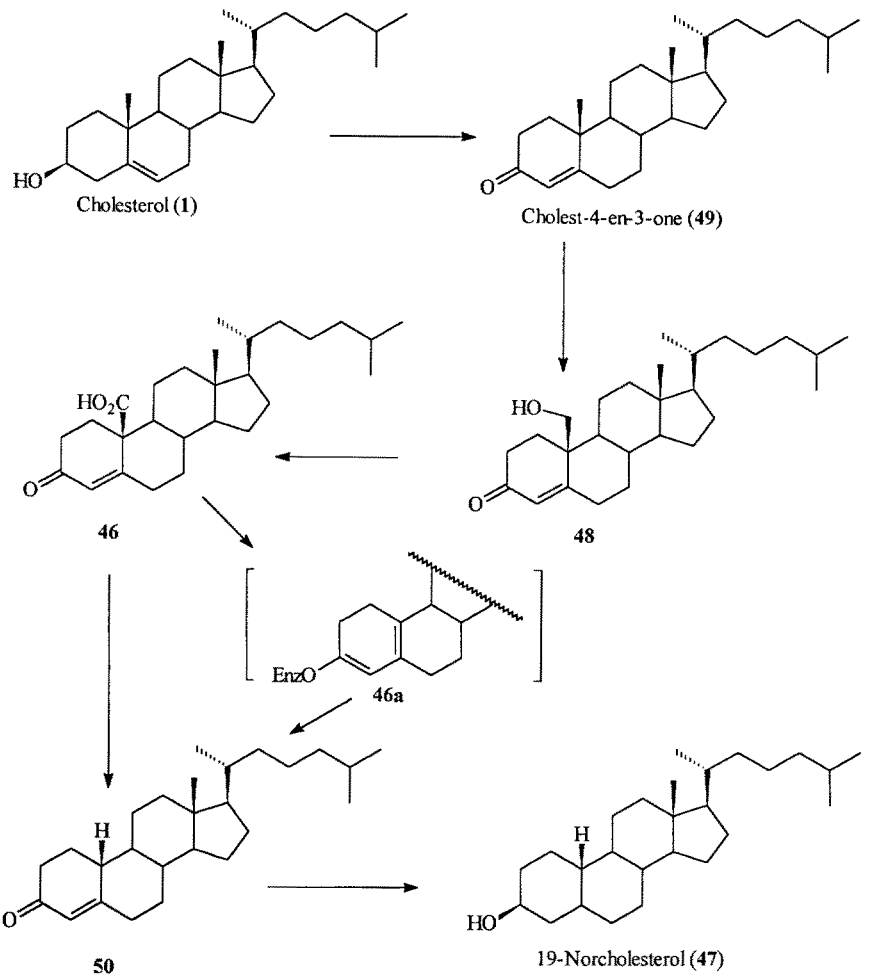


Scheme 5. Origin of the A-*nor* nucleus in sponges

4.2 Side-Chain Modifications

4.2.1 Alkylation

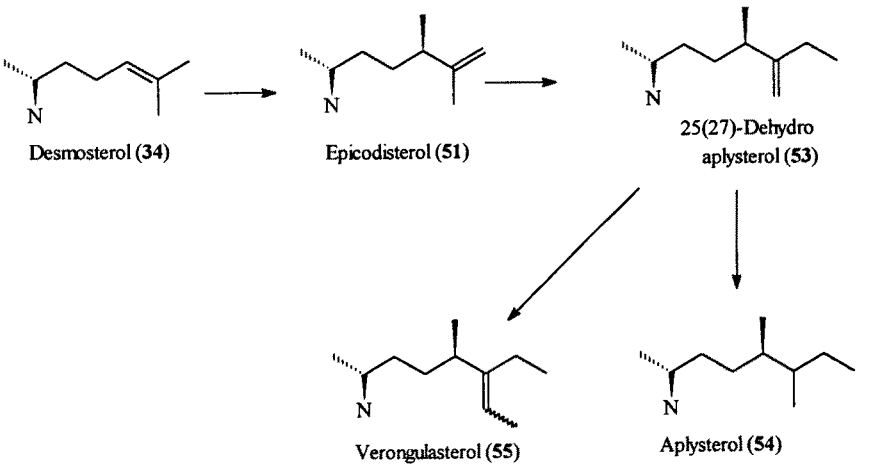
Unquestionably, the greatest amount of research activity in sterol biosynthesis has been devoted to the elucidation of metabolic origins of unconventional



Scheme 6. Biosynthesis of 19-*nor* stanols in sponges

side-chains, particularly in sponges and dinoflagellates. There are now numerous structurally intriguing sterols whose biosynthetic origin has been determined; the following discussion is not comprehensive, but is intended to give the reader an appreciation of the pathways operating in these systems. The vast majority of this work has been performed by the Djerassi group.

One of the first biosynthetic investigations undertaken in the Djerassi laboratory dealt with the sponge *Aplysina fistularis*. This sponge contains sterols with side-chain alkylations at C-24 and C-26, the aplysterols [37]. The biosynthesis of these unconventional sterols was shown (Scheme 7) to proceed by a conversion of desmosterol (**34**) to epicodisterol (**51**) [but not its C-24 epimer, codisterol (**52**)] followed by methylation to 25(27)-dehydroaplysterol (**53**). Sterol

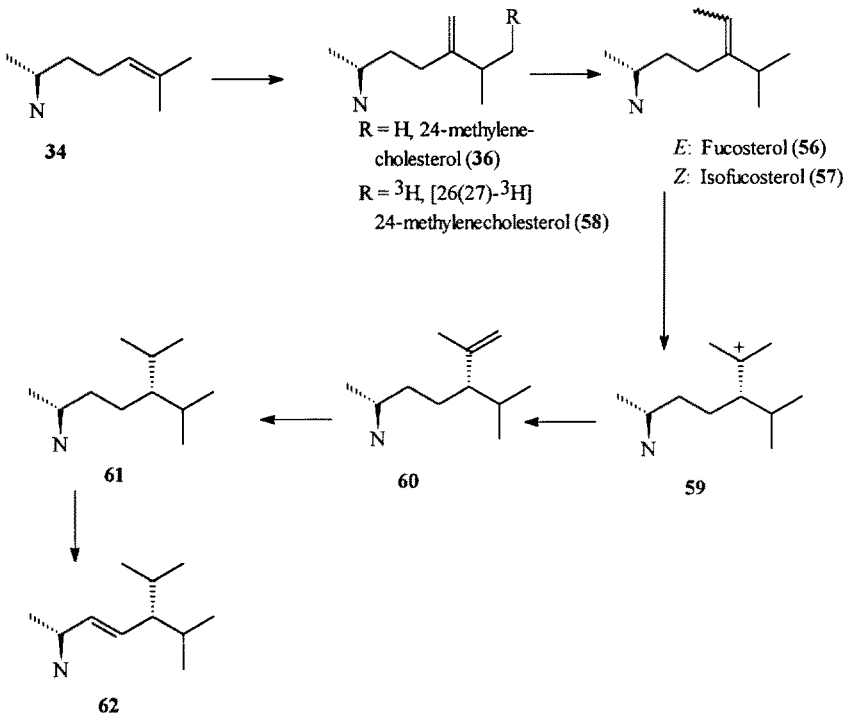


Scheme 7. Biosynthesis of the Aplysterols in *Aplysia fistularis*

53 is subsequently hydrogenated to aplysterol (**54**) or methylated to the trace sterol verongulasterol (**55**).

The Great Barrier Reef sponge *Pseudaxinyssa* sp. attracted the attention of the Djerassi laboratory as > 98% of its sterol mixture is composed of three unconventional sterols with 24-isopropyl or 24-isopropenyl side-chains [38, 39]. Furthermore, analysis of membrane fractions confirmed that these three sterols are almost exclusively associated with membranes and are thus likely to play a role in membrane function [10]. The 24-isopropylcholesterols were shown to be the result of three successive bioalkylations. The first of these two transformations occur with desmosterol (**34**) and 24-methylenecholesterol (**36**) as shown in Scheme 8. The stereospecificity in the third biomethylation is intriguing; both *E* and *Z* isomers of 24-ethylidenecholesterol [fucosterol (**56**) and isofucosterol (**57**) respectively] were transformed to the isopropylcholesterols, thus indicating no preference for configuration of the 24(28) double bond. However, biomethylation at C-28 occurred exclusively on the α-face. The regioselectivity of the proton migration (see Scheme 8) was determined by feeding of [26(27)-^{3}H]24-methylenecholesterol (**58**).

The Indo-Pacific sponge *Strongylophora durissima* is noteworthy as it contains essentially one sterol, (24*R*)-24-ethyl-27-methylcholesta-5,25-dien-3β-ol (strongylosterol, **63**). Four possible biosynthetic routes to this unconventional sterol have been proposed [41–44]. Incorporation experiments with ten radiolabeled precursors were carried out, which demonstrated that only one of the four hypothetical biosynthetic routes is operational. As summarized in Scheme 9 [44], this pathway involves three successive bioalkylations of desmosterol (**34**), codisterol (**52**) and 24(28)-dehydroaplysterol (**64**). No stereoselectivity at C-25 was observed, yet high selectivity at C-24 was found, as epicodisterol (**51**) was not metabolized.

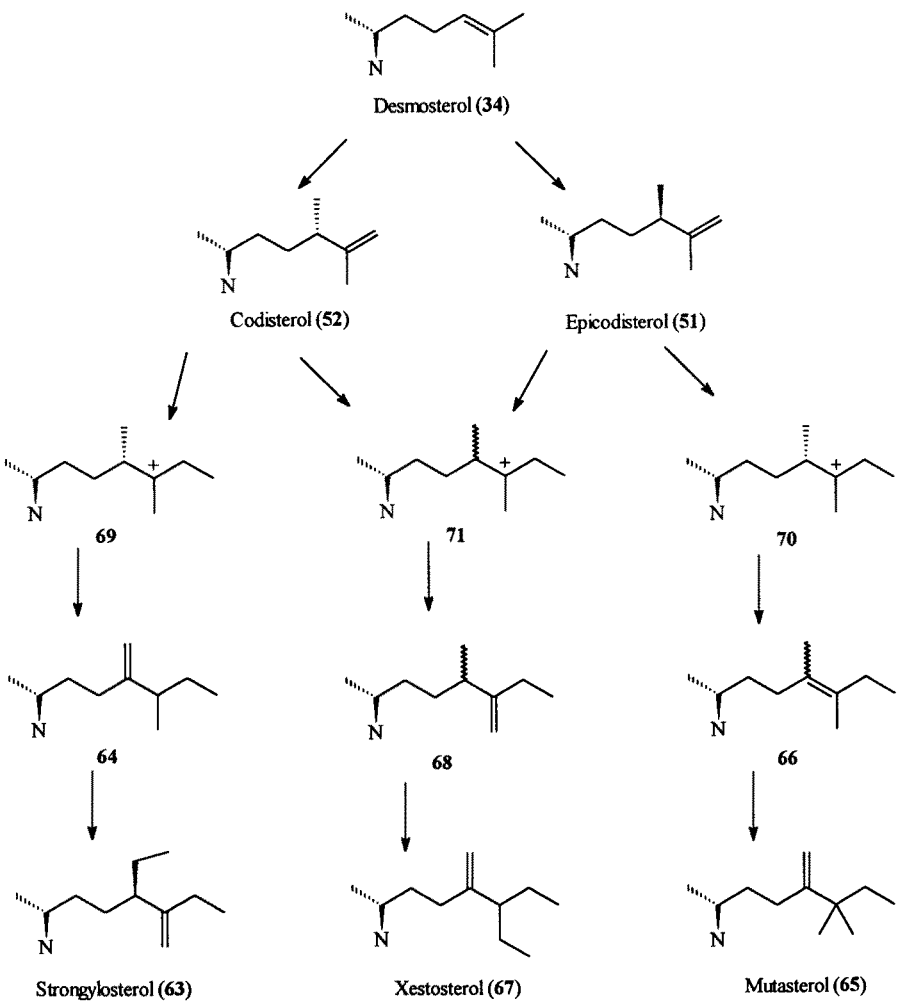


Scheme 8. Biosynthesis of 24-isopropylcholesterol (**62**) in *Pseudaxinyssa* sp.

The biosynthesis of mutasterol (**65**) [45] has recently been determined [46] and, as found with strongylosterol (**63**), considerable stereoselectivity at C-24 was observed. The metabolism of mutasterol was shown to proceed via three biomethylations of desmosterol (**34**), epicodisterol (**51**) (rather than the epimeric codisterol (**52**) in strongylosterol biosynthesis) and 24,26-dimethyldesmosterol (**66**) as described in Scheme 9.

Xestosterol (**67**), another unconventional sterol, was isolated as the major sterol of the Caribbean and Great Barrier Reef *Xestospongia* spp. [44]. This sterol (Scheme 9) possesses a unique symmetrical double extension at C-26 and C-27 and a 24-methylene substituent. The biosynthesis again involves three successive biomethylations of desmosterol (**34**), epicodisterol (**51**) and 25(26)-dehydroaplysterol (**68**) (Scheme 9), a very similar sequence to that found for strongylosterol (**63**) and mutasterol (**65**). However, in this case, complete lack of stereospecificity was observed in the bioalkylation of codisterol (**52**) and epicodisterol (**51**).

The origin of sterols with four and five biomethylations in the side-chain has not been investigated. Sutinasterol (**72**), the result of four biomethylations, was isolated as essentially the only sterol (> 98% of the sterol mixture) of a *Xestospongia* species from Puerto Rico [47]. A trace sterol, **73**, is the product of an

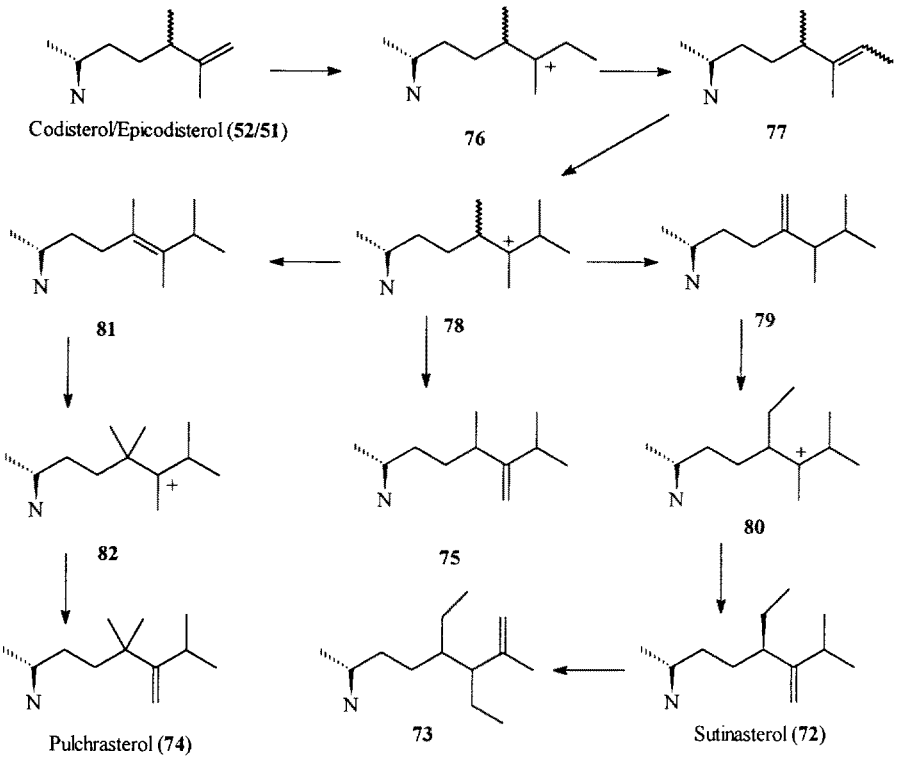


Scheme 9. Biosynthesis of Strongylosterol (**63**), Mutasterol (**65**), and Xestosterol (**67**)

unprecedented five biomethylations. A plausible biosynthesis of sutinasterol has been suggested (Scheme 10). This proposal is attractive as it invokes a common intermediate for the production of sutinasterol as well as two structurally related steroids pulchrasterol (**74**) and **75** from other sponges.

4.2.2 Dealkylation

A recent and surprising finding in sponge sterol biosynthesis is the observation that certain 24-alkyl sterols can be transformed to cholesterol [46, 48, 50]. This is particularly unusual, as this pathway operates in organisms which are also

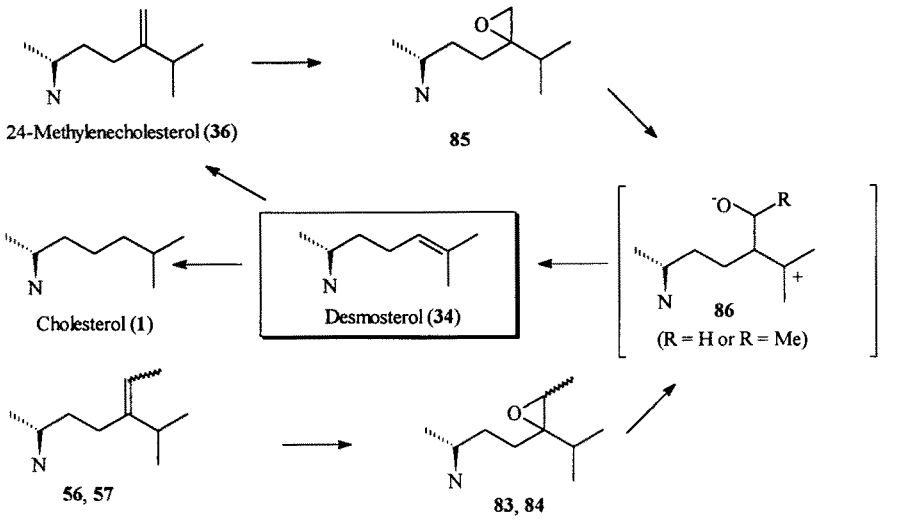


Scheme 10. Proposed biosynthesis of Sutinasterol (**72**) and related sterols

capable of the reverse process, i.e. *S*-adenosylmethionine-mediated bioalkylation and de novo biosynthesis. Sterol side-chain dealkylation is known to occur in phytophageous insects [5, 51], which require cholesterol (**1**) yet are incapable of producing it de novo.

The mechanism and scope of side-chain dealkylation in sponges has been examined in the sponge *Tethya aurantium* with tritium-labeled sterols [49]. Fucosterol (**56**), isofucosterol (**57**), their corresponding 24,28-epoxides (**83**, **84**), and 24-methylenecholesterol (**36**) were transformed to desmosterol (**34**) and cholesterol (**1**) (Scheme 11). This indicates that the mechanism in sponges is parallel to that observed in insects. Also, lack of stereospecificity for the 24(28) double bond is evident as both **56** and **57** were metabolized. Further, sterols with saturated side-chains, 24-methylcholesterol (**2**) and 24-ethylcholesterol (**3**), were not metabolized, thus indicating an apparent inability to dehydrogenate the C-24(28) linkage.

The authors reported that fucosterol (**56**) and isofucosterol (**57**) were transformed not only to desmosterol (**34**) and cholesterol (**1**), but also to 24-methylenecholesterol (**36**) [49]. The unexpected production of 24-methylenecholesterol



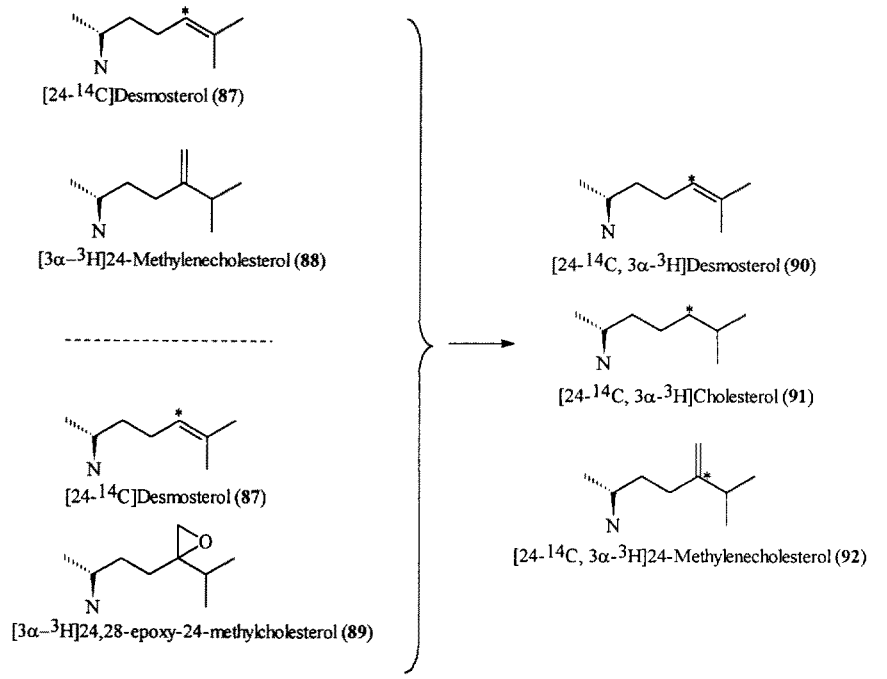
Scheme 11. Demethylation and methylation of Desmosterol (**34**) in sponges

was investigated in a series of double-labeling experiments. 24-Methylenecholesterol was shown to arise by dealkylation of isofucosterol to desmosterol, followed by subsequent alkylation. The concurrent operation of enzymes responsible for alkylation and dealkylation (Scheme 11) was demonstrated in two double labeling experiments. One involved the feeding of [24-^{14}C]desmosterol (**87**) and [3α-^{3}H]24-methylenecholesterol (**88**), while in the other, [24-^{14}C]desmosterol and [3α-^{3}H]24,28-epoxy-24-methylenecholesterol (**89**) were fed (Scheme 12) to *Tethya aurantium*. In both experiments, ^{3}H and ^{14}C activity was recovered in desmosterol (**90**), cholesterol (**91**) and 24-methylenecholesterol (**92**). The dealkylation of sterol side-chains in sponges has been shown to be a general property of sponges, as cell-free extracts of eight different sponges transformed 24-methylenecholesterol to its 24,28-epoxide (**85**), desmosterol and cholesterol [46].

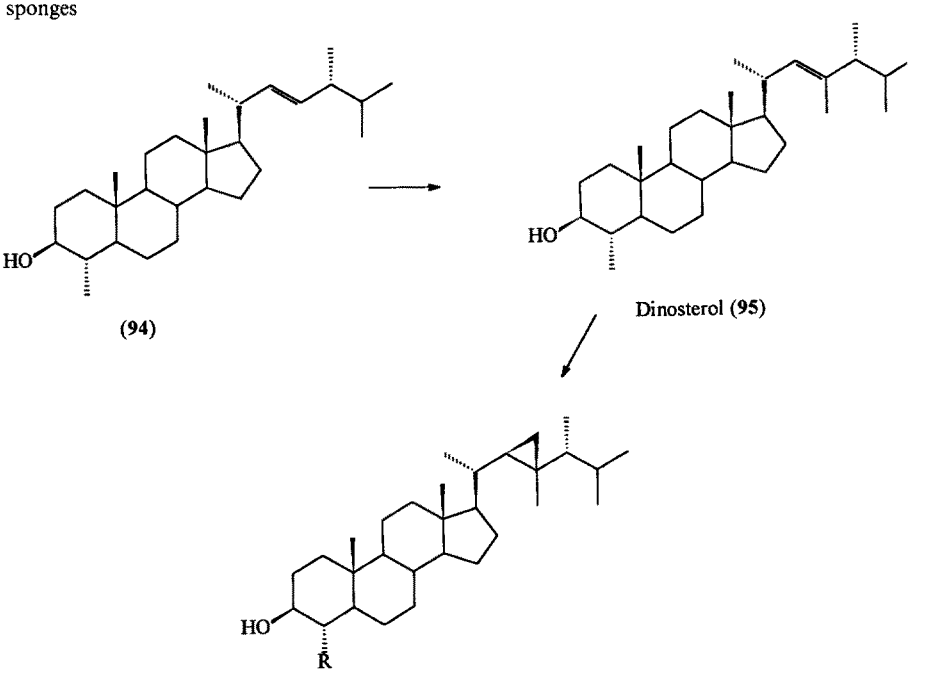
There is a single report of the dealkylation of a sterol side-chain in the whelk *Buccinum undatum* [52]. This organism also transformed ^{3}H-lanosterol (**33**) to cholesterol (**1**), thus indicating its capability for de novo sterol biosynthesis.

4.2.3 Formation and Utilization of Sterol Cyclopropanes and Cyclopropenes

More than twenty sterols with cyclopropanes or cyclopropenes have been isolated from marine organisms [53]. The majority of these come from sponges, although significant numbers have been isolated from dinoflagellates and coelenterates. There are no terrestrial organisms known to contain such sterols and thus their biosynthesis is purely a marine problem.



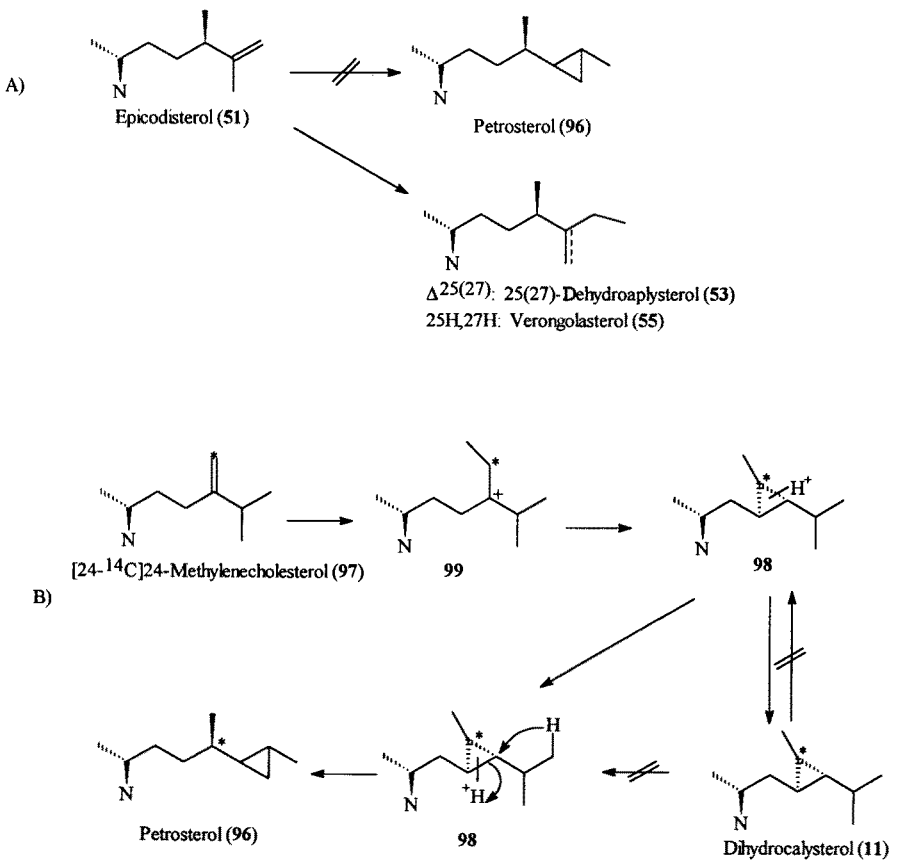
Scheme 12. Double-labelling experiments demonstrating concurrent alkylation and dealkylation in sponges



R = H: Gorgosterol (**93**)
R = CH_3: 4α-Methylgorgosterol (**93a**)

Scheme 13. Biosynthesis of the gorgosterol side-chain in dinoflagellates

In 1943, Bergmann [54] reported the isolation of gorgosterol (**93**) from the gorgonian *Plexaura flexuosa*. However, it was not until 1970 that its structure was elucidated and the presence of a side-chain cyclopropane was demonstrated by Djerassi and co-workers [55, 56]. This sterol is particularly unusual in that, in addition to the presence of a side-chain cyclopropane, there is alkylation at C-22, C-23 and C-24. Since its initial isolation, gorgosterol (**93**) has been found in many coelenterates and molluscs, all of which harbor symbiotic zooxanthellae [57]. Subsequent to these isolations, gorgosterol was found in a number of dinoflagellates [58]. It has been suggested that the production of gorgosterol is somehow related to the coexistence of the invertebrate host and zooxanthellae. Recently, gorgosterol has been isolated from the eggs and larvae of the symbiont-free giant clam (*Tridachna gigas* and *Hippopus hippopus*), which is the first documentation of the occurrence of this cyclopropane sterol in symbiont-free cells of an invertebrate [59].

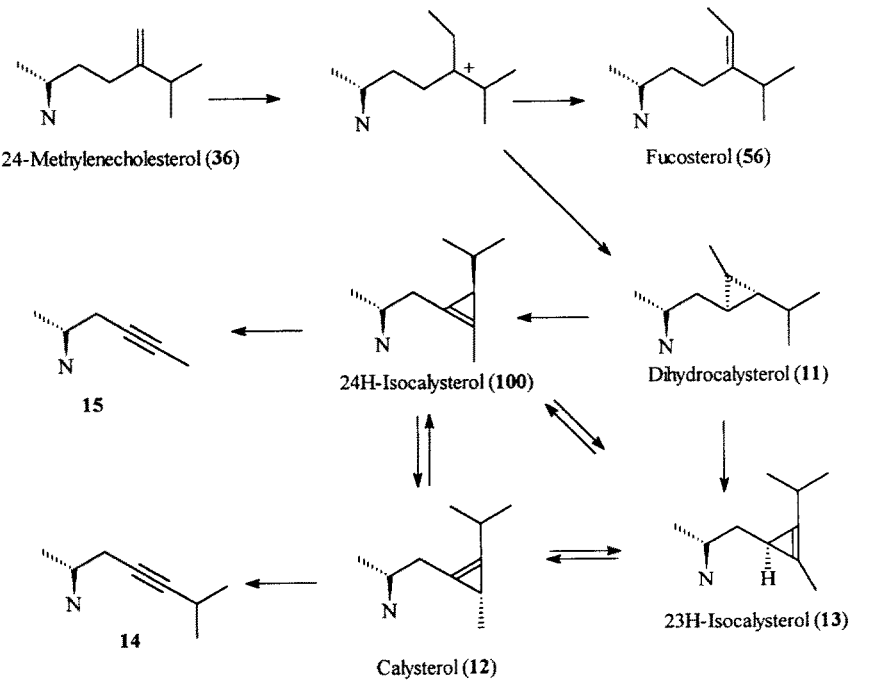


Scheme 14. Biosynthesis of Petrosterol (**96**)

The biosynthesis of gorgosterol (**93**) has been the subject of much discussion and speculation, yet it was not until very recently that its origin was elucidated [60]. This study was performed by feeding radiolabeled sterols to a cell-free extract of the heterotrophic dinoflagellate *Crypthecodinium cohnii*. As was predicted [55] in 1970, the gorgosterol side-chain (in **93a**) is produced from dinosterol (**94**) as shown in Scheme 13.

Petrosterol (**96**) was initially isolated as the major sterol of the sponge *Petrosia ficiformis* [61] and subsequently found in *Petrosia hebes* [62], *Halichondria* sp. [63] and *Cribrochalina vasculum* [64]. The most likely biosynthetic route (Scheme 14A) to petrosterol [65] is by SAM methylation of epicodisterol (**51**) as is known to occur for aplysterol (**54**). Feeding radiolabeled epicodisterol to *P. ficiformis* resulted in no incorporation of radioactivity in petrosterol; however [28-^{14}C]24-methylenecholesterol (**97**) was efficiently transformed to the target sterol [65]. Experiments with *C. vasculum* [64] demonstrated that conversion of 24-methylenecholesterol to petrosterol proceeded via an unprecedented cyclopropane-cyclopropane rearrangement involving a protonated dihydrocalysterol intermediate (**98**) (Scheme 14B). A key feature of this rearrangement is that C-28 of 24-methylenecholesterol becomes C-24 of petrosterol. This was unambiguously demonstrated through degradation studies.

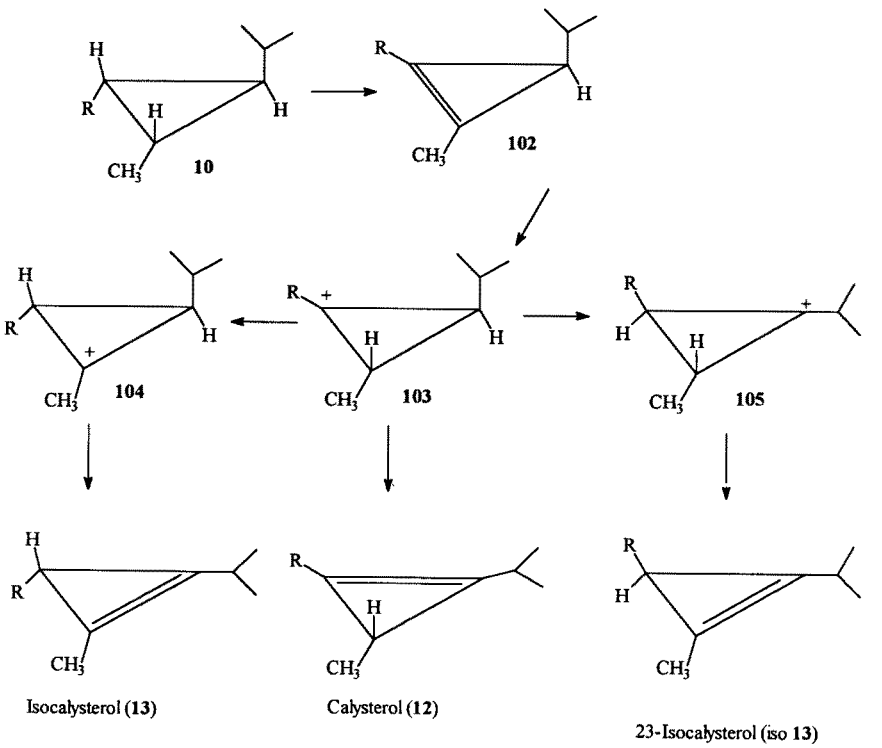
Calysterol (**12**) and its isomers **13** and **100** are the only known steroidal cyclopropenes. Calysterol was first isolated by Fattorusso and co-workers [66]



Scheme 15. Biosynthesis of Calysterol (**12**)

as the predominant sterol of the Mediterranean sponge *Calyx nicaensis*. Subsequently, Djerassi and co-workers [67] isloated 23H-isocalysterol (**13**), 24H-isocalysterol (**100**), 23,24-dihydrocalysterol (**10**), fucosterol (**56**), 23-dehydro-24-ethylcholesterol (**101**) and acetylenes **14** and **15** from the same sponge. Feeding experiments by the Italian group [24] showed that fucosterol is a precursor to calysterol. Subsequently, Djerassi and co-workers [68] demonstrated that 24-methylenecholesterol (**36**) and dihydrocalysterol are transformed to all three cyclopropenes and the two acetylenes (Scheme 15). Calysterol and its isomers were not hydrogenated to dihydrocalysterol, but the existence of cyclopropene isomerization was demonstrated. Feeding experiments with [3,28-^{3}H]-labeled dihydrocalysterol demonstrated that calysterol is not the initial dehydrogenation product, but is produced by isomerization. A mechanism for the isomerization of isocalysterol to calysterol [69] has been proposed and is summarized in Scheme 16.

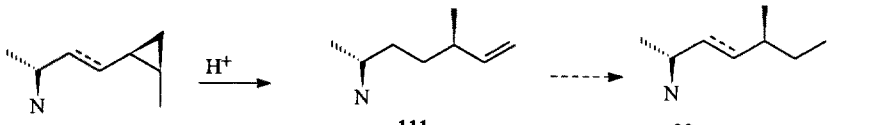
Cyclopropane ring openings have been proposed to account for the biosynthesis of several unconventional sterols. Ficisterol (**106**) [70, 71] is of the 26-norergostane type and contains the rare 23-ethyl substituent. Its biosynthesis became apparent following the isolation of the trace cyclopropane sterol,



Scheme 16. Isomerization of the Calysterols

Hebesterol (**107**) Ficisterol (**106**)

Scheme 17. Biomimetic conversion of Hebesterol (**107**) to Ficisterol (**106**)



Δ^{22}: Papakusterol (**108**)
22,23-dihydro (**109**)

111

Δ^{22}: Occelasterol (**110**)

Scheme 18. Biomimetic conversion of cyclocholesterol (**109**) to **111**

hebesterol (**107**) [72]. All diastereomers of hebesterol were prepared and their conversion to ficisterol demonstrated in vivo by acid-catalyzed isomerization (Scheme 17).

Papakusterol (**108**) and its 22,23-dihydro analog 24,26-cyclocholesterol (**109**) have been isolated from deep-sea gorgonians [73, 74], a shallow-water sponge [75] and a deep-sea sponge [76]. These sterols may represent the unknown precursors to the 26-norergostane sterols such as occelasterol (**110**). In fact, in a biomimetic acid-catalyzed ring opening [77], cyclocholesterol (**109**) was transformed into the Δ^{25} sterol **111** (Scheme 18). The occelasterol side-chain is believed to be the most likely precursor to the 24-norcholesterols.

5 Biosynthesis of Polyoxygenated Marine Sterols

Polyoxygenated sterols and steroid hormones have been reported to occur in marine invertebrates since the early 1960s; they have been of interest to researchers for a number of reasons, especially for their potential to be convertible to hormones of interest in human biochemistry [78]. Isolation of these multioxygenated sterols and sterol sulfates has been from sponges, coelenterates, bryozoans, molluscs, echinoderms, arthropods, and tunicates [2]. Such compounds have been shown to exhibit a range of biological activities (including cytotoxic, antimicrobial, hemolytic and antileukemia) which seem to play a role in the chemical defense of the host; little is known of the biosynthetic origin of many of these compounds.

A number of pregnanes have recently been isolated from marine invertebrates. Pregnenolone (5-pregnen-3β-ol-20-one) (**112**) and pregnane diols **113**

and **114** (Fig. 4) were isolated from the Caribbean sponge *Amphimedon compressa* (= *Haliclona rubens*) [79] and **112** was also found in the Hawaiian sponge *Damiriana hawaiiana* [80]. *Stylopus australis* from New Zealand was shown to contain the novel pregnane sulfate **115** [81]. A series of unusual pregnane derivatives with vinyl substituent at C-17 (**116–119**) have been isolated from soft corals [82–85]. In addition, a pregnane with an unusual $\Delta^{9(11)}$ double bond (**120**) has been reported from the starfishes *Acanthaster planci* [86], *Asterias forbesi* [87] and *Asterias amurensis* [88]. Existence of the rare $\Delta^{9(11)}$ unsaturation poses an interesting biosynthetic problem.

The biosynthesis of pregnenelone (**112**) in mammals has been well documented [1]; it is known to be produced from cholesterol (**1**) via its 20,22-dihydroxy derivative (**121**) (Scheme 19A). The origin of **112** has been investigated in the sponge *Amphimedon compressa*. At the present time it is not known whether cholesterol and its 20,22-dihydroxy derivative serve as precursors for this pregnane in *A. compressa*; however a new pathway (Scheme 19B) has been found to operate [89]. This involves the conversion of 22-dehydro sterols (e.g. stigmasterol, **122**) to pregnenelone. The mechanism is believed to

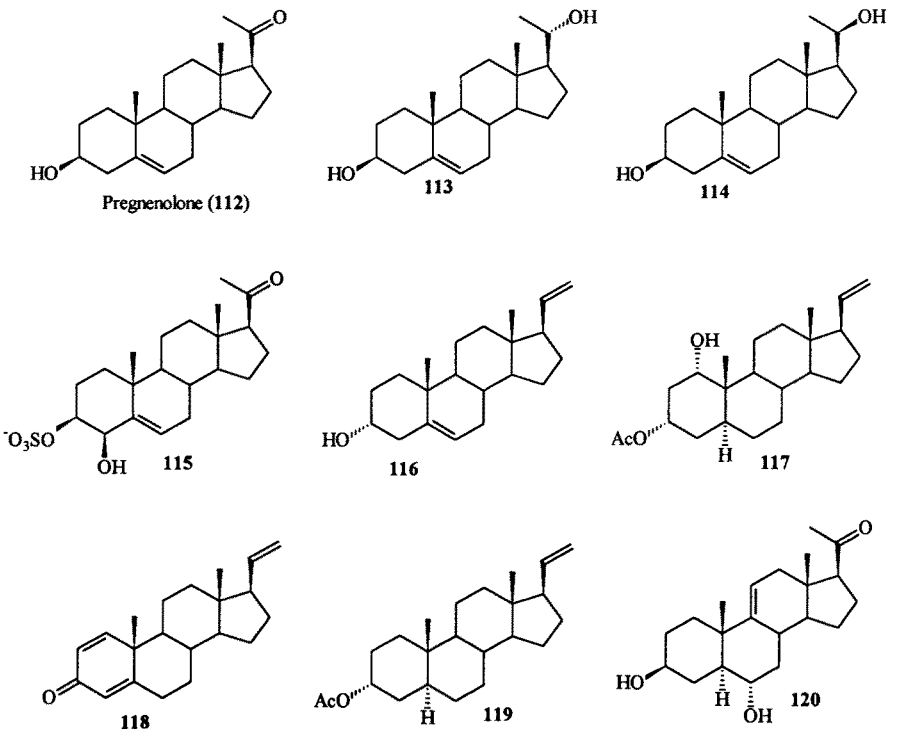
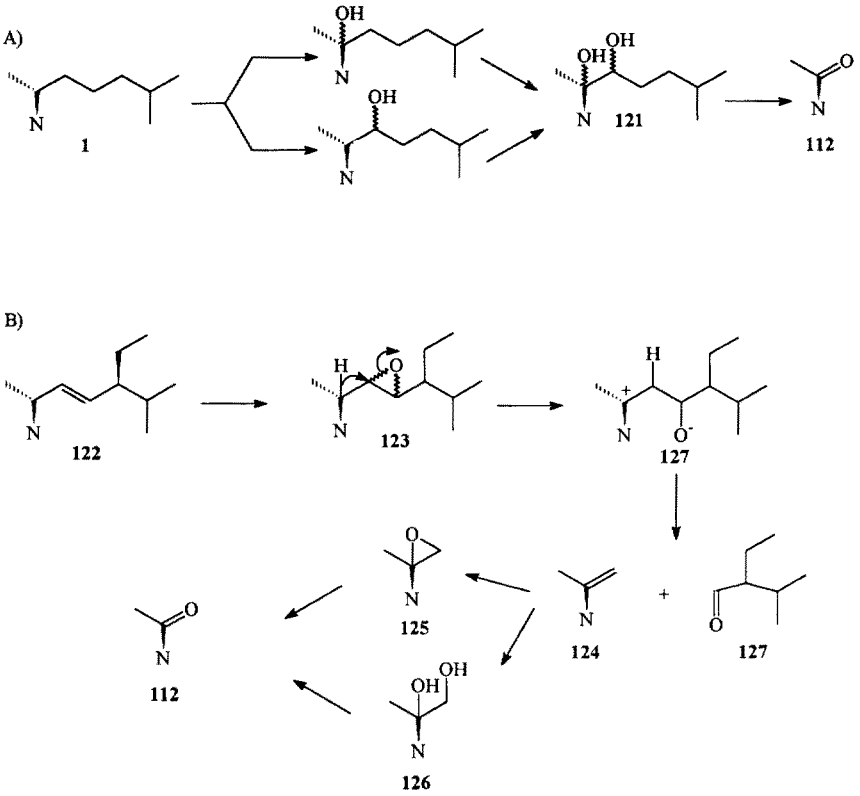


Fig. 4. Some marine invertebrate pregnane steroids



Scheme 19. Biosynthesis of Pregnenolone (**112**) in *Amphimedon compressa*

involve epoxidation of the Δ^{22} double bond, followed by degradation to 20-methylene sterol **124**. Loss of this terminal methylene group could then proceed via the epoxide (**125**) or the 20,22-diol (**126**), paralleling the degradation of 24-alkyl groups (see Sect. 4.2.2).

In his review of steroid hormone interconversions, Goad [4] addressed the metabolism of *Asterias rubens* on which much work has been performed. Briefly, gonad and pyloric caeca tissue homogenates of this sea star were responsible for converting cholesterol (**1**) to pregnenolone (**112**) and progesterone (**129**) [90]; moreover, progesterone was converted into 17α-hydroxyprogesterone (**130**), androstenedione (**131**), testosterone (**132**), 20α-hydroxyprogesterone (**133**), 11-deoxycorticosterone (**134**), and 5α-androstan-3,20-dione (**135**) (Fig. 5). In vivo experiments showed conversion of cholesterol into 3β-hydroxy-5α-pregnan-20-one (**136**) and 5α-pregnane-3β,20ξ-diol (**137**), as well as several polyhydroxy stanols. Conversion of progesterone into 5α-pregnan-3,20-dione (**138**), 3β-hydroxy-5α-pregnan-20-one, 5α-pregane-3β,20ξ-diol and 3β,6α-dihydroxy-pregan-20-one (**139**) has been demonstrated. The sea star also metabolizes

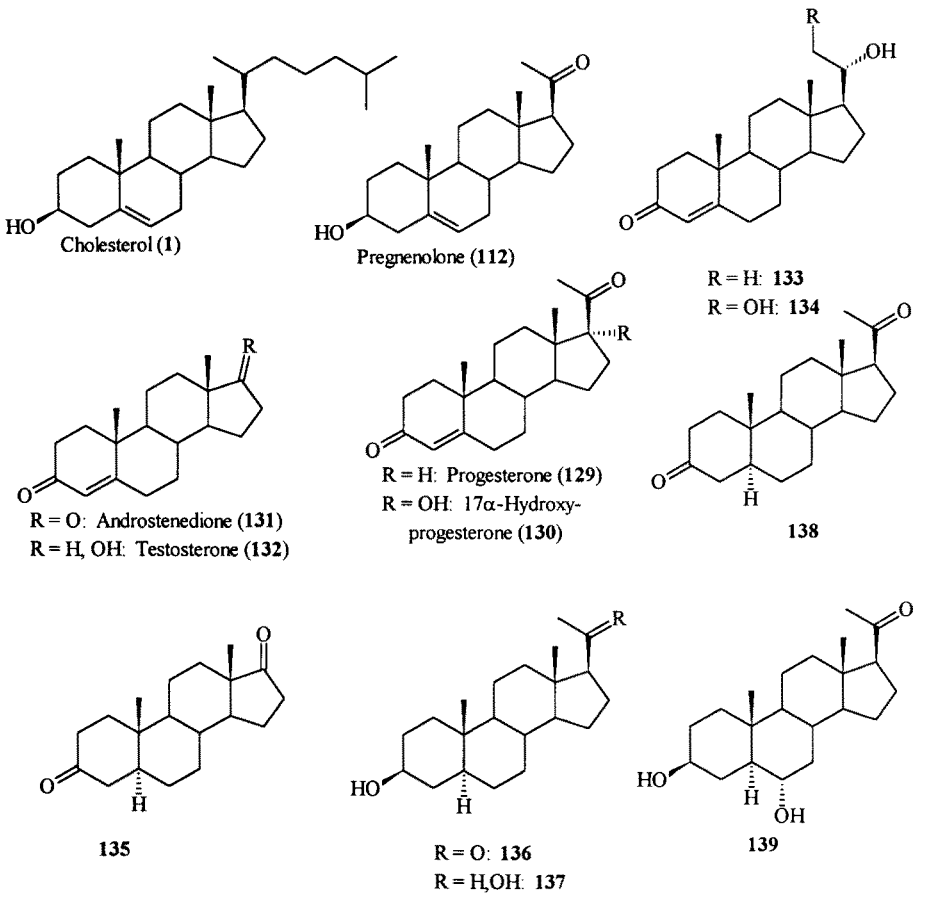
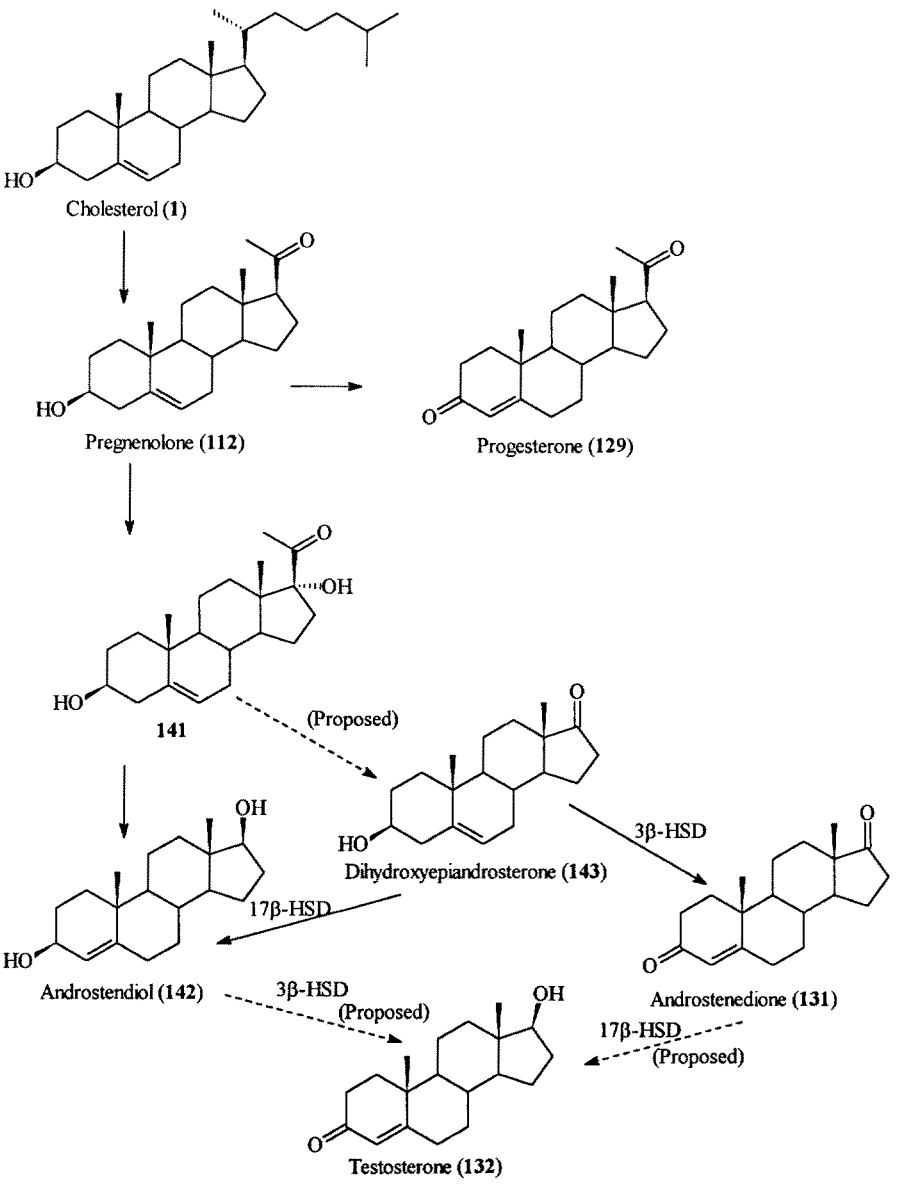


Fig. 5. Steroid hormones in *Asterias rubens*

androstenedione to a number of androstanes and to testosterone. Male sea stars have a tendency to produce fatty acyl esters of the 3β-hydroxy-5α-progesterones, 17α-hydroxypreogesterones and androstenedione.

Recently, Voogt et al. [91] have reported on the Δ^5-pathway in steroid metabolism of *Asterias rubens*. These workers established the existence of the Δ^5-pathway (Scheme 20), analogous to the pathway found in mammals; this conclusion was based on the observation that radiolabeled cholesterol (**1**) was converted to pregnenolone (**112**), 17α-hydroxypregnenolone (**141**), and androstenediol (**142**). Labeled pregnenolone was converted additionally to progesterone (**129**). Androstenediol (**142**) was the main metabolite of dehydroepiandrosterone (**143**), a reaction catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD). The metabolic conversion of androstenedione (**131**) to testosterone (**132**) is also mediated by 17β-HSD and is related to



Scheme 20. The Δ^5 pathway in *Asterias rubens*

dehydroepiandrosterone metabolism by 3β-HSD. That **131** and **132** were produced from **143** is evidence that both of these enzymes are operative in *Asterias rubens*, but does not provide evidence as to whether testosterone originates directly from androstenediol [by action of 3β-HSD] or via dehydroepiandrosterone and androstenedione (see Scheme 20).

There have been no recent reports concerning ecdysone biosynthesis. For two reviews on this subject, see Goad [3] and Ikekawa [5].

6 Techniques

Sterol biosynthetic experimentation has encompassed a variety of techniques. A number of choices have to be made before one can begin a biosynthetic experiment, such as the use of stable or radioactive isotopes, in vivo or in vitro techniques, etc. [11, 92]. Often it is the organism or the nature of the biosynthetic experiment itself which dictates the technique. This has been the case with dinoflagellates [93], in which precursors labeled with stable isotopes are administered to axenic cultures in the traditional manner, in which microorganisms have been treated in biosynthetic experimentation. Not all marine organisms can be cultured; the feeding mechanisms are quite diverse; and the organisms span a range of developmental sophistication. Thus, more general techniques have been developed to address various biosynthetic problems.

Recently, Silva et al. have compared several techniques that have been applied to colonial marine invertebrates [13]. Catalan et al. [37] developed a technique in which sponges maintained in aquaria are attached to a plastic plaque. On the plaque, the sponge can be transferred, first to a smaller, aerated, vessel for treatment with an ethanolic or ethereal solution of the desired precursor. Then, after an incorporation period for uptake of the precursor, the sponge is returned to the sea, where metabolism is allowed to proceed in the animal's natural habitat. Silva et al. [13] found that optimal incubation time depended on the sponge, but generally was 20 to 90 days. These authors also reported on the effectiveness of lipophilic compared to hydrophilic precursors; the former were taken up and metabolized more efficiently in sponges than hydrophilic ones.

To facilitate the uptake of hydrophilic precursors in filter-feeding organisms, and generally to enhance precursor incorporation, a variety of methods are available. Silva et al. [13] have compared several methods. They found that liposome encapsulation, where the precursor is warmed with an aqueous solution of dipalmitophosphatidyl choline and fed as in Catalan's method [37], yields improved incorporation [94]. The use of an osmotic pump to provide slow injection of the precursor into, for example, a sponge was examined. These miniature pumps inject their contents over a 12 to 24 hour period, depending on water temperature and type of pump. Gelatin capsules, in which a precursor is placed into the capsule [95] and the capsule surgically inserted into a sponge in situ also were compared by Silva et al. [13]. For water-soluble precursors, the osmotic pump and gelatin capsule techniques were ineffectual as compared with liposomes and Catalan's method. Water-soluble precursors were only poorly incorporated in all of Silva's experiments.

The usefulness of liposomes, gelatin capsules and osmotic pumps should not be underestimated; when lipophilic precursors are used, these methods proved comparable to Catalan's technique [37]. Alternatively, gelatin capsules may be placed with the organism into a sealed plastic bag for 24 hours, after which the organism is transferred to the ocean for the incorporation period. Liposomes and gelatin capsules have proven effective in other biosynthetic applications [94, 95] and have, in two separate studies of sterol biosynthesis, yielded results comparable to the standard aquarium method [13, 46]. While the in situ methods do not offer great advantages in terms of incorporation levels, an advantage is gained by not subjecting the organism to the stress of being removed from the ocean. Some tropical sponges do not survive in an aquarium even for a few hours.

Direct injection of a precursor into an organism is a traditional technique that has had limited use in marine invertebrates. Typically, this technique is used for more highly evolved organisms such as echinoderms [15, 96] and nudibranchs [97], but it has also been used on sponges [46]. In the latter case, the precursor is generally added in a number of "micro injections" [46]. The advantage of the technique for organ-bearing organisms is that the precursor is not exposed to the digestive organs.

Preparation and utilization of crude enzyme preparations to study sterol biosynthesis has found increasing popularity [13, 98–100]. An advantage of these preparations is that they can provide selective evidence for one or two enzymatic transformations, thereby allowing one to study otherwise reactive intermediates. Kerr et al. [50] found an epoxide intermediate in dealkylation of sterol side-chains using cell-free extracts, an intermediate which could only be postulated [48] by analogy with insect dealkylation [5]. Anderson [101] demonstrated the ability of enzyme systems in zooxanthellae to biosynthesize squalene (**26**) but not sterols, which leaves the question of the participation of the gorgonian host in gorgosterol biosynthesis unresolved. Voogt [91] utilized tissue sections from sea star gonads and pyloric caeca, where steroid hormone biosynthesis takes place, and reported that diffusion of the precursor from the incubation medium to the cell organelle can limit the utilization of a particular precursor. Especially troublesome was cholesterol, which had the opportunity to exchange with cell-wall sterols before reaching the site of biosynthesis.

Tissue culture is an attractive technique in biosynthetic experimentation, since many advantages of microorganism culture become available, including the use of stable isotopes. Tissue culture could also address a major limitation of virtually all of the above techniques. This major limitation is the uncertainty as to whether the invertebrate is performing the biosynthesis or whether the biosynthesis is a product of a symbiont or other associated organism; in a pure cell culture one can be assured that biosynthesis is being performed by the organism from which the cells were isolated. While tissue culture of marine invertebrates is in its infancy, at least one example of the technique has been attempted and the result was that the oyster *Crassostrea virginica* was incapable

of de novo sterol biosynthesis [96]; it should be noted that other oysters have been shown to be capable of transforming dietary sterols [102].

7 References

1. Nes WK, McKean ML (1977) Biochemistry of steroids and other isopentanoids, University Park, Baltimore
2. Kerr RG, Baker BJ (1991) Nat Prod Rep 8:465 and cited references
3. Goad LJ (1978) In: Scheuer PJ (Ed.) Marine natural products, chemical and biological perspectives, vol 2, Academic, New York, chap 2
4. Goad LJ (1981) Pure Appl Chem 51:837
5. Ikekawa N (1985) In: Danielsson H, Sjovall J (eds) Sterols and bile acids, Elsevier, Amsterdam, chap 8
6. Djerassi C, Silva CJ (1991) Acct Chem Res 24:371
7. Carlson RMK, Tarchini C, Djerassi C (1980) In: Ananchenko SN (ed) Frontiers in bioorganic chemistry and molecular biology, p 211
8. Djerassi C, Lam W-K (1991) Accts Chem Res 24:69
9. D'Auria MV, Paloma LG, Minale L, Riccio R (1991) Tetrahedron Lett 2149
10. Lawson MP, Stoilov IL, Thompson JE, Djerassi C (1988) Lipids 23:750
11. Herbert RB (1989) The biosynthesis of secondary metabolites, 2nd edn, Chapman and Hall, New York
12. Voogt PA (1976) Nether J Zoo 26:84
13. Silva CJ, Wunsche L, Djerassi C (1991) Comp Biochem Physiol 99B:763
14. De Rosa M, Minale L, Sodano G (1975) Experientia 31:758
15. Cordeiro ML, Djerassi C (1990) J Org Chem 55:2806
16. Walton MJ, Pennock JF (1972) Biochem J 127:471
17. Ferezou JP, Devys M, Allais JP, Barbier M (1974) Phytochem 13:593
18. Voogt PA (1975) Comp Biochem Physiol 50B:505
19. Teshima S, Kanazawa A (1974) Comp Biochem Physiol 47B:555
20. Beastall GH, Rees HH, Goodwin TW (1971) FEBS Lett 18:175
21. Giner J-L, Wunsche L, Andersen RA, Djerassi C (1991) Biochem Syst Ecol 19:143
22. Cordeiro ML, Kerr RG, Djerassi C (1988) Tetrahedron Lett 2159
23. De Rosa M, Minale L, Sodano G (1973) Comp Biochem Physiol 45B:883
24. Minale L, Ricco R, Scalano O, Fattorusso E, Magno S, Mayol L, Santacroce C (1977) Experientia 33:1550
25. Kerr RG, Stoilov IL, Thompson JE, Djerassi C (1989) Tetrahedron 45:1893
26. Silva CJ, Wunsche L, Djerassi C (1991) Comp Biochem Physiol 99B:763
27. Stoilov IL, Bladocha-Moreau M, Thompson JE, Djerassi C (1987) Tetrahedron 43:1987
28. John V, Stoilov IL, Djerassi C, Karuso P, Poiner A, Scheuer PJ (1989) J Org Chem 54:1642
29. De Rosa M, Minale L, Sodano G (1976) Experientia 32:1112
30. Garson MJ, Partali V, Liaaen-Jensen S, Stoilov IL (1988) Comp Biochem Physiol 91B:293
31. Biblino L, Minale L, Sodano G (1978) J Chem Soc Chem Commun 524
32. De Stefano A, Sodano G (1980) Experientia 36:630
33. Malik S, Stoilov IL, Djerassi C (1988) Tetrahedron Lett 4081
34. Rabinowitz MH, Djerassi C (1992) J Am Chem Soc 114:304
35. Papov S, Carlson RMK, Wegmann A-M, Djerassi C (1976) Tetrahedron Lett 3491
36. a) Bortolotto M, Braekman JC, Daloze D, Losman D, Tursch B (1976) Steroids 28:461. b) Kashman Y, Carmely S (1980) Tetrahedron Lett 4939
37. Catalan CAN, Thompson JE, Kokke WCMC, Djerassi C (1985) Tetrahedron 41:1073
38. Stoilov IL, Thompson JE, Djerassi C (1986) Tetrahedron 42:4147
39. Stoilov IL, Back TG, Thompson JE, Djerassi C (1986) Tetrahedron 42:4156
40. Djerassi C, Theobald T, Kokke WCMC, Pak CS, Carlson RMK (1979) Pure Appl Chem 51:1815
41. Djerassi C (1981) Pure Appl Chem 53:873

42. Li LN, Sjostrand U, Djerassi C (1981) J Org Chem 46:3867
43. Gebreyesus TG, Stoilov IL, Luo F-T, Djerassi C (1985) Steroids 45:447
44. Stoilov IL, Thompson JE, Cho J-H, Djerassi C (1986) J Am Chem Soc 108:8235
45. Li LN, Sjostrand U, Djerassi C (1981) J Am Chem Soc 103:115
46. Kerr RG, Kerr SL, Djerassi C (1991) J Org Chem 56:63
47. Kerr RG, Kerr SL, Pettit GR, Herald DL, Groy TL, Djerassi C (1991) J Org Chem 56:58
48. Malik S, Kerr RG, Djerassi C (1988) J Am Chem Soc 110:6895
49. Kerr RG, Kerr SL, Malik S, Djerassi C (1992) J Am Chem Soc 114:299
50. Kerr RG, Baker BJ, Kerr SL, Djerassi C (1990) Tetrahedron Lett 5425
51. Svoboda JA (1984) In: Nes WD, Fuller G, Tsai L, (eds) Isopentenoids in plants, Marcel Dekker, New York, p 367
52. Khalil MW, Idler DR (1976) Comp Biochem Physiol 55B:239
53. Djerassi C, Doss GA (1991) In: Rahman A (ed) Studies in Natural Products Chemistry, Vol. 9, Elsevier, Amsterdam, p 35
54. Bergmann W, McLean MJ, Lester D (1943) J Org Chem 8:271
55. Hale RL, Leclercq J, Tursch B, Djerassi C, Gross RA, Westheimer AJ, Gupta KC, Scheuer PJ (1970) J Am Chem Soc 92:2179
56. Ling NC, Hale RL, Djerassi C (1970) J Am Chem Soc 92:5281
57. Ciereszko LS (1991) Biol Ocean 6:363
58. Ciereszko LS, Johnson MAW, Schmidt RW, Koons CB (1968) Comp Biochem Physiol 24B:899
59. Kerr RG, Southgate P (1992) Comp Biochem Physiol B in press
60. Giner J-L, Djerassi C (1991) J Org Chem 56:2357
61. Sica D, Zollo F (1978) Tetrahedron Lett 837
62. Cho J-H, Djerassi C (1987) J Chem Soc Perkin Trans 1 1307
63. Ravi BN, Kokke WCMC, Delseth C, Djerassi C (1978) Tetrahedron Lett 4379
64. Doss GA, Proudfoot JR, Silva CJ, Djerassi C (1990) J Am Chem Soc 112:305
65. Djerassi C, Theobald T, Kokke WCMC, Pak CS, Carlson RMK (1979) Pure Appl Chem 51:1815
66. Fattorusso E, Magno S, Mayol L, Santacroce C, Sica D (1975) Tetrahedron 31:1715
67. Li LN, Li J-T, Lang RW, Itoh T, Sica D, Djerassi C (1982) J Am Chem Soc 104:6726
68. Margot C, Catalan CAN, Proudfoot JR, Sodano G, Sica D, Djerassi C (1987) J Chem Soc Chem Commun 1441
69. Doss GA, Djerassi C (1988) J Am Chem Soc 110:8124
70. Khalil MW, Durham LJ, Djerassi C, Sica D (1980) J Am Chem Soc 102:2133
71. Shu AYL, Djerassi C (1987) J Chem Soc Perkin Trans 1 1291
72. Cho J-H, Djerassi C (1987) J Chem Soc Perkin Trans 1 1307
73. Bonini C, Kinnel RB, Scheuer PJ, Djerassi C (1983) Tetrahedron Lett 277
74. Kobayashi M, Mitsuhashi H (1982) Steroids 40:665
75. Catalan CAN, Lakshami V, Schmitz FJ, Djerassi C (1982) Steroids 40:455
76. Kerr RG, Kerr SL (unpublished results)
77. Catalan CAN, Djerassi C (1983) Tetrahedron Lett 3461
78. Gurst JE, Sheikh YM, Djerassi C (1973) J Am Chem Soc 95:628
79. Ballantine JA, Williams K, Burke BA (1977) Tetrahedron Lett 1547
80. Delseth C, Carlson RMK, Djerassi C, Erdman TR, Scheuer PJ (1978) Helv Chim Acta 61:1470
81. Prinsep MR, Blunt JW, Munro M (1989) J Nat Prod 52:657
82. Bandurraga MM, Fenical W (1985) Tetrahedron 41:1057
83. Higgs MD, Faulkner DJ (1977) Steroids 30:379
84. Kingston JF, Gregory B, Fallis AG (1979) J Chem Soc Perkin Trans 1 2064
85. Ross RA, Scheuer PJ (1979) Tetrahedron Lett 4701
86. Sheikh YM, Tursch BM, Djerassi C (1972) J Am Chem Soc 94:3278
87. ApSimon JW, Buccini JA, Badripersand S (1973) Can J Chem 51:850
88. Ikegami S, Kamiya Y, Tamura S (1972) Tetrahedron Lett 1661
89. Kerr RG, Schulman AL (1992) 1992 Annual Meeting – Florida Sections, American Chemical Society, April 30–May 2, 1992, abstract 19
90. Schoenmakers HJN (1979) Comp Biochem Physio 63B:179
91. Voogt PA, Den Besten PJ, Jansen M (1990) Comp Biochem Physiol 97B:555
92. Garson MJ (1989) Nat Prod Rep 6:143
93. Withers N (1983) In: Scheuer PJ (ed) Marine natural products, chemical and biological perspectives, vol 5, Academic, New York, chap 3

94. Tymiak AA, Rinehart KL (1981) J Am Chem Soc 103:6763
95. Hagadone MR, Scheuer PJ, Holm A (1986) J Am Chem Soc 106:2447
96. Barnett D, Dean PW, Hart RJ, Lucas JS, Salathe R, Howden MEH (1988) Comp Biochem Physiol 90B:141
97. Avila C, Ballesteros M, Cimino G, Crispino A, Gavagnin M, Sodano G (1990) Comp Biochem Physiol 97B:363
98. Giner J-L, Djerassi C (1990) Tetrahedron Lett 5421
99. Voogt PA, Van Rheenen JWA, Lambert JGD, de Groot BF, Mollema C (1986) Comp Biochem Physiol 84B:397
100. Voogt PA, Van Rheenen JWA (1986) Comp Biochem Physiol 85B:497
101. Anderson DG (1985) Comp Biochem Physiol 81B:423
102. Saliot A, Barbier M (1973) J Exp Mar Bio Ecol 13:207

Marine Isocyano Compounds

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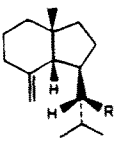
Marine isocyano (–NC) terpenoids are often accompanied by isothiocyanates (R–NCS) and formamides (R–NHCHO). Recent work has uncovered the less common cyanates (R–NCO), thiocyanates (R–SCN), amines ($R–NH_2$) and carbonimidic dichlorides ($R–N=CCl_2$). A majority of sesquiterpenes bear one of the nitrogenous functional groups of the related –NC/–NCS/–NHCHO triad. More complex substitution exists in diterpenoid skeletons. Most complex are the kalihinols which are polyfunctional diterpenoid tetrahydrofurans or pyrans elaborating multiple isocyano- and isocyano-related functions together with hydroxyl, chloro, and olefinic groups. More recent discoveries of sesquiterpenoid isonitiles with hydroxyl and chloro substituents reinforce the speculative role of cationic intermediates in the biosynthesis of these compounds. Cyanide is incorporated into the isocyano function of terpenoids in sponges (Porifera), the phylum from which the majority of marine isocyano-related compounds have been obtained. Marine isonitriles from nudibranchs are of dietary origin. The diversity of marine isocyano-related compounds and their degree of substitution is limited by the fact that so far only isoprenoids (sesqui- and diterpenes) in certain taxa of the Porifera are involved.

1 Introduction

The genesis of the isocyano ($-\overset{+}{N}\equiv\overset{-}{C}$) group as a naturally occurring function in marine natural products remains enigmatic. Prior to 1973, natural products bearing this function were rare [1]. In light of the chemical reactivity of the isocyano group with various nucleophiles [2] and of the medium (sea water) from which the organisms were obtained, the surprising discovery by Fattorusso's group in Naples of a marine isocyano compound signaled the existence of this unexpected class of marine metabolites [1]. Concurrent investigations by researchers, Minale in Arco Felice [3] and Scheuer in Hawaii [4], resulted in the characterization of additional isocyano metabolites.

The first marine isocyano metabolite was appropriately named axisonitrile-1 (**1**) based on the trivial name "isonitrile" and the genus of the sponge, *Axinella cannabina*, which also furnished axisothiocyanate-1 (**2**) [1] and, subsequently, a minor constituent, axamide-1 (**3**) [5]. This observation that isonitriles are often accompanied by isothiocyanates (–N=C=S) and formamides (–NHCHO) was later duplicated by the Hawaiian researchers working with a *Halichondria* sp. In



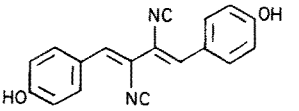
1 R = NC
2 R = NCS
3 R = NHCHO

addition to the cyclic C_{15} compounds, an acyclic diterpenoid isonitrile [4, 6, 7] was also isolated.

Since the discovery of this new class or marine metabolites, the origin of the isocyano group and its relation to its congeners has continued to draw the interest of investigators. While the chemical transformations of these functions are firmly established, progress in understanding the biogenetic details within the –NC/–NCS/–NHCHO series has been slow.

$$\text{R–NCS} \overset{\leftarrow \text{biogenesis} \rightarrow}{\underset{\leftarrow \text{S}—}{}} \text{R–NC} \overset{\leftarrow \text{biogenesis} \rightarrow}{\underset{—\text{H}_2\text{O} \rightarrow}{}} \text{R–NHCHO}$$

Unlike xanthocillin (**4**), which is an amino acid derivative and was the first terrestrial isonitrile isolated from *Penicillium notatum* Westling [8], compounds **1–3** are sesquiterpenes whose structures imply a mevalonate origin. This chapter reviews the chemistry and examines the progress of naturally occurring marine isonitriles and their congeners. Several publications on the chemistry [9] and, in part, biosynthesis [10] of naturally occurring isonitriles have appeared together with specific reviews on the biosynthesis of marine isocyanoterpenoids in sponges [11] and the chemistry of organic sulfur compounds including marine isothiocyanates [12]. The progress of isocyanides and cyanides as natural products was recently surveyed [13]. Within the past year, reports of several marine isonitrile-related compounds having possible biogenetic links were published (cf. Sect. 7).



4

2 Distribution

Considering the vast spectrum of marine biota, it is perhaps surprising that even twenty years after the initial report the phylum Porifera continues to be the

Table 1. Taxonomic classification of some marine sponges (I) and nudibranchs (II) containing isonitriles

I. Phylum: Porifera, Class: Demospongiae Subclass	Order	Family	Genus	Location	Terpenoid type	Ref.
Tetractinomorpha	Axinellida	Axinellidae	*Axinella*	Naples, Italy	C_{15}	[1]
			Axinella	Southern California	C_{15}	[18]
			Acanthella	Naples	C_{15}	[3]
			Acanthella	Guam, Fiji	C_{20}	[19]
			Acanthella	Australia	C_{15}	[20]
			Acanthella	Japan	C_{20}	[21]
Ceractinomorpha	Halichondrida	Halichondriidae	*Halichondria*	Hawai'i	C_{15}, C_{20}	[7]
		Hymeniacidonidae	*Hymeniacidon*	Australia	C_{20}	[22]
	Haplosclerida	Adociidae	*Amphimedon*	Australia	C_{20}	[23]
II. Phylum: Mullusca, Class: Gastropoda						
Opisthobranchia	Aplysiomorpha	Phyllidiidae	*Phyllidia*	Hawai'i, Guam	C_{15}	[15]
	Nudibranchia	Cadlinidae	*Cadlina*	Southern California	C_{15}	[18]

major source of isonitriles. Studies to-date reveal that these natural products are rather restricted to a few genera in the class Demospongiae. These sponges are sessile, filter-feeding organisms possessing siliceous skeletal elements [14]. None have so far been isolated from other classes in Porifera, the Calcarea, Hexactinellida, or Sclerospongiae.

2.1 Porifera

Of the relatively few sponges that contain isonitrile-related metabolites, no more than twenty have been fully identified and some of these are known only at the genus level. All belong to one of three orders, Axinellida, Halichondrida and Haplosclerida, with the majority of isonitriles being associated with sponges of the orders Axinellida and Halichondrida. The few isocyano-bearing sponges from the Haplosclerida have yielded predominantly C_{20} isonitriles (Table 1). The Mediterranean *Axinella cannabina* of the order Axinellida has been the most prolific source of isonitriles, elaborating at least five skeletal types (cf. Sect. 4.1).

In addition to genetic factors evidenced by sponge systematics, environmental parameters, such as time and site of collection, appear to play a part in the isonitrile inventory of a given taxon. Examples from the genera *Ciocalypta* and *Acanthella* will be discussed in the following sections.

2.2 Mollusca

There exists considerable evidence to indicate that isonitriles isolated from mollusks result chiefly from their diet. Relevant field observations were substantiated by chemical analyses of the involved organisms [15]. While several blue-green algal (cyanophytes) metabolites contain the isocyano function [16], these organisms are terrestrial and hence these isonitriles are not of marine origin. However, subsequent work using electron microscopy revealed abundant cyanobacteria in the outer 0.5 mm of a haplosclerid sponge [17], an observation that needs to be followed up.

Table 1 lists some taxa, sponges and mollusks from which isonitrile-related compounds were isolated. The partial list identifies selected sponges at the genus level with the geographical origin and compound type.

3 Isolation Methodology

Bioactivity-guided separation has in recent years become the prevalent mode of separation. Typically, it involves extraction of the whole animal with aqueous

alcohol, although lyophilized material may be used. The crude extract is then subjected to the so-called Kupchan scheme, which partitions constituents on the basis of polarity [24]. This is accomplished by sequential solvent extractions of the crude aqueous extract using, for example, hexane, carbon tetrachloride, chloroform, and methanol [19].

In those cases where quantities of the test organisms are limited or non-aqueous conditions are preferred, blending of the lyophilized sponge with organic solvents is employed. The latter option is prudent, if one suspects the presence of reactive isocyano and isothiocyano functions. Although these isolation methods may not be unduly harsh, artifacts derived from isonitriles and isothiocyanates may arise.

We have used the Kupchan scheme successfully in the separation of the multifunctional diterpenoid kalihinols from various *Acanthella* species (cf. Sect. 4.2.2). These compounds were distributed between the carbon tetrachloride and chloroform layers [19, 25]. No isocyanosesquiterpenes were present in the hexane layer. By contrast, separate experiments with the Australian *A. klethra* revealed that sesquiterpenoid isonitriles were found exclusively in the hexane extract. No kalihinols were present in the carbon tetrachloride or chloroform extracts [26].

Solvent-partitioned mixtures are fractionated further by column chromatography involving Sephadex LH-20, silica gel, or a combination [19]. Quantitative recovery of constituents is often possible with Sephadex chromatography. With a $MeOH/CHCl_3$ (1:1) eluent, pigmented and other polar material is removed in the early fractions, while the nonpolar isonitrile-related compounds elute later.

Thick-layer silica gel chromatography can also be employed [7], although most separations are now accomplished by high-performance liquid chromatography. Resolution of complex mixtures often requires both normal and reverse phase modes [19]. A further dimension is added, when bioactivity is correlated with spectroscopically-monitored chromatographic profiles.

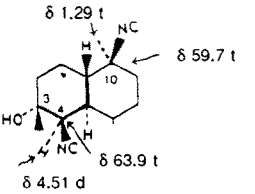
3.1 Spectroscopic Evaluation

Infrared spectroscopy is the preferred spectral method to detect isocyano and isothiocyano functions, which absorb in the same region (2150–2110 cm^{-1}), although the –NCS group absorbs at slightly lower wavenumber (2150–2050 cm^{-1}) [27]. Isonitrile absorption appears as a sharp band [27a] in contrast to the broad band (due to Fermi resonance) of isothiocyano compounds [27b]. Recent reports of the thiocyano (R–SCN) group were characterized by the sharp band in the 2170–2135 cm^{-1} region [27a] – in contrast to the broad band for organo-isothiocyanates [19]. This methodology is particularly useful and has been employed effectively. Preliminary screening employing IR data for a number of sponges has been used as a valuable diagnostic tool for preliminary sponge spectrotaxonomy [28].

Ultraviolet spectroscopy is not as useful in detecting the –NC function. Despite its limitation, coeluting isothiocyano compounds are UV active (~ 250 nm, $\varepsilon \sim 1200$) [27c]. Thus, a UV monitor can be interfaced with an LH-20 or silica column to detect column fractions containing –NCS compounds. Final resolution of enriched mixtures of previously fractionated isonitrile-related compounds is achieved by examining the responses generated by UV and RI detectors coupled in liquid chromatography.

While high-field and two-dimensional FT-NMR methods have simplified immensely the structural determination of these compounds, early progress in marine isonitrile chemistry was facilitated by the observation that protons on carbon bearing the isonitrile function may be detected readily since these protons couple with the isonitrile ^{14}N ($J =$ ~ 2–3 Hz) and appear as a weak triplet in the ^1H NMR spectrum [29]. For example, in kalihinol-A (partial structure **5**) the C-10 methyl, which is geminal to the isonitrile function appeared as a 3-H triplet at δ 1.29 ($J \sim 2$ Hz) and the C-4 methine proton as a broad doublet at δ 4.51 [25]. The lower than normal and broad resonance of the latter signal is due to a combination of effects, which include weak coupling with the isonitrile function and the inductive effect of the neighboring tertiary hydroxy function. Similarly, the isocyano-bearing carbon in the ^{13}C NMR spectrum also shows weak coupling ($J \sim 5$ Hz); these broad resonances are typically found in the 55–65 ppm region (in the absence of inductive effects by other hetero atoms) [19]. For example, the isocyano-bearing carbons, C-10 and C-4, resonate at δ 59.7 and 63.9 respectively. The signal for the *sp*-hybridized isocyano carbon is generally weak and found near 150 ppm.

5



Protons on carbon geminal to –NCS or –NHCHO functions do not experience the weak coupling with nitrogen due to the normal hybridization of the nitrogen atom. In the case of formamides, however, both *E*- and *Z*-rotamers are possible as revealed by resonances in the ^1H NMR spectrum.

4 Marine Isocyano/Isothiocyano/Formamido Compounds

Naturally occurring marine isonitriles are either sesquiterpenes or diterpenes with one, two, or three isocyano (or isocyano-related) functions. As mentioned previously, it usually is the same carbon that bears the –NC, –NCS, or –NHCHO function. Commonly encountered in sesqui- and diterpenes are

Table 2. Sesquiterpenoids – Monoisonitriles with Formula $C_{16}H_{25}N$

Compound (Structure)[#]	Organism	Origin	$[\alpha]_D^1$	mp °C	Spectral Data[2]	Refs.
axisonitrile-1 (**1**)[#]	*Axinella cannabina*	Bay of Taranto	+ 22.6	43–45	H-NMR	[1, 30]
					X-ray deriv	[31]
	Phyllidia pulitzeri	Mediterranean			GC	[32]
(±)-axisonitrile-1				45–46	syn, H-NMR	[76]
axisonitrile-4 (**7**)[#]	*Axinella cannabina*	Bay of Taranto	+ 51.4	56–58	X-ray, H-NMR	[31, 33]
(±)-axisonitrile-4				61–63	syn, H- & C-NMR	[78]
acanthellin-1 (**14**)	*Acanthella acuta*	Bay of Naples	− 41.2		MS	[3]
	Axinella cannabina	Bay of Taranto				[35]
(**17**)	*Axinella cannabina*	Bay of Taranto	+ 39.6		H-NMR, MS	[35]
6α-isocyano-5α-H,7α-H,10-α eudesm-4(14)-ene (**20**)	*Axinella cannabina*	Bay of Taranto	+ 92.9	78–79	H-& C-NMR, MS	[40, 41]
	Acanthella acuta	Bay of Naples				
stylotelline (**23**)[#]	*Stylotella* sp.	New Caledonia	− 47		H- & C-NMR, MS	[42]
11-isocyano-7β-H-eudesm-5-ene (**25**)	*Axinella cannabina*	Bay of Taranto	− 85.7		H- & C-NMR, MS	[36]
	Acanthella pulcherrima	Darwin, Austl.				[20]
(−)-10-isocyano-4-amorphene (**31**)[#]	*Halichondria* sp.	north shore, O'ahu	− 75[ccl]	40–42	H- & C-NMR, MS	[6, 7]
10α-isocyano-4-amorphene (**34**)	*Acanthella* cf. *cavernosa*	Hachijo-jima Island	+ 101		2-D NMR, MS	[34]
	Phyllidia ocellata					[34]
4α-isocyano-9-amorphene (**35**)	*Phyllidia pustulosa*	Hachijo-jima Island	− 13.7		2-D NMR, MS	[43]
(**38**)	*Axinella cannabina*	Bay of Taranto	− 65.7		H- & C-NMR, MS	[37]
axisonitrile-3 (**48**)	*Axinella cannabina*	Bay of Taranto	+ 68.4	101–103	X-ray, H-NMR	[46]
	Acanthella acuta	Banyuls, France			H-NMR, MS	[47]
	Acanthella klethra	Austl.			H- & C-NMR, MS	[26, 38]
	Axinyssa aplysinoide	Palau		99		[49]
	Topsentia sp.	Thailand		100–102	C-NMR	[45]
	Acanthella cf. *cavernosa*	Hachijo-jima Island			2D- NMR, MS	[34]
(**51**)	*Acanthella acuta*	Bay of Naples	− 31.5		H- & C-NMR, MS	[41]
axisonitrile-2 (**53**)	*Axinella cannabina*	Bay of Taranto	+ 29.0		H-NMR, MS	[5, 36, 50]
	Phyllidia pustulosa	Hachijo-jima Island			2-D NMR, MS	[43]

	Acanthella cf. *cavernosa*	Hachijo-jima Island			2-D NMR, MS	[34]
	Phyllidia ocellata	Hachijo-jima Island			2-D NMR, MS	[34]
10α-isocyanoalloaromadendrane (**58**)	*Axinella cannabina*	Bay of Taranto	− 17.2		H- & C-NMR, MS	[36]
	Acanthella acuta	Bay of Naples				
	Acanthella pulcherrima	Darwin, Austl.				[20]
1-isocyanoaromadendrane (**62**)	*A. acuta*	Banyuls, France	− 9[mc]	65–66	H- & C-NMR, MS	[47]
	A. pulcherrima	Darwin, Austl.				[20]
(**64**)	*A. acuta*	Bay of Naples	− 13.7	65–66	H- & C-NMR, MS	[41]
(**68**)	*Cadlina luteomarginata*	southern CA	+ 36		H- & C-NMR	[18]
	Axinella sp.	southern CA				[18]
(**73**)	*A. cannabina*	Bay of Taranto	− 34.4		H- & C-NMR	[52]
9-isocyanopupukeanane (**76**)#	*Ciocalypta* sp.	north shore, O'ahu	− 5[ccl]		X-ray, H-NMR, MS	[15, 53]
	Phyllidia varicosa					[15, 53]
	Phyllidia bourguini	Hachijo-jima Island	− 51		2D NMR, MS	[56]
	Phyllidia pustulosa	Hachijo-jima Island			2D NMR, MS	[43]
(±)-9-isocyanopupukeanane					syn, H-NMR	[73, 74]
9-*epi*-9-isocyanopupukeanane (**77**)#	*P. bourguini*	Hachijo-jima Island	+ 31		2D NMR, MS	[43, 56]
	P. pustulosa	Hachijo-jima Island			2D NMR, MS	[43]
2-isocyanopupukeanane (**78**)#	*Ciocalypta* sp.	north shore, O'ahu		81–82	X-ray, H- & C-NMR	[53, 54]
					syn	[75]
2-isocyanoallopupukeanane (**79**)	*P. pustulosa*	Hachijo-jima Island	+ 16.2		2D NMR, MS	[43]
9-isocyanoneopupukeanane (**80**)	*Ciocalypta* sp.	south shore, O'ahu	+ 33		H- & C-NMR, MS	[54]
3-isocyanotheonellin (**85**)	*Phyllidia* sp.	Sri Lanka	0		H- & C-NMR, MS, UV	[55]
	P. pustulosa	Hachijo-jima Island			2D NMR, MS	[43]
					syn	[80]
7-isocyano-7,8-dihydro-α-bisabolene (**88**)	*Ciocalypta* sp.	Kaneohe, O'ahu	− 49.9[h]		H- & C-NMR, MS, UV	[55]
	P. pustulosa	Hachijo-jima Island			2D NMR, MS	[43]
	A. cf. *cavernosa*	Hachijo-jima Island			2D NMR, MS	[34]
	P. ocellata	Hachijo-jima Island			2D NMR, MS	[34]

[1] rotations in chloroform unless indicated otherwise: [ccl], CCl_4; [mc], $[\alpha]_{579}$ CH_2Cl_2; [h], hexane. A blank represents no report.
[2] data provided for methods indicated; IR for –NC absorption given in all cases; syn = synthetic. # absolute stereochemistry

Table 3. Sesquiterpenoids – Isothiocyanates and Formamides. R = –NCS and –NHCHO

Structure (Compound)	Organism	Origin	$[\alpha]_D^1$	Spectral Data[2]	Refs.
(2) R=NCS axisothiocyanate-1[#]	*Axinella cannabina*	Bay of Taranto	+ 5.9	H-NMR, MS	[1, 30]
(3) R = NHCHO axamide-1[#] (±) axamide-1			+ 10	H-NMR, MS	[5, 31]
				syn, H- & C-NMR	[76]
(8) R=NCS axisothiocyanate-4[#]	*A. cannabina*	Bay of Taranto		H-NMR	[31, 33]
(±) axisothiocyanate-4				syn, H- & C-NMR	[78]
(9) R=NHCHO axamide-4[#]			+ 63.3	GC	[33]
(±) axamide-4				syn, H- & C-NMR	[78]
(15) R= NCS	*A. cannabina*	Bay of Taranto	– 24.4	H-NMR	[3, 35]
(16) R=NHCHO			– 24.0	H-NMR	[35]
(18) R=NCS	*A. cannabina*	Bay of Taranto	+ 41.0	H-NMR	[35]
(19) R=NCHO			+ 48.5	H-NMR	[35]
(21) R=NCS 6α-isothiocyanato-5α-*H*,7α-*H*,10α-eudesm-4(14)-ene	*A. cannabina*	Bay of Taranto	+ 88.4	H- & C-NMR, MS	[40]
(22) R=NHCHO 6α-formamido-5α-*H*,7α-*H*,10α-eudesm-4(14)-ene	*Acanthella acuta*	Bay of Naples		H-NMR, MS	[40]
(26) R=NCS 11-isothiocyanato-7β-*H*-eudesm-5-ene	*A. cannabina*	Bay of Taranto	– 89.7	H-NMR	[36]
	Acanthella pulcherrima	Darwin, Austl.		H- & C-NMR, MS	[20]
	Acanthella klethra	Queensland, Austl.			[38]
	Acanthella cf. *cavernosa*	Hachijo-jima Island		2-D NMR, MS	[54]
(27) R=NHCHO 11-formamido-7β-*H*-eudesm-5-ene	*A. cannabina*	Bay of Taranto		H-NMR, MS	[36]
(28) (1*R*,5*R*,6*R*,8*S*)-Dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethenyl)-5-isothiocyanate	*A. klethra*	Queensland, Austl.	+ 142.9	X-ray, 2D-NMR, MS	[26, 27, 38]
(29) (1*R*,5*R*,6*R*,8*R*)-Dec[4.4.0]ane-1,5-dimethyl-8-(1′methylethenyl)-5-isothiocyanate	*A. klethra*	Queensland, Austl.	+ 180	2D-NMR, MS	[38]
(32) R=NCS[#] (–)-10-isothiocyanato-4-amorphene	*Halichondria* sp.	north shore, O'ahu	– 63[ccl]	H-NMR, MS	[4, 6, 7]
(33) R=NHCHO[#] (–)-10-formamido-4-amorphene			– 50[ccl]	H-NMR, MS	
(39) R=NCS	*A. cannabina*	Bay of Taranto	– 91.2	H- & C-NMR, MS	[39]
(40) R=NHCHO	*A. cannabina*	Bay of Taranto		H-NMR, MS	[39]
(41) R=epi-NCS	*A. pulcherrima*	Darwin, Austl.	+ 9.1	H- & C-NMR, MS	[20]

(43) (+)-10-isothiocyanato-4-amorphene#	*Axinyssa fenestratus*	Fiji	+ 100[ccl]	H- & C-NMR, MS	[45]
(44) (+)-4-isothiocyanato-9-amorphene#	*A. fenestratus*	Fiji	+ 111	H- & C-NMR, MS	[45]
(45) (+)-10-isothiocyanato-4,6-amorphadiene#	*A. fenestratus*	Fiji	+ 74	H- & C-NMR, MS	[45]
(49) R=NCS axisothiocyanate-3	*Axinella cannabina*	Bay of Taranto	+ 165.2	H-NMR, MS	[46]
	Acanthella klethra	Austl.		H- & C-NMR, MS	[47, 26, 38]
	A. cf. *cavernosa*	Hachijo-jima Island		2D NMR, MS	[34]
(50) R=NHCHO axamide-3	*A. cannabina*	Bay of Taranto	− 6.9	H-NMR	[46]
(52) R=NCS	*Acanthella acuta*	Bay of Naples	− 12.9	H- & C-NMR, MS	[41]
(54) R=NCS axisothiocyanate-2#	*A. cannabina*	Bay of Taranto	+ 12.8	H-NMR	[5, 36, 50]
(55) R=NHCHO axamide-2			+ 37.5	H-NMR	[5, 36, 50]
(59) R= NCS 10α-isothiocyanoalloaromadendrane	*A. cannabina*	Bay of Taranto	− 7	H- & C-NMR	[36]
	A. pulcherrima	Darwin, Austl.		H- & C-NMR, MS	[20]
(60) R= NHCHO 10α-formamidoalloaromadendrane	*A. cannabina*	Bay of Taranto		H-NMR, MS	[36]
(61) R=NCS epipolasin B#	*Epipolasis kushimotoensis*	no location specified	+ 91.2 & 76.7?	H-NMR, MS	[51]
(63) 1-isothiocyanatoaromadendrane	*A. aplysinoides*	Pohnpei	+ 128	H- & C-NMR, MS	[49]
(65) R=NCS	*A. acuta*	Bay of Naples	− 32.8	H- & C-NMR, MS	[41]
(69) R=NCS	*Cadlina luteomarginata*	San Diego, CA	− 12	H-NMR, MS	[18]
(69) R=NCS epipolasin A#	*E. kushimotoensis*		+ 7.6	H-NMR, UV	[51]
(72) R=NCS	*A. pulcherrima*	Darwin, Austl.	− 8.0	H- & C-NMR, MS	[20]
(74) R=NCS	*A. cannabina*	Bay of Taranto	− 56	H-NMR, MS	[52]
(75) R=NHCHO			+ 14	H-NMR, MS	
(86) R=NCS 3-isothiocyanato-theonellin	*Theonella* cf. *swinhoei*	Okinawa		H- & C-NMR, UV	[57]
	Phyllidia pustulosa	Hachijo-jima Island			[43]
(87) R=NHCHO 3-formamido-theonellin	*Theonella* cf. *swinhoei*			H-NMR, MS	[57]
(90) R=NCS (6*R*,7*S*)7-isothiocyano-7,8-dihydro-α-bisabolene	*Halichondria* sp.	Ponape, Marshall Is.	+ 60.5	H- & C-NMR, MS	[58]

[1] rotations in chloroform unless indicated otherwise: [ccl], CCl_4; [mc], CH_2Cl_2; [h], hexane. A blank represents no report.
[2] data provided for methods indicated; IR for –NCS and/or –NHCHO absorptions given in all cases; syn = synthetic

olefins or olefinic equivalents, e.g. cyclopropyl functions. Atypical are compounds with other hetero functions.

The isocyano and isothiocyano functions are much less polar than the formamido group. Thus, it is not surprising that sponges bearing isothiocyanosesquiterpenes, which are usually oils, possess a characteristic pungent odor. Predictably, sponges containing the more polar, often crystalline, terpenoid formamides have less pronounced odors.

In the following sections, we will review the marine isonitriles by skeletal types. This permits comparison of their differences and may suggest biogenetic clues. Skeletal frameworks are generally revealed by reducing the isonitrile with lithium/ethylamine to the corresponding hydrocarbon. Where trivial names have been assigned for skeletal types and for compounds, we shall use these as reported in the literature. In some cases, when structures related to previously mentioned compounds are discussed, formula numbers will be employed.

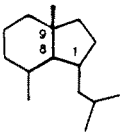
4.1 Sesquiterpenoids

The majority of marine isonitriles are sesquiterpenes with the molecular formula, $C_{16}H_{25}N$. Often cyclic, these are alkanes or alkenes possessing only a single isocyano-related functional group. In the mass spectrum, they exhibit a molecular ion at *m/z* 231, or an intense fragment ion at *m/z* 204, indicative of M^+-HCN. Some are crystalline (see Table 2). With few exceptions most of the isothiocyano and formamido analogs are minor noncrystalline metabolites (see Table 3).

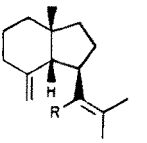
4.1.1 Axane

Axisonitrile-1 (**1**) and axisothiocyanate-1 (**2**) were the first pair of NC/NCS compounds isolated from *Axinella cannabina*, see Introduction [1]. That both compounds possessed a new skeleton was evident, when **1** was reduced ($Li/EtNH_2$) to axane (**6**). Other transformations involving the exocyclic methylene which survived selective reduction (Na/NH_3) of **1**, coupled with evaluation of the ^1H NMR data, supported its gross structure. Confirmation of **2** was secured when **1** was heated with sulfur and the resultant purified product found to be identical to the natural product.

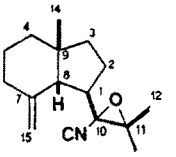
Further examination of the extracts of *A. cannabina* revealed axisonitrile-4 (**7**), axisothiocyanate-4 (**8**) and axamide-4 (**9**) [33]. A vinylic isonitrile function was supported by ^1H NMR signals at δ 1.67 and 1.89, which were assigned to the two isopropylidene methyls of **7**. Difficulty in isolating the natural product **9** was circumvented, when isonitrile **7** was transformed to **9**, mp 81–84 °C, by acetic acid in anhydrous ether. The absolute configurations of both axanes **1** and **7** and their analogs were later established [31] by studies including X-ray diffraction of the *p*-bromoaniline derivative of **2** and by CD data of (+)-10-methyldecalone-1 obtained from ozonolysis of the reduction (Na/NH_3) product of **1** [1].



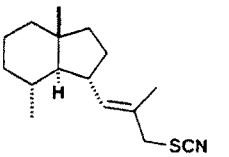
6 axane



7 R = NC
8 R = NCS
9 R = NHCHO



10



11

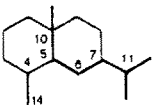
Recent studies on sponges and several nudibranchs in Japan revealed a number of isocyano-related compounds, among which are cavernoisonitrile (**10**) and cavernothiocyanate (**11**) [34]. Discussion of the possible roles of axanes **10** and **11** in chemical ecology and biogenesis will be deferred.

4.1.2 Eudesmane

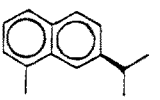
About two dozen isocyano- and isocyano-related compounds with a eudesmane (**12**) skeleton are known. Although most of these were isolated from *Axinella cannabina*, the first isocyanoeudesmane, acanthellin-1 (**14**) [3], was obtained from a related sponge, *Acanthella acuta*. (cf. Table 2). Eudalene (**13**) resulting after two steps (*i.* $Li/EtNH_2$, *ii.* Pd/C) from **14**, proved the eudesmane skeleton. ^1H NMR data including those observed by double resonance techniques, allowed assignment of the relative stereochemistry of **14**. This was accomplished after comparing the corresponding ^1H NMR data with those obtained from the hydrogenated products of β-eudesmene.

A minor isonitrile named acanthellin-2 with a specific rotation of $-24.1°$ was reported with no further details except some IR and MS data [3]. Isothiocyanate **15** and formamide **16** were also isolated from *Axinella cannabina* in a later study, in which isonitrile **14** and isomers **17–19** were also secured [35]. That **17–19** are $C_{4(14)}$-ene isomers of **14** was concluded after extensive double resonance ^1H NMR experiments. Coupling constants and nOe effects between the affected protons and various substituents of **17** established the relative stereochemistry of this series.

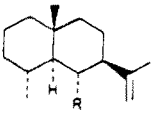
Compounds **20–22** are further examples of minor metabolites of *Axinella cannabina* [40]. When *Acanthella acuta* was reinvestigated by the same researchers, isocyano compound **20**, but not acanthellin-1 (**14**), was found to be the



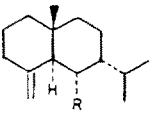
12 eudesmane



13 eudalene



14 R = NC
15 R = NCS
16 R = NHCHO



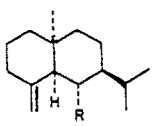
17 R = NC
18 R = NCS
19 R = NHCHO

major isonitrile-related compound. The collection sites for both sponges were identical to those of previous studies; i.e., *Axinella cannabina* from the Bay of Taranto and *Acanthella acuta* from the Bay of Naples.

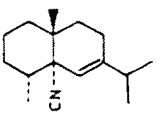
Both ^{13}C- and ^{1}H NMR experiments using 2D NMR techniques established that **20** had a *cis*-eudesmane ring juncture. This was based on a 12% nOe effect between the C-10 methyl and H-5 proton. Based on this and other evidence, the relative stereochemistry as shown in **20** [6α-isocyano-5α-H, 7α-H, 10α-eudesm-4(14)ene] and its analogs (**21**–**22**) was proposed [40].

Stylotelline (**23**) is a constituent of a *Stylotella* sp. collected offshore in New Caledonia. ^{13}C NMR spectra involving 2D NMR techniques provided the bulk of information leading to its structure. The absolute configuration was demonstrated after the tertiary isocyano group was removed to yield the known conjugated diene, (+)-δ-selinene (**24**). Optical rotation and spectral data of the transformation product were identical in all respects to those of the corresponding product obtained from eudesmols [42]. Although neither the isothiocyanato nor the formamido compounds were isolated, the latter was prepared, which allowed an nOe observation between the axial C-3 and the amide protons.

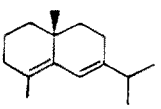
The structures and relative stereochemistry of *epi*-eudesmanes **25**–**27** from *Axinella cannabina* were formulated from 2D NMR and chemical correlation experiments [36]. The 500 MHz ^{1}H NMR spectrum of 11-formamido-7β *H*-eudesm-5-ene (**27**) measured in $CDCl_3$ showed a 2:3 *cis*:*trans* ratio of the –NHCHO group (*cis*: δ 8.06 d, $J = 2$ Hz; *trans*: δ 8.20 d, $J = 12$ Hz). Isothiocyanate **26** was one of several sesquiterpenes reported from *Acanthella pulcherrima* [20].



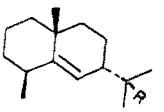
20 R = NC
21 R = NCS
22 R = NHCHO



23



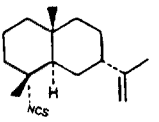
24 δ-selinene



25 R = NC
26 R = NCS
27 R = NHCHO

During our studies of the kalihinols (Sect. 4.2.2), isothiocyanate **28** was one of several isocyano-related compounds isolated from *Acanthella klethra* from Australia. Its structure was also published independently from a *Ciocalypta* sp. in Hawaii [37] and reported as a poster at the 1988 Gordon Research Confer-

ence on marine natural products. The structures of compounds **28** and **29**, which is the C-7 epimer of **28**, obtained from the same sponge (*A. klethra*) were published recently by another group [38].



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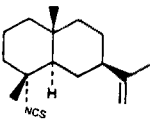
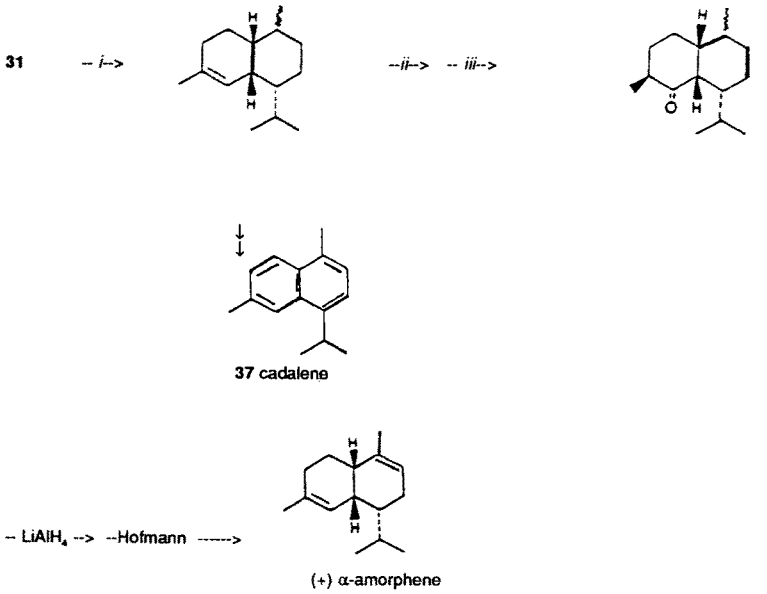


29

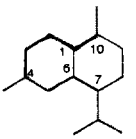
4.1.3 Amorphane

From a *Halichondria* species collected by dredging at -200 m off the north shore of O'ahu, two series of isocyano-related triads with diverse skeletal types were discovered [6, 7]. Possessing the amorphane (**30**) skeleton, sesquiterpenoids **31–33** comprised about 80% of the mixture, of which approximately equal amounts were compounds **31** and **33**. The remaining 20% were acyclic diterpenoid isonitriles (cf. Sect. 4.3). Several elegant transformations of **31** secured the structures of the isonitrile and its analogs. Conversion (Scheme 1) of **31** to the hydrocarbon (*i.* $Li/EtNH_2$) and the ketone (*ii.* *m*-chloroperbenzoic acid, *iii.* BF_3 etherate) permitted unambiguous assignment of functionality and

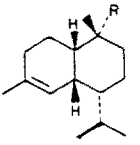


Scheme 1. Transformations of **31** and **33**

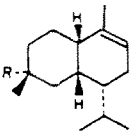
relative stereochemistry. The amorphane skeleton was confirmed by its transformation product, cadalene (**37**). The Hofmann degradative procedure applied to formamido compound **33** resulted in a non-conjugated diene and comparison with authentic (+)-α-amorphene provided the absolute stereochemistry for this series. A C-7 epimer of **31**, compound **34**, was recently reported from *Acanthella* cf. *cavernosa* [34].



30 amorphane



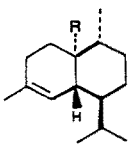
31 R = NC
32 R = NCS
33 R = NHCHO
34 C_7- *epi*-**31**



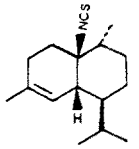
35 R = NC
36 R = SCN

From the nudribranch, *Phyllidia pustulosa*, the *cis*-decalin **35**, an analog of **31**, was one of seven isonitriles isolated [43]. In 1989 an unusual thiocyanate **36**, mp 67–68 °C, was reported to occur in a Palauan sponge, *Axinyssa* (= *Trachyopsis*) *aplysinoides*, from which several other marine isonitriles were obtained [44]. The corresponding isonitrile, which was not present in the sponge, was later obtained from *P. pustulosa* [43].

Amorphanes **38**–**40** were also isolated from *Acanthella cannabina*. These are *trans*-fused and functionalized at C-1 rather than C-10 which was the case with the *Halichondria* metabolites. The carbocyclic skeleton was demonstrated when **39** was dehydrogenated to yield cadalene (**37**). Relative stereochemistry assignments were secured by extensive ^{13}C- and ^{1}H NMR experiments [39], in which ^{13}C data were particularly useful, as illustrated in the case of isothiocyanate **41**, one of several isothiocyanates obtained from *Acanthella pulcherrima* [20]. The *cis*-fused ring was corroborated by comparing the corresponding chemical shifts with those of *ent*-epicubenol (**42**) [20], which has the same relative stereochemistry.



38 R = NC
39 R = NCS
40 R = NHCHO



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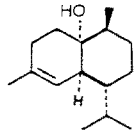
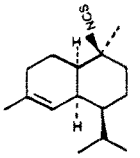


42 ent-epicubenol

In a recent study, a Fijian sponge, *Axinyssa fenestratus*, was found to elaborate four isothiocyanatoamorphanes (**43**–**46**) [45]. That this series of compounds may be antipodal to **32** from the Hawaiian *Halichondria* sp. was indicated by the spectral and optical data of **43** [(1*R*,6*S*,7*S*,10*S*)-10-isocyanato-4-amorphene].

The antipodal relationship appears secure despite differences in the magnitude of the rotation values ($[\alpha]_D$: **32**, $-63°$; **43**, $+100°$), both of which were measured in CCl_4. Of particular interest is the isothiocyanato-alcohol **46**, which is the first example of an oxygenated marine sesquiterpenoid possessing a function unrelated to the –NC/–NCS/–NHCHO triad.



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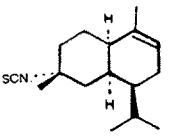

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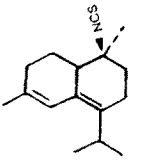

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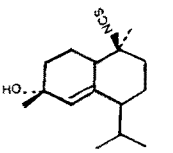
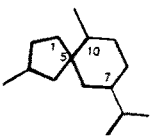


46

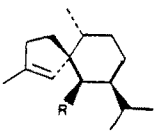
4.1.4 Spiroaxane

Continued studies on *Axinella cannabina* by researchers at the University of Naples resulted in the isolation of a novel series of metabolites with a spiroaxane (**47**) skeleton [46]. These may be biogenetically related to the amorphanes **38**–**40**, which were subsequently isolated from the same sponge [39, 46]. Axisonitrile-3 (**48**) and its analogs, **49** and **50**, are *spiro*[4.5]decanes whose carbocyclic framework was deduced by chemical and spectral evidence. A single crystal X-ray study of **48** confirmed the structure and provided its relative stereochemistry. Isonitrile **48** appears to be ubiquitous, having been isolated, for example, from *Acanthella acuta* collected off Banyuls, France [47], *Acanthella klethra* from Australia [26, 38], and *Axinyssa* (= *Trachyopsis*) *aplysinoides* from Palau [44, 49] (cf. Table 2). Isothiocyanate **49** was also isolated from this sponge [26, 38].

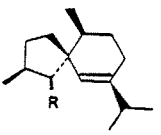
Fattorusso's group, which earlier had discovered **48**–**50** in *Axinella cannabina*, reported the occurrence of another spiroaxane series, **51** and **52**, from *Acanthella acuta*. The habitat of the latter sponge is the Bay of Naples. As was the case with other metabolites reported in their recent studies, the investigators deduced the structures of isonitrile **51** and isothiocyanate **52** chiefly by 2D NMR methods.



47 spiroaxane



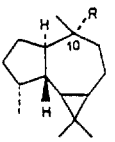
48 R = NC
49 R = NCS
50 R = NHCHO



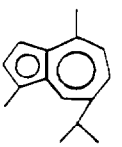
51 R = NC
52 R = NCS

4.1.5 Aromadendrane

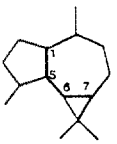
Yet another skeletal type is represented by axisonitrile-2 (**53**), which was isolated from the ether-soluble fraction of the acetone extract of *A. cannabina* [50]. The cyclopropyl moiety gave rise to the high field complex resonances between 0.8–0.4 ppm in the ^{1}H NMR spectrum. Selenium dehydrogenation of **53** yielded the aromatic compound, guaiazulene (**56**), thereby implicating the aromadendrane (**57**) ring system. Both **54** and **55**, constituents of a complex mixture from which axanes **1–3** were obtained, were verified by known transformations of **53** [5]. The question of the stereochemistry about C-10 and, hence, the relative stereochemistry of this series (**53–55**) [49] was ascertained ten years later by extensive use of ^{1}H- and ^{13}C NMR spectroscopy with particular attention given to nOe data [36]. Interestingly, axisonitrile-2 (**53**) was one of the isonitrile compounds from *Phyllidia pustulosa* [43].



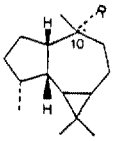
53 R = NC
54 R = NCS
55 R = NHCHO



56 guaiazulene

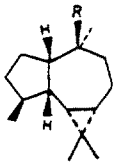


57 aromadendrane

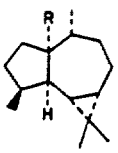


58 R = NC
59 R = NCS
60 R = NHCHO

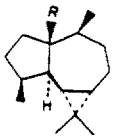
Along with *epi*-eudesmanes, **25–27**, alloaromadendranes **58–60** were separated from the chloroform solubles of a methanol extract of *A. cannabina* [36]. As was the case with axisonitrile-2 (**53**), extensive spectroscopic analyses including Eu(fod)$_3$ shift reagent experiments in ^{1}H- and ^{13}C NMR permitted assignment of relative stereochemistry. *Acanthella pulcherrima* was also a source of isothiocyanate **59**, although both isonitrile and formamido compounds appear to be absent [20].



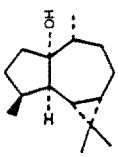
61 R = NCS



62 R = NC
63 R = NCS

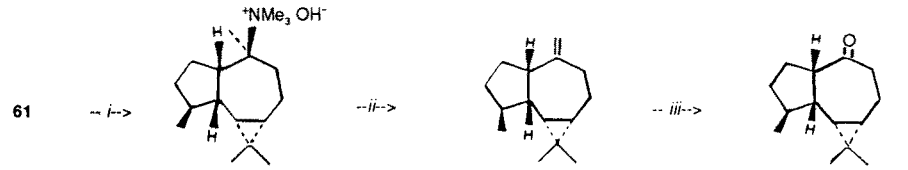


64 R = NC
65 R = NCS



66 palustrol

Epipolasin-B (**61**), mp 92 °C, was one of two isothiocyanates obtained from the sponge *Epipolasis kushimotogensis*. Neither the isonitrile nor formamido analog were found with the isothiocyanate. Instead, the thiourea adduct of β-phenylethylamine was isolated along with another isothiocyanate-thiourea pair [51]. The absolute configuration of **61** was determined by appropriate



i: (1) $LiAlH_4$, (2) CH_3I, (3) Ag_2O; *ii*: heat ; *iii*: HIO_4

Scheme 2. Transformation of **61** to norketone, **67**

degradations (Scheme 2) and comparisons with known compounds. Both transformed exocyclic olefin and norketone **67** were identical with the known compounds, (−)-aromadendrene and (+)-apoaromadendrone, respectively. Accordingly, on the basis of the optical rotations of axisothiocyanate-2 (**54**, $[\alpha]_D$ + 12.8°) and epipolasin-B (**61**, $[\alpha]_D$ + 76.7°, **54** and **61** differ in stereochemistry [51].

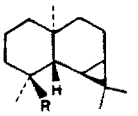
Two isocyano-alloaromadendranes with identical melting points and similar rotations were isolated from *Acanthella acuta*, albeit, collected from different regions. The structure of **62** (mp 65–66 °C, $[\alpha]_D$ − 9°) was correlated with the corresponding alcohol, palustrol (**66**), with tentative stereochemistry assigned at C-1 and C-10 by the Belgian group [47]. The corresponding isothiocyanate **63** was obtained upon reinvestigation of the Palau sponge *Axinyssa aplysinoides* [49]. In a separate study, extensive spectroscopic studies of the isonitrile **64** (mp 65–66 °C, $[\alpha]_D$ − 13.7°) and isothiocyanate **65** led the Italian workers to the proposed relative stereochemistry [41]. Whether the samples were compared is not known, but it is interesting to note that another isothiocyanate from an Australian *Acanthella pulcherrima* was reported in a later study to be identical to **62** in all respects [20].

4.1.6 Epimaaliane

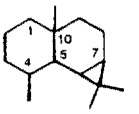
In an elegant field study performed in southern California, a variety of metabolites were obtained from the nudibranch *Cadlina luteomarginata* [18]. Isolated compounds were assessed as possible defensive substances against predators. Though several isonitriles and isothiocyanates were separated on a Florisil column using an extract from approximately 100 animals, only two compounds (**68** and **69**) were chemically defined. Compound **69** was the major isonitrile-related constituent among a complex mixture of other isocyano- and isothiocyano-compounds. The relative stereostructure of **69** was secured by NMR methods and confirmed by single crystal X-ray diffraction of the formamide obtained from **68** [18]. Both **68** and **69** are epimaalianes (**70**) which result in eudalene (**13**) upon dehydrogenation.

Subsequent to the California study, epipolasin-A, mp 49–50 °C, was shown to have structure **69** by Japanese workers [51]. A thiourea derivative was also obtained from its source, *Epipolasis kushimotoensis*. Both structures were deduced independently by the same strategy of chemical transformations used on **61**. Reduction of the isothiocyanate **69** to form the amine, followed by methylation and Hofmann degradative procedures, resulted in the exocyclic olefinic product. Ozonolysis led to the ketone which on comparison of the circular dichroism data with those obtained from the natural hydroxy analog, maaliol (**71**), permitted the assignment of its structure and absolute configuration [51]. The related isothiocyanate **72** from *Acanthella pulcherrima*, whose spectroscopic properties are identical to those of epipolasin-A, was shown to be the enantiomorph on the basis of their nearly equal but opposite optical rotations measured in chloroform (Table 3) [20]. While other minor metabolites, epipolasins -C, -D, and -E, were reported from the extract of *E. kushimotoensis* only partial data based primarily on IR spectroscopy [51] suggest that these were isothiocyanates.

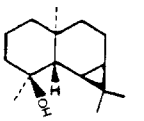
Continued studies by the Naples group of *Axinella cannabina* resulted in another epimaaliane series of isocyano-related compounds, **73–75**. Dehydrogenation of **73** with Pd/C at 350 °C afforded eudalene (**13**). The cyclopropyl moiety was evidenced by both proton and carbon resonances in the NMR spectra and relative stereochemistry was assigned by coupling constants and nOe data [52].



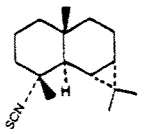
68 R = NC
69 R = NCS



70 epimaaliane



71 maaliol



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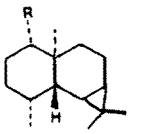


73 R = NC
74 R = NCS
75 R = NHCHO

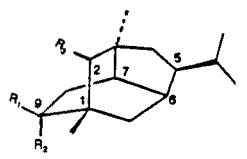
4.1.7 Pupukeanane

A novel series of marine isonitriles is represented by three related tricyclic compounds, **76–78**. 9-Isocyanopupukeanane (**76**) was isolated chiefly from a *Ciocalypta* sp. after the nudibranch *Phyllidia varicosa* was observed feeding on the off-white sponge [15]. Most of the sponges which at first had been identified

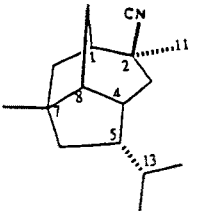
as *Hymeniacidon* sp. were collected off the Pupukea site. It is an area on the north shore of O'ahu, which is still popular with associates of Scheuer's research group. Burreson's key observation of the nudibranch-sponge connection resulted in the isolation of the isonitrile from the sponge [15]. That the isonitrile was tricyclic was suggested by failure of the parent hydrocarbon (obtained by Li, $EtNH_2$) to undergo Pd/C or Se dehydrogenation. Several transformations designed to permit comparison with known tricyclic sesquiterpene hydrocarbons led to the conclusion that **76** possesses a novel carbocyclic skeleton, which was named pupukeanane. Conversion of **76** to the amine and derivatization with phenylisothiocyanate produced the corresponding phenylthiourea derivative suitable for X-ray crystallography [15]. Hence, compound, **76**, skeleton is 1,3-dimethyl-5-isopropyl-tricyclo[4.3.1.$0^{3,7}$]decane.

Isomeric 2-isocyanopupukeanane (**78**), which was not resolved by a variety of preparative TLCs (e.g., argentate silica gel), crystallized on standing in the refrigerator. The isomer had been anticipated since carbon NMR data strongly suggested its existence by displaying 32 instead of 16 anticipated signals in the purified fraction. Single crystal X-ray diffraction analysis of **78** confirmed its structure as a regioisomer of **76**. Transformation of isonitrile **76**, but not the hindered **78**, to the corresponding 9-ketone permitted assignment of the absolute configuration of both compounds [53]. Both isocyano-pupukeananes **76** and **78** were the first marine isonitriles to be synthesized (cf. Sect. 6).

The most recent isonitrile in this series is the rearranged pupukeanane, isocyano*neo*pupukeanane (**80**), from a *Ciocalypta* sp. found in the south shore of O'ahu. Ample evidence suggested the tricyclic compound was similar to **76** and **78**, but the new compound **80** possessed the isocyano group at the quaternary C-9 carbon (δ 64.5 t, $J \sim 4$ Hz) instead of a methine carbon expected for **76** and **78**. Extensive NMR spectral studies including long-range carbon–hydrogen, nOe, and COSY connectivities were necessary to establish that **80** from the south shore *Ciocalypta* sp. was indeed different from **76** [54]. Isonitrile **80** is 3,6-dimethyl-9-isopropyltricyclo[4.3.1.$0^{3,7}$]decane.



76 R_1 = NC R_2 = H R_3 = H
77 R_1 = H R_2 = NC R_3 = H
78 R_1 = H R_2 = H R_3 = NC



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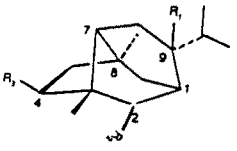


80 R_1 = NC R_2 = H R_3 = H
81 R_1 = H R_2 = SCN R_3 = H
82 R_1 = H R_2 = H R_3 = SCN

The novel isonitriles having the pupukeanane and the *neo*pupukeanane skeletons are generally not accompanied by the isothiocyano and formamido analogs, though recent studies have uncovered the thiocyanate function among these compounds [48, 49]. Investigations on an Okinawan sponge, *Phycopsis terpnis*, and an unidentified Pohnpei sponge revealed the uncommon –SCN

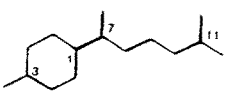
function in **81** and **82**, having the *neo*pupukeanane framework [48] (cf. Sect. 4.3). The rare thiocyanate function was encountered earlier from a Palauan *Axinyssa aplysinoides* [44]. Reinvestigation of another sample from Pohnpei resulted in the isolation of **81** and other isonitrile-related fractions [49]. Extensive nOe studies resulted in the reassignment of 2-thiocyanato*neo*pupukeanane as shown in **81** [49], which had previously been assigned as the C-2 epimer [48].

The C-9 epimer (**77**) of 9-isocyanopupukeanane (**76**) was isolated from the Japanese nudibranchs *Phyllidia bourguini* [56] and *P. pustulosa* [43]. The differences in W-coupling of the appropriate protons of **76** (H-9 and H-2) and **77** (H-9 and H-2) and carbon resonances were verified by NOESY experiments, thereby confirming the epimeric relationship at C-9 [56]. In a subsequent study, 2D NMR methods were again critical for determining the structure of 2-isocyanoallopupukeanane (**79**) from *P. pustulosa*, also collected off Hachijo-jima Island. The rearranged **79** may be biogenetically related to the amorphenes [43] as noted previously [55].

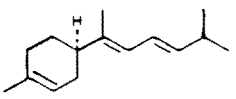
4.1.8 Bisabolane

Several examples of the monocyclic isothiocyano sesquiterpenoids having the bisabolane (**83**) skeleton are known. Along with the hydrocarbon theonellin (**84**), isothiocyanate **86** and formamide **87** were obtained from the Okinawan sponge *Theonella* cf. *swinhoei*. It seems remarkable, but not unusual, that not only was the amide the major constituent, but the isonitrile **85** was the missing member of the triad [57]. Relative stereostructures were indicated by NMR analysis of theonellin formamide (**87**) and its transformation products.

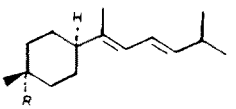
In several related studies that involved *Ciocalypta* and *Phyllidia* spp., optically inactive isonitrile **85** was part of the mixture from a Sri Lankan *Phyllidia* sp. The mixture also contained a minor isothiocyanate which was not identified [55]. The observation that 7-isocyano-7,8-dihydrobisabolene **88** and isocyanato **89** were obtained from separate collections of *Ciocalypta* spp. was surprising



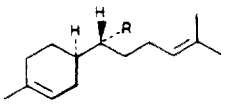
83 bisabolane



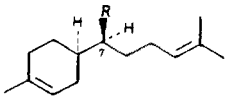
84 theonellin



85 R = NC
86 R = NCS
87 R = NHCHO



88 R = NC
89 R = NCO

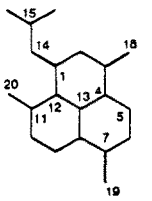


90 R = NCS

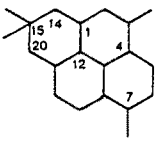
with the initial discovery of a naturally occurring isocyanate. Several other metabolites including the amino analogs were derived from different O'ahu sites, the north shore (Pupukea) and Kaneohe [55]. In a separate study, investigation of a Pohnpei *Halichondria* sp. resulted in the isolation of (6*R*,7*S*)-isothiocyano-7,8-dihydro-α-bisabolene (**90**), along with several amino-related compounds. Although isonitriles were detected, they were unstable under the chromatographic separation conditions [58]. Compounds related to **88** and **90** are interesting, since the epimeric functionality at C-7 raises the question of a common intermediate in their biogenesis [55]. Compound **88** was also a minor constituent isolated from the mollusk, *Phyllidia pustulosa* [43] and *Acanthella* cf. *cavernosa* [34]. The synthesis of 3-isocyanotheonellin (**85**) was reported recently [80].

4.2 Diterpenoids

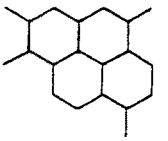
With the exception of the acyclic diterpenoids, e.g., **131–133** (cf. Sect. 4.3), isolated earlier with **31–33** from a *Halichondria* sp. [4], the majority of isocyano-related diterpenoids are complex. Their skeletal framework may be tri- or tetracyclic and multiple nitrogenous functions of the now familiar trio are common. Marine isocyanoditerpenoids generally are of two types: those related to the (*i*) amphilectane (**91**)-cycloamphilectane (**92**) skeleton and others having the (*ii*) kalihinol-type (**94-A** and **94-F**) skeletons. For example, tetracyclic adociane (**95**) may be considered a "modified cycloamphilectane" (**93**) with a rearranged methyl group. Recent investigations of an Okinawan sponge in the



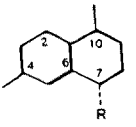
91 amphilectane



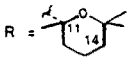
92 cycloamphilectane



93 modified cycloamphilectane



94- kalihinol-type



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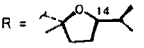

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Table 4. Diterpenoid Isocyano/Isothiocyano/Formamido Compounds

Compound (Structure) [Molecular Formula]#	Organism	Origin	$[\alpha]_D^1$	mp °C	Spectral Data[2]	Refs.
diisocyanoadociane* (**95**) [$C_{22}H_{32}N_2$]	*Amphimedon* sp.	Townsville, Austl.	+ 47.4[mc]		X-ray, H-NMR, MS	[23, 61]
(±)-diisocyanoadociane			+ 23.0		syn, H-NMR	[63]
	Adociidae	Miyako Isl.				[59]
8,15-diisocyano-11(20)-amphilectene	*Hymeniacidon*	Glover Reef,			X-ray, H- & C-NMR, MS	[60]
R=NC (**96**) [$C_{22}H_{32}N_2$]	*amphilecta*	Belize	− 56	105–106		[60]
(±)-(**96**)				84–86	syn, H- & C-NMR	[79]
R=NHCHO (**97**) [$C_{22}H_{34}N_2$]			− 24		H- & C-NMR, MS	[60]
7-isocyano-11(20),14-epiamphilectadiene	*Amphimedon* sp.	Townsville, Austl.	+ 116.8	113–115	X-ray, H- & C-NMR, MS	[61]
(**98**) [$C_{21}H_{31}N$]	*Adociidae*	Miyako Isl.				[59]
7-isocyano-11(20),15-epiamphilectadiene	*Amphimedon* sp.	Townsville, Austl.			H-NMR, MS	[61]
(**99**) [$C_{21}H_{31}N$]						
7,15-diisocyano-11(20)-epiamphilectene	*Amphimedon* sp.	Townsville, Austl.	+ 3.6		H-NMR, MS	[61]
(**100**) [$C_{22}H_{32}N_2$]	*Adociidae*	Miyako Isl.				[59]
7,15-diisocyanoadociane (**101**)	*Amphimedon* sp.	Townsville, Austl.	+ 45.8	123	X-ray, H- & C-NMR, MS	[61]
[$C_{22}H_{32}N_2$]						
8-isocyano-10-cycloamphilectene (**102**)	*Amphimedon* sp.	Townsville, Austl.	− 21.7	88–89	X ray, H- & C-NMR, MS	[61]
	Adociidae	Miyako Isl.				[59]
8-isocyano-1(12)-cycloamphilectene (**103**)	*Halichondria* sp.	Palau			X-ray deriv	[22]
	Adociidae	Miyako Isl.				[59]
7-isocyano-11-cycloamphilectene (**104**)	*Halichondria* sp.	Palau		134	H-NMR, MS	[22]
[$C_{21}H_{31}N$]						
7-isocyano-1-cycloamphilectene (**105**)	*Halichondria* sp.	Palau	− 14	182–183	H- & C-NMR, MS	[22]
[$C_{21}H_{31}N$]	*Adociidae*	Miyako Isl.				[59]
8-isocyano-10,14-amphilectadiene (**106**)	*Halichondria* sp.	Palau	− 79.8		H- & C-NMR, MS	[22]
kalihinol-A (**107**) [$C_{22}H_{33}ClN_2O_2$]	*Acanthella* sp.	Guam	+ 16	233	X-ray	[19, 25]
	A. cavernosa	Fiji			H- & C-NMR, MS	[65]
	A. klethra	Satsunan Arch.				[21]

kalihinol-E (**108**) [$C_{22}H_{33}ClN_2O_2$]	*Acanthella* sp.	Fiji	+ 4	197–199	H- & C-NMR, MS	[19, 64]
kalihinol-X (**109**) [$C_{22}H_{33}N_2O_2S$]	*Acanthella* sp. *Acanthella cavernosa*	Fiji Fiji	– 22	199–200	H- & C-NMR, MS	[19] [65]
kalihinol-Y (**110**) [$C_{21}H_{32}ClNO_2$]	*Acanthella* sp. *A. cavernosa*	Fiji Fiji	– 34	176–179	H- & C-NMR, MS	[19] [65]
kalihinol-Z (**111**) [$C_{22}H_{33}ClN_2O_2$]	*Acanthella* sp. *A. cavernosa*	Fiji Fiji	– 10	228–230	H- & C-NMR, MS	[19] [65]
kalihinol-B (**113**) [$C_{22}H_{33}ClN_2O_2$]	*Acanthella* sp.	Guam	+ 10		H- & C-NMR, MS	[19, 64]
kalihinol-C (**114**) [$C_{22}H_{32}N_2O_2$]	*Acanthella* sp.	Guam	+ 6		H- & C-NMR, MS	[19, 64]
kalihinol-D (**115**) [$C_{22}H_{33}N_2O_2$]	*Acanthella* sp.	Guam	+ 8	183–184	H- & C-NMR, MS	[19]
kalihinol-F (**112**) [$C_{23}H_{33}N_3O_2$]	*Acanthella* sp. *A. cavernosa*	Guam Fiji	+ 8	176–178	X-ray H- & C-NMR, MS	[19, 64] [65]
kalihinol-G (**116**) [$C_{23}H_{33}N_3O_2S$]	*Acanthella* sp.	Fiji	– 12		H- & C-NMR, MS	[19]
kalihinol-H (**117**) [$C_{23}H_{33}N_3O_2S$]	*Acanthella* sp.	Fiji	+ 98		H- & C-NMR, MS	[19]
kalihinol-I (**121**) [$C_{22}H_{33}ClN_2O_2$]	*A. cavernosa*	Thailand		180	H- & C-NMR, MS	[45]
kalihinol-J (**122**) [$C_{22}H_{35}ClN_2O_3S$]	*A. cavernosa*	Thailand		199–200	H- & C-NMR, MS	[45]
isokalihinol-F (**118**) [$C_{23}H_{33}N_3O_2$]	*A. cavernosa*	Fiji	+ 13.6	180–182	H- & C-NMR, MS	[65]
kalihinene (**119**) [$C_{22}H_{32}N_2O$]	*A. klethra*	Satsunan Arch.	0		X-ray, H- & C-NMR, MS	[21]
isokalihinol-B (**120**)# [$C_{22}H_{33}ClN_2O_2$]	*A. klethra*	Satsunan Arch.	0		H- & C-NMR, MS	[21]
10-isothiocyanatobiflor-5,15-diene (**123**) [$C_{21}H_{33}NS$]	family: *Adociidae*	Miyato Island	+ 97		H- & C-NMR, MS	[59]
7-isocyano*neo*amphilecta-11,15-diene (**124**)# [$C_{21}H_{31}N$]	family: *Adociidae*	Miyato Island	+ 31.8	109–110	X-ray, H- & C-NMR, MS	[59]

[1] rotations in chloroform unless indicated otherwise: [mc], CH_2Cl_2. A blank represents no report.
[2] data provided for methods indicated; IR for –NC/–NCS/and/or/–NHCHO bands given in all cases.
absolute stereochemistry.

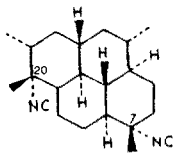
family *Adociidae* revealed two unusual diterpenes which resemble the kalihinols and cycloamphilectanes [59].

Marine isocyanoditerpenoids are generally crystalline. In those instances where melting points were not reported, we believe that the trace amounts involved in those studies often precluded cystallization.

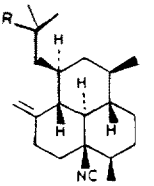
4.2.1 Amphilectane–Cycloamphilectane

Amphilectane and cycloamphilectane are trivial names proposed [60, 61] for the compounds derived from two sponges, an *Amphimedon* sp. from Australia and *Hymeniacidon amphilecta* (order: Halichondrida) from the Caribbean. Isolated from an *Amphimedon* sponge, which was identified initially as an *Adocia* sp., the first tetracyclic C_{20} marine isonitrile was named diisocyanoadociane (**95**) [23]. Highly crystalline **95** may be considered a rearranged cycloamphilectane bearing two isocyano groups. While mild hydrolysis of **95** confirmed the presence of both isocyano functions, the relative stereostructure of the unique perhydropyrene ring system was ascertained by X-ray crystallographic determination of the natural product, **95** (1,8-diisocyano-1,2,5,8-tetramethylperhydropyrene) [23]. The absolute configuration of **95**, which was the first metabolite from *Amphimedon*, was achieved subsequently by single crystal X-ray analysis of a *p*-bromobenzamide derivative [62]; it was later synthesized (cf. Sect. 6).

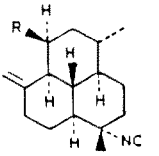
Two related tricyclic diterpenes, diisocyano **96** and isocyanoformamido **97**, were discovered as a result of antibacterial monitoring during workup of *H. amphilecta* [60]. Both compounds upon hydrolysis yielded the expected diformamide. Spectral data, including ^{13}C NMR, contributed to the proposed structures, which were confirmed by X-ray analyses [60].



95 adociane



96 R = NC
97 R = NHCHO

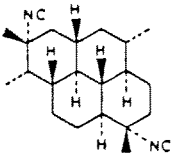


98 R = -CH=CMe$_2$
99 R = -CH$_2$C(Me)=CH$_2$
100 R = -CH$_2$C(NC)Me$_2$

Shortly after amphilectanes **96** and **97** were reported, other metabolites from *Amphimedon* were isolated, with the majority of their structures supported by the corresponding spectral and X-ray data. Although the gross structures of the series, **98–100**, are regio isomers with respect to one of the isocyano functions, they also exhibit minor differences in the C_4 moiety attached to C-1. X-ray determination of **98** led to assignment of its relative stereochemistry, thereby securing assignments for **99** and **100** by comparison of spectroscopic data [61].

The 7,15-diisocyano isomer (**101**) of **95** (–NC substituents at C-7 and -20), and 8-isocyano-10-cycloamphilectene (**102**) are also *Amphimedon* metabolites.

From the mixture, a minor component whose double bond was at first assigned incorrectly [61], was isolated as the major metabolite from a *Halichondria* sp. and identified as 8-isocyano-15,20-cycloamphilect-1(12)-ene (**103**) [22]. X-ray crystallography of **103** and its minor co-metabolites, **104–106**, provided relative stereostructures.



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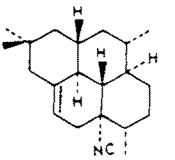

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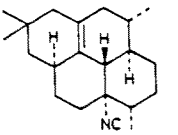

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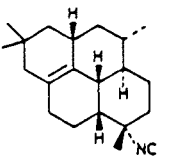
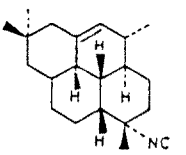


104



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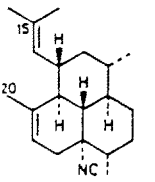


106

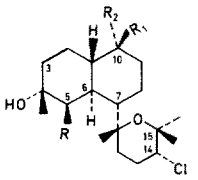
The configurational differences between **104** and **105** with the partial *cis*-fused junctions and those of the all *trans*-fused **95** and **101** (and related compounds) were pointed out [22] and resolved [62] with the determination of the absolute stereochemistry of diisocyanoadociane (**95**) (vide supra). Selective conversion to, and preparation of, an appropriate formamido-(*p*-bromobenzamide) derivative for X-ray determination [62], coupled with total synthesis [63], support the 1*R*,2*R*,3a*S*,5*S*,6*R*,8*S*,8a*S*,10a*S*,10b*S*,10c*S* absolute configuration.

4.2.2 Kalihinols

Sponges of the genus *Acanthella* are widely distributed in the Mediterranean Sea and the Pacific Ocean. Marine isonitriles of the European *A. acuta* have various C_{15} terpenoid skeletons [3, 41, 47]. Isocyano-related sesquiterpenoids are also natural products of *A. pulcherrima* [20] and *A. klethra* [26, 38] from Australia (cf. Sect. 5.1). From *Acanthella* spp. collected in the north (Guam) and south (Fiji) Pacific, eleven highly functionalized isonitriles were reported in a series of communications [19, 25, 64]. The compounds have in common a highly substituted *trans* decalin ring attached to either a substituted (*i*) tetrahydropyran (type A, **94-A**) or (*ii*) tetrahydrofuran (type F, **94-F**) as part of an unrearranged C_{20} isoprenoid structure. As a class, the kalihinols are unique since they

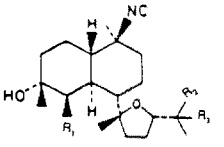
represented the first examples of marine isocyano-related compounds having functionality other than a double bond or cyclopropyl group.

Preliminary data from IR, ^{1}H- and ^{13}C NMR measurements conducted on HPLC-resolved fractions indicated that some of the compounds were di- and tri-isocyanoditerpenols. From a complex mixture rich in isonitriles obtained from LH-20 Sephadex separation, initial HPLC experiments afforded the two major types, -A (**94-A**) [25] and -F (**94-F**) [64], arbitrarily coded according to the HPLC reversed-phase elution pattern. Compounds in the A–H series of kalihinols were obtained from crude extracts of the Guam *Acanthella* sp. [19]. Solvent partition of the extracts by the Kupchan scheme indicated the absence of sesquiterpenoid isonitriles in the hexane layer and presence of diterpenoid isonitriles distributed in both carbon tetrachloride and chloroform partition fractions. By contrast, from the Fijian *Acanthella* sp., the less polar series of kalihinols, designated X–Z, were found in the carbon tetrachloride layer [19]. Cursory examination of small specimens of Australian *A. pulcherrima* and *A. klethra* revealed no kalihinols. Instead, isothiocyanates were noted in the hexane fraction as inferred from the diagnostic broad bands in the IR spectra [26]. These compounds were sesquiterpenes bearing mainly –NC and NCS functions, and were the subject of later communications by separate groups [20, 38].



Kalihinol type-A			R_1	R_2	R_3
107	A =		NC	Me	NC
108	E =	epi-A			NC
109	X =		Me	NCS	NC
110	Y =		$=CH_2$		NC
111	Z =		Me	NC	NC
121	I =		Me	NCS	NCS
122	J =		Me	NCS	NHCHO

During the early studies of the kalihinols, the first to be examined were diisocyano kalihinol-A (**107**) [25] and triisocyano-F (**112**) [64]. Although the number of isocyano groups in kalihinol-A and -F was indicated clearly from NMR data, the nature of the heterocyclic ring could only be inferred. After repeated HPLC resolutions involving both reverse and normal modes followed by crystallization trials, suitable crystals for X-ray analysis led to the structural assignments of all diterpenoids belonging to the two kalihinol types. Circumstantial evidence for the furanyl and pyranyl moieties – gleaned from prominent



Kalihinol type-F			R_1	R_2	R_3
112	F =		NC	Me	NC
113	B =		NC	Me	Cl
114	C =		NC	$=CH_2$	
115	D =		Cl	Me	NC
116	G =		NC	Me	NCS
117	H =	10-NCS-F (structure **112**)			

^{13}C-NMR resonances in the 60–80 ppm region – was confirmed by X-ray determinations.

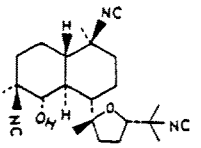
To separate homogeneous compounds from co-eluting metabolites, repeated HPLC experiments with changes in column and solvent systems were necessary. For example, kalihinol-A (**107**) sharing similar retention times with kalihinol-C (**114**), and kalihinol-F (**112**) with kalihinol-E (**108**), were resolved successfully on an ODS reverse phase column. Crystallization experiments were repeatedly undertaken. The sample of kalihinol-F (**112**) prepared for X-ray analysis had two $C_{22}H_{33}N_3O_2$ molecules in its asymmetric unit.

Separation of trace constituents, kalihinol-D (**115**), -G (**116**), and -H (**117**), was achieved only after repeated chromatographies. Regio-placement of groups and assignment of relative stereochemistry was facilitated by observing the relative and affected chemical shifts in both proton and carbon NMR spectra and by considering the presence or absence of coupling with the ^{14}N of the –NC (but not –NCS) group [19].

Independent studies involving two separate collections of *A. cavernosa* from Fiji afforded kalihinol-A (**107**), -F (**112**), -X (**109**), and a new compound, isokalihinol F (**118**) [65]. The latter was obtained from a batch, in which kalihinol-A was the major component. Recollection of the same sponge a year later, however, resulted in -X (**109**), -Y (**110**), and -Z (**111**) as the only kalihinols [65]. It is unclear, whether seasonal variation is the determining factor of the chemical changes noted in *A. cavernosa*. Our sample from Fiji identified only at the genus level contained kalihinol-X, -Y, -Z, and minor kalihinols, none of which were found identical (TLC examination) to the A–H types of the Guam *Acanthella* [19].

A subsequent study of *A. cavernosa* from Thailand by California workers revealed two additional F-type kalihinols in addition to kalihinol-X (**109**) and -Y (**110**) [45]. The structures of kalihinol-I (**121**) and -J (**122**) were secured by low resolution chemical ionization mass spectral and NMR data. Both ^{1}H-^{1}H COSY and ^{13}C NMR techniques were used extensively. Furthermore, hydrolysis of compound **109** to kalihinol-J (**122**) confirmed the assignments.

Slight, yet significant, variations were evident when *A. klethra* from Kuchinoerabu Island of the Satsunan Archipelago was studied by a Japanese research group [21]. In addition to kalihinol-A (**107**), kalihinene (**119**) and isokalihinol-B (**120**) were obtained. In contrast to the typical *trans*-fused kalihinol **107** or **120**, NMR data clearly showed a *cis*-fused octalin of kalihinene (**119**). Decoupling and nOe experiments revealed a large coupling constant of 11 Hz between H-5 and H-6 while irradiation of H-7 showed that H-1 was equatorial and H-6 and H-7 were axial. X-ray determination confirmed the relative stereochemistry initially assigned by rigorous NMR experiments. In similar fashion, the structure of isokalihinol-B (**120**) was established by COSY and nOe experiments. The Japanese *A. klethra* metabolites differ in the decalin junctions of **119** and **120** and by the fact that both kalihinols from this sponge are optically inactive. The optical rotation of kalihinol-A, also a constituent of this sponge, was not reported [21].



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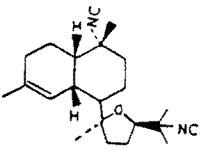

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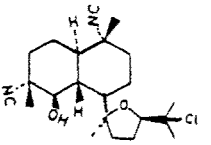

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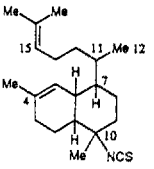
Both 10-isothiocyanatobiflora-4,15-diene (**123**) and 7-isocyano*neo*amphilecta-11,15-diene (**124**), isolated from a sponge in the family *Adociidae* [59], appear to bridge the amphilectane-cycloamphilectane and kalihinol-type skeletons (Sect. 4.2). Metabolite **123** from *Adociidae* is interesting since the kalihinols isolated from several *Acanthella* species (vide supra) were first recognized to possess the biflorin skeleton [25]. The recent discovery of **123** and **124**, the latter structure having been confirmed by X-ray crystallography [59], forms a link to the known tri- and tetracyclic amphilectanes (**91**)/cycloamphilectane (**92**) series.



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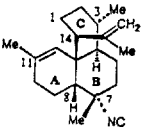

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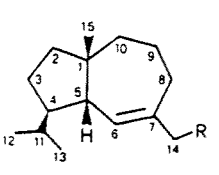
4.3 Other Marine Isocyano-Related Terpenoids and Nonterpenoids

Included in this section are (*i*) isocyanoterpenes having skeletons previously undescribed, (*ii*) other marine compounds related to the –NC/–NCS/–NHCHO triad and (*iii*) relatives of the –NC function: cyano (–CN), cyanates (–NCO) and thiocyanates (–SCN).

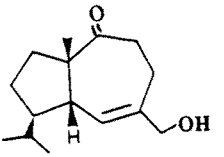
An isocyano- and isothiocyano pair of sesquiterpenes, **125** and **126**, is included in this section. Upon reexamination of *Acanthella acuta*, both **125** and **126** were discovered as part of the nitrogenous sesquiterpenes in the sponge [41]. The relative configuration of the sesquiterpene compounds was deduced from extensive 2D NMR experiments and shown to be similar, and, perhaps, biogenetically related, to the aphanamols (**127**) [41].

13-Isothiocyanatocubebane (**128**) and 10-isothiocyanatoguaia-6-ene (**129**) were isolated from a Palau sponge, *Axinyssa aplysinoides*, which is rich in isocyano-related sesquiterpenoids [44, 49]. The former (**128**) is apparently related to the amorphanes (Sect. 4.1.3) and the latter (**129**) to the aromadendranes [Sect. 4.1.5].

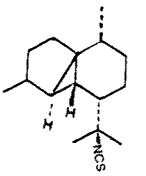
Sesquiterpenoid **130** and the diterpenoids **131–133** are some of the simplest acyclic marine isocyano-related compounds. The latter series represent the



125 R = NC
126 R = NCS



127
aphanamol I



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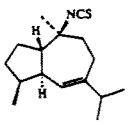

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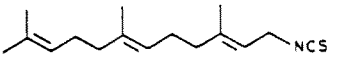

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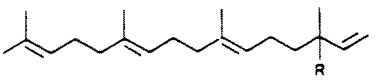
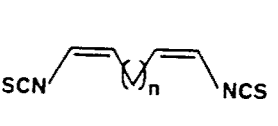


131 R = NC
132 R = NCS
133 R = NHCHO

earliest C_{20} mevalonate-based compounds which were isolated along with the cyclic sesquiterpenoids **31**–**33** from a *Halichondria* sp. [4, 7]. Although farnesyl isothiocyanate (**130**) was obtained from *Pseudaxinyssa pitys* (Axinellidae, Halichondrida) [Table 5], the corresponding isonitrile was not detected [66]. *P. pitys* is a unique sponge in this respect. Based on the presence of vinyl carbonimidic dichlorides ($R–N{=}CCl_2$), the suggestion was made that the carbonimidic dichlorides may have a biosynthetic role involving the isocyano function [66, 67].

An unusual series of nonterpenoid aliphatic isothiocyanates from a Fijian *Pseudaxinyssa* sp. was evident by their strong IR absorption [68]. After silica gel and reverse-phase HPLC experiments, the structures of (*Z*,*Z*)-1,18-diisothiocyanooctadeca-1,17-diene (**134**, $n = 14$), mp ~ 15 °C, and seven other di-olefinic α,ω-bisisothiocyanates (**135**), $n = 8$–13) were elucidated. Ten mono-olefinic α,ω-bisisothiocyanates (**135**, $n = 9$–18) and three α-isothiocyano-ω-formyl mono-olefins (**136**, $n = 9$, 15, 16) were also identified by spectral data



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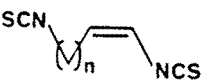

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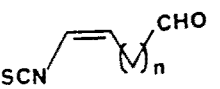

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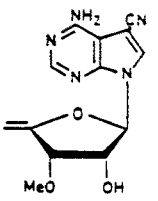

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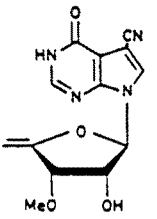

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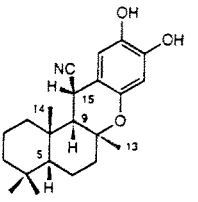

139

Table 5. Selected Examples of Other Terpenoids and Nonisoprenoids – R=–NC, –NCS, –NHCHO, –NCO, –SCN, and –CN

(Structure) compound	Organism	Origin	$[\alpha]_D^1$	Spectral Data[2]	Refs.
Terpenoids					
(10) cavernoisonitrile [$C_{16}H_{23}NO$]	*Acanthella* cf. *cavernosa*	Hachijo-jima Island	$+27^{MeOH}$	H- & C-NMR, MS	[34]
(11) cavernothiocyanate [$C_{16}H_{25}NS$]	*A.* cf. *cavernosa*	Hachijo-jima Island	−37.8	H- & C-NMR, MS	[34]
(36) 4-thiocyanato-9-cadinene	*Axinyssa aplysinoides*	Palau	−13.7	H- & C-NMR, MS, UV X-ray	[44]
(46) (+)-10-isothiocyanato-amorphen-4-ol#	*Axinyssa fenestratus*	Fiji		H- & C-NMR, MS	[45]
(81) 2-thiocyanato*neo*pupukeanane	*Phycopsis terpnis*	Okinawa	−80.3[3]	H- & C-NMR, MS	[48]
	A. aplysinoides	Pohnpei	−71.5	H- & C-NMR, MS	[49]
(82) 4-thiocyanato*neo*pupukeanane	*Phycopsis terpnis*	Okinawa	−121	H- & C-NMR, MS	[48]
(89) R=NCO 7-isocyanato-7,8-dihydro-α-bisabolene	*Ciocalypta* sp.	Kaneohe, O'ahu	-49.9^{h}	H- & C-NMR, MS, UV	[55]
(125) R=NC	*Acanthella acuta*	Bay of Naples, Italy	+44	H- & C-NMR, MS	[41]
(126) R=NCS	*A. acuta*	Bay of Naples	+36.2	H- & C-NMR, MS	[41]
(128) 13-isothiocyanatocubebane	*A. aplysinoides*	Palau	−19.9	H- & C-NMR, MS, UV	[49]
(129) 10-isothiocyanatoguaia-6-ene	*A. aplysinoides*	Palau	−33.9	H- & C-NMR, MS, UV	[44]
(130) farnesyl isothiocyanate [$C_{16}H_{25}NS$]	*Pseudaxinyssa pitys*	Indo-Pacific		H-NMR, MS	[66]
(131) R=NC [$C_{21}H_{33}N$]	*Halichondria* sp.	north shore, O'ahu, Hawai'i	$+15^{ccl}$	H-& C-NMR, MS	[4, 7]
(132) R=NCS					
(133) R=NHCHO					
Nonterpenoids					
(135) (*Z*,*Z*)-1,18-diisothiocyanooctadeca-1,17-diene [$C_{20}H_{32}N_2S_2$]	*Pseudaxinyssa* sp.	Fiji		H- & C-NMR, MS, UV	[68]
Mixed Biogenesis					
(137) mycalasine A [$C_{13}H_{13}N_5O_3$]	*Mycala* sp.	Japan	-88^{EtOH}	H- & C-NMR, MS, UV	[84]
(138) mycalasine B [$C_{13}H_{12}N_4O_4$]	*Mycala* sp.	Japan	-75.9^{EtOH}	H- & C-NMR, MS, UV	[84]
(139) 15-cyanopuupehenol [$C_{22}H_{29}NO_3$]	order: *Verongida* (?)	Moloka'i Hawai'i	-22^{MeOH}	H- & C-NMR, MS, UV	[85]

[1] rotations in chloroform unless indicated otherwise: [ccl], CCl_4; [h], hexane. A blank represents no report.
[2] data provided for methods indicated; IR for –NC/–NCS/and/or/–NHCHO bands given in all cases.
[3] an incorrect rotation was reported in the original paper [48].

[68]. These twenty-one compounds are unique among marine isothiocyanates as their biosyntheses do not implicate a mevalonic acid origin [68].

The cyano (–CN) function is relatively rare in marine metabolites [13]. Two cyanonucleosides, mycalisines A (**137**) and B (**138**), were isolated from a shallow water sponge *Mycale* sp. [84]. 15-Cyanopuupehenol (**139**) is of mixed biogenesis involving terpenoid-shikimate metabolic pathways. Interestingly, **139** could also be obtained *in vitro* by 1,6 conjugate addition of hydrogen cyanide at 0 °C to puupehenone, which co-occurs in the Verongida sponge collected off the island of Moloka'i, Hawai'i [85].

5 Chemical Ecology – Biological Activity

While secondary metabolites of plants and animals have been the subject of many chemical investigations, their associations and roles in their host organism are at times controversial; this is particularly so, when insufficient observations exist. Nevertheless, natural products provide fruitful areas of research [69]. There is little doubt that chemical defense against predators is an important aspect of survival. In the marine environment, early observations of nudibranch–sponge relationships were reported and those relating to isocyano compounds are summarized in Table 6.

Table 6. Chemical Ecology – Sponge ↔ Nudibranch

Compound (Structure)	Sponge [Nudibranch]	Location	Refs.
9-isocyanopupukeanane (**76**)	*Ciocalypta* sp. [*Phyllidia varicosa*]	north shore, O'ahu, Hawai'i	[15, 53]
9-*epi*-9-isocyano-pupukeanane (**77**)	—	Hachijo-jima Isl., Japan	[43, 56]
(**76**, **79**)	[*P. bourguini*, *P. pustulosa*]		
(**68**)	*Axinella* sp. [*Cadlina luteomarginata*]	Southern California	[18]
3-isocyanotheonellin (**85**)	*A.* cf. *cavernosa* [*P. ocellata*]	Hachijo-jima Isl.	[34]
	[*Phyllidia* sp.]	Sri Lanka	[55]
axisonitrile-1 (**1**)	*A. cannabina* [*P. pulitzeri*]	Mediterranean	[32]
axisonitrile-2 (**53**) cavernoisonitrile (**10**) 10-α-isocyano-4-amorphene (**35**) 7-isocyano-7,8-dihydro-α-bisabolene (**88**)	*A.* cf. *cavernosa* [*P. ocellata*]	Hachijo-jima Isl.	[34]

"—" indicates the sponge has not been identified.

Growth and diet are subject to seasonal changes. The presence (or absence) and relative concentrations of secondary metabolites in the extracts of organisms are factors which chemists should document along with observations made during collection. Often, in preliminary experiments guided by bioassay, mixtures, rather than individual compounds, are tested. Association of a certain activity with structurally related compounds may serve as a lead for further tests, but should not be considered definitive.

5.1 Chemical Ecology

In the early 1960s, the secretion of a shell-less, brightly colored Hawaiian mollusk, *Phyllidia varicosa* Lamarck, was investigated by a marine biologist [70]. It seemed fitting that our interest in marine toxins led to an examination of the chemical constituents.

When stressed, some nudibranchs may release a toxic secretion from their mantle for defense against predators. Following field and laboratory observations by Johannes [70], Burreson linked the dietary source of *P. varicosa* to a sponge (15, 53) later identified as *Ciocalypta* sp. In view of the small size and scarcity of the nudibranch, this crucial observation eventually resulted in isolation and GC comparison of the constituents derived from *Ciocalypta* and *P. varicosa*. The unpleasant smelling volatile substances of the secretion contained the isocyanopupukeananes (**76**, **78**, **80**), which at that time were the most complex marine sesquiterpenoids [15, 53, 54]. Recently, compound **76** and 9-*epi*-9-isocyanopupukeanane (**77**), possessing ichthyotoxic properties, have been reported from *P. bourguini* [56]. Whether the nudibranch or an unidentified sponge is involved in the production of the epimers is not known. The pupukeananes do not seem to elaborate the corresponding isothiocyanates and formamides, for these were not detected in *P. varicosa*, *P. bourguini*, and *Ciocalypta*. However, the rare thiocyano function [44] was discovered in two sponges containing *neo*pupukeananes [48, 49].

Field studies of the dorid nudibranch *Cadlina luteomarginata* and ten sponges on which the mollusks grazed were conducted off the La Jolla (San Diego) coast of Southern California [18]. From three separate collections of *C. luteomarginata* with sample sizes ranging from 25 to 109 animals per collection, analyses of extracts revealed the varied nature of the chemical constituents. Isocyano-related compounds were not present in all batches of the mollusk. When detected, they were associated with the sponge's siliceous spicules in the gut contents of the mollusk. These, in turn, implicated an unidentified *Axinella* sp., which is uncommon to the region [18]. Gas chromatographic analyses of the extracts from the nudibranch and sponge, however, revealed nearly identical profiles. Of the six isocyano-related metabolites from *C. luteomarginata*, a pair of new isocyano- and isothiocyano-compounds (**68** and **69**) was identified. In an experiment unrelated to the habitat or ecosystem of *C. luteomarginata* and *Axinella* sp., isonitrile mixtures exhibited antifeedent activity against goldfish at 10 μg/mg [18].

Similar investigations in the Mediterranean involved extracts from the skin and digestive gland of *P. pulitzeri.* Along with three other nudibranchs, *P. pulitzeri* was collected while grazing on *Axinella cannabina* [32]. Ethereal extracts of the digestive glands, mantles and secretion were chromatographically identical with the ethereal extract of the sponge. The major constituent, axisonitrile-1 (**1**) from the sponge, although ineffective as an antifeedant, was toxic in the fish bioassay at a concentration of 8 ppm. The bioassays involved the marine fish *Chromis chromis* and the freshwater *Carassius carassius* (Crucian Carp) [32].

Attributed to seasonal variations was the noted absence of isocyano-related metabolites in one batch of *C. luteomarginata* [18]. Similarly, in previous studies on *Acanthella acuta*, acanthellin-1 (**14**) was the major constituent of the isonitrile fraction [3], but a subsequent reinvestigation by a different group showed that **14** was only a minor constituent [32].

Diversity in the isocyanosesquiterpenes from *Ciocalypta*, whose habitats on one island (O'ahu) influence the nature and quantities of the secondary metabolites, has been demonstrated by the Hawaiian group. Biosynthetic studies involving *Ciocalypta* sponges from Pupukea (north shore of O'ahu) were conducted on 2-isocyanopupukeanane (**78**), the main isocyano constituent cooccurring with **76** [71]. During the winter season, when high surf precludes studies at the north shore site, field experiments on *Ciocalypta* species from Ala Moana beach located on the south shore were conducted. It was demonstrated that the distribution of the pupukeananes varies (vide supra) and a rearranged compound, **80**, was the principal isonitrile [71]. Moreover, another *Ciocalypta* species collected from Kane'ohe bay located in east O'ahu afforded a 1:1 mixture of 9-isocyanopupukeanane (**71**) and 7-isocyano-7,8-dihydrobisabolene (**88**). The isocyano-related compounds were not the major secondary metabolites [55]. Given the subtle differences in the nature of both compounds and variation of concentration with locality, it would seem worthwhile to compare the distribution of these compounds on a seasonal basis.

Of the nitrogenous triad, the European *Acanthella acuta* [3, 36, 41, 47], the Australian *A. pulcherrima* [20] and *A. klethra* [26, 38] elaborate chiefly sesquiterpenes. From *A. pulcherrima* the C_{15} compounds are exclusively isothiocyanates, while in *A. klethra* both isocyano and isothiocyano sesquiterpenes coexist (Tables 2 and 3). Our collections of *Acanthella* spp. from Guam [19, 25, 64] and Fiji [24] yielded distinct diterpenoids, kalihinols A–H (**107**, **108**, **112–117**) from the Guam sponge and kalihinols X–Z (**109–111**) from the Fijian sponge. Yet, in a separate independent study, constituents of *A. cavernosa* from 1986 and 1987 Fijian collections contained not only "mixed" but also different kalihinols [65]. No seasonal factor for this phenomenon was suggested [66]. The differences in site, where *A. klethra* was collected, and their constituents were noted previously (cf. Sect. 4.2.2).

In a continuing study in the vicinity of Hachijo-jima Island of nudibranchs and a sponge, subsequently identified as *A.* cf. *cavernosa* [34, 43], a variety of isonitrile-related metabolites were identified from as few as two nudibranchs [34]. Other than ecological implications, the constituents (**10**, **11**, **34**, **48**, and **53**)

from the sponge are interesting in view of the uncommon isocyano-oxiran (**10**) and thiocyanate (**11**) groups.

5.2 Biological Activity

In this review, we use the terms, "biologically active" or "bioactive" for any substance which elicits some biological response in another organism. Early investigations on bioactive marine isonitriles focussed on compounds that might serve as chemical defense agents (cf. Sect. 5.1). As chemists began to resolve mixtures into individual components, other tests related to antiviral, antimicrobial, or antiparasitic activity, were reported. Often, bioassays were performed

Table 7. Biological Activity of Marine Isocyano and Isocyano-Related Compounds

Compound	Organism	Activity[1,2]	Refs.
(**1**) axisonitrile-1	*Axinella cannabina*	cytotoxic: KB, P388	[52]
(**48**) axisonitrile-3	*Topsentia* sp.	antimicrobial: CA, SA, TM; antithelmintic	[45]
	Ciocalypta sp.	antiviral: KB, LOVO	[48]
(**43–46**)	*Axinyssa fenestratus*	anthelmintic	[35]
(**61, 69**) epipolasin-B & -A	*E. kushimotoensis*	inactive, but thiourea derivatives are antibacterial, antiviral, and cytotoxic	[51]
(**85**) 3-isocyanotheonellin	*Phyllidia* sp.	antimicrobial: BS	[55]
(**74**)	*Axinella cannabina*	cytotoxic: KB, P388	[52]
(**96**) 8,15-diisocyano-11(20)-amphilectene	*Hymeniacidon amphilecta*	antibiotic: BS, CA, SA	[60]
(**97**)	*H. amphilecta*	antibiotic: BS, CS, SA	[60]
(**103**) 8-isocyano-1 (12)-cycloamphilectene	*Halichondria* sp.	antimicrobial: BS, SA	[22]
(**106**) 8-isocyano-10(14)-amphilectadiene	*Halichondria* sp.	antimicrobial: BS, SA	[22]
(**107–111, 112–117**) kalihinols	*Acanthella* sp.	antimicrobial: BS, CA, SA	[19]
(**119** and **120**) kalihinine, isokalihinol B	*Acanthella klethra*	antifungal: MR, PC; cytotoxic: P388	[21]
(**107, 109–110**) kalihinol A, X–Z	*Acanthella cavernosa*	antithelmintic	[45, 65]
(**109**) kalihinol X	*A. cavernosa*	antimicrobial: CA, SA, TM	[45]
(**139**) 15-cyanopuupehenol	order: Verongida (?)	cytotoxic: KB, antiviral: Herpes simplex II; antimicrobial: TM, SC, TM	[85]

[1] Unless indicated otherwise, in vitro results. References should be consulted for concentrations, etc.

[2] Abbreviations used:

BS, *Bacillus subtilis*; CA, *Candida albicans*; SA, *Staphylococcus aureus*; SC, *Saccharomyces cerevisiae*; TM, *Trichophyton mentagrophytes*; PN, *Penicillium nonatum*

KB, LOVO (colon cancer), P388 are cell lines in cytotoxic activity testings.

MR, *Mortierella ramannianus*; PC, *Penicillium chrysogenum* are used as antifungal agents.

during early isolation steps as limited sample size makes structure determination a top priority.

Anthelmintic screens against parasitic stages of *Nippostrongylus brasiliensis* have been used extensively by a California group [45, 65]. Table 7 summarizes some of the reported activities other than those listed in Table 6. Because of the preliminary nature of these reports, the summary is presented without comment.

6 Synthesis

Reviews of the syntheses of marine natural products, including marine isonitriles, have appeared recently [72]. After the early synthetic challenges of the tricyclic 9-isocyanopupukeanane (**76**) [73, 74] and 2-isocyanopupukeanane (**78**) [75] were met in 1979, additional syntheses of marine isonitriles were reported during 1986–1991. These were: (±)-7,20-diisocyanoadociane (**95**) [63], (±)-axisonitrile-1 (**1**) and (±)-axamide-1 (**3**) [76], the axisonitrile-4 triad (**7–9**) [78], and (±)-8,15-diisocyano-11(20)-amphilectene (**96**) [79], and theonellin isocyanide (**85**) [80].

During the latter period the absolute configurations of some classes of isocyano compounds were secured. More importantly, the synthesis of 7,20-diisocyanoadociane (**95**) [63] permitted the resolution of some concerns that had been raised earlier.

7 Biosynthesis

Early observations by Italian and Hawaiian investigators – that marine isocyano compounds are often accompanied by –NCS and –NHCHO analogs – provided a biogenetic speculation, that the formamide function might lead to the isonitrile from which the corresponding isothiocyanate may result [5, 7, 40]. In an early attempt, ^{14}C-labeled axamide-1 (**3**), was fed to *Axinella cannabina* in aerated sea water aquaria for five days. Under these conditions failure by the sponge to incorporate radio-labeled formamide implied that the formamido function is not the precursor to the –NC group [30]. The Italian investigators were the first to mention that their negative, hence inconclusive, results may be attributed to several factors such as slow incorporation rate [30]. Field experiments on a *Ciocalypta* sp. using the labeled 2-formamido (–NH^{13}CHO) compound of pupukeanane **78**, and the corresponding ^{13}C-labeled isothiocyanate revealed upon workup that neither compound was converted to the corresponding isonitrile (**78**). Furthermore, ^{13}C-formate was not incorporated and strong evidence showed that the –NC function was the precursor to the other functions of the triad [81].

Garson's work on the *Amphimedon* sp. provided direct evidence that cyanide is a feasible precursor to the isonitrile function [62, 82]. Both [^{14}C]-cyanide and labeled acetate were incubated separately with the *Amphimedon* sponge under identical conditions. Only the former was incorporated into diisocyanoadociane (**95**). Like formate, radio-labeled acetate was not transformed into the isonitrile function, but was found to be a precursor for carotenoids in *Amphimedon* sp. [82]. Extended studies on several labeled amino acids including [U-^{14}C]alanine, [U-^{14}C]leucine, [2-^{14}C]glycine, [guanidino-^{14}C]arginine showed assimilation into the sponge, but the presence of the ^{14}C-label was not established in diisocyanoadociane (**95**) itself [62]. Although no link between these labeled amino acids and the source of cyanide ion for the biosynthesis of marine isonitriles was established, it remains a viable hypothesis that some amino acid precursor(s) under some appropriate metabolic conditions may have a biosynthetic connection. Application of the radio-precursors was performed in aquaria, and the sponge was returned to its habitat after feeding.

Use of encapsulated labeled precursors in lipid vesicles enabled the Hawaiian group to conduct the biosynthetic studies – with the exception of workup of the sponge – entirely in the field. Incorporation of doubly labeled [^{13}C, ^{15}N]cyanide into a *Ciocalypta* sp. and an *Acanthella* sp. produced labeled 9-isocyanoneopupukeanane (**77**) and kalihinol-F (**112**) respectively [71]. Detection of incorporation was followed by ^{13}C NMR experiments.

The intermediacy of a carbocation or complex-equivalent is attractive, if one considers that the nucleophilic ambident cyanide ion may be accomodated on secondary or tertiary cationic sites. Where exceptions (e.g., **125**, **126**, **134–136**; cf. Sect. 4.3) exist, the cationic intermediate resides on a primary allylic carbon. The following skeletal types are examples of some biogenetic schemes offered in conjunction with the structural determination of isocyanoterpenoids:

- amorphanes and spiroaxanes [39, 46]
- pupukeananes [43, 54]
- adocianes [23, 82]
- kalihinols [11, 19]

Chemically, all of these apparently involve skeletal rearrangements and uptake of the isocyano function at some stage during biosynthesis.

Biogenetic implications in connection with the interrelation among nitrogenous functions, epimers, etc. are noted below.

1. That farnesyl isothiocyanate (**130**), and not the corresponding isocyano compound, was isolated together with several carbonimidic dichlorides (structures not shown) suggested a possible biosynthetic relationship among the nitrogenous functions [60].
2. The epimeric compounds, e.g., **88** and **89** from a Hawaiian *Ciocalypta* sponge [55] and **90** from a Pohnpei *Halichondria* sp., may share a common sp^2-carbon center in the biosynthesis of the bisabolane skeleton of these compounds.

3. With the isolation of both 9-isocyanopupukeanane (**76**) and its epimer (**77**) from *P. bourguini*, coupled with the results of ichthyotoxicity assays, the suggestion was made that the origin of these compounds may not be exclusively dietary; i.e., partial metabolism of **76** to **77** by the nudibranch may be operative [56].
4. Additional reports of multifunctional kalihinols implicate a polar and stepwise biosynthetic cationic mechanism [21, 45].

Although use of radio and stable isotope labels involving the trio of covalently-bonded nitrogenous functions in **3** and in **78**, provided evidence that isocyano is the precursor of the isothiocyano and formamido groups [30, 81], it remains to be shown that a biosynthetic equivalent of the in vitro chemically-proven fusion process between isocyano and free sulfur (e.g., cf. Introduction) exists in the cells of sponges. In marine biota, various ionic forms of sulfur in a number of oxidation states, as well as organo-polysulfides are known. However, any association with the isonitrile group and a sulfated species has yet to be established.

Assuming that the metabolic pathways are similar in the biosynthesis of related isocyanoterpenes, these studies remain difficult, due in part to the competitive formation of other secondary metabolites. In addition to the common trio (–NC, –NCS, –NHCHO) of the nitrogenous functions found attached to these skeletons, analogs such as –CN, –CNO, and –SCN foreshadow the complexity of identifying and selecting specific precursors to be targeted for incorporation into the family of marine isonitriles.

8 Discussion and Summary

Since the first marine isocyano compound, axisonitrile-1 (**1**), was reported 20 years ago, approximately twenty isocyano- and some forty isothiocyanato and formamido sesquiterpenoids have been described. Most of these co-occur in a few sponges (Phylum Porifera) primarily in the class Demospongiae. As first reported in 1973, and confirmed shortly thereafter, the C_{15} compounds bear either the uncommon isocyano or an isocyano-related function. Noteworthy is a sponge of the genus *Axinella* of European origin, from which isocyano-related compounds of seven skeletal types – represented by **1**, **14**, **30**, **48**, **58**, **68**, and **125** – have been isolated. Biogenetically, the various skeletal types, including the complex tricyclic pupukeananes (eg., **76**), may be derived from cationic or cationic-equivalent intermediates of terpene origin. Recent isolation of five previously known isonitriles, **53**, **76**, **77**, **85**, **88**, coupled with the isomeric amorphene, **36**, and allopupukeanane, **79**, by Fusetani et al. [43] is consistent with the biogenetic relationship of the pupukeananes with the amorphane type skeletons [54].

Biosynthetically, the isocyano function appears to be the precursor of the isothiocyano and formamido groups. Questions arise, however, when only isothiocyantes or the rare cyanates or thiocyanates are reported from certain sponges.

Though the first reported isocyano diterpenoid was acyclic, the majority are cyclic and possess the amphilectane-cycloamphilectane or kalihinol skeletons. The latter are the most highly substituted, bearing two or three isocyano or isocyano-related functions and a cyclic ether. Moreover, hydroxy, chloro, and/or alkene functions occur in the kalihinols. The kalihinols constitute slightly over 50% of the approximately 30 known isocyano diterpenes. These compounds have been isolated exclusively from sponges of the genus *Acanthella*, mainly from the Pacific Ocean.

Clear evidence that cyanide can be a precursor of the –NC/–NCS/–NHCHO triad has been reported by two groups [62, 68, 82]. However, the origin of cyanide in the marine environment has not been demonstrated unambiguously. Nor have the precursors of the isocyanoterpenes been identified. Efforts to date have shown that precursors that are fed to sponges are incorporated poorly – cyanide is an exception – or have been shunted to other pathways and products, e.g. carotenoids, etc. No doubt, the biosynthesis of isocyano terpenes is a complex problem awaiting solution.

Occurrence of positional isomers such as 1-isocyanoaromadendrane (**62**) and 10α-isocyanoalloaromadendrane (**58**), compounds **95** and **101**, or kalihinol-F (**112**) and isokalihinol-F (**118**) suggest an olefinic precursor. Kalihinene (**119**) with the alkene moiety (C_3–C_4) in place of the hydroxy and isocyano functions of kalihinol-F and isokalihinol-F lends credence to its possible intermediacy in the biosynthesis of compounds such as **112** and **118**. While this is an attractive hypothesis, one may also argue that a stronger case could be made if all three, **112**, **118**, and **119**, were found in the same organism. On the other hand, such may not be the case, since the biological process may be construed as an inefficient (or efficient) metabolic process depending on the identity of the sponge.

Another premise cannot be excluded and will be investigated eventually. As in the case of cyanide ion, isothiocyanate ion may be a precursor in the biosynthesis of some marine isothiocyanates. Like cyanide, thiocyanate ion is an ambident nucleophile. Specific incorporation of this ion by a carbocationic site – if indeed, such intermediates are involved – is predicted, on steric grounds, to yield isothiocyanates. Table 3 identifies the more prevalent marine isothiocyanates discovered thus far.

With few exceptions (**125** or **126**), marine isocyano-related, cyclic compounds generally have their nitrogenous functions attached to secondary or tertiary carbon sites. Without exception, cationic centers which are allylic, secondary, or tertiary are reasonable transient intermediates. Marine thiocyanates, of which few (**11**, **36**, **81** and **82**) are known, may accomodate the bent –S–C≡N moiety. The resultant quarternary carbon of thiocyanate **36** (R–S–C≡N), though less favorable than that of the absent isothiocyanate

compound (R–N=C=S), may be viewed speculatively as a biospecific capture of thiocyanate ion.

Biological specificity is a paramount consideration if one considers two examples, *Acanthella pulcherrima* [20] and *Pseudaxinyssa* sp. [68]. That thiocyanate ion may be the precursor to marine isothiocyanates should be tested on these organisms since isothiocyantes were the primary representative of the triad. Also, should incorporation of thiocyanate ion by *Phycopsis terpnis* yield **81** and **82** [48], rather than the unreported corresponding isothiocyanates, the biospecificity of marine organisms will be demonstrated.

Before the discovery of the multifunctional kahilinols (cf. Sect. 4.2.2), sesquiterpenoid isonitriles and related analogs were found distinctly void of other functions. Recent reports of isocyano analogs bearing hydroxy (**46**) [45], oxiran (**10**) [34], and chloro groups [86], for example, indicate the continued interest in the chemistry of marine isocyano compounds.

9 References

1. Cafieri F, Fattorusso E, Magno S, Santacroce C, Sica D (1973) Tetrahedron 29:4259
2. Ugi I (1971) Isonitrile chemistry. Academic, New York
3. Minale L, Riccio R, Sodano G (1974) Tetrahedron 30:1341
4. Burreson BJ, Scheuer PJ (1974) J Chem Soc Chem Commun 1035
5. Fattorusso E, Magno S, Mayol L, Santacroce C, Sica D (1975) Tetrahedron 31:269
6. Burreson BJ, Christophersen C, Scheuer PJ (1975) J Am Chem Soc 97:201
7. Burreson BJ, Christophersen C, Scheuer PJ (1975) Tetrahedron 31:2015
8. Rothe W (1950) Chem Abst 44:8063e. [Pharmazie 5:190]
9. Edenborough MS, Herbert RB (1988) Nat Prod Rep 5:229
10. Garson MJ (1989) Nat Prod Rep 6:142
11. Chang CWJ, Scheuer PJ (1990) Comp Biochem Physiol 97B:227
12. Christophersen C, Anthoni U (1986) Sulfur Rep 4:365
13. Scheuer PJ (1992) Acc Chem Res 25:433
14. Bergquist PR, Wells RJ (1983) Chemotaxonomy of the porifera: The development and current status of the field. In: Scheuer PJ (ed) Marine Natural Products. Chemical and biological perspectives. Academic, New York, vol 5
15. Burreson BJ, Scheuer PJ, Finer J, Clardy J (1975) J Am Chem Soc 97:4763
16. Moore RE, Cheuk C, Yang X-QG, Patterson GML, Bonjouklian R, Smitka TA, Mynderse JS, Foster RS, Jones ND, Swartzendruber JK, Deeter JB (1987) J Org Chem 52:1036
17. Garson MJ, Larsen R, Thompson JE, Bergquist PR (1988) Aust Mar Sci Ass (Jubilee Conference 1963–1988), 149
18. Thompson JE, Walker RP, Wratten SJ, Faulkner DJ (1982) Tetrahedron 38:1865
19. Chang CWJ, Patra A, Baker JA, Scheuer PJ (1987) J Am Chem Soc 109:6119
20. Capon RJ, MacLeod JK (1988) Aust J Chem 41:979
21. Fusetani N, Yasumuro K, Kawai H, Natori T, Brinen L, Clardy J (1990) Tetrahedron Lett 31:3599
22. Molinski TF, Faulkner DJ, Van Duyne GD, Clardy J (1987) J Org Chem 52:3334
23. Baker JT, Wells RJ, Oberhänsli WE, Hawes GB (1976) J Am Chem Soc 98:4010
24. Kupchan SM, Britton RW, Ziegler MF, Sigel CW (1973) J Org Chem 38:178
25. Chang CWJ, Patra A, Roll DM, Scheuer PJ, Matsumoto GK, Clardy J (1984) J Am Chem Soc 106:4644
26. Chang CWJ, Scheuer PJ, unpublished work reported, in part, at the 1988 Gordon Research Conference

27. Pretsch E, Clerc T, Seibl J, Simon W (1981) Tabellen zur Strukturaufklarung organischer Verbindungen, Springer, Berlin Heidelberg New York, a p I65; b p I80; c p U15
28. Karuso P, Hagadone MR, Scheuer PJ, Bergquist PR (1990) Chemotaxonomy of the Porifera by infrared spectroscopy. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution, Washington, DC, p 55
29. Kuntz Jr ID, Schleyer PvR, Allerhand A (1961) J Chem Phys 35:1533
30. Iengo A, Santacroce C, Sodano G (1979) Experientia 35:10
31. Adinolfi M, De Napoli L, Di Blasio B, Iengo A, Pedone C, Santacroce C (1977) Tetrahedron Lett 18:2815
32. Cimino G, De Rosa S, De Stefano S, Sodano G (1982) Comp Biochem Physiol 73B:471
33. Iengo A, Mayol L, Santacroce C (1977) Experientia 33:11
34. Fusetani N, Wolstenholme HJ, Shinoda K, Asai N, Matsunaga S, Onuki H, Hirota H (1992) Tetrahedron Lett 33:6823
35. Ciminiello P, Fattorusso E, Magno S, Mayol L (1984) J Org Chem 49:3949
36. Ciminiello P, Fattorusso E, Magno S, Mayol L (1987) Can J Chem 65:518
37. Gulavita NK, Ph.D. Thesis, University of Hawaii, 1987, pp 53–61
38. König GM, Wright AD, Sticher O, Fronczek FR (1992) J Nat Prod 55:633
39. Ciminiello P, Fattorusso E, Mágno S, Mayol L (1986) Experientia 42:625
40. Ciminiello P, Magno S, Mayol L, Piccialli V (1987) J Nat Prod 50:217
41. Mayol L, Piccialli V, Sica D (1987) Tetrahedron 43:5381
42. Pais M, Fontaine C, Laurent D, La Barre S, Guittet E (1987) Tetrahedron Lett 28:1409
43. Fusetani N, Wolstenholme HJ, Matsunaga S, Hirota H (1991) Tetrahedron Lett 32:7291
44. He HY, Faulkner DJ, Shumsky JS, Hong K, Clardy J (1989) J Org Chem 54:2511
45. Alvi KA, Tenenbaum L, Crews P (1991) J Nat Prod 54:71
46. Di Blasio B, Fattorusso E, Magno S, Mayol L, Pedone C, Santacroce C, Sica D (1976) Tetrahedron 32:473
47. Braekman JC, Daloze D, Deneubourg F, Huysecom J, Vandevyver G (1987) Bull Soc Chim Belg 96:539
48. Pham AT, Ichiba T, Yoshida WY, Scheuer PJ, Uchida T, Tanaka J-i, Higa T (1991) Tetrahedron Lett 32:4843
49. He HY, Salva J, Catalos RF, Faulkner DJ (1992) J Org Chem 57:3191
50. Fattorusso E, Magno S, Mayol L, Santacroce C, Sica D (1974) Tetrahedron 30:3911
51. Tada H, Yasuda F (1985) Chem Pharm Bull 33:1941
52. Ciminiello P, Fattorusso E, Magno S, Mayol L (1985) J Nat Prod 48:64
53. Hagadone MR, Burreson BJ, Scheuer PJ, Finer JS, Clardy J (1979) Helv Chim Acta 62:2484
54. Karuso P, Poiner A, Scheuer PJ (1989) J Org Chem 54:2095
55. Gulavita NK, de Silva ED, Hagadone MR, Karuso P, Scheuer PJ (1986) J Org Chem 51:5136
56. Fusetani N, Wolstenholme HJ, Matsunaga S (1990) Tetrahedron Lett 31:5623
57. Nakamura H, Kobayashi J, Ohizumi Y, Hirata Y (1984) Tetrahedron Lett 25:5401
58. Sullivan BW, Faulkner DJ, Okamoto KT, Chen MHM, Clardy J (1986) J Org Chem 51:5134
59. Sharma HA, Tanaka J-i, Higa T, Lithgow A, Bernardinelli G, Jefford CW Tetrahedron Lett 33:1593
60. Wratten SJ, Faulkner DJ, Hirotsu K, Clardy J (1978) Tetrahedron Lett 19:4345
61. Kazlauskas R, Murphy PT, Wells RJ, Blount JF (1980) Tetrahedron Lett 21:315
62. Fookes CJR, Garson MJ, MacLeod JK, Skelton BW, White AH (1988) J Chem Soc Perkin Trans I 1003
63. Corey EJ, Magriotis PA (1987) J Am Chem Soc 109:287
64. Patra A, Chang CWJ, Scheuer PJ, Van Duyne GD, Matsumoto GK, Clardy J (1984) J Am Chem Soc 106:7981
65. Omar S, Albert C, Fanni T, Crews P (1988) J Org Chem 53:5971
66. Wratten SJ, Faulkner DJ, Van Engen D, Clardy J (1978) Tetrahedron Lett 19:1391
67. Wratten SJ, Faulkner DJ (1977) J Am Chem Soc 99:7367
68. Karuso P, Scheuer PJ (1987) Tetrahedron Lett 28:4633
69. Williams DH, Stone J, Hauck PR, Rahman SK (1989) J Nat Prod 52:1189
70. Johannes RE (1963) Veliger 5:104
71. Karuso P, Scheuer PJ (1989) J Org Chem 54:2092
72. Albizati KF, Martin VA, Agharahimi MKR, Stolze DA (1992). In: Scheuer PJ (ed) Synthesis of marine natural products 1. Terpenoids. Bioorganic Marine Chemistry, vol 5, Springer, Berlin Heidelberg New York
73. Corey EJ, Behforouz M, Ishiguro M (1979) J Am Chem Soc 101:1608

74. Yamamoto H, Sham HL (1979) J Am Chem Soc 101:1609
75. Corey EJ, Ishiguro M (1979) Tetrahedron Lett 2745
76. Piers E, Yeung BWA (1986) Can J Chem 64:2475
77. Piers E, Yeung BWA, Rettig SJ (1987) Tetrahedron 43:5521
78. Hart DJ, Lai CS (1989) Synlett 1:49
79. Piers E, Llinas-Brunet M (1989) J Org Chem 54:1483
80. Ichikawa Y (1991) Synlett 3:715
81. Hagadone MR, Scheuer PJ, Holm A (1984) J Am Chem Soc 106:2447
82. Garson MJ (1986) J Chem Soc Chem Commun 35
83. Garson MJ, Partali V, Liaaen-Jensen S, Stoilov I (1989) Comp Biochem Physiol 91B:293
84. Kato Y, Fusetani N, Matsunaga S, Hashimoto K (1985) Tetrahedron Lett 26:3483
85. Hamann MT, Scheuer PJ (1991) Tetrahedron Lett 32:5671
86. Andersen RJ (personal communication)

Biosynthesis of Secondary Metabolites in Marine Molluscs

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The studies on the biosynthetic origin of secondary metabolites in marine molluscs are reviewed. Although the majority of natural products found in marine molluscs appears to have a dietary origin, de novo biosynthetic ability towards particular classes of compounds has been demonstrated. Marine molluscs are also able to modify metabolites sequestered from their specific prey.

1 Introduction

The phylum Mollusca comprises eight classes: Caudofoveata, Solenogastres, Placophora, Monoplacophora, Gastropoda, Bivalvia, Scaphopoda, Cephalopoda [1]. Among the most extensively studied, from the natural product chemistry point of view, are the Opisthobranchia, a subclass of Gastropoda, and chiefly the order Nudibranchia which is exclusively marine. The reason for the interest in the chemistry of these invertebrates resides in the fact that they possess an array of exotic secondary metabolites, most of which take part in the defense mechanisms enabling their survival and compensating for the partial or total evolutionary loss of the shell [2–5]. Therefore it is not surprising that many studies have been devoted to the investigation of the origin of the defense metabolites in opisthobranchs.

It is well established that the principal source of secondary metabolites in marine molluscs resides in the sometimes selective concentration of chemicals contained in their food. The chemical connection between molluscan predator and its algal or invertebrate diet has been confirmed as a result of many investigations of the chemistry of herbivorous [6] and carnivorous [3] molluscs and their dietary sources. However, the assumption often reported, as in a recent review [7], that marine molluscs invariably obtain their metabolites from dietary sources, should be regarded as an oversimplification. In fact, active biosynthesis of secondary metabolites has been ascertained in several cases, as documented here.

Throughout this chapter a major emphasis is given to recent work, since the biosynthesis of molluscan secondary metabolites has been partly covered in previous reviews [8, 9] devoted to the entire field of the biosynthetic studies on marine natural products.

2 Methodology

The problems associated with biosynthetic studies on marine organisms have been elaborated by Garson [9]. They essentially derive from our still limited understanding of metabolic processes in the marine environment.

Virtually all biosynthetic studies with molluscs have been conducted using precursors labelled with radioisotopes. In general, experiments are carried out

with whole animals, but sometimes also selected organs or tissues have been used, and the precursor has been delivered by injection or, when a filtering ability was present, by addition to the sea water in which the animal is placed. In several papers it is reported that the precursor has been injected into the digestive gland. This seems unlikely since the digestive gland is a small organ contained in the hepatopancreas; to inject inside it would represent a very difficult task, especially with small molluscs. It should therefore be reasonable to assume that precursors have been simply injected inside the animal, probably into the hepatopancreas.

The scope of the majority of the papers is limited to the ascertainment of the existence of de novo biosynthesis of a particular compound. In this respect it has recently been proposed that in many cases it would be possible to predict the origin of secondary metabolites in nudibranch molluscs by examining their geographical variations in a given species [10]. Those molluscs that exhibit considerable variation in their chemical constituents undoubtedly obtain these from dietary sources, while those that have the same substances wherever they are collected are most likely capable of de novo biosynthesis.

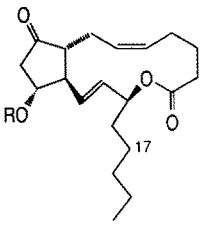
3 Biosynthesis of Prostaglandins and Other Eicosanoids

Until the discovery of prostaglandin A_2 derivatives in the gorgonian coral *Plexaura homomalla* [11] prostaglandins (PGs) had been reported only in association with higher vertebrates. Subsequently, PGs and other biologically active derivatives of polyunsaturated fatty acids have been detected, mainly by bioassay, in a large number of invertebrate species [12] including marine molluscs. PGs appear to be important in basic physiological functions in molluscs, including ion regulation, possible renal functions and reproductive biology. For example PGE_2 has been reported to be involved in ion regulation in the marine bivalve *Modiolus demissus* [13]. When isolated gills were subjected to hypo-osmotic stress by incubation for 60 min in 25% seawater, there was a 10-fold increase in PGE_2 released into the medium, suggesting increased biosynthesis and release of prostaglandin. PGs induce spawning in two marine molluscs, the abalone *Haliotis refescens* and the mussel *Mytilus californianus* [14].

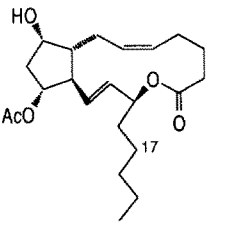
However, in spite of reports suggesting that eicosanoids play fundamental physiological roles in representatives of many invertebrate phyla, careful identification of the compounds and data on their biosynthesis and metabolism are generally lacking. A paper on comparative aspects of prostaglandin biosynthesis in animal tissues [15] claims that prostaglandin synthetase activity has been detected in several animals, including a mussel, after incubation of tissue homogenates with all-*cis*-[8,9,11,12,14,15-$^3H_6$]eicosatrienoic acid, followed by isolation of labelled prostaglandin E and prostaglandin F. Surprisingly, in the

paper it is reported that the "all-*cis*-[8,9,11,12,14,15-$^3H_6$]eicosatrienoic acid (spec. act. 1.7×10^5 dpm/μg) was prepared by reduction of 8,11,14-eicosatrienoic acid with tritium gas". Obviously the tritium reduction of 8,11,14-eicosatrienoic acid cannot lead to [8,9,11,12,14,15-$^3H_6$]eicosatrienoic acid as stated, but only to the saturated C-20 fatty acid, eicosanoic acid, which is not a direct precursor of prostaglandins.

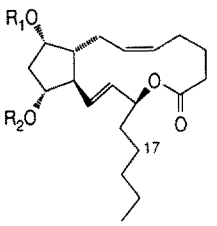
Recently, a novel class of prostaglandin derivatives, PG-1,15-lactones (Fig. 1) of both E [16] and F [17] series, has been reported to occur in the opisthobranch mollusc *Tethys fimbria*, along with smaller amounts of some of the corresponding PGs. Some lactones were present mainly in the mucous secretion and dorsal appendages known as cerata, while others were more abundant in the mantle. Fatty acid esters of PGF-1,15-lactones (C_9 and C_{11}) were found only in *T. fimbria* eggmasses and reproductive glands (ovotestis). The structural variety of the lactones and the data on their distribution in the body



R = H; PGE_2-1,15-lactone
R = Ac; PGE_2-1,15-lactone 11-acetate
R = H, $\Delta^{17}Z$; PGE_3-1,15-lactone
R = Ac, $\Delta^{17}Z$; PGE_3-1,15-lactone 11-acetate

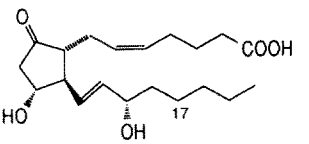


$PGF_{2\alpha}$-1,15-lactone 11-acetate
$\Delta^{17}Z$; $PGF_{3\alpha}$-1,15-lactone 11-acetate



R_1 = long chain acyl groups, R_2 = H;
$PGF_{2\alpha}$(or $PGF_{3\alpha}$)-1,15-lactone-9-fatty acid esters

R_1 = H, R_2 = long chain acyl groups;
$PGF_{2\alpha}$(or $PGF_{3\alpha}$)-1,15-lactone-11-fatty acid esters



PGE_2
$\Delta^{17}Z$; PGE_3

Fig. 1. Prostaglandin 1,15-lactones and prostaglandins isolated from *Tethys fimbria*

of *T. fimbria* suggested a range of different biological functions for this novel class of prostanoids.

The biosynthesis of PG-lactones was investigated using tritiated precursors [18, 19]. Injection of [^{3}H]arachidonic acid in the mantle of *T. fimbria* resulted in the isolation of labelled PGE_2 and PGE_2-1,15-lactone, which reached satisfactory radioactivity levels after 7 days, thus insuring that the mollusc was able of de novo synthesis of PGs and of the corresponding lactones. Moreover, injection of [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ produced the corresponding labelled lactones and their acyl derivatives. Interestingly, injection of [^{3}H]$PGF_{2\alpha}$ also yielded strongly labelled PGE_2-1,15-lactone, thus indicating the occurrence of an unusual oxidative step, whereby PG derivatives of the F series are converted into those belonging to the E series.

The radiolabelling experiments provided also some insight into the dynamics of the PG derivatives in *T. fimbria* and into their possible biological functions [18]. Following the radioactivity levels of PG derivatives at various times into mantle and cerata of *T. fimbria* it was possible to conclude that conversion of both PGE_2 and $PGF_{2\alpha}$ into PGE_2-1,15-lactone and of $PGF_{2\alpha}$ into $PGF_{2\alpha}$-1,15-lactone occur mainly in the mantle and that the lactones of the E series are specifically transported and accumulated into the cerata. The cerata are dorsal appendages which in a typical defense behavior of *T. fimbria* and of other molluscs, known as autotomy, are released when the mollusc is molested by predators. The released cerata carry on contracting and secreting mucus for long periods of time, thus engaging the predator while the mollusc escapes. It was found that in the cerata, after detachment, PGE_2- and PGE_3-1,15-lactones are reconverted into the corresponding PGs, thus suggesting that the latter might have a role in the contraction of the body appendages after detachment. Furthermore, by quantitating the levels of $PGF_{2\alpha}$-1,15-lactone 11-acetate and of 9-,11-fatty acid esters of $PGF_{2\alpha}$-1,15-lactones at the three sexual phases of the mollusc (i.e. immature, mature and immediately after egg-laying), it was possible to infer [20] that during the process of sexual maturation of the mollusc the PGF lactones produced in the mantle are specifically transferred to the ovotestis as 9-,11-fatty acid esters. The latter, in turn, are partially transferred into the eggmasses, while immediately after ovodeposition, the synthesis of their precursors, PGF lactones, starts again in the mantle. Finally, incorporation experiments carried out by injection of [^{3}H]-$PGF_{2\alpha}$ into the mantle of sexually mature *T. fimbria* specimens established that during the early oocyte developmental stages, in correspondence with the formation of larvae (veligera) and immediately before hatching, 9-,11-fatty acid esters of PGF 1,15-lactones are partly transformed into PGE-1,15-lactone 11-acetates. Although these findings, taken altogether, indicate that PG lactone derivatives are implicated in the oocyte maturation, development and hatching, their exact role remains to be assessed.

Detailed studies on the metabolism of arachidonic acid (AA) in the nervous system of the opisthobranch mollusc *Aplysia californica* were carried out [21]. *A. californica* provides a very useful model for neurobiological studies since its

relatively simple nervous system is composed of large and easily identifiable neurons. The metabolites of AA identified in nervous tissue of *A. californica* are shown in Fig. 2.

Incubation of *A. californica* neuronal cell bodies and synaptosomes with ^{3}H-AA yielded labelled metabolites of both the lipoxygenase and cyclo-oxygenase pathways [22]. In particular, the identified lipoxygenase products were 12- and 5-hydroxyeicosatetraenoic acids (12-HETE, 5-HETE). The identity of these compounds was established by several methods, including HPLC and GC/MS. The cyclo-oxygenase products were identified as PGE_2 and $PGF_{2\alpha}$ by radioimmunoassay, after purification by reversed-phase HPLC. Interestingly, it was observed that the ^{3}H-labelled 12-HETE, but not ^{3}H-5-HETE or prostaglandins, was released from ganglia previously labelled with ^{3}H-AA after a 1 min applica-

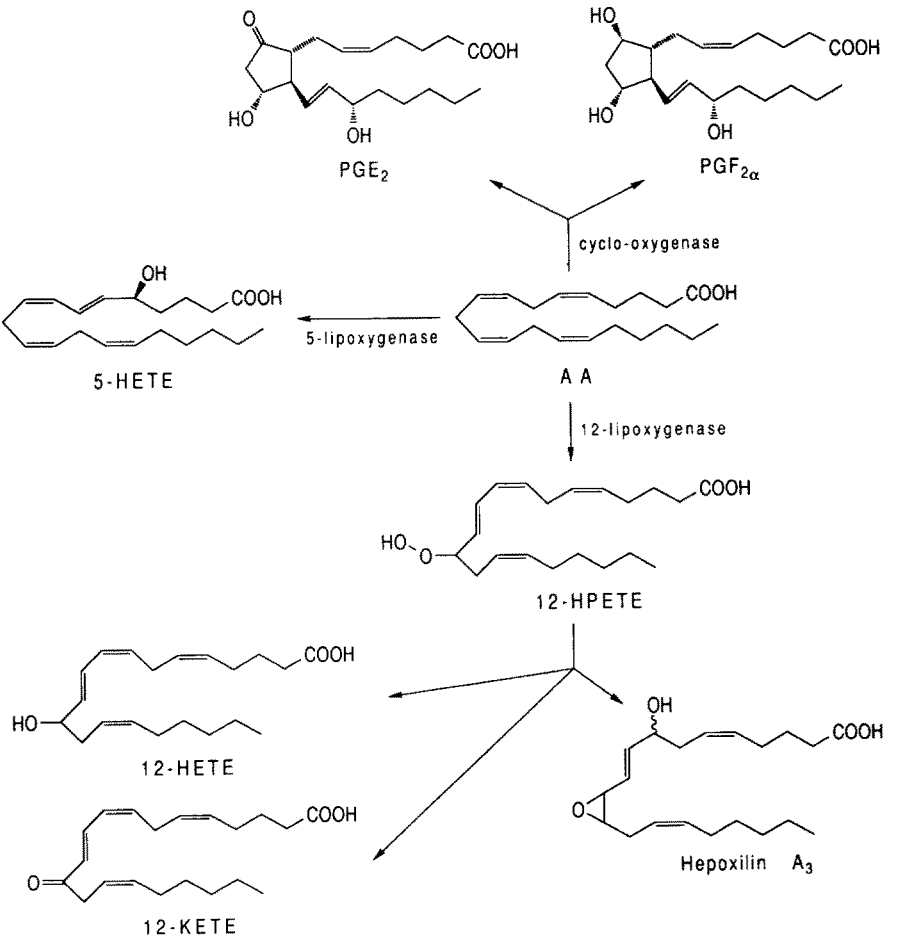


Fig. 2. Arachidonic acid metabolites identified in *Aplysia californica* nervous tissue

tion of histamine or by application of phenylalanylmethionylarginylphenylalaninamide (FMRFamide) [23], two modulatory neurotransmitters characterized in *Aplysia* that produce presynaptic inhibition. Electrical stimulation of L32 cells, a group of identified neurons in the abdominal ganglion, also resulted in the release of radioactive 12-HETE.

Production of 12-HETE after treatment with neurotransmitters or after intracellular stimulation of L32 neurons suggests a potential physiological role for metabolites of the 12-lipoxygenase pathway. However, when 12-HETE was applied to L14 neurons, which are histaminoreceptive followers of L32 cells and control inking, a defensive behavior of the animal, no effect was seen [23, 24]. It is known that 12-HETE is formed through 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE). Application of 12-HPETE to *Aplysia* histaminoreceptive neurons caused a response similar to that caused by histamine [25] and in addition mimicked the electrophysiological response of sensory cells to the tetrapeptide, FMRFamide. FMRFamide-induced opening of K^+ channels, membrane hyperpolarization and shortening of the action potential were reproduced by application of AA and 12-HPETE [23, 26, 27]. These findings suggest that 12-HPETE may be the second messenger underlying the response to histamine and FMRFamide in *Aplysia* L14 and sensory cells.

Further metabolism of 12-HPETE in *Aplysia* was also investigated, since it could be possible that the biological effects of 12-HPETE may be carried out by one or more of its metabolites. 12-HPETE is known to undergo a complex metabolism giving rise to two isomeric epoxy alcohols, hepoxilin A_3 (8-hydroxy-11,12-epoxyeicosatrienoic acid) and hepoxilin B_3 (10-hydroxy-11,12-epoxyeicosatrienoic acid). These epoxy alcohols can in turn be opened to the corresponding trihydroxyacids (trioxilin A_3 and B_3) by a specific epoxide hydrolase. In addition, 12-HPETE can also be converted to the ketoacid 12-KETE. From homogenates of *Aplysia* nervous tissue incubated with ^{3}H-AA, [^{3}H]hepoxilin A_3 was isolated and identified by GC/MS, HPLC and conversion to a mixture of trihydroxy acids with rat lung epoxide hydrolase [25]. The production of hepoxilin A_3 is specific, as its positional isomer hepoxilin B_3, which is produced in nearly equal amounts as hepoxilin A_3 by the nonenzymic rearrangement of 12-HPETE, could not be detected in the experimental samples that contained significant amounts of hepoxilin A_3. In similar experiments with *Aplysia* nervous tissue labelled by incubation with ^{3}H-AA, the formation of [^{3}H]-labelled 12-keto-5,8,10,14-eicosatetraenoic acid (12-KETE) was observed [28]. Identification of 12-KETE rests on UV spectra, GC/MS and $NaBH_4$ reduction of the methyl ester to a compound having the same HPLC retention time of 12-HETE methyl ester. Application of histamine to [^{3}H]AA-labelled *Aplysia* neural tissue caused a $\sim$ 10-fold increase in radioactivity associated with 12-KETE, as compared with controls.

As far as the biological action in *Aplysia* neurons of hepoxilin A_3 and 12-KETE is concerned, application of hepoxilin A_3 to L14 neurons results in a marked membrane hyperpolarization, accompanied by an increased membrane ion conductance, while application of 12-KETE to the same cells produced

membrane hyperpolarization. The findings summarized here were believed to indicate that products of the 12-lipoxygenase pathway may act as intracellular second messengers in *Aplysia* neurons.

4 Biosynthesis of Navenones in *Navanax inermis*

Cephalaspidea molluscs [29] are opisthobranchs characterized by a prominent head shield. Species in the Aglajidae family are of great interest, from both an ecological and chemical points of view. These molluscs are extremely voracious predators of other opisthobranchs [30], and, in particular, of cephalaspideans belonging to the Bullidae and Haminoeidae families. They locate their prey following the slime-trails deposited by the molluscs.

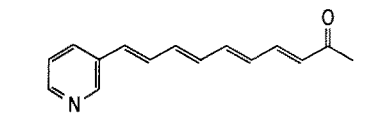
Chemical studies [31, 32] of the yellow secretion of the Pacific *Navanax inermis* led to the characterization of three main conjugated methyl ketones: navenone-A (**1**), navenone-B (**2**), navenone-C (**3**), together with some minor related metabolites with either an additional methyl at C-3, methyl navenone-A (**4**) and methylnavenone-B (**5**), or two *cis* double bonds, 3,5-di-*cis*-navenone-A (**6**) and 3,5-di-*cis*-navenone-B (**7**).

All navenones were secreted by *Navanax* from a specialized gland, the "yellow gland", when molested, and also exhibited the capacity to induce an immediate escape reaction to other trail-following *Navanax*. A series of rigorous bioassays [32] carefully documented the intraspecific "alarm pheromone" properties of the navenones.

A single biosynthetic experiment [33] was also performed to clarify the origin of the navenones. Labelled ^{14}C-acetate was injected into the food of *N. inermis*. After two days, a preparative silica gel TLC of the yellow secretion revealed significant incorporation (0.28%) in navenone-C (**3**), modest (0.05%) in navenone-B (**2**), not measurable in navenone-A (**1**). This experiment was reputed to support a phenol → benzene → pyridine biosynthetic pathway. But as navenone-C (**3**) was difficult to purify as the free phenol [32], misleading interference by co-occurring lipids might have occurred. Further biosynthetic experiments need to clarify fully the origin of this relevant group of metabolites. Probably, navenones A–C (**1–3**) are derived via the acetogenin pathway, in which the precursors would be nicotinic, benzoic and *p*-hydroxybenzoic acid, respectively.

A series of interesting observations was made [32] while maintaining *N. inermis* in a laboratory aquarium for some months in the presence of selected prey, mainly *Haminoea virescens* and *Bulla gouldiana*.

1) The relative ratio between **1** and **3** (usually 4:1) was completely modified in favor of **3** in regenerated secretions; 2) the minor methylated navenones (**4, 5**) were produced in higher yields under stress. According to these results, *N. inermis* under laboratory conditions could modify its secondary metabolites,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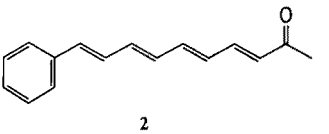
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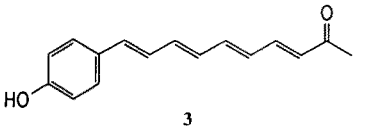
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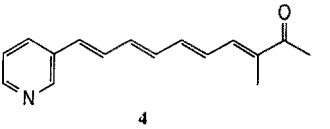
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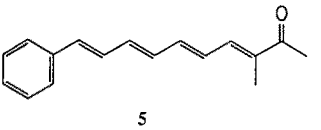
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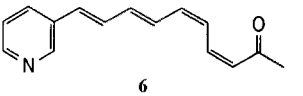
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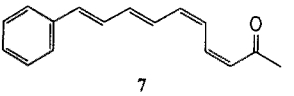
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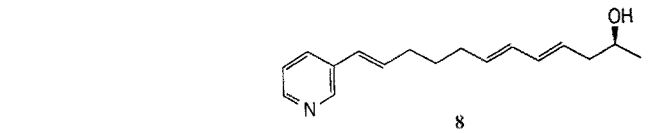
but these anomalies could also be ascribed to the different metabolic contents of the preys offered to *N. inermis*.

Analogously with *N. inermis*, the Mediterranean *Philinopsis* (= *Aglaja*) *depicta* [34] is known as a predator of other cephalaspidean molluscs [35], *Bulla striata* and *Haminoea hydatis*. When *P. depicta* lives in habitats populated by *B. striata*, its secondary metabolic pattern, consisting of a series of uncommon polypropionates, is almost identical to that of its prey [36, 37]. On the contrary, if the mollusc, under maintenance in an aquarium, feeds on *Haminoea navicula*, it discharges [5] in the form of a yellow secretion two *Haminoea* metabolites, the haminols (**8**, **9**), structurally related to navenone-A (**1**), which possess alarm pheromone properties [38] for *H. navicula*. Studies on a third Aglajidae mollusc [39, 40], *Philinopsis speciosa*, led to the characterization of a 2-alkyl-pyridine, pulo'upone (**10**), as a minor constituent co-occurring with two polypropionates closely related to the metabolites from the pair *P. depicta-B. striata*.

According to Faulkner et al. [10] comparison of organisms living in distinct geographical areas can sometimes make a significant contribution to clarifying the origin of secondary metabolites from opisthobranchs. Comparative analysis of the secondary metabolites of three Aglajidae molluscs, living in very different habitats, offers an extraordinary parallelism that prompts further studies on related cephalaspidean species. Recently [41] metabolites related to navenone-C, lignarenone-A (**11**) and -B (**12**), have been found in the Mediterranean cephalaspidean *Scaphander lignarius*.



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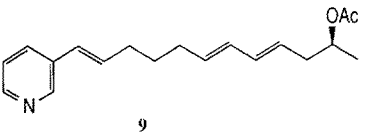

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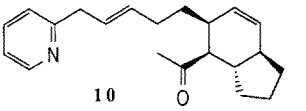

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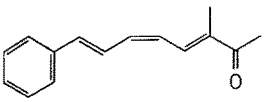

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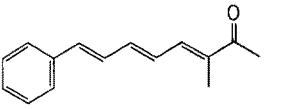

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5 Biosynthesis of Polypropionate Metabolites

Secondary metabolites generated via the propionate route are quite unusual in nature. Relevant exceptions are some antibiotic macrolides from Streptomycetes [42], but wholly propionate-derived macrolides are rare. This biosynthetic pathway has been well proved for erythromycin (**13**), where the aglycone is produced by assembling seven propionate units [43, 44], and for a few related antibiotics [45]. However, very sophisticated biosynthetic experiments [46] have established that some "apparent propionate" units in other macrolides (e.g., aplasmomycin [46]) from Streptomycetes could be formed either by C-methylation through *S*-adenosylmethionine or from glycerol.

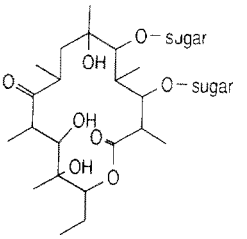
Surprisingly, the propionate pathway seems to be a biosynthetic route which is quite developed among marine molluscs. In fact, metabolites exhibiting formal polypropionate skeletons have been isolated from ascoglossans, cephalaspideans and pulmonates [47]. Their origin (from epibionts or from endobionts, de novo from propionate or from acetate), their biological function (protection against predators, protection against microorganisms, ecological mediators) open intriguing research areas.

Ascoglossan molluscs are herbivorous opisthobranchs [48], which are subdivided into three superfamilies: Oxynoidea, Elysioidea and Polybranchioidea [49]. The most ancestral conchoid Oxynoidea species retain a relic shell that disappears in the other two aconchoid superfamilies. All the aconchoid molluscs, with the exception of those belonging to the genus *Cyerce* [50], show diverticula of the digestive glands in the parapodia and in the cerata. Chloroplasts sequestered from algae are transferred into the diverticula, where they can

remain active for many days synthesizing molecules useful to the mollusc both for nutrition and for defense.

The ability of chloroplasts in Elysioidea to synthesize primary organic metabolites was ascertained by a series of biosynthetic experiments. Some ascoglossans were incubated with $NaH^{14}CO_3$ in Millipore-filtered sea water. Radio-autography of sections of mollusc tissues proved that the labelled precursor was first fixed by chloroplasts and then moved into various parts of the animals, in particular into the podal glands of *Tridachia crispata* [51] and *Tridachiella diomedea* [52]. Labelled organic material, including ^{14}C-hexoses, was also recovered from the mucus secreted from the podal gland of *Placobranchus ianthobapsus* (*ocellatus*) [53]. Careful dissection of *P. ocellatus*, after two series of experiments (in the light and in the dark) for different times (from 12 to 60 h) of incubation with labelled bicarbonate, led to the quantification [54] of the radioactivity of both chloroplast-free tissues and chloroplast-bearing tissues. In all experiments carried out in the light, almost 20% of the total radioactivity fixed by the molluscs was passed to the chloroplast-free tissue. Of course, from parallel experiments in the dark, lacking the photosynthesis by chloroplasts, very small amounts of fixed ^{14}C were recovered. A series of labelled primary metabolites (amino acids, sugars and organic acids) were identified by co-electrophoresis and co-chromatography with known compounds after incubation (2.5 h, $NaH^{14}CO_3$, in the light) of *Elysia hedgpethi*, *Placida dendritica* and *P. ianthobapsus* [55]. After comparing the metabolism of the green alga *Codium fragile* the authors concluded that animal-cell chloroplasts lack the ability to synthesize lipids. The first in vivo incorporation into secondary metabolites of ascoglossans was reported in 1979 [56]. Further chemical studies on *P. ocellatus* led to the characterization of two γ-pyrones: 9,10-deoxytridachione (**14**), previously characterized from *T. diomedea* [57], and photodeoxytridachione (**15**) already isolated from *T. crispata* [58].

By biosynthetic experimental procedures similar to those previously reported [56], four specimens of *P. ocellatus* were placed in aerated seawater (16 mL) enriched with ^{14}C-labelled sodium bicarbonate at 20 μ Ci/mL. After 8 h in sunlight and 18 h in the dark the animals were sacrificed. The ether soluble metabolites from the methanol extract of the mollusc were fractionated on silica gel TLC recovering good incorporation in the band associated with a mixture containing the two γ-pyrones **14** and **15** and an unknown compound **X**, which were resolved by reversed phase HPLC. Replicate experiments in the dark showed that the total incorporation was 50-fold smaller. However, parallel shorter incubations (0.5 and 1 h) in the light and in the dark proved that light favors major incorporation into photodeoxytridachione (**15**). The authors, observing an easy rearrangement of **14** into **15** in sunlight, suggested a "sunscreen" non-enzymatic protective function for **14**. The mechanism of the rearrangement, analogous to that described by Barton and Kende [59] for the transformation of dehydroergosterol acetate to photodehydroergosterol, has been suggested by Ireland and Faulkner and also proved by rigorous photochemical experiments [60].



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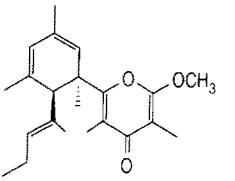

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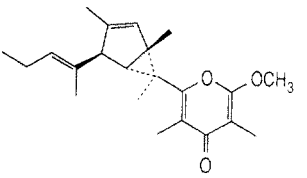

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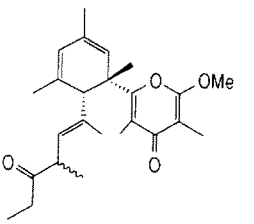

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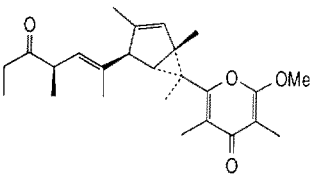

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It seems that these unusual propionate metabolites might be taxonomical marker of many Elysioidea molluscs. In fact, they have also been found in *Elysia chlorotica* [61], where the enantiomer of deoxytridachione (**14**) co-occurs with elysione (**16**) (= tridachiapyrone-A [62]) which could be the formal precursor of crispatene (**17**) found in populations of *T. crispata* from the Caribeean [58, 62]. It seems well supported that the ascoglossan-chloroplast pair is able to synthesize these molecules de novo, but further experiments with less general precursors need to ascertain the propionate origin.

More recently, some metabolites (Fig. 3) related to the Elysioidea polypropionates, even though exhibiting very unusual demethylation, have also been found in two Polybranchioidea molluscs of the genus *Cyerce*; the Pacific *C. nigricans* [63, 64] and the Mediterranean *C. cristallina* [65, 66]. The species belonging to this genus have adopted a defensive strategy that includes the autotomy of the dorso-lateral appendages (cerata). Even minor stress triggers within a few seconds the detachment of all cerata, which continue to wiggle vigorously while secreting a conspicuous defensive mucus. *Cyerce* is the only Polybranchioidea genus without diverticula of the digestive gland in the cerata

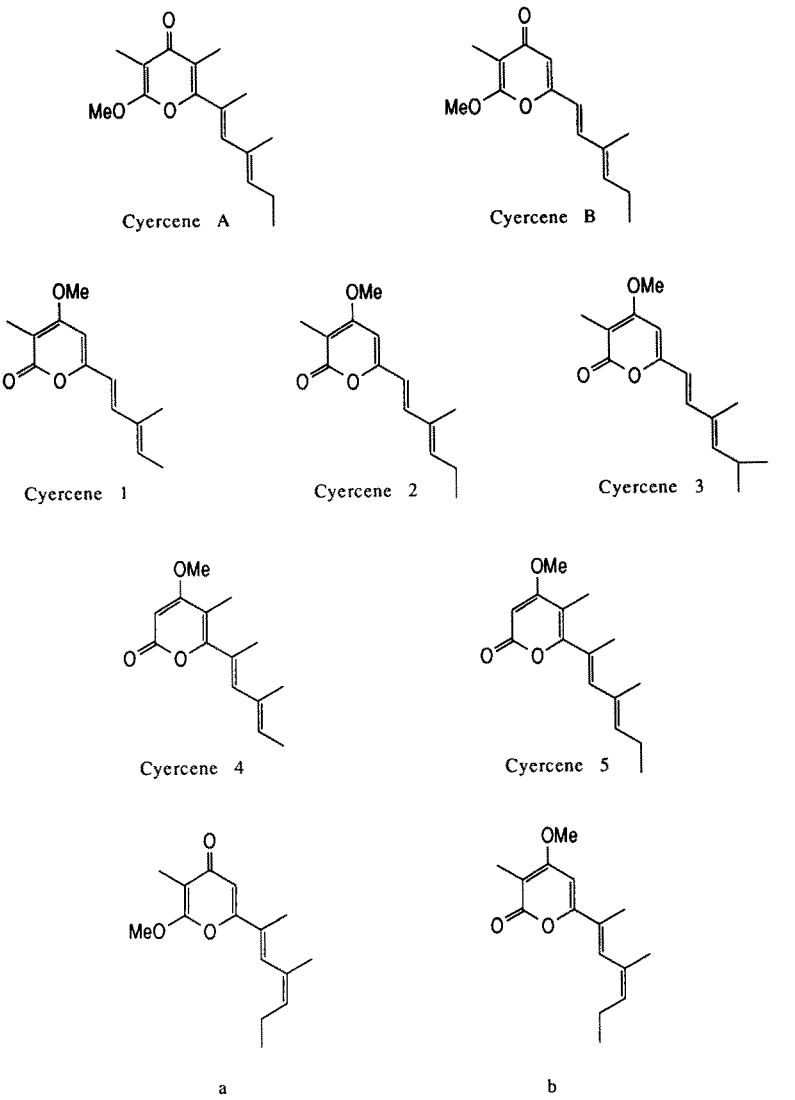


Fig. 3. Polypropionates from *Cyerce cristallina* (cyercenes) and from *Cyerce nigricans* (a, b)

[50] and without symbiotic algal chloroplasts (A. Marin, personal communication).

As the mollusc is able to regenerate the missing cerata at an extraordinarily high speed, usually within 7–10 days, an in vivo biosynthetic experiment was performed by dissolving 25 μCi of sodium [2-^{14}C]propionate in 1.5 L of aerated sea water containing four *C. cristallina* completely devoid of cerata [66]. The molluscs were incubated for five days, until regeneration of the cerata was

achieved attaining at least 50% of their original volume. Significant radioactivity was recovered from the detached cerata. Specific incorporation into individual cyercenes was monitored by recording the radioactivity of a series of fractions obtained from both preparative SiO_2 TLC and reversed phase HPLC. All cyercenes exhibited good incorporation, thereby strongly supporting de novo biosynthesis from five propionate units. However, further experiments need to exclude rigorously other biosynthetic pathways, which, in analogy with those described for some Streptomycetes macrolides, could lead to propionate units. Cyercenes might play different biological functions (defense, chemical mediators of the tissue regeneration, etc.) in *C. cristallina*. In fact, their tissue distribution revealed interesting specific compartmentalization. All cyercenes

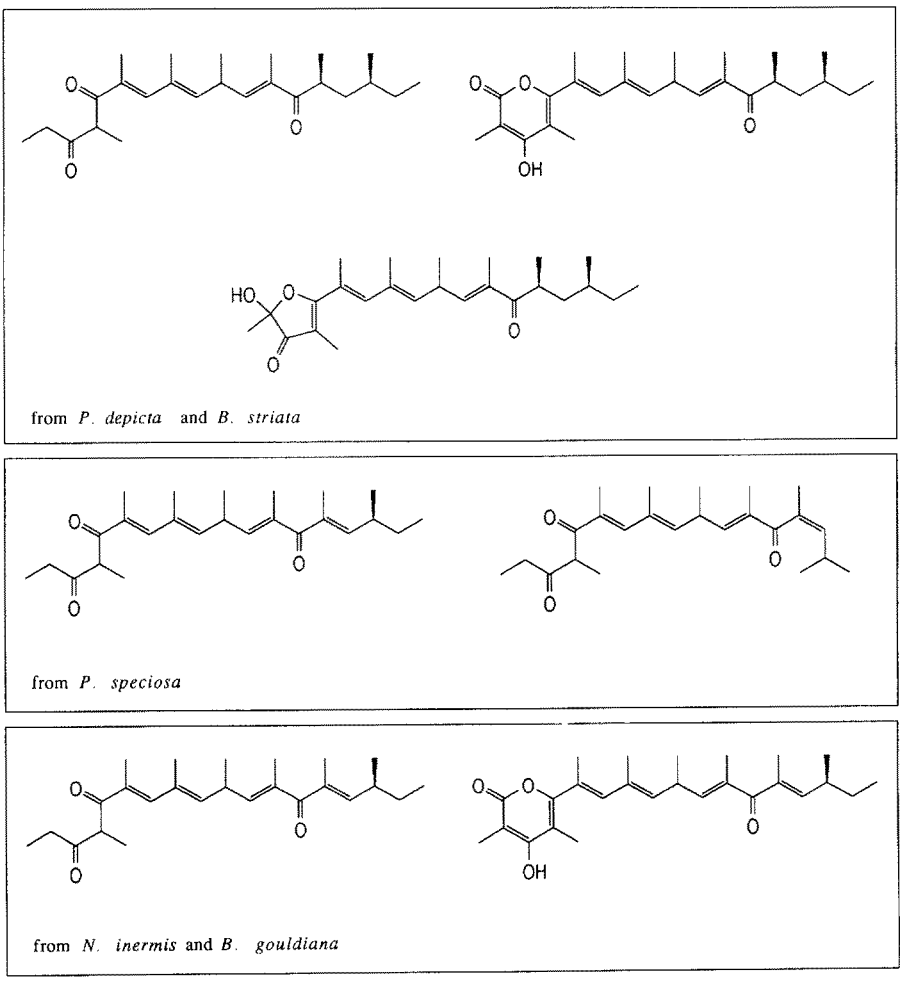
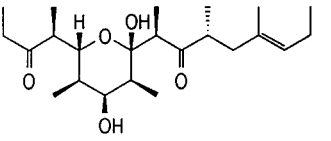


Fig. 4. Polypropionates from Mediterranean and Pacific cephalaspideans

were present in the cerata and, with the exception of cyercene-A, in the copious mucous secretion. By contrast, they were completely absent in the hepatopancreas, whereas only cyercenes 1–3 (Fig. 3) were recovered from the mantle. Specifically, cyercene-A was present only in the cerata, thus raising intriguing hypotheses on its biological role during the tissue regeneration of *C. cristallina.* The biosynthesis of related propionate metabolites from ascoglossans with and without symbiotic chloroplasts, supports the possibility that this biosynthetic ability has to be ascribed to the molluscs and not to their algal endobionts. Metabolites with a supposedly propionate skeleton have also been isolated from some cephalaspidean molluscs (Fig. 4): *Philinopsis* (= *Aglaja*) *depicta* [36], *B. striata* [37], *Philinopsis speciosa* [39], *N. inermis*, *B. gouldiana* [67]. The



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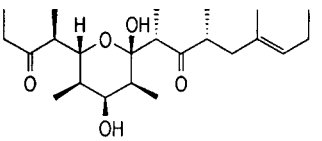

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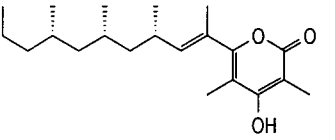

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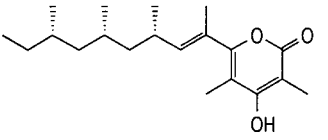

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presence of these metabolites in *P. depicta*, *P. speciosa* and *N. inermis*, carnivorous molluscs that are tremendously voracious, should be linked to their food chain. The Mediterranean *P. depicta* and the Pacific *N. inermis* exhibited the same metabolite pattern as *B. striata* and *B. gouldiana*, when they were collected at places populated by *Bulla* species. According to Garson's suggestion [9], biosynthetic studies should be performed to establish the true origin of propionate metabolites in cephalaspidean molluscs.

Pulmonates are air-breathing molluscs that do not include many families and genera [68]. *Siphonaria* species from many distinct geographical areas possess a series of secondary metabolites with polypropionate skeletons characterized by the presence of ketal, pyrone and furanone moieties [47]. A rigorous biosynthetic experiment [69] proved that *Siphonaria denticulata* is able to

biosynthesize de novo its potential defensive metabolites, denticulatins-A (**18**) and -B (**19**) [70].

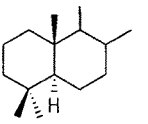
A preliminary, anatomical dissection allowed localization of the denticulatins only in the foot mantle and in the mucous secretion. Because of this finding, labelled sodium (1-^{14}C) propionate was injected into the foot of 16 molluscs resulting in recovering significant incorporation (0.1%) into denticulatins after 6 days. Alternatively, the radioactive precursor was directly added to the aquarium water and absorbed through the skin of the molluscs, leading to labelled denticulatins (0.02%) after 7 days. Simultaneously, an experiment with an identical amount of labelled [1-^{14}C] acetate led to practically unlabelled denticulatins, thus excluding synthesis via acetate and subsequent methylation. In order to establish, whether the [1-^{14}C] propionate was uniformly incorporated into the denticulatins, a denticulatin A/B mixture was subjected to Kuhn–Roth degradation. The carbon atoms of the denticulatin framework arising from C-1 of propionate were recovered as labelled $BaCO_3$, while those arising from C-2 and C-3 were recovered as unlabelled acetic acid. These results show that no randomization of the labelled propionate occurred during the biosynthetic experiment; however, since only 41% of the anticipated radioactivity was recovered as $BaCO_3$, it was not possible to assess whether the denticulatins were uniformly labelled. The authors proposed further experiments with ^{13}C and ^{2}H precursors to clarify this point. Finally, a defensive role of denticulatins was doubtful on the basis of the observation that *S. denticulata* is commonly eaten by some marine predators.

Biogenetic considerations [71], and a correlation of all polypropionates from pulmonate molluscs led to revision of the relative stereochemistry of pectinatone (**20**) and nor-pectinatone (**21**), metabolites of the pulmonate *Siphonaria pectinata* [72, 73]; the point had already been questioned by Oppolzer's synthesis [74] and was definitively confirmed by two independent X-ray diffraction studies [71, 75] of pectinatone (**20**).

6 Biosynthesis of Drimane Sesquiterpenoids in Dendrodoridídae Nudibranchs

Sesquiterpenoids possessing the drimane skeleton (**22**) exhibit an impressive series of biological properties. Recently, all aspects relative to occurrence [76], biological activity [76], insect antifeedant properties [77] and synthesis [78] of these terpenoids have been exhaustively reviewed.

Drimane sesquiterpenoids occur in many terrestrial and marine organisms, such as higher plants, fungi, sponges, and marine molluscs belonging to the order Nudibranchia. Specifically, drimanes were isolated from nudibranchs *Archidoris montereyensis* (**23**, **24**) [79], *Hypselodoris porterae* (**25**) [80], *Cadlina luteomarginata* (**26**, **27**) [81], *Chromodoris albonotata* (**28**) [82], and *Dendrodoris* and *Doriopsilla* species (**29–34**) [83–88]. The fish antifeedant properties of many of these metabolites were supported by a series of positive responses in bioassays



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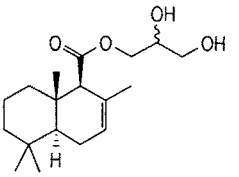

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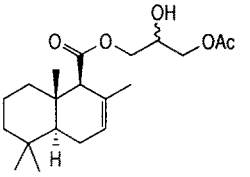

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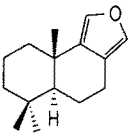

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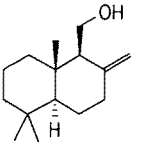

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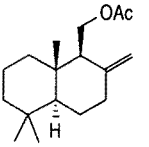

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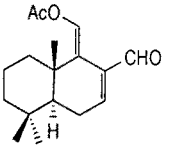

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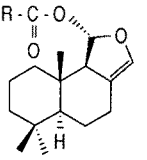
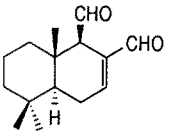


29 R = various methylene chains



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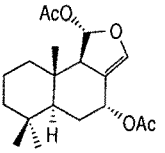

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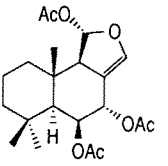

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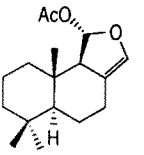

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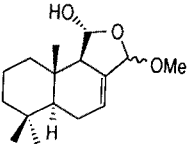

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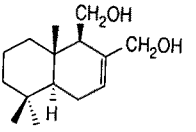

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with the drimane acid glyceride (**23**) [79], albicanol (**26**) [81], pu'ulenal (**28**) [82], polygodial (**30**) [84], olepupuane (**31**) [88] and 6β-acetoxy-olepupuane (**32**) [85].

Drimanes in nudibranchs could derive either from a sponge diet or by biosynthesis de novo. Biosynthetic experiments [79] have conclusively proven the ability of *A. montereyensis* to synthesize its own drimane glyceride (**23**). However, major studies have been performed on *Dendrodoris* molluscs where drimanes, which have also taxonomic significance, play a defensive role. In fact, terpenoids with a drimane skeleton are present in all species that were studied and, moreover, some of them are strongly antifeedant to fish. Specifically, the marine fish *Chromis chromis* and the freshwater fish *Carassius auratus* vigorously refused food pellets that had been treated with polygodial at a concentration of 30 $\mu g/cm^2$ [84]. Polygodial (**30**), a known plant metabolite [89], was first isolated from the Mediterranean *Dendrodoris limbata* [84], then from three Pacific *Dendrodoris* spp. (*nigra, tubercolosa, krebsii*) [88] and finally, from the Mediterranean *Dendrodoris grandiflora* [85].

Labelled mevalonic acid dibenzylethylenediamine (DBED) salt (14 μCi) was injected into the digestive glands of *D. limbata* [90]. After 24 h, the animals were sacrificed and labelled polygodial (**30**) was recovered from the skin of the mollusc. After two subsequent chromatographic purifications, the dialdehyde was reduced to the corresponding diol (**35**) which was significantly labelled. The ability of *D. limbata* to synthesize de novo its own defensive allomone raised a series of questions linked to

1) the origin and biological role of some co-occurring drimanes;
2) the precursor-product relationships among *Dendrodoris* sesquiterpenoids;
3) the mechanism of the action of polygodial;
4) the auto-protection of the mollusc to the dangerous effects of its own allomone;
5) the anatomical distribution of drimanes in *Dendrodoris* species.

Some drimane esters (**29**), related to polygodial but without fish anti-feedant properties, co-occur in *D. limbata* [83], where they are localized in the hermaphrodite glands [87]. Analogously to polygodial, they are biosynthetized de novo by the mollusc. In fact, biosynthetic experiments [90] led to labelled esters which after two chromatographic steps had lost contaminating colored products but retained radioactivity. Chemical degradation by thermolysis to euryfuran (**25**) placed the radioactivity in the sesquiterpenoid skeleton.

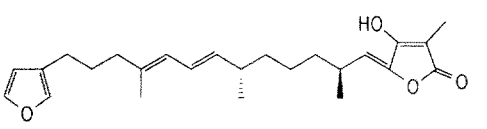
Further biosynthetic experiments were performed with the aim to clarify metabolic relationships between polygodial and the esters, as well as to ascertain whether or not polygodial was the precursor of the esters. Two series of experiments [86] were performed injecting labelled [2-^{14}C] mevalonic acid into nine (4.5 μCi) and fourteen (7 μCi) animals, which were sacrificed at various times from 3 to 77 h. The two experiments, performed during the months of March and April, 1984, led to apparently conflicting but not contradictory results. In fact, in the first series of experiments the major specific recovered radioactivity was always associated with polygodial (**30**). On the other hand, the

esters retained more of the label in the second experiment. This apparent anomaly would exclude a precursor-product relationship between polygodial (**30**) and its esters (**29**) and suggests [86] that the drimanes are biosynthetised by independent pathways linked to the developmental stage of the animal. A recent anatomical analysis [87] of the distribution of drimanes in *D. limbata*, localizing the esters only in the hermaphrodite glands and in the eggmasses, supports the above hypothesis. Of course, in a sexually mature mollusc the amount of drimane esters is more relevant since they are located in the abundant eggmasses.

A comparable metabolic pattern was found in all *Dendrodoris* spp., where polygodial co-occurred with olepupuane in the skin of the *D. nigra*, *D. tuberculosa* and *D. krebsii* [88] and with 6-β-acetoxy-olepupuane [85] in the skin of the Mediterranean *D. grandiflora*. In addition, a series of compounds (**36**–**44**) already isolated from sponges, or closely related to sponge metabolites, were isolated from the digestive glands of *D. grandiflora* [85].

Biosynthetic experiments were performed injecting 14 μCi of [2-^{14}C] mevalonic acid – DBED salt into the digestive glands of seven *D. grandiflora* specimens [85]. After 24 h, by the same experimental procedures previously adopted for *D. limbata*, all metabolites were recovered, but good incorporation was observed only in the drimane sesquiterpenoids. Moreover, the minor radioactivity associated with all co-occurring terpenoids dropped to almost background values after some purification steps. Most probably, all *Dendrodoris* species are able to synthetize de novo molecules useful for the defense against predators and for the protection of the eggmasses during reproduction. The impressive biological properties of polygodial can be ascribed to the reactivity of the aldehyde groups with primary amines [91]. Biomimetic experiments monitored by ^{1}H-NMR [92] established formation of an immonium ion (**45**) intermediate in the reaction between polygodial (**30**) and methylamine. With opposite stereochemistry at C-9, no reaction was observed for the inactive 9-*epi*-polygodial (**46**). Proximity of the two aldehyde groups and a small steric bulk appear to be the two structural prerequisites for a terpenoid dialdehyde to possess polygodial-like properties (antifeedant to fish, anorexic to insects, hot tasting to humans) [93, 94]. This behavior is independent of absolute stereochemistry [93].

Because of the toxicity of polygodial (**30**) to *D. limbata*, [84], research was aimed at characterizing a potential masked form of polygodial (**30**). Careful extraction [95] of *D. limbata* and *D. grandiflora* and rapid chromatographic purification ascertained the presence of olepupuane (**31**) in the extracts. As protected form of polygodial, it can easily deliver the active dialdehyde. More recently [87], without any chromatographic steps, two live specimens of *D. limbata* were extracted with deuteriated benzene. The ^{1}H-NMR spectrum of the crude extract revealed complete absence of polygodial signals, whereas those of olepupuane were easily recognizable. Anatomical studies [87] revealed the compartmentalization of all drimanes in selected *D. limbata* tissues: olepupuane along the dorsum edge, the drimane ester only into the hermaphrodite glands,



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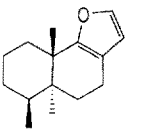

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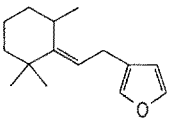

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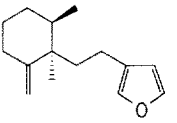

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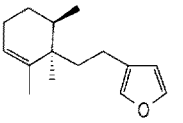

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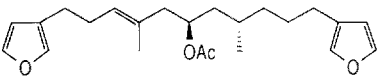

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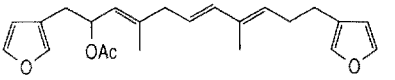

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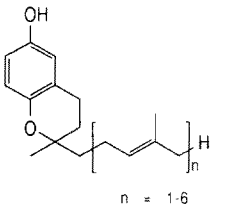

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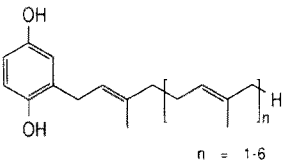

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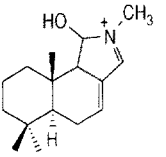

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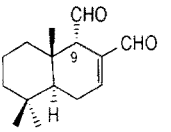

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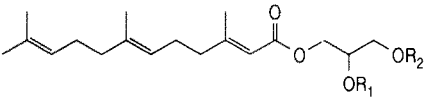
and a new metabolite, 7-deacetoxy-olepupuane (**33**), in the gill. The latter might play a key role in the biosynthesis of drimanes in *Dendrodoris* molluscs. In fact, it or its deacetyl derivative, could be the formal precursor of all other drimanes. However, the recent isolation of **33** from a *Dysidea* sponge [96] might support a dietary origin of **33**, prompting further biosynthetic experiments.

In conclusion, the soft-bodied porostome (without radula) nudibranchs have elaborated a very effective defensive strategy by synthesizing de novo multifunctional protected unsaturated dialdehydes. There is an extraordinary parallelism with the defensive strategy of some Chlorophyta algae [97] and of some pungent fungi belonging to Russulaceae and Clavicoronaceae [98].

7 Biosynthesis of Unusual Acylglycerols

Several glycerides having unusual acyl substituents have been isolated from skin extracts of opisthobranch molluscs and apparently endowed with ichthyotoxicity and feeding deterrent activity. The farnesic acid glycerides **47**, **48** and **49** were isolated from the nudibranch *Archidoris odhneri* [99], which also contained as minor metabolites the monocyclofarnesic acid glyceride **50**, the drimane acid glyceride **23** and the diterpene acid glyceride **51** [79]. Glyceride **23** was reported to be active as an antifeedant. *Archidoris montereyensis* also contained **50**, **23** and **51** (major metabolite) [100], along with monoacetylated derivatives **52** and **53** and the known ether 1-0-hexadecylglycerol **54** [79]. The ether **54**, which is toxic to fish and inhibits feeding behavior, was also found as a minor component of the extracts of the dorid nudibranch *Aldisa sanguinea cooperi* and of the sponge *Halichondria panicea*, on which *A. montereyensis* feeds.



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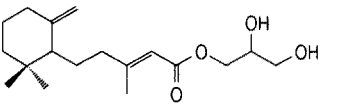
48: $R_1 = Ac.\ R_2 = H$
49: $R_1 = H.\ R_2 = Ac$



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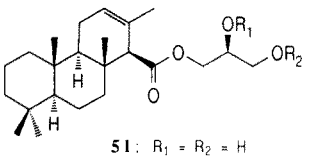
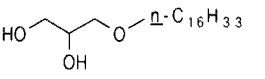


51: $R_1 = R_2 = H$
52: $R_1 = H.\ R_2 = Ac$
53: $R_1 = Ac.\ R_2 = H$



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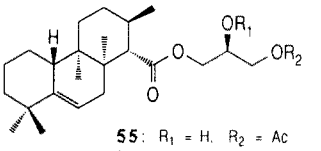


55: $R_1 = H.\ R_2 = Ac$
56: $R_1 = Ac.\ R_2 = H$

In order to investigate whether the above glycerides are derived from the animal's diet or by biosynthesis, [2-^{14}C]*RS*-mevalonic acid DBED salt was injected into the digestive gland of *A. montereyensis* and *A. odhneri* specimens, which were placed in a running sea-water aquarium for 24 h and then extracted [79]. Incorporation of the label was checked in the major metabolites of the two nudibranchs, namely **23** and **51** in *A. montereyensis* and **47** in *A. odhneri*, after HPLC purification and conversion to derivatives that were also purified via

HPLC before being crystallized and counted. The authors [79] do not disclose the specific radioactivity (dpm/mg) after each purification step, but only the final activity of the derivatives. Incorporation levels, ranging from 550 dpm/mg for the derivative of **51** to 3260 dpm/mg for the derivative of **23**, should be considered indicative of the ability of the two nudibranchs to biosynthesize their acylglycerols de novo.

From the nudibranch *Doris verrucosa* two diterpene acid glycerides, verrucosin-A (**55**) and -B (**56**), were isolated along with minor amounts of the farnesic acid glyceride **49** [101, 102]. The diterpene acyl moiety of verrucosin-A and -B, which may arise by Wagner–Meerwein rearrangement of an isocopolane skeleton as in **51**. **55** and **56**, but not **49**, were very toxic to the assay fish *Gambusia affinis*. Existence of de novo biosynthesis of verrucosins was investigated by injection into the *D. verrucosa* hepatopancreas of both [2-^{14}C] mevalonic acid DBED salt and [u-^{14}C]glycerol in a series of separate experiments at different times [103]. Although the verrucosins were found labelled after HPLC purification, the results were inconclusive to establish de novo biosynthesis. In fact, in all experiments radioactivity of the crude verrucosin mixture dropped to low levels after isolation of individual components. Lack of material prevented further purification of verrucosin-A and -B to constant specific radioactivity. In an attempt to explain the poor levels of incorporated radioactivity, if any, it was conjectured that the precursors were administered in the hepatopancreas, while verrucosins have been found only in the mantle: it might be possible that the verrucosins, which act as defense allomones, are synthesized in specialized skin glands, which are poorly accessible to the precursors injected into the hepatopancreas. However, in spite of the questionable results of the radiolabelling experiments, *D. verrucosa* was believed to be able to biosynthesize the verrucosins; this is based on the observation that comparative analysis of the verrucosin mixture of *D. verrucosa* specimens collected in the Bay of Naples and along the Spanish coasts, showed very similar patterns. In addition, the sponge *Hymeniacidon sanguinea*, which is a common prey for both specimens living in the two different habitats, lacks the verrucosins. These findings parallel those reported for *A. montereyensis* and *A. odhneri*, which when collected from different locations contain the same array of glycerides, thus supporting the results of the biosynthetic studies with labelled precursors [10].

Other unusual diacylglycerols that are highly toxic to fish have been isolated from the rare opisthobranch *Umbraculum mediterraneum* [102, 104].

8 Biosynthesis of Steroids

The sterol composition of marine organisms, including molluscs, has been reviewed by Goad [105] and more recently by Kerr and Baker [106]. A general picture emerges that molluscs do not possess the spectrum of exotic sterols

found in other marine invertebrates, and that their sterols are characterized by conventional nuclei and side-chains. As pointed out by Goad [105], there are four possible contributing factors that determine the final sterol content of an animal: (a) de novo biosynthesis; (b) absorption of dietary sterols; (c) modification of dietary sterols; (d) passage of sterols from symbiotic algae, fungi or bacteria to the host animal. There is experimental evidence that in molluscs factors a, b and c are operative.

Many papers have appeared which report investigations on the de novo biosynthesis of sterols in molluscs. Apart from the problems of the nature of the administered labelled precursor (usually acetate or mevalonate) and with the methods of feeding, also reviewed by Goad [105], one major limitation in the earlier studies lies in the fact that they were performed before the advent of HPLC; therefore, individual sterols were not isolated and counted, as purification was achieved in most cases by crystallization of the whole sterol mixture to constant specific radioactivity. Since de novo biosynthesis might be operative only for one or more sterols of the mixture, while the others originate from the diet or from symbionts, the above method clearly does not allow to establish, which sterol of the mixture, if any, is radioactive. In addition, although sterols have a tendency to co-crystallize, a minor labelled constituent might be lost after several crystallizations, thus leaving only unlabelled dietary sterols. However, beside other failures not reviewed here, de novo biosynthesis has convincingly been reported in several molluscs as, for example, in the chitons *Liolophura japonica* [107] and *Lipodochitona cinerea* [108], and in the whelk *Buccinum undatum* [109].

In more recent studies the use of HPLC allowed isolation and counting of individual sterols after administration of labelled precursors. The sterols isolated from mantles and viscera of the nudibranch *Doris verrucosa* were identified as cholestanol, cholesterol, 24-dehydrocholesterol and 7-dehydrocholesterol [103]. After injection of DL-[2-^{14}C]-mevalonic acid DBED salt, cholesterol (**57**) and 7-dehydrocholesterol (**58**) were isolated as the acetates by reversed phase HPLC. Both sterols were found significantly labelled: specific radioactivity associated with 7-dehydrocholesterol was higher by one order of magnitude than that associated with cholesterol. This fact would indicate either that the reduction of the Δ^7 double bond of 7-dehydrocholesterol to afford cholesterol occurs at a low rate, or that the cholesterol found in *D. verrucosa* comes partly from a dietary source.

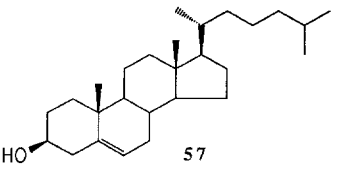
The ability of bivalve molluscs to synthesize sterols is questioned [106]. Approximately forty sterols have been identified from the oyster *Crassostrea virginica* and, since it appeared that many of the sterols identified must be of dietary origin, the ability of the oyster to incorporate injected radioactive acetate was studied [110]. Of the forty sterols naturally occurring in the oyster, only four were labelled by injection of labelled acetate: cholesterol, desmosterol, 24-methylenecholesterol and fucosterol. However, when an oyster hearth tissue culture was grown aseptically with addition of labelled acetate, the sterols were found to be non-radioactive [111]. This finding does not rule out the possibility

of sterol synthesis in other oyster tissues, but can also raise the suspicion that the labelled sterols found in the previous study [110] might have arisen from the biosynthetic ability of symbionts associated with the animal.

Establishment of the dietary or symbiotic origin of sterols could be a difficult task, unless the tracer sterol has an unusual structure and restricted occurrence. There is little doubt, for example, that petrosterol (**59**) found in the nudibranch *Peltodoris atromaculata* [112] arises from predation of the mollusc on the sponge *Petrosia ficiformis*, from which the sterol was first isolated [113]. However, the origin of gorgosterol (**60**) in *Tridacna* species has not yet been established [114] despite its unusual structure.

The ability of molluscs to modify dietary sterols is exemplified by the dealkylation of phytosterols to cholesterol. This pathway was first demonstrated in arthropods (insects; crustaceans) and then in other invertebrates, including molluscs. ^{3}H-Labelled fucosterol (**61**) and β-sitosterol (**62**) were injected into the hepatopancreas of the limpet *Patella vulgata* [115] and the sterols were isolated as the propionyl esters by preparative GLC and counted. Desmosterol (**63**) and cholesterol (**57**) were found significantly labelled. Injection of [1-^{14}C]acetate and [5-^{14}C]mevalonic acid also resulted in the isolation of labelled cholesterol and desmosterol, thus showing that in *Patella vulgata*, and for the first time in a mollusc, two biosynthetic pathways to C_{27} sterols are operative: de novo biosynthesis and dealkylation of phytosterols, presumably coming from the diet. Similarly, *Ostrea gryphea* [116] and *Buccinum undatum* [109] were reported to be able to dealkylate 24-alkylsterols.

Occurrence in molluscs of steroidogenesis along a pathway very similar to that found in vertebrates has been claimed. Testosterone (**64**) – androstenedione (**65**) interconversion has been demonstrated in gonads of the male and female bivalve – *Mytilus edulis* [117] and of the gastropod *Crepidula fornicata* [118], by using labelled testosterone and androstenedione. Moreover, several steroids have been identified in the gonads and hepatopancreas of the opisthobranch



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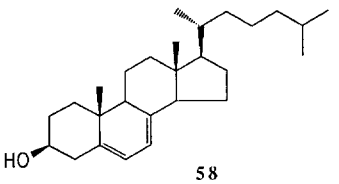

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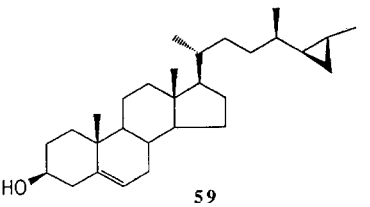

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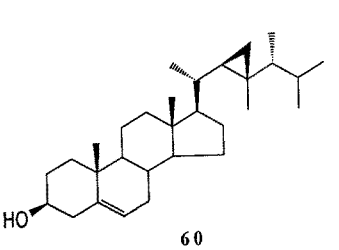

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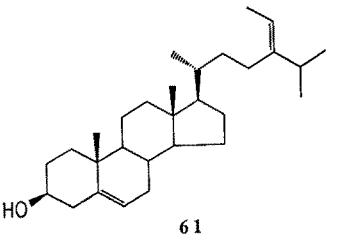

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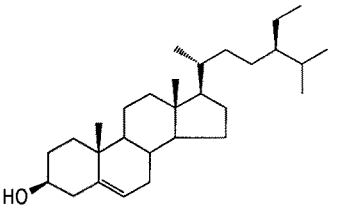

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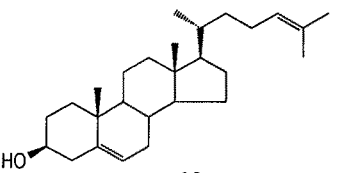

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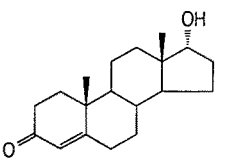

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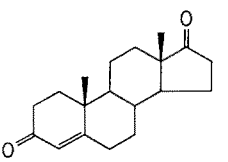

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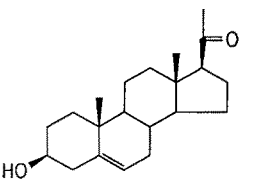

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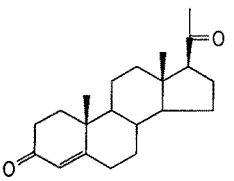

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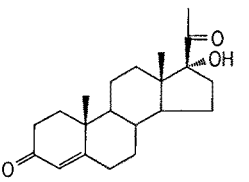

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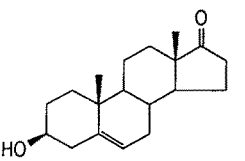

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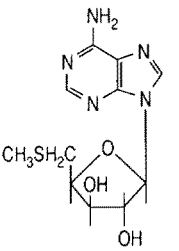
Aplysia depilans [119]. Incubation of gonads and hepatopancreas of *A. depilans* with [1-^{14}C]acetate and [4-^{14}C]cholesterol allowed isolation of several steroids with pregnane and androstane frameworks labelled to various degrees. The steroids were isolated by TLC and identified by crystallization to constant specific activity after addition of cold carrier. In subsequent work [120], the same *A. depilans* tissues were incubated with a mixture of [7α-^{3}H]pregnenolone (**66**) and [4-^{14}C]progesterone (**67**). After isolation and identification of the metabolites as above, it appeared that the major metabolites in the gonads were progesterone (**67**), 17α-hydroxyprogesterone (**68**) and testosterone (**64**), while in the hepatopancreas they were dehydroepiandrosterone (**69**) and androstenedione (**65**). Some aspects of the overall pathway leading to steroids in *A. depilans* remain unexplained.

9 Biosynthesis of Xylosyl-MTA in the Nudibranch *Doris verrucosa*

Xylosyl-MTA (9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine; **70**), isolated from the nudibranch *Doris verrucosa* [121], represents the first described naturally occurring analog of MTA (5′-deoxy-5′-methylthioadenosine; **71**), which is a sulfur-containing nucleoside ubiquitously distributed in micromolar amounts in nature. MTA originates from *S*-adenosylmethionine metabolism through several metabolic pathways and exerts significant inhibition in vitro on several enzymic systems; among these inhibition of spermine synthase is probably the most important [122]. In mammalian and other tissues MTA does not accumulate intracellulary, because of its rapid cleavage by MTA phosphorylase into adenine and 5-methylthioribose-1-phosphate. By contrast, in *D. verrucosa* the cellular concentration of xylosyl-MTA ranges from 330 to 380 μmol/g of wet tissue and is two orders of magnitude higher than that of MTA [123].



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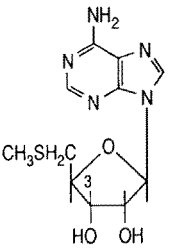

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The natural occurrence of xylosyl-MTA poses intriguing questions concerning its biosynthesis and physiological role. Assuming that the xylosyl thioether does not derive directly from a dietary source, one can envisage three possible pathways for its biogenesis. (1) The molecule could originate from the xylofuranosyl analog of adenosylmethionine through known enzymic reactions involved in MTA biosynthesis; (2) MTA could be enzymically isomerized at the 3′-position affording xylosyl-MTA; or (3) an independent biosynthetic pathway, unrelated to adenosylmethionine and its metabolites could be operative. Biosynthetic experiments with labelled precursors [124] revealed that xylosyl-MTA originates in *D. verrucosa* by isomerization at C-3′ of endogenous MTA and that the overall pathway probably involves an oxidation-reduction step at C-3′, since the 3′ proton of MTA is lost during the process.

In an initial series of experiments, [Me-^{14}C]methionine and [8-^{14}C]adenine were injected into *D. verrucosa* hepatopancreas. After HPLC isolation, xylosyl-MTA was found consistently labelled; in particular, in the experiment with labelled methionine 28.0% of the recovered radioactivity was found associated with xylosyl-MTA, while 12.1% was recovered in the adenosylmethionine

fraction and only 0.57% in the MTA fraction. When [Me-^{14}C]MTA was injected, 72% of the recovered radioactivity was found after 24 h in the xylosyl-MTA fraction, while MTA was found non-radioactive because of its high turnover rate. These results clearly show that xylosyl-MTA arises by isomerization of endogenous MTA. In order to ascertain, whether an oxidation-reduction process at C-3′ is involved in the MTA/xylosyl-MTA conversion, the fate of the 3′-proton of MTA was investigated by injection in *D. verrucosa* of a mixture (1:3; ^{14}C/^{3}H ratio) of [Me-^{14}C]MTA and [3′-^{3}H]MTA. The latter compound was prepared along with [3′-^{3}H]xylosyl-MTA by $NaBT_4$ reduction of the parent 3-ketone [125]. Recovered xylosyl-MTA was labelled to a considerable extent and retained only ^{14}C radioactivity, the ^{3}H label of the precursor having been lost during the MTA/xylosyl-MTA conversion. This evidence strongly favors a 3′-oxo derivative of MTA as an intermediate during the conversion. On the other hand, recovered MTA showed a decreasing ^{3}H/^{14}C ratio from 2.67:1 after 1 h to 0.46:1 after 24 h, thereby suggesting that reduction of the hypothetical ketone intermediate proceeds *in vivo* with low stereospecificity, giving rise to both xylosyl-MTA and MTA. MTA is transformed into xylosyl-MTA by an iterative process, since 60.4% of ^{14}C radioactivity after 24 h was found associated with xylosyl-MTA and only 6.2% with MTA.

Experiments were also carried out injecting [3′-^{3}H]xylosyl-MTA. The results indicated that the molecule has a very low turnover rate in *D. verrucosa*, since 96% of the recovered radioactivity after 24 h was associated with xylosyl-MTA. Accordingly, it was observed [126] that xylosyl-MTA is resistant to the enzyme MTA-phosphorylase which cleaves MTA but not the xylose analog, which therefore accumulates in the animal. Since xylosyl-MTA is mainly concentrated in the hermaphrodite gland of *D. verrucosa* and is very abundant in the eggmasses [103], it may play a role in the reproductive biology of *D. verrucosa*.

10 Further Metabolism of Dietary Compounds

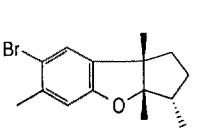
The presence in molluscs of molecules structurally related to typical dietary metabolites could be ascribed either to selective accumulation of minor compounds acquired through the diet, or to an in vivo chemical transformation of major metabolites acquired from the prey. However, all reports on this topic have to be carefully evaluated before drawing hurried conclusions. In particular, interaction among molecules from different organs could favor formation of artifacts when the secondary metabolites are extracted from the whole mollusc and not from individual dissected tissues. Only some cases, where the ability of the molluscs to modify dietary metabolites seems to be well supported, are reported in this chapter.

It is generally accepted [47] that sea hares, opisthobranch molluscs belonging to the order Anaspidea, are protected by dietary metabolites obtained by

feeding upon algae and which are transferred from the digestive gland into some parts of the mantle which are exposed to the attacks of predators. However, until now no adequate bioassays have definitively supported this hypothesis.

Some unique compounds from sea hares, closely related to algal metabolites, are likely to be derived from the algal diet. Aplysin (**72**), debromoaplysin (**73**) and aplysinol (**74**) were found almost thirty years ago in *Aplysia kurodai* [127] and two of them are the first bromosesquiterpenoids found in nature. After some years, the same metabolites were also isolated from the red alga *Laurencia okamurai* [128], while some related compounds, laurinterol (**75**) and debromolaurinterol (**76**) were found in *Laurencia intermedia* [129, 130]. Treatment of **75** with *p*-toluensulfonic acid resulted in good yields of **72** [131], thus suggesting an acid-catalyzed biotransformation of **75** to **72** in *A. kurodai*. A biosynthetic experiment [132] on *Aplysia californica*, which contains **72**, **73**, **75** and **76** [133], confirmed this hypothesis in vivo. Labelled [^{3}H] laurinterol (**75**) was dissolved in ether and the solution was applied to the surface of dried Japanese "nori". The coated seaweed was offered to a single specimen of *A. californica*. After three days the animal was sacrificed and dissected. The metabolites from the digestive glands were fractionated by a series of chromatographic steps, which yielded aplysin (**72**) that had retained significant label, after three crystallizations.

In vitro experiments [132] also supported the ability of *A. californica* to modify chemically a second algal metabolite, pacifenol (**77**), characterized from *Laurencia pacifica* [134]. Treatment of **77** with ethereal hydrogen chloride yielded other molecules co-occurring in *A. californica*, first pacifidiene (**78**) by acid-catalyzed dehydration and then two epimeric dichlorides (**79**) arising by 1,4-addition of hydrogen chloride to **78**. The slight acidity (pH 5–6.1) of the digestive gland explains the easy dehydration, while formation of the 1,4 adducts should require enzymatic control. Aplysin (**72**), pacifidiene (**78**) and the chlorides (**79**) were not detected in the extracts from the algae on which *A. californica* [132] feeds.



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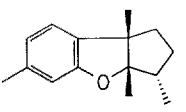

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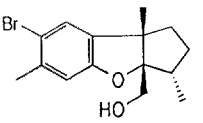

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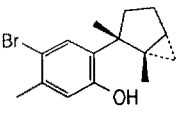

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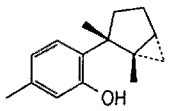

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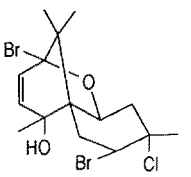

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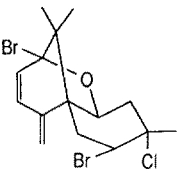

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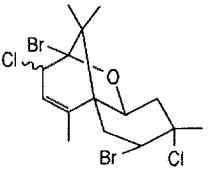

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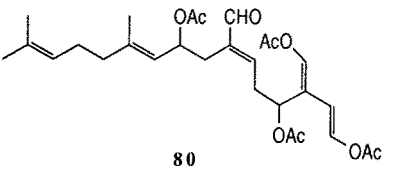
More recently [135], **72** and **73** were also found in the sea hare *Stylocheilus longicauda* but, surprisingly, only dehalogenated **73** was present in the algal food source, *Lyngbya majuscula*. This metabolic comparison suggested an uncommon *in vivo* bromination of **73**, but further experiments need to ascertain whether *L. majuscula* is the unique food source of *S. longicauda*.

The ability to modify dietary compounds has also been well documented for some ascoglossan molluscs. Specifically, the shell-less *Elysia halimedae* (Elysioidea) feeds on the green seaweed *Halimeda macroloba*, which elaborates two main fish antifeedant metabolites, halimedatetraacetate (**80**) and halimedatrial (**81**) [136, 137, 138]. Both molecules were absent in the lipophilic extracts of *Elysia halimedae*, where a single related diterpenoid **82** was present in large amounts (7% of dried mollusc) [139]. The authors suggest that *E. halimedae* is able to selectively sequester from the alga only halimedatetraacetate (**80**) a protected, and presumably, less toxic 1,4-dialdehyde and to reduce the C-7 aldehyde group of **80**, yielding the corresponding alcohol **82**. Feeding deterrent properties of **80** and **82** towards herbivorous fishes were shown by rigorous ecological experiments to be comparable but inferior to those of halimedatrial (**81**), since **80** and **82** contain the protected 1,4-conjugated dialdehyde. Further research needs to ascertain how *E. halimedae* selectively retains **80**, and to show that **81** is not generated during the usual work-up. It is also worthwhile to demonstrate how the reduction of **80** to **82** modifies its biological properties. Probably, the presence of the aldehyde group favors the deprotection of the masked 1,4 dialdehyde group.



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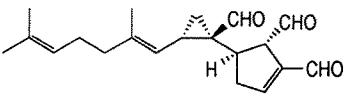

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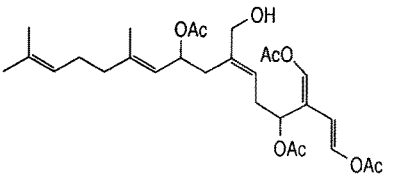

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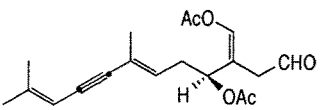

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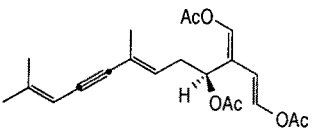

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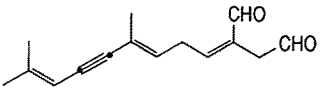

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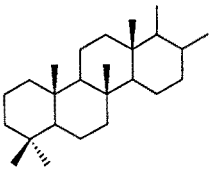
Oxynoe olivacea lives in close association with its algal prey *Caulerpa prolifera*. Extraction of intact frozen animals [140] with deuteriobenzene yielded an extract that, without any chromatographic procedure, was analyzed by ^{1}H-NMR and revealed signals easily assignable to oxytoxin-1 (**83**), and closely related to those of caulerpenyne (**84**), the main metabolite of *C. prolifera* [141]. Therefore, *O. olivacea*, in analogy with *E. halimedae*, is able to modify a dietary metabolite (**84**) by selective hydrolysis of the enol acetate to give **83**. The authors [140] attempting to explain the high toxicity of the mucous secretion of *O. olivacea*, applied the same experimental procedure to fresh mucus and characterized a second metabolite, oxytoxin-2 (**85**), closely related to caulerpenyne (**84**), and most probably responsible for the high toxicity of the mucus toward the mosquito fish *Gambusia affinis* [142, 143]. In conclusion, *O. olivacea*, modifying the algal metabolite caulerpenyne (**84**), first to oxytoxin-1 (**83**), and then to oxytoxin-2 (**85**), has elaborated a very effective defensive strategy that displays an extraordinary analogy with the ability of Dendrodoridídae nudibranchs to biosynthetize de novo olepupuane (**31**), the protected form of the active polygodial (**30**) [87] (vide supra).

Good evidence is at hand which shows that other nudibranchs belonging to the Chromodorididae family can biomodify dietary metabolites. Sesterterpenoids with the scalarane (**86**) skeleton have been isolated from three nudibranchs living in distinct geographical areas, *Chromodoris youngbleuthi* from the Pacific Ocean [144], *Hypselodoris orsini* (= *Glossodoris tricolor*) from the Mediterranean Sea [84] and *Glossodoris pallida* from Guam [145]. All molluscs live in close association with dictyoceratid sponges, *Spongia oceania*, *Cacospongia mollior* and *Hyrtios erecta* (sample A), respectively, all of which contain scalaradial (**87**) as their major metabolite. Surprisingly, scalaradial (**87**) was absent in *C. youngbleuthi* and *H. orsini*; conversely, some related terpenoids were found in large amounts: 12-deacetyl-12-*epi*-scalaradial (**88**) and 12-deacetyl-18-*epi*-12-oxoscalaradial (**89**) in *C. youngbleuthi*, and deoxoscalarin (**90**) in *H. orsini*. The compounds **88** and **89** were completely absent in *S. oceania*, whereas minor amounts of **90** were found in *C. mollior*, thereby supporting a possible selective accumulation of dietary metabolites in *H. orsini*. Further experiments need to rule out unambiguously the accidental presence of some specimens of the very small mollusc in the oscula of the sponge. On the basis of these close analogies observed in the two sets of prey-predator organisms living in very different locations, it is highly likely that, starting from **87**, *C. youngbleuthi* epimerizes the hydroxy function at C-12, whereas *H. orsini* selectively reduces the aldehyde group at C-17. In *G. pallida*, **87** co-occurs with **90** which is completely absent in the extracts of the sponge *H. erecta* A. 24-Methylscalarane derivatives (**91**–**95**) were isolated from *Glossodoris* (= *Chromodoris*) *sedna* [146]. Their structural analogies to known sponge metabolites [147, 148] further support the ability of some Chromodorididae molluscs to modify dietary metabolites. Surprisingly, diet-modified scalarane sesterterpenoids have been found in Chromodorididae molluscs belonging to three different genera, *Chromodoris*, *Hypselodoris* and *Glossodoris*. However, the reported systematics might be in doubt, as the



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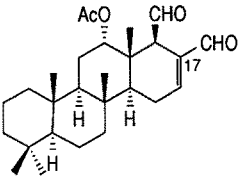

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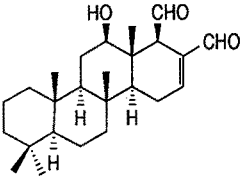

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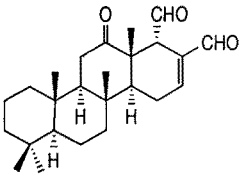

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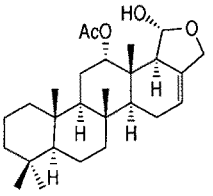

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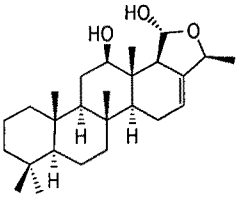

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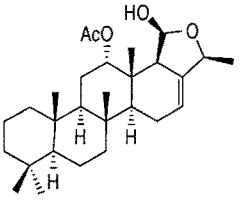

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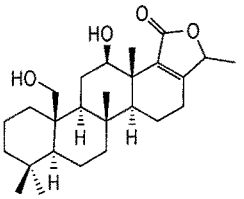

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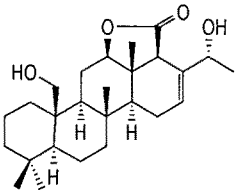

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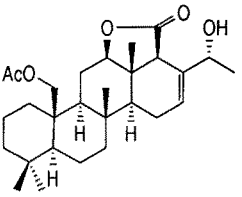

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taxonomic characters in the Chromodorididae family are very complex; only recently, some diagnostic anatomical features have been well described [149].

The ability to selectively reduce vinyl aldehyde groups of sesterterpenoids also seems to exist in the nudibranch *Chromodoris funerea*, collected from a Palauan marine lake [150]; *C. funerea* contains two sesterterpenoids, luffariellin-C (**96**) and -D (**97**) closely related to luffariellin-A (**98**) and -B (**99**), which are potent antiinflammatory agents isolated from the marine sponge *Luffariella variabilis* [151]. Reduction of the carbonyl groups of conjugated aldehydes might be linked to a detoxification process. However, some molluscs, as e.g.,

Oxynoe olivacea [140], modify dietary metabolites toward more toxic compounds. Surprisingly, the same *C. funerea*, but living in shallow Palauan waters, 1.6 km distant from the above lake, displayed [152] a metabolite pattern without sesterterpenoids, but instead characterized by oxidized sesquiterpenoid derivatives of furodysinin (**100**), and furodysin (**101**), metabolites of a *Dysidea* sponge [153]. It was suggested [152] that *C. funerea* modifies furodysinin (**100**) to generate more effective allomones. This hypothesis was experimentally supported by singlet oxygen oxidation of **100**, which yielded a series of photoproducts some of which were also present in the extracts of *C. funerea*. Specifically, hydroperoxide **102** was active in a fish feeding inhibition assay with the "spotted kelpfish" *Gibbonsia elegans* [154] at a concentration at least ten fold smaller (1–5 μg/mg pellet) than that of furodysinin (50 μg/mg). Analogously, oxidation of furodysin (**101**) by singlet oxygen, according to Fig. 5, explained the origin of γ-hydroxybutenolide **103** in *C. funerea*. Even though no experiments with newly collected molluscs were reported, analysis of the extracts of the *Dysidea* sponge, stored for two years in methanol, failed to detect hydroperoxide **102**, γ-hydroxybutenolide **103**, and other oxidation products, whereas furodysinin (**100**) and furodysin (**101**) were present in very large amounts (0.9% and 0.3% of the dry weight of the sponge).

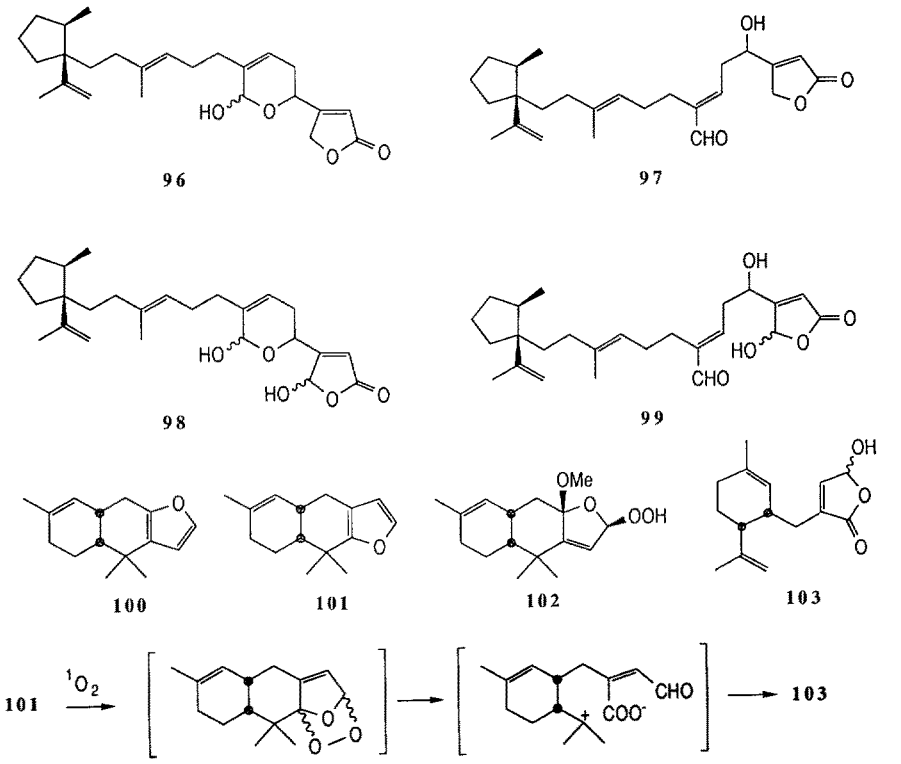
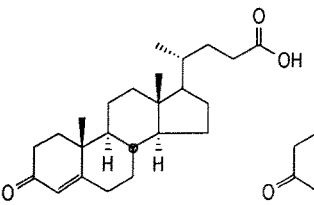


Fig. 5. Oxidation with singlet oxygen of furodysin (**101**) to **103** [148]

Another example of a nudibranch, which probably modifies dietary metabolites to obtain more effective allomones, is seen in *Aldisa cooperi* (= *A. sanguinea cooperi*) [155]. It elaborates two fish antifeedant bile acids (**104, 105**) that are absent in its prey, the sponge *Anthoarcuata graceae*, where the main steroid is 4-cholesten-3-one (**106**). Biosynthetic experiments starting from both labelled mevalonic acid and labelled 4-cholesten-3-one would definitely clarify, whether, the two allomones (**104–105**) are biosynthetized de novo by the mollusc, or if they are derived from a food source.



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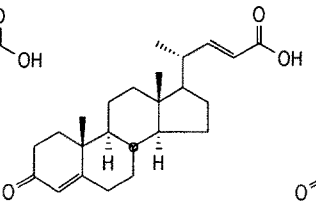

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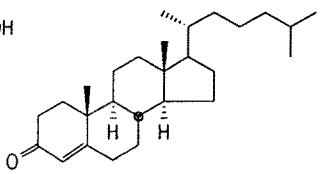

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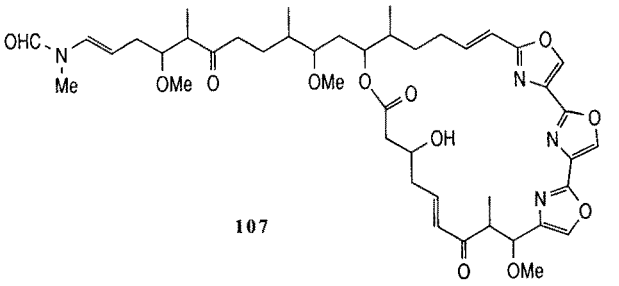
A rigorous comparative study [156] clarified the origin of some unusual macrolides found in the nudibranch *Hexabranchus sanguineus* (Spanish dancer) and in its eggmasses. *Hexabranchus* specimens were maintained for 2 months eating on thawed pieces of *Halichondria* sponge whose major metabolites are halichondramide (**107**), (0.350% of dry sponge) and dihydrohalichondramide (**108**, 0.030%). When the animals were sacrificed, chemical analysis of the



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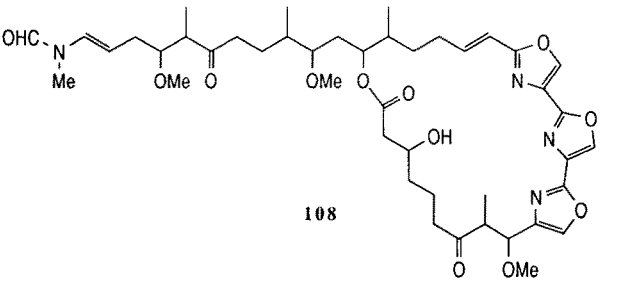

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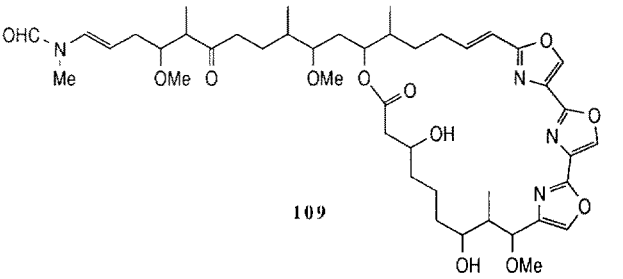

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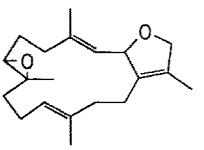
extracts of the molluscs revealed complete absence of **107**, whereas **108** and a third macrolide, tetrahydrohalichondramide (**109**), were present in large amounts. Both macrolides were strong feeding inhibitors of the Indo-Pacific reef fish, *Thalassoma lunare*. Anatomical dissection revealed that the macrolides were mainly concentrated in the dorsal mantle and in the combined digestive gland/gonad of the mollusc. Finally, they were also found in the eggmasses. In conclusion, *H. sanguineus* first sequesters macrolides from dietary sponges, then during digestion modifies them by selective reduction, and finally transfers the strong fish antifeedant metabolites to the mantle and into the eggmasses, presumably to protect both from the attacks of predators.

Lastly, the ovulid egg cowrie *Ovula ovum* offers a significant example of how easy it is to draw misleading conclusions. The prosobranch *Ovula ovum* is known to ingest terpenes from many alcyonocean corals [157]. Two *O. ovum* specimens were found [158] on *Sarcophyton glaucum*, known to contain copious amounts of the toxin sarcophytoxide (**110**) [159]. Surprisingly, the digestive glands and the feces of the molluscs contained large amounts of 7,8-deoxysarcophytoxide (**111**) markedly less toxic than **110**, and reported to be completely absent among the metabolites of *S. glaucum*. Because of this, it was suggested that *O. ovum* has the ability to detoxify the highly toxic sarcophytoxide by transforming it into deoxysarcophytoxide [**111**]. However, a series of rigorous ecological experiments [160] revealed that 1) some colonies of *Sarcophyton*, including the original one, contain moderate amounts of **111**; 2) sarcophytoxide (**110**) passes unaltered through the digestive tract of *O. ovum*; and 3) in vitro incubation of digestive gland homogenates of *O. ovum* under varied conditions failed to convert sarcophytoxide (**110**) to deoxysarcophytoxide (**111**). As *O. ovum* retains dietary metabolites for a long time (at least 6 weeks), this apparent anomaly was rationalized by suggesting that the large amounts of deoxysarcophytoxide (**111**)



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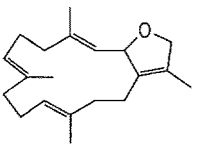


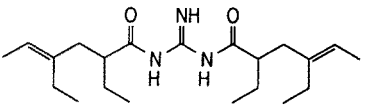
111

found in *O. ovum* might derive from predation on an unidentified *Sarcophyton* colony before migration of the mollusc to *S. glaucum.*

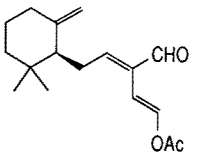
11 Conclusion

The examples reported in this chapter well document the ability of marine molluscs to biosynthetize secondary metabolites; cases that are predominantly linked to primary metabolism (e.g., polyamines from the nudibranch *Phestilla sibogae* [161], dienoic acids from the bivalve mollusc *Scapharca broughtoni* [162], peptides from *Aplysia californica* [163]) have been excluded from this review. This biosynthetic capability seems to be restricted mainly to molluscs belonging to the Gastropoda, in particular to the order Opisthobranchia. However, as negative experiments are rarely reported in the literature, most probably biosynthetic studies have until now been mainly limited to molluscs of ecological interest. Recently, a strategy to identify potential candidates for further biosynthetic experiments has been suggested by Faulkner *et al.* [10]. Comparative analysis of chemical components of geographically diverse collections of molluscs can be useful in selecting biosynthetic experiments, when a particular metabolite seems ubiquitous.

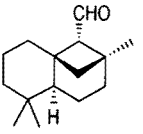
Applying this strategy, the authors [10] suggested investigation of the origin of triophamine (**112**) in the nudibranch *Triopha catelinae* [164], of onchidal (**113**)



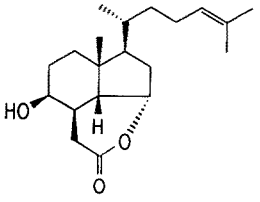
112



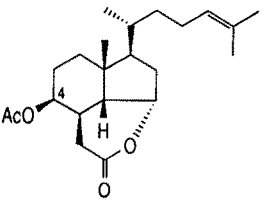
113



114



115



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in the pulmonates of the genus *Onchidella* [165], and of acanthodoral (**114**) in nudibranchs of the genus *Acanthodoris* [166]. An additional candidate might be the degraded sterol skeleton of aplykurodin-B (**115**) and 4-acetylaplykurodin-B (**116**) found in *Aplysia kurodai* [167] and in the Mediterranean *Aplysia fasciata* [168].

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12 References

1. Riedl R (1986) Fauna y Flora del Mar Mediterraneo, Edicione Omega, Barcelona
2. Faulkner DJ, Ghiselin MT (1983) Mar Ecol Prog Ser 13:295
3. Karuso P (1987) Chemical ecology of the nudibranchs. In: Scheuer PJ (ed) Bioorganic Marine Chemistry (vol 1), Springer, Berlin Heidelberg New York, p 31
4. Faulkner DJ (1988) Feeding deterrents in molluscs. In: Fautin DG (ed) Biomedical importance of marine organisms, California Academy of Sciences, San Francisco, p 29
5. Cimino G, Sodano G (1989) Chem Scr 29:389
6. Scheuer PJ (1982) Naturwissenschaften 69:528
7. Faulkner DJ (1991) Nat Prod Rep 8:97
8. Barrow KD (1983) Biosynthesis of marine metabolites. In: Scheuer PJ (ed) Marine natural products (vol V), Academic, New York, p 51
9. Garson MJ (1989) Nat Prod Rep 6:143
10. Faulkner DJ, Molinski TF, Andersen RJ, Dumdei EJ, de Silva ED (1990) Comp Biochem Physiol 97C:233
11. Weinheimer AJ, Spraggins RL (1969) Tetrahedron Lett 59:5185
12. Stanley-Samuelson DV (1987) Biol Bull 173:92
13. Freas W, Grollman S (1981) J Exp Zool 216:225
14. Morse DE, Duncan H, Hoover K, Morse A (1977) Science 196:298
15. Christ EJ, Van Dorp DA (1972) Biochim Biophys Acta 270:537
16. Cimino G, Spinella A, Sodano G (1989) Tetrahedron Lett 30:3589
17. Cimino G, Crispino A, Di Marzo V, Spinella A, Sodano G (1991) J Org Chem 56:2907
18. Di Marzo V, Cimino G, Crispino A, Minardi C, Sodano G, Spinella A (1991) Biochem J 273:593
19. Cimino G, Crispino A, Di Marzo V, Sodano G, Spinella A, Villani G (1991) Experientia 47:56
20. Di Marzo V, Minardi C, Vardaro RR, Mollo E, Cimino G (1992) Comp Biochem Physiol 101B:99
21. Piomelli D (1991) Am J Physiol 260 (Regulatory Integrative Comp Physiol 29): R844
22. Piomelli D, Shapiro E, Feinmark SJ, Schwartz JH (1987) J Neurosci 7:3677
23. Piomelli D, Volterra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH, Belardetti F (1987) Nature (London) 328:38
24. Shapiro E, Piomelli D, Feinmark SJ, Vogel S, Chin G, Schwartz JH (1988) Cold Spring Harbor Symp Quant Biol 53:425
25. Piomelli D, Shapiro E, Schwartz JH, Feinmark SJ (1989) Proc Natl Acad Sci USA 86:1721

26. Belardetti F, Campbell WB, Falck JR, Demontris G, Rosolewski M (1989) Neuron 3:497
27. Buttner N, Siegelbaum S, Volterra A (1989) Nature (London) 342:553
28. Piomelli D, Feinmark SJ, Shapiro E, Schwartz JH (1988) J Biol Chem 263:16591
29. Thompson TE (1976). Biology of opisthobranch molluscs, vol 1. The Ray Society, London
30. Paine RT (1963) The Veliger 6:1
31. Sleeper HL,. Fenical W (1977) J Am Chem Soc 99:2367
32. Sleeper HL, Paul VJ, Fenical W (1980) J Chem Ecol 6:57
33. Fenical W, Sleeper HL, Paul VJ, Stallard MO, Sun HH (1979) Pure Appl Chem 51:1865
34. Sabelli B, Giannuzzi-Savelli R, Bedulli D (1990) Annotated check-list of Mediteranean marine mollusks, Libreria Naturalistica Bolognese, Bologna
35. Fasulo G, Izzillo F, Villani G (1982) Boll Malacologico 18:97
36. Cimino G, Sodano G. Spinella A, Trivellone E (1985) Tetrahedron Lett 26:3389
37. Cimino G, Sodano G, Spinella A (1987) J Org Chem 52:5326
38. Cimino G, Passeggio A, Sodano G, Spinella A, Villani G (1991) Experientia 47:61
39. Coval SJ, Schulte GR, Matsumoto GK, Roll DM, Scheuer PJ (1985) Tetrahedron Lett 26:5359
40. Coval SJ, Scheuer PJ (1985) J Org Chem 50:3024
41. Cimino G, Spinella A, Sodano G (1989) Tetrahedron Lett 30:5003
42. Bu'Lock JD (1979) Biological Compounds. In: Barton DHR, Ollis WD (eds) Comprehensive Organic Chemistry, Pergamon Press, New York vol. 5, p 927
43. Grisebach H, Achenbach H, Hofheinz W (1960) Z Naturforsch 15b:560
44. Grisebach H, Achenbach H, Hofheinz W (1962) Z Naturforsch 17b:64
45. Omura S (ed) (1984) Macrolide antibiotics. Academic, New York
46. Lee JJ, Dewick PM, Gorst-Allman CP, Spreafico F, Kowal C, Chang CJ, McInnes AG, Walter JA, Keller PJ, Floss HG (1987) J Am Chem Soc 109:5426
47. Ref. 7 and previous reports in the same series
48. Gascoigne T (1985) J Molluscan Studies 51:8
49. Ref. 34 p 242
50. Marcus EBR (1982) J Molluscan Studies Suppl. 10:1
51. Trench RK (1969) Nature (London) 222:1071
52. Trench RK, Green RW, Bystrom BG (1969) J Cell Biol 42:404
53. Trench ME, Trench RK, Muscatine L (1970) Comp Biochem Physiol 37B:113
54. Green RW (1970) Malacologia 10(2):369
55. Green RW, Muscatine L (1972) Mar Biol 14:253
56. Ireland C, Scheuer PJ (1979) Science 205:922
57. Ireland C, Faulkner DJ, Solheim B, Clardy J (1978) J Am Chem Soc 100:1002
58. Ireland C, Faulkner DJ, Finer J, Clardy J (1979) Ibid 101:1275
59. Barton DHR, Kende AS (1958) J Chem Soc 688
60. Ireland C, Faulkner DJ (1981) Tetrahedron 37 (Suppl 1): 233
61. Dawe RD, Wright JLC (1986) Tetrahedron Lett 27:2559
62. Ksebati MB, Schmitz FJ (1985) J Org Chem 50:5637
63. Hay ME, Pawlik JR, Duffy JE, Fenical W (1989) Oecologia 81:418
64. Roussis V, Pawlik JR, Hay ME, Fenical W (1990) Experientia 46:327
65. Vardaro RR, Di Marzo V, Crispino A, Cimino G (1991) Tetrahedron 47:5569
66. Di Marzo V, Vardaro RR, De Petrocellis L, Villani G, Minei R, Cimino G (1991) Experientia 47:1221
67. Spinella A, Alavarez LA, Cimino G (1993) Tetrahedron (in press)
68. Ref. 34 p 261
69. Manker DC, Garson MJ, Faulkner DJ (1988) J Chem Soc Chem Commun 16:1061
70. Hochlowski JE, Faulkner DJ, Matsumoto GK, Clardy J (1983) J Am Chem Soc 105:7413
71. Garson MJ, Small CJ, Skelton BW, Thinapong P, White AH (1990) J Chem Soc Perkin Trans I 805
72. Biskupiak JE, Ireland CM (1983) Tetrahedron Lett 24:3055
73. Capon RJ, Faulkner DJ (1984) J Org Chem 49:2506
74. Oppolzer W, Moretti R, Bernardinelli G (1986) Tetrahedron Lett 27:4713
75. Norte M, Cataldo F, Gonzales AG, Rodrigues ML, Ruiz-Perez C (1990) Tetrahedron 46:1669
76. Jansen BJM, de Groot A (1991) Nat Prod Rep 8:309
77. Van Beek TA, de Groot A (1986) Recl Trav Chim 105:513
78. Jansen BJM, de Groot A (1991) Nat Prod Rep 8:319
79. Gustafson K, Andersen RJ (1985) Tetrahedron:1101
80. Hochlowski JE, Walker RP, Ireland C, Faulkner DJ (1982) J Org Chem 47:88

81. Hellou J, Andersen RJ, Thompson JE (1982) Tetrahedron 38:1875
82. Schulte GR, Scheuer PJ (1982) Tetrahedron 38:1857
83. Cimino G, De Rosa S, De Stefano S, Sodano G (1981) Tetrahedron Lett 22:1271
84. Cimino G, De Rosa S, De Stefano S, Sodano G (1982) Comp Biochem Physiol 73B:471
85. Cimino G, De Rosa S, De Stefano S, Morrone R, Sodano G (1985) Tetrahedron 41:1093
86. Cimino G, De Rosa S, De Stefano S, Sodano G (1985) Experientia 41:1335
87. Avila C, Cimino G, Crispino A, Spinella A (1991) Experientia 47:306
88. Okuda RK, Scheuer PJ, Hochlowski JE, Walker RP, Faulkner DJ (1983) J Org Chem 48:1866
89. Barnes CS, Loder JW (1962) Aust J Chem 15:322
90. Cimino G, De Rosa S, De Stefano S, Sodano G, Villani G (1983) Science 219:1237
91. D'Ischia M, Prota G, Sodano G (1982) Tetrahedron Lett 23:3295
92. Cimino G, Spinella A, Sodano G (1984) Tetrahedron Lett 25:4151
93. Caprioli V, Cimino G, Colle R, Gavagnin M, Sodano G, Spinella A (1987) J Nat Prod 50:146
94. Cimino G, Sodano G, Spinella A (1987) Tetrahedron 43:5401
95. Cimino G, Sodano G, Spinella A (1988) J Nat Prod 51:1010
96. Garson MJ, Dexter AF, Lambert LK, Liokos V (1992) J Nat Prod 55:364
97. Paul VJ, Fenical W (1987) Natural Products Chemistry and Chemical Defense in Tropical Marine Algae of the Phylum Chlorophyta. In: Scheuer PJ (ed) Bioorganic Marine Chemistry – Springer-Verlag (vol 1) Berlin Heidelberg New York, p 1
98. Hansson T, Sterner O (1991) Tetrahedron Lett 32:2541
99. Andersen RJ, Sum FW (1980) Tetrahedron Lett 21:797
100. Gustafson K, Andersen RJ, Chen MHM, Clardy J, Hochlowski JE (1984) Tetrahedron Lett 25:11
101. Cimino G, Gavagnin M, Sodano G, Puliti R, Mattia CA, Mazzarella L (1988) Tetrahedron 44:2301
102. Gavagnin M, Spinella A, Cimino G, Sodano (1990) Tetrahedron Lett 31:6093
103. Avila C, Ballesteros M, Cimino G, Crispino A, Gavagnin M, Sodano G (1990) Comp Biochem Physiol 97B:363
104. Cimino G, Crispino A, Spinella A, Sodano G (1988) Tetrahedron Lett 29:3613
105. Goad LJ (1978) The sterols of marine invertebrates. In: Scheuer PJ (ed) Marine natural products, vol II. Academic, New York, p 76
106. Kerr RG, Baker BJ (1991) Nat Prod Rep 8:465
107. Teshima S, Kanazawa A (1973) Comp Biochem Physiol 44B:881
108. Voogt PA, Van Rheenen JWA (1974) Comp Biochem Physiol 47B:131
109. Khalil MW, Idler DR (1976) Comp Biochem Physiol 55B:239
110. Teshima S, Patterson GW (1981) Lipids 16:234
111. Holden MJ, Patterson GW (1991) Lipids 26:81
112. Castiello D, Cimino G, De Rosa S, De Stefano S, Izzo G, Sodano G (1979) Colloq Int C.N.R.S. 291 (Biol Spongiaires):413
113. Mattia CA, Mazzarella L, Puliti R, Sica D, Zollo F (1978) Tetrahedron Lett 3953
114. Teshima S, Kanazawa A, Shimamoto R (1985) Mem Fac Fish Kagoshima Univ 34:53
115. Collignon-Thiennot F, Allais JP, Barbier M (1973) Biochimie 55:579
116. Saliot A, Barbier M (1973) J Exp Mar Biol Ecol 13:207
117. de Longcamp D, Drosdowsky M, Lubet P (1970) C R Acad Sci Paris D 271:1564
118. Bardon O, Lubet P, Drosdowsky MA (1971) Steroidologia 2:366
119. Lupo Di Prisco C, Dessì Fulgheri F, Tomasucci M (1973) Comp Biochem Physiol 45B:303
120. Lupo Di Prisco C, Dessì Fulgheri F (1975) Comp Biochem Physiol 50B:191
121. Cimino G, Crispino A, De Stefano S, Gavagnin M, Sodano G (1986) Experientia 42:1301
122. Pajula RL, Raina A (1979) FEBS Lett 99:343
123. Porcelli M, Cacciapuoti G, Oliva G, Zappia V (1988) J Chromatogr 440:151
124. Porcelli M, Cacciapuoti G, Cimino G, Gavagnin M, Sodano G, Zappia V (1989) Biochem J 263:635
125. Gavagnin M, Sodano G (1989) Nucleosides and Nucleotides 8:1319
126. Porcelli M, Cacciapuoti G, Cimino G, Gavagnin M, Sodano G, Zappia V (1988) Characterization and biogenesis of 5′-methylthioixylofuranosyladenine, a new natural analog of 5′-methylthioadenosine. In: Zappia V, Pegg AE (eds) Progress in polyamine research, Plenum, New York, p 219
127. Yamamura S, Hirata Y (1963) Tetrahedron 19:1485
128. Irie T, Suzuki M, Hayakawa Y (1969) Bull Chem Soc Japan 42:843
129. Irie T, Suzuki M, Kurosawa E, Masamune T (1966) Tetrahedron Lett 1837

130. Irie T, Suzuki M, Masamune T (1970) Tetrahedron 26:3271
131. Suzuki M, Hayakawa, Irie T (1969) Bull Chem Soc Japan 42:3342
132. Stallard MO, Faulkner DJ (1974) Comp Biochem Physiol 49B:37
133. Stallard MO, Faulkner DJ (1974) Comp Biochem Physiol 44B:25
134. Sims J, Fenical W, Wing R, Radlick P (1971) J Am Chem Soc 93:3774
135. Naylor S (1984) Chem Br 118
136. Paul VJ, Fenical W (1983) Science 221:747
137. Paul VJ, Fenical W (1984) Tetrahedron 40:3053
138. Paul VJ, Van Alstyne KL (1987) Coral Reefs 4:269
139. Paul VJ, Van Alstyne KL (1988) J Exp Mar Biol Ecol 119:15
140. Cimino G, Crispino A, Di Marzo V, Gavagnin M, Ros JD (1990) Experientia 46:767
141. Amico V, Oriente G, Piattelli M, Tringali C, Fattorusso E, Magno S, Mayol L (1978) Tetrahedron Lett 3593
142. Coll JC, La Barre S, Sammarco PW, Williams WT, Bakus GT (1982) Mar Ecol Prog Ser 8:271
143. Gunthorpe L, Cameron AM (1987) Mar Biol 94:39
144. Terem B, Scheuer PJ (1986) Tetrahedron 42:4409
145. Rogers SD, Paul VJ (1991) Mar Ecol Prog Ser 77:221
146. Hochlowski JE, Faulkner DJ, Bass LS, Clardy J (1983) J Org Chem 48:1738
147. Kazlauskas R, Murphy PT, Wells RJ, Daly JJ (1980) Aust J Chem 33:1783
148. Kazlauskas R, Murphy PT, Wells RJ (1982) Aust J Chem 35:51
149. Rudman WB (1984) Zool J Lin Soc 81:115
150. Kernan MR, Barrabee EB, Faulkner DJ (1988) Comp Biochem Physiol 89B:275
151. Kernan MR, Faulkner DJ (1987) J Org Chem 52:3081
152. Carté B, Kernan MR, Barrabee EB, Faulkner DJ (1986) J Org Chem 51:3528
153. Kazlauskas R, Murphy PT, Wells RJ, Daly JJ, Schonholzer P (1978) Tetrahedron Lett 4951
154. Pawlik JR, Albizati KF, Faulkner DJ (1986) Mar Ecol Prog Ser 30:25
155. Ayer SW, Andersen RJ (1982) Tetrahedron Lett 23:1039
156. Pawlik JR, Kernan MR, Molinski TF, Harper MK, Faulkner DJ (1988) J Exp Mar Biol Ecol 119:99
157. Bowden BF, Coll JC, Hicks W, Kazlauskas R, Mitchell SJ (1978) Aust J Chem 33:681
158. Coll JC, Tapiolas DM, Bowden BF, Webb L, Marsh H (1983) Mar Biol 74:35
159. Ciereszko LS (1962) Trans NY Acad Sci 24:502
160. Webb L (1988) Comp Biochem Physiol 90C:155
161. Manen CA, Hadfield MG, Russe DH (1977) Dev Biol 57:454
162. Zhukov NV (1986) Biochim Biophys Acta 878:131
163. Kelner KL, Nagle GT, Painter SD, Blankenship JE (1984) Comp Biochem Physiol B 154:435
164. Gustafson K, Andersen RJ (1982) J Org Chem 47:2167
165. Ireland C, Faulkner DJ (1978) Bioorg Chem 7:125
166. Ayer SW, Andersen RJ, He CH, Clardy J (1984) J Org Chem 49:2653
167. Miyamoto T, Higuchi R, Komori T, Fujioka T, Mihashi K (1986) Tetrahedron Lett 27:1153
168. Spinella A, Gavagnin M, Crispino A, Cimino G, Martinez E, Ortea J, Sodano G (1992) J Nat Prod 55:989
169. Kobayashi R, Chavakula O, Murata O, Sharma NS (1992) J Chem Res Synop 11:366
170. Zubia E, Gavagnin M, Crispino A, Martínez E, Ortea J, Cimino G (1993) Experientia 49:268

Oxylipins from Marine Invertebrates

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Marine invertebrates are a surprisingly rich source of oxidized fatty acid-derived substances known as oxylipins. They occur in many phyla of marine invertebrates, including Porifera, Coelenterata, Crustacea, Mollusca, Echinodermata, and Urochordata. Many of the oxylipins obtained from these life forms are structurally unique and have no direct parallel to oxylipins from other sources. Consequently, the biosynthetic pathways responsible for their formation are also unique. Even those oxylipins of comparable structure to those occurring terrestrially are produced by novel biosynthetic pathways in marine organisms. Hence, biosynthetic studies of marine oxylipins are providing fundamental new insights into the scope and nature of oxylipin chemistry and metabolism. Further, study of the ecological and potentially useful pharmacological properties of these unique substances continues to provide new opportunities in medicine and biology.

Symbols and Abbreviations

AA, eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoic acid = arachidonic acid; ATP, adenosine triphosphate; CD, circular dichroism; COSY, correlation spectroscopy; diHETE, dihydroxyeicosatetraenoic acid; DMP, 4,6-dimethylpyrimidyl; EPA, eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-pentaenoic acid; FAB, fast atom bombardment; FMRF, Phe-Met-Arg-Phe; FT-IR, Fourier transform infrared spectroscopy; GC, gas chromatography; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HPLC, high performance liquid chromatography; 5-HT, 5-hydroxytryptamine; Hz, Hertz; IC_{50}, concentration required for 50 percent inhibition; IR, infrared spectroscopy; KETE, ketoeicosatetraenoic acid; LPO, lipoxygenase; LSC, liquid scintillation counting; LTB_4, leukotriene B_4; MC, (−)-menthoxycarbonyl; MHFA, monohydroxy fatty acids; MS, mass spectrometry; NDGA, nordihydroguaiaretic acid; NMDA, *N*-methyl-D-aspartate; NMR, nuclear magnetic resonance spectroscopy; ORD, optical rotatory dispersion; PC, preclavulone; PG, prostaglandin; ppb, parts per billion; R_f, retention factor; RIA, radioimmunoassay; T/C, (treatment/control) × 100; THFA, trihydroxy fatty acid; TLC, thin layer chromatography; TMS, trimethylsilyl; UV, ultraviolet; W, watt.

1 Introduction

It has been a relatively recent development in the study of marine-derived natural products to recognize that creatures inhabiting the world's oceans are rich in eicosanoids and related fatty acid derivatives, collectively known as oxylipins[1] [1]. Because metabolites of this class play a crucial role in both

[1] The term "oxylipin" is redefined here as an encompassing term for oxidized compounds which are formed from fatty acids by reaction(s) involving at least one step of mono- or dioxygenase-dependent oxidation [1]. Thus, this term includes eicosanoids as well as biosynthetically related compounds of longer and shorter chain length.

mammalian physiology and disease, interest in the structural chemistry, biosynthesis, and pharmacological activities of these marine products has been intense. Although this field traces its origins to the pioneering discovery that the Caribbean gorgonian, *Plexaura homomalla*, is exceptionally rich in prostaglandin A_2 esters [2], it is now established that oxylipins are produced in a broad spectrum of life forms that inhabit the sea, including bacteria [3], algae [4, 5], and diverse animals which are the subject of this review. Due to the wealth of novel oxylipin structures encountered in marine organisms, the uniqueness of the biosynthetic pathways which lead to their formation, and the potency of their biological effects [6], much is to be learned from study of this metabolism in the marine environment [7].

This review is an attempt to cover comprehensively the available literature to October 1992 on the chemistry, biosynthesis, ecology, and pharmacology of oxylipins from marine invertebrates. This material is presented phylogenetically in the following order: Porifera, Coelenterata, Crustacea, Mollusca, Echinodermata, and Urochordata. As such, it serves as a companion to a recent review on the oxylipins from marine algae [8]. It has been our intention in some cases to provide the reader with sufficient experimental detail to allow critical evaluation of the original authors' conclusions, particularly as it relates to structural elucidation and stereochemical determination. Discussions of biosynthetic, ecological and pharmacological investigations of marine animal oxylipins have similarly sought to provide enough detail to communicate both the difficulties associated with this type of investigation as well as some of the clever ideas which have been used to overcome these challenges. Further, we hope that throughout this review the reader will critically evaluate the nature of these experiments, and recognize that in many cases we have yet to learn the entire story. It is our hope that this review will stimulate additional interest in this area from scientists with diverse backgrounds by pointing out several areas ripe for further exploration.

It has been necessary to exclude several groups of oxylipin-related topics from consideration in this chapter. Upon compiling the literature, it became apparent that it would be much longer than originally anticipated if we included a meaningful discussion of the methodologies used in structure elucidation, biosynthesis, and ecology. Therefore, we have chosen to exclude several specific topics that we felt were peripheral to the central focus of the chapter, including prostaglandins from fish [9, 10], polyacetylenes from sponges [11], and most of the butenolides and furanoids from corals [12] and sponges [11].

Many reviews have appeared on prostaglandin metabolites from various sources, including several dealing with marine organisms [9, 13, 14]. Furthermore, there have been several surveys of the animal kingdom, including marine species, for prostaglandin metabolites employing a number of methodologies [15–19].

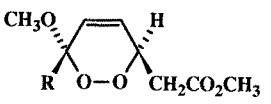
2 Porifera

Marine sponges have long been recognized as a source of novel lipids including unique branched and elongated fatty acids, often found incorporated into phospholipids, triglycerides, and sphingolipids [20–23].

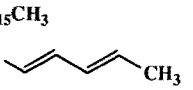
2.1 Cyclic Peroxides

2.1.1 *Chondrilla* sp.

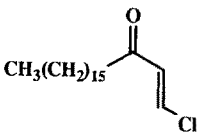
The first evidence of unusual oxidative fatty acid metabolism in the Porifera was provided by an investigation of an Australian sponge of the genus *Chondrilla* [24]. A combination of spectroscopic and degradative techniques, including deoxygenation with triphenylphosphine, was used to determine the structure of chondrillin (**1**). The optical activity of this novel peroxyketal suggested that it was formed by an enzymatic process, presumably acting upon an unsaturated C_{22} fatty acid precursor. CD and ORD studies of the hydrogenated derivative suggested a 3*S* configuration in **1**. An interesting acid-catalyzed cleavage of chondrillin was observed that slowly produced the chloro-enone (**2**).



1) R = $-(CH_2)_{15}CH_3$



4) R = $-(CH_2)_{13}$



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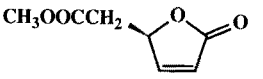

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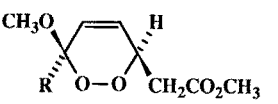
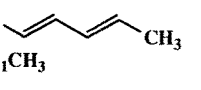
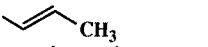


3) R = $-(CH_2)_{13}$

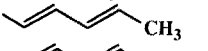


6) R = $-(CH_2)_{11}CH_3$

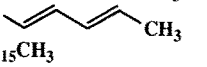
7) R = $-(CH_2)_9$



8) R = $-(CH_2)_7$



9) R = $-(CH_2)_9$



10) R = $-(CH_2)_{15}CH_3$

2.1.2 *Xestospongia* sp.

Subsequently, cytotoxic fractions from a Fijian sponge, *Xestospongia* sp., were demonstrated to contain the related unsaturated-1,2-dioxanes, xestin A (**3**) and xestin B (**4**) [25]. A weakly cytotoxic but-3-enolide (**5**) was also isolated. The relative stereochemistries of the ring substituents were established from a combination of chiroptic analysis of reduction products and ^1H NMR coupling

constants. It was suggested that a biological singlet oxygen Diels–Alder cycloaddition to the methoxy diene of a proposed tetraene ester precursor could result in the formation of both xestins A and B. The butenolide (**5**) was envisioned as the final product formed from either xestin following cleavage of the peroxide and C6-7 bonds and subsequent lactonization. While attempts to synthetically produce xestin and chondrillin analogs by reaction of a methoxy diene with singlet oxygen were unsuccessful, photooxygenation of an acetoxy diene precursor in the presence of rose bengal and oxygen has produced peroxy hemiketals which were easily converted to their corresponding peroxy ketals [26]. The xestins A (**3**) and B (**4**) were found to be highly cytotoxic to P388 cells in vitro (xestin A – $IC_{50} = 0.3\ \mu g\,ml^{-1}$; xestin B – $IC_{50} = 3\ \mu g\,ml^{-1}$) [25]. Xestin A was also shown to inhibit other tumor cells lines including lung (A459), colon (HCT-8), and mammary (MDAMB) cell lines at $5\ \mu g\,ml^{-1}$.

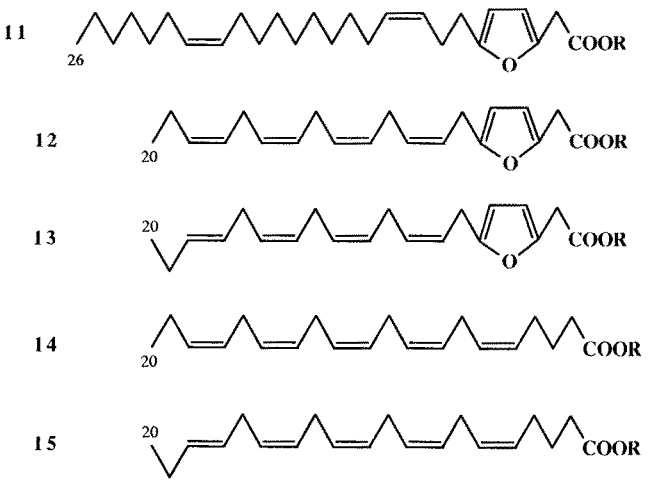
2.1.3 *Plakortis lita*

Four new cyclic peroxides (**6–9**) and the known compound chondrillin (**1**) were isolated from an Okinawan collection of the sponge *Plakortis lita* [27]. Compounds **6–9** appear to derive from shorter chain fatty acid precursors than xestins A (**3**) and B (**4**). It is interesting to note that the $[\alpha]_D$ value reported for chondrillin isolated from *P. lita* is $+40.0°$, while that of chondrillin isolated from *Chondrilla* sp. is $+144°$. Plakorin (**10**), another peroxy ketal isolated from an Okinawan *Plakortis* sp., appears to possess a different stereochemistry than chondrillin (**1**) as well as being derived from a shorter chain [28]. Its structure (**10**) was assigned from ^1H and ^{13}C NMR data. No absolute stereochemical information was available for plakorin. The relationship of the cyclic peroxide epidioxy ring stereochemistry to P388 cytotoxicity was demonstrated by a comparison of several *P. lita* metabolites [27]. Chondrillin (**1**) showed an IC_{50} of $5\ \mu g\,ml^{-1}$, while compounds **6–9** exhibited IC_{50} values ranging from 0.05 to $0.1\ \mu g\,ml^{-1}$. Plakorin (**10**) was weakly active against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro, with IC_{50} values of 0.85 and $1.8\ \mu g\,ml^{-1}$, respectively [28]. Plakorin (**10**) has also been shown to act as a potent activator of sarcoplasmic reticulum Ca^{2+}-ATPase, 30% activation at 10^{-5} M [28]. Other biologically active but-3-enolides and related compounds which have been isolated from various *Plakortis* spp. are not covered in this review [28, 29].

2.2 Furan Fatty Acids

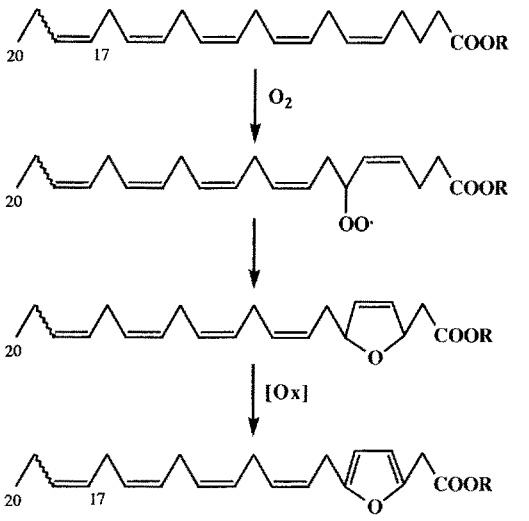
Three pro-inflammatory steryl esters of furan fatty acids (**11–13**) have recently been isolated from the Mediterranean sponge *Dictyonella incisa* [30]. The furan moiety was clearly established by ^1H and ^{13}C NMR signals corresponding to

a 2,5 disubstituted furan and was further supported by an observed C3 to C4 furan $^3J_{HH}$ value of 3 Hz. These furan fatty acid steryl esters (**11**–**13**) were shown to be highly inflammatory using an in vivo rat paw edema assay [30]. The inflammatory response was significantly reduced in rats pre-treated with mepyramine and methysergide, pharmacological antagonists of histamine and 5-hydroxytryptamine (5-HT), respectively. These findings were confirmed by showing a 300% increase in histamine release from rat peritoneal cells following exposure to the furan fatty acids. While little is actually known about the biological role of furan fatty acids previously isolated from various animals and cod liver oil [31, 32], it has been suggested that their inflammatory activity may indicate their ability to serve as feeding deterrents to potential predators of *D. incisa*. EPA (**14**) and an unusual polyunsaturated fatty acid eicosa-5*Z*,8*Z*,11*Z*,14*Z*,17*E*-pentaenoic acid (**15**) were also found in *D. incisa*.



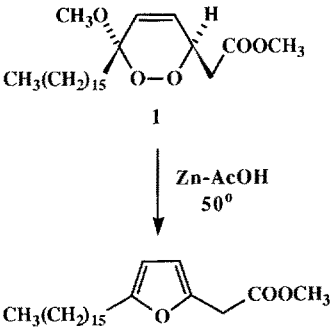
R = cholesta-5,7,22-trien-3β-ol, 24-methylcholesta-5,7,22-trien-3β-ol, or 24-ethylcholesta-5,7,22-trien-3β-ol

As depicted (Scheme 1), the furan fatty acids (e.g., **12**, **13**) have been suggested to arise from a biogenesis initiated by a lipoxygenase-type reaction acting upon **14** and **15**, followed by ring closure, and oxidation to the furan. It is highly unlikely that a lipoxygenase (LPO) is involved, since there is no evidence for the conjugated diene functionality which is typically produced during LPO initiated events. More likely, an alternative type of oxidase is involved. It is possible that a polyunsaturated cyclic peroxide intermediate may undergo biochemical conversion to the furan involving a peroxidase, similar to the chemical process observed with chondrillin (**1**) (Scheme 2). Peroxidase activity has been demonstrated in the sponge *Iotrochota birotulata* from Florida [33, 34].



14) R = H or steryl, Δ17 = Z
15) R = H or steryl, Δ17 = E
12) R = H or steryl, Δ17 = Z
13) R = H or steryl, Δ17 = E

Scheme 1. Proposed biogenesis of furan fatty acids **12** and **13**, modified from Ciminiello and coworkers [30]



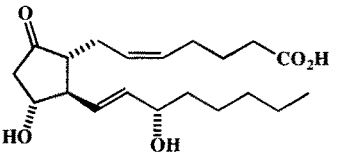
Scheme 2. Chemical conversion of peroxyketal **1** to a furan fatty acid [24]

2.3 Prostaglandins and Hydroxy-Fatty Acids

2.3.1 *Terpios zeteki*

Both acyclic and cyclic metabolites were detected in the sponge *Terpios zeteki* using radioimmunoassay (RIA) [35]. Radiolabelled haptens, specific for arachidonic acid metabolites including prostaglandins, thromboxanes, and hydroxyeicosanoids, were used to establish the extent of oxylipin metabolism in the sponge. Aqueous extracts of *T. zeteki* reacted with anti-PGE_2, anti-$PGF_{2\alpha}$, anti-6-keto-$PGF_{1\alpha}$, and anti-12-HETE. Immunochromatographic analyses of prostaglandins in *T. zeteki* extracts were consistent with authentic PGE_2 (**16**),

$PGF_{2\alpha}$ (**17**) and 6-keto-$PGF_{1\alpha}$ (**18**) standards. The majority of compounds reacting with anti-12-HETE, which is known to react with heterologous mono- and dihydroxyeicosanoids, were more lipophilic than authentic 12-HETE (**19**) or leukotriene B_4 (LTB_4) (**20**). It is conceivable that rather than producing hydroxyeicosanoids as in mammalian systems, sponges may utilize their stores of unusual long-chain and branched fatty acids to synthesize new and as yet undescribed oxylipins.



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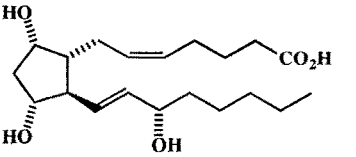

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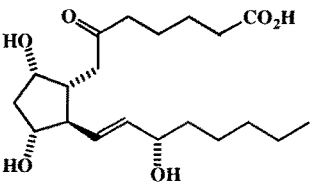

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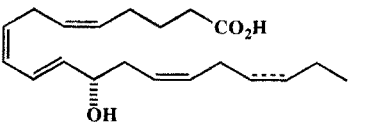
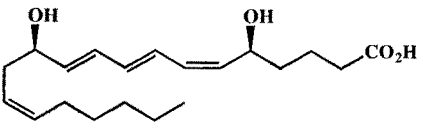


19 ω6
21 ω3



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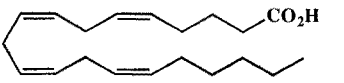

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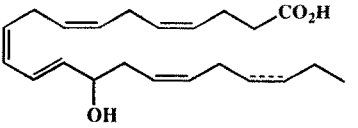
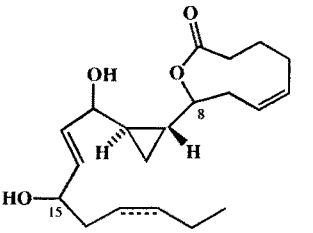
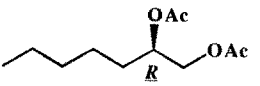


22 ω6
23 ω3



25) ω7; 15*R*
26) ω3; 8*S**,9*R**,11*R**,12*R**,15*R**



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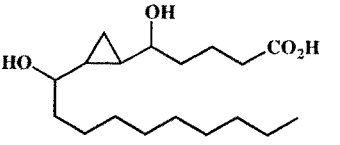

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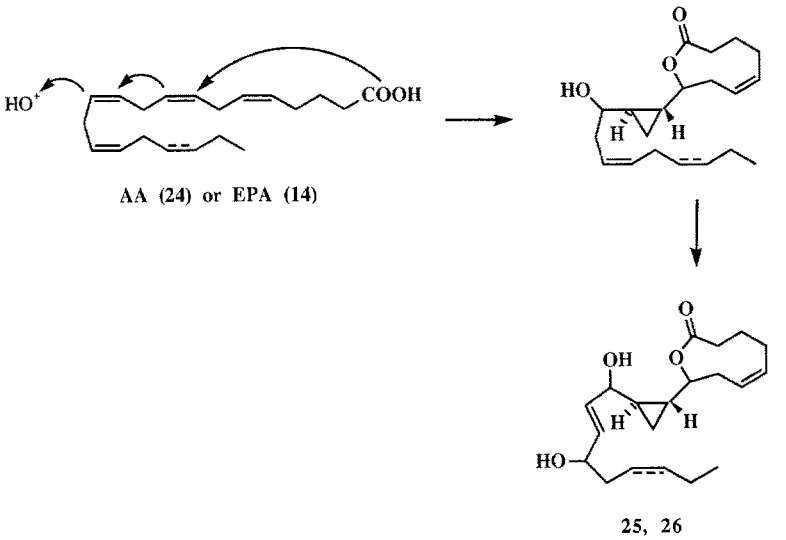
2.3.2 *Echinochalina mollis*

Evidence for the oxidative metabolism of long-chain fatty acids in sponges is provided by the isolation of four optically active oxylipins from *Echinochalina mollis* as their methyl esters [36]. A combination of 1D and 2D NMR techniques, as well as mass spectral fragmentation patterns, were used to deduce these structures (**19**, **21**–**23**). The 12-hydroxy eicosanoids (**19**) and (**21**) are typically produced by the action of a 12-LPO acting upon AA (**24**) and EPA (**14**) respectively. Compounds (**22**) and (**23**) would similarly arise as a result of the same LPO acting upon polyunsaturated C_{22} precursors if directed by $\omega 6$ recognition, in analogy to some soybean lipoxygenases [37].

2.4 Cyclopropyl Lactones

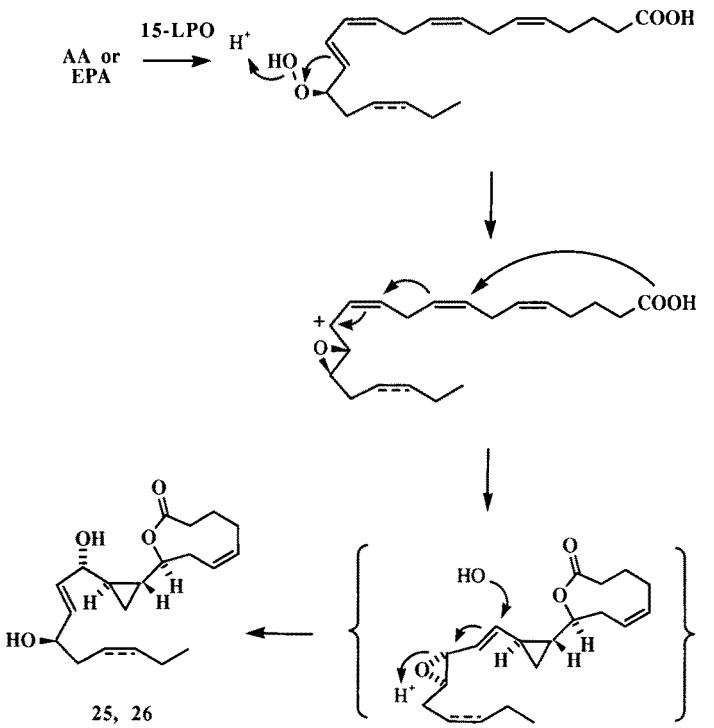
Perhaps the most unusual oxylipins found to date in the Porifera have been isolated from *Halichondria okadai* collected off the coast of Japan [38]. Both spectroscopic and degradative chemical evidence were used to elucidate the structures of two cyclopropyl and lactone containing eicosanoids: halicholactone (**25**) and neohalicholactone (**26**). Following acetylation, halicholactone (**25**) was treated with OsO_4, oxidatively cleaved with $NaIO_4$, reduced with $NaBH_4$, and re-acetylated to produce 1,2*R*-diacetoxyheptane (**27**). This fragment was compared with an authentic standard. Although no stereochemical information is presented in the structural representation of halicholactone, the diacetate (**27**)



Scheme 3. Biogenesis of halicholactones (**25**, **26**) from arachidonic acid and eicosapentaenoic acid, as proposed by Niwa and coworkers [38]

should establish a 15*R* stereochemistry. Subsequently, the relative configuration of neohalicholactone (**26**) was determined as 8*S**, 9*R**, 11*R**, 12*R**, 15*R** by X-ray crytallography [39].

While little biosynthetic information is available, it has been suggested [38] that **25** and **26** may be formed from AA (**24**) and EPA (**14**) via a cyclization mechanism (Scheme 3) similar to that which forms *trans*-cyclopropyl-containing diol **28** upon treatment of linoleic acid with performic acid [40]. An alternative biogenetic mechanism (Scheme 4), based upon that proposed for the structurally related red algal metabolites constanolactone A and B [41], would involve the formation and opening of an allylic epoxide intermediate created as a result of a 15-*R*-LPO acting on either AA or EPA. Related compounds have been isolated from the coral *Plexaura homomalla* and the mollusc *Aplysia kurodai* (see below).



Scheme 4. Alternative biogenesis of halicholactones (**25**, **26**)

Halicholactone (**25**) was reported to exhibit weak inhibitory activity against the 5-lipoxygenase from guinea pig polymorphonuclear leukocytes (IC_{50} = 630 μM) [38]. A subsequent report detailing the three-dimensional structure of the ω3 unsaturated neohalicholactone (**26**) described it as a "potent lipoxygenase inhibitor", however, no additional experimental data were provided [39].

2.5 Function of Sponge Oxylipins

Little is known concerning the biochemical, ecological or physiological significance of oxylipin metabolism in sponges. However, several interesting correlations have emerged. Over 80 years ago Wilson demonstrated that when a living sponge was strained through a gauze cloth, the dissociated sponge cells would move with amoeboid motion and form new sponge colonies [42–44]. When two distinct sponge species were similarly dissociated and mixed they reformed as homogeneous single sponges [45, 46]. Inclusion of rabbit antiserum specific to only one sponge inhibited the aggregation of only that sponge species, similar to an antigen-antibody type reaction [47]. It was reported that the aggregation of dissociated marine sponge cells is a calcium-dependent process resembling the immunological response of human neutrophils and platelets [48, 49]. The 5-LPO product leukotriene B_4 (LTB_4) (**20**), a known calcium ionophore [50] was shown to cause sponge cell aggregation in *Microciona prolifera* [51]. Hence, it has been suggested that an endogenous LTB_4 analog may be involved in sponge cell aggregation phenomena.

3 Coelenterata

3.1 Overview

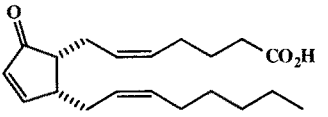
As the first phylum of marine organisms to be identified as containing prostaglandin derivatives [2], the corals are now the best examined group of marine invertebrates for oxylipin chemistry. Following the initial studies with *Plexaura homomalla*, there was a surge of attention given to the distribution of prostanoids in marine life. This was fueled in part by interest of the Upjohn Company in the commercial-scale extraction of prostaglandins from corals [52–54]. In this regard, a major symposium was held in the early 1970s to discuss the biology, ecology and conservation of *P. homomalla* relative to its potential utilization as a commercial source of prostaglandin derivatives [55]. Efforts in the early and mid-1970s utilized various bioassays and enzyme screens in these evaluations of hard and soft coral species from around the world.

Homogenates of several marine and terrestrial animals were examined for their ability to convert exogenous ^{3}H-labeled arachidonic acid to prostaglandins, as evaluated by TLC, UV following treatment with base (i.e. formation of PGBs from PGE or PGA), and gas chromatography [15]. Although this methodology provides a less than rigorous proof of structure, several marine species identified as containing prostaglandins in that study were subsequently re-investigated and these findings essentially confirmed. The homogenate of one coral, *Anthoplexaura* sp., was shown to have a slight capacity to biosynthesize prostaglandins. A survey of 7 Caribbean gorgonians (the number of gorgonians screened has been erroneously reported as 17 in a number of sources) [56], using

a suite of eight bioassays for prostaglandin-type activity, showed that two gorgonians not previously known to produce prostaglandins, *Gorgonia flabellum* and *Muriceopsis flavida*, may contain this class of metabolite [16]. While the former species has never been reinvestigated for oxylipins, a homogenate of the latter was subsequently shown to convert exogenous AA to 8*R*-HPETE (**29**) and preclavulone A (**30**, see below) [57]. A broad screen of the prostaglandin-forming ability of Caribbean and Pacific coral species utilized a prostaglandin biosynthesis assay which was coupled to the formation of adrenochrome from L-epinephrine [18]. While many of the species examined gave a positive result for prostaglandin biosynthesis, it has subsequently been shown that this assay is not specific for prostaglandin H synthase (= cyclooxygenase) [58]. Although this certainly limits the interpretation of these survey results of Morse et al. [18], follow-up of the nature of the oxidase system in at least one case, *Pseudoplexaura porosa*, has yielded some interesting results [58].

3.2 *Leiopathes* sp.

Studies of the natural products of the antipatharian (Hexacorallia), *Leiopathes* sp., obtained during an expedition to the South Indian Ocean, led to the isolation of one novel oxylipin, (+)-10*R*-hydroxydocosa-7*Z*,11*E*,13*Z*,16*Z*,19*Z*-pentaenoic acid (= leiopathic acid, **31**), as well as two known compounds,



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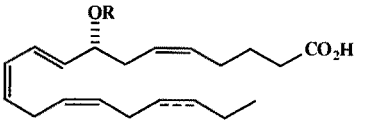
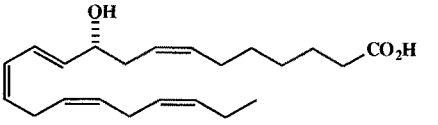


29) R = OH; ω6
32) R = H; ω3
33) R = H; ω6



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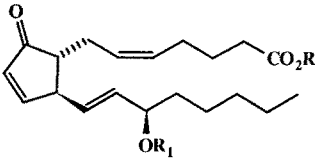
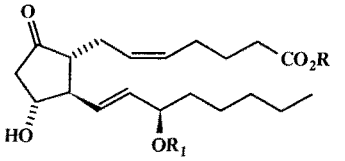


34) R = H, R_1 = H
35) R = H, R_1 = Ac
36) R = CH_3, R_1 = Ac
37) R = CH_3, R_1 = H



38) R = H, R_1 = H
39) R = CH_3, R_1 = H
40) R = CH_3, R_1 = Ac

(+)-(8*R*-hydroxyeicosa-5*Z*,9*E*,11*Z*,14*Z*,17*Z*-pentaenoic acid (8*R*-HEPE, **32**) and (+)-8*R*-hydroxyeicosa-5*Z*,9*E*,11*Z*,14*Z*-tetraenoic acid (8*R*-HETE, **33**) [59]. The planar structure of the new compound was established by 1D and 2D NMR strategies in combination with MS and UV data, while the absolute stereochemistry was determined by degradation to malic acid derivatives. Benzoate and urethane derivatives of this malate fragment were compared with authentic samples by NMR in the presence of a chiral shift reagent and by HPLC. Estimated yields (combination of natural products and ethyl esters, presumed products from storage of the animal in EtOH) indicate the $\omega 3$ unsaturated compounds (**31** = 0.25%, **32** = 0.22%) predominate over the $\omega 6$ unsaturated compound (**33** = 0.06%). Co-isolation of these C_{20} and C_{22} metabolites, all oxidized at the $\omega 13$ position, suggests the operation of a lipoxygenase with substrate specificity determined relative to the ω-terminus. The hydroperoxide analog of compound **33**, 8*R*-hydroperoxyeicosatetraenoic acid (8*R*-HPETE, **29**) has been isolated from biosynthetic experiments with several octocorals (see below) and is likely an important intermediate in the formation of coral "prostanoids". However, the biological function of these metabolites in *Leiopathes* sp. remains unknown. While this represents an interesting first report from the Hexacorallia, the full significance of these findings is uncertain until more representatives of this group are evaluated for oxylipins.

3.3 *Plexaura homomalla*

Although a good review has appeared on the prostaglandin chemistry and biochemistry of *Plexaura homomalla*, complete to 1984 [14], as well as a recent more general review on coral chemistry and its ecological implications [60], it seems most meaningful to describe current findings in the context of earlier studies. Hence, a comprehensive and complete chronology of the discovery of prostaglandins in *P. homomalla* and ensuing biochemical and ecological studies are presented.

3.3.1 Natural Products

The discovery of prostaglandin metabolism in marine corals, and the marine environment in general, was initiated with the discovery of 15*R*-PGA_2 (**34**) and methyl 15*R*-acetoxy-PGA_2 (**36**) in *Plexaura homomalla* forma *homomalla* by Weinheimer and Spraggins [2]. It is important to note that the C15 hydroxyl group in these prostaglandins is epimeric with those found from mammalian systems. Further, the quantities of prostanoids found in this Caribbean octocoral far exceed those found in higher animals, reaching approximately 1.5% of the dry weight of the cortex, or about 20–30% of the hexane extract. Interestingly, in subsequent work the yield of prostaglandin derivatives from *P. homomalla* was reported to vary from a low of about 1% to a high of 3.5% of the wet weight of extracted coral [61]. Reports that prostaglandins can amount to

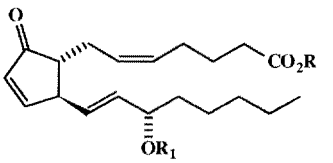
8% of the wet weight of the gorgonian appear to be in error [62] although a yield of 8.5% of the dry weight has been reported [63]. In the initial discovery of prostaglandins from *P. homomalla* [2], considerable effort was expended to chemically degrade these metabolites to structurally-informative fragments; however, the questions of double bond geometry and stereochemistry at C8 and C12 were less firmly established. In later studies [64, 65] detailed examination of these points confirmed the double bond geometry and C8 and C12 stereochemistry postulated earlier [2].

Subsequently, it was shown that if the gorgonian is handled with extreme care (frozen with liquid N_2 at the time of collection and then extracted with organic solvents) only the bis-ester of PGA_2 (**36**) is isolable [64]. If the coral is allowed to stand in water or methanol at room temperatures before extraction, a mixture of methyl 15*R*-acetoxy-PGA_2 (**36**), methyl 15*R*-PGA_2 (**37**), and 15*R*-PGA_2 (**34**) is obtained. These findings help to explain the wide array of acetate (**35**) and methyl esters that have been isolated in the many studies of *Plexaura* chemistry.

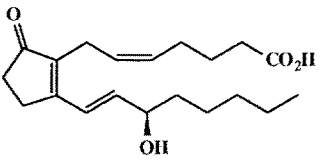
For example, Light and Samuelsson [65] reported the isolation of not only the previously isolated bis-ester of 15*R*-PGA_2 (**36**) and free 15*R*-PGA_2 (**34**), but also the simple methyl ester of 15*R*-PGA_2 (**37**). In addition to prostanoids of the PGA_2 series, an analogous series of free acids and esters of the PGE_2 (**38–40**) series was also isolated. Florida collections of *P. homomalla* used in that work were stored frozen at $-20\,^{\circ}C$ or $-80\,^{\circ}C$, cut into small pieces and then blended in $CHCl_3$/MeOH (2:1). Despite relatively careful handling, partial hydrolysis of these esters likely occurred at some stage of the collection or extraction process.

A more significant point of these two early efforts [64, 65] was the isolation of the 15*S*-isomers of these coral prostaglandins. Both groups reported the isolation of 15*S*- and 15*R*-PGA_2 and 15*S*- and 15*R*-PGE_2, as free acids (**41**, **34**, **16**, **38**) and as methyl esters (**42**, **37**, **43**, **39**). Some individual colonies of *P. homomalla* have been found to produce both 15*R*- and 15*S*-prostanoids while other individuals produce only 15*R*-(Florida) or 15*S*-(Cayman Islands) prostanoids [53]. It was subsequently shown that, while arachidonic acid was a major component of both the 15*S*-PGA_2 and 15*R*-PGA_2 producing forms of *P. homomalla* (33.9% and 22.6% respectively), neither contains substantial quantities of arachidonic acid with *trans* olefins (2–3%) [66]. This suggested that a mechanism other than simple incorporation of 14,15-*trans*-arachidonic acid into a mammalian-type prostaglandin biosynthetic pathway was reponsible for the formation of 15*R*-prostaglandins in the coral.

Additionally, 15*R*-PGB_2 (**44**) and 15*S*-PGB_2 (**45**) were reported from a single dried specimen of *P. homomalla*, although no experimental details were provided [64]. An artifactual source (e.g. rearrangement of PGA_2) for these two C15 stereoisomers is likely. A second report by the Upjohn group described another new prostanoid from *P. homomalla* as a minor component [67]. Its structure (**46**), determined by synthesis of derivatives which were compared with those obtained from PGE_2 and $PGF_{2\alpha}$ (5-*trans*-PGE_2 and 5-*trans*-$PGF_{2\alpha}$), was found



41) R = H, R_1 = H
42) R = CH_3, R_1 = H
54) R = CH_3, R_1 = Ac
119) R = Et, R_1 = H



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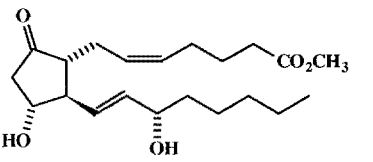

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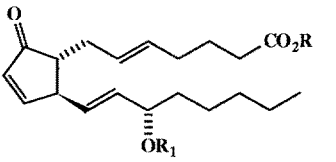
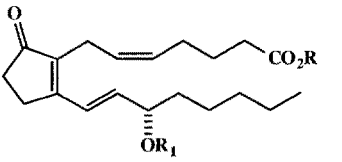


46) R = H, R_1 = H
47) R = CH_3, R_1 = Ac



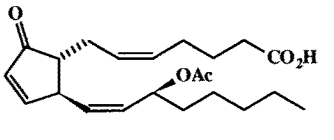
45) R = H, R_1 = H
52) R = CH_3, R_1 = H
53) R = CH_3, R_1 = Ac
120) R = Et, R_1 = H

to differ from mammalian PGA_2 only in the geometry of the C5 double bond. While the original structure work was accomplished with 5-*trans*-15*S*-PGA_2 (**46**), it was subsequently found that the majority of this prostanoid resided in the coral as the 15-acetoxy methyl ester (**47**) [53].

In a detailed study of the prostaglandins from *P. homomalla* [53], the esterase activity associated with coral homogenates was used to aid in the isolation of prostaglandins from other *P. homomalla* lipids. From Cayman Island Collections, 15*S*-PGE_2 (**16**) and 15*S*-$PGF_{2\alpha}$ (**17**) were also isolated as minor constituents. Details are included in this report, including spectroscopic data, for the isolation of 5-*trans*-15*S*-PGA_2 (**46**) from *P. homomalla*, its conversion to 5-*trans*-15*S*-PGE_2 and its comparison with synthetically formed 15*S*-PGE_2.

Additional minor prostaglandin derivatives were subsequently reported from large-scale collections of *P. homomalla* (from a 78 g column chromatography fraction!) [54]. Four new compounds were obtained, in multi-gram quantities, and characterized by a combination of ^{1}H NMR spectroscopy and chemical conversion to known compounds. By chemical conversion of one to PGE_2 and PGF_2 derivatives, and consideration of the lack of any IR stretch at 970 cm^{-1}, it was shown to be 13-*cis*-15*S*-acetoxy-PGA_2 (**48**). 15*S* Stereochemistry was established by ORD analysis of the 2*S*-acetoxyheptanoic acid obtained from ozonolysis of **48**. The second new substance, following hydrogenation, was shown by comparison with authentic synthetic materials, to be a mixture of

epimeric ethers (**49**, **50**), presumably formed by internal Michael addition of the C15 hydroxyl to the enone of PGA_2. The major natural product (**49**), following catalytic hydrogenation and methylation, was identical by NMR and TLC mobility to the synthetic 11α-epimer, while the very minor natural product (**50**) had shifts comparable to the 11β-epimer. Finally, the fourth new product was identified as 13,14-dihydro-PGA_2 acetate (**51**) by spectroscopic evaluation and conversion to the above obtained 11α-cyclic ether (**49**). Stereochemistry was not rigorously established for compounds **49**–**51**.



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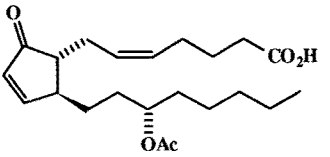

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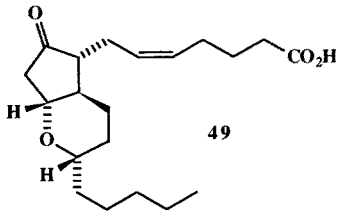

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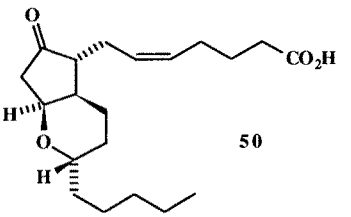

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Consideration of the potential to confuse dried specimens of *P. homomalla* forma *homomalla* and *P. homomalla* forma *kukenthali* led Ciereszko and co-workers to investigate the prostanoids of this latter form [63]. Apparently, these species are easily separated in the field, although they occur in the same habitat side-by-side. Three Caribbean collections of *P. homomalla* forma *kukenthali* were preserved either frozen, in alcohol, or by drying. One sample was investigated in detail and found to possess only 15*S*-PGA_2 (absolute stereochemistry by optical rotation of the corresponding 15*S*-PGB_2), either as the methyl ester (**52**) or 15-acetoxy methyl ester (**53**). Other samples were apparently only investigated by TLC. The suggestion [60] that there may be a taxonomic division (subspecies or species) which distinguishes the 15*R*- and 15*S*-PGA-producing specimens seems premature given the limited scope of the above work. Indeed, this appears not to be the case considering that single *P. homomalla* specimens have been found to contain both 15*R*- and 15*S*-prostanoids [53, 64]. The best preserved samples of *P. homomalla* forma *kukenthali* (fresh-frozen) gave largely the bis-ester (**54**), while those which had been allowed to sit at room temperature or were preserved by drying or in alcohol gave mainly the simple methyl ester (**42**), in agreement with earlier findings [53].

This investigation also separately analyzed the eggs of *P. homomalla* forma *kukenthali* for prostanoids; however, none were detected [63]. Since immature

eggs were harvested in this study (December), it remains possible that mature eggs (July–September) [68] could contain prostaglandin derivatives [60]. Methyl 15-acetoxy-PGA_2 has been reported, without experimental details, as a constituent of the eggs from an unidentified alcyonacean coral. However, production occurs only about one month before egg release [60]. It is interesting to note that feeding on *P. homomalla* by the butterfly fish, *Chaetodon capistratus*, one of the few fish predators of this gorgonian, is greatest during the summer spawning cycle of the gorgonian [68].

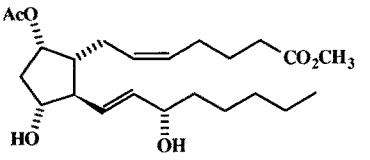
More recently, the prostanoid metabolites of *P. homomalla* have been reinvestigated relative to their potential natural function [69]. A minor new metabolite was isolated and characterized in this work by high field NMR studies. Its structure, the 9-*O*-acetate of methyl $PGF_{2\alpha}$ (**55**), seems secure given the opportunity to compare ^1H and ^{13}C NMR data sets with an earlier isolate of the 11-*O*-acetate of $PGF_{2\alpha}$ (**56**) [70].

P. homomalla prostaglandins have been utilized a number of times as starting materials in the synthesis of other prostaglandins of physiological relevance or theoretical interest [53, 71, 72]. In other studies aimed at discovering constituents that might be useful as starting materials for prostaglandin syntheses, a number of fatty acids and waxes were obtained from several corals (*Turbinaria bifrons, T. peltata, Favia favus, Acropora, Pocillopora damicornis*) [73–76].

3.3.2 Biosynthesis

The collection of papers stemming from investigation of how *P. homomalla* synthesizes prostaglandins from polyunsaturated fatty acids forms one of the more interesting detective stories in the scientific literature. While it was realized nearly 20 years ago that the coral uses a route to construct prostaglandins that is fundamentally different than that employed by mammalian systems (prostaglandin H synthetase = prostaglandin endoperoxide synthetase = cyclooxygenase), it is still uncertain exactly how the coral accomplishes this transformation. There has been a slow evolution in thought on this point, with pieces of the puzzle coming from disparate sources, including other coral species (i.e., *Clavularia viridis*, see below) and various plant systems (most notably, flaxseed) [77]. Several papers reviewing some aspects of this subject have appeared [7, 60, 78].

The first indication that corals employ pathways fundamentally different from those used by mammalian systems to construct prostaglandins came in 1973 with the finding that the two systems responded quite differently to various inhibitors (aspirin, indomethacin) and cofactors [79]. In that work, the microsomal pellet from centrifugation of *P. homomalla* buffer homogenate was said to metabolize exogenous ^3H-labeled arachidonic acid when resuspended; however only, when the buffer contained 1 M NaCl. Prostaglandin A_2 (**41**) was reported as a minor (ca. 10%) product of these incubations based on chromatographic and co-crystallization comparisons with authentic PGA_2 and several derivatives. Two additional and more major products were uncharacterized. Incubations of



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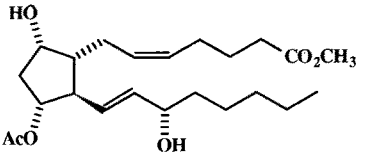

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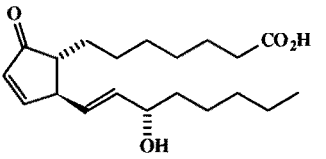

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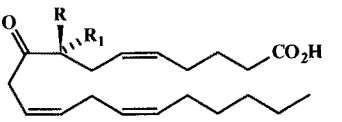
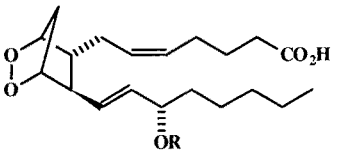
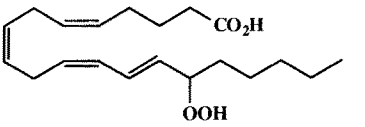


58) R = OH, R_1 = H
63) R = H, R_1 = OH



59) R = H
60) R = OH



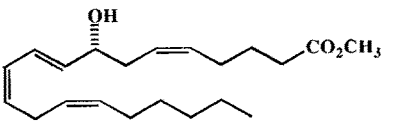
61

8,11,14-eicosatrienoic acid were reported to yield PGA_1 (**57**). As the ability of *P. homomalla* cell-free preparations to convert exogenous arachidonic acid to PGA_2 has not been repeated, it may be that the original analytical methodology did not discriminate between PGA_2 and other products containing hydroxy and ketone functionalities (i.e., α-ketols such as **58**, see below). Further, this report found that exogeneously supplied ^{3}H-labeled PGE_1 was not converted to PGA_1, in contrast to the established pathway in mammals in which PGE is an intermediate. Additionally, it was shown that biosynthetically active preparations of the coral "PGA_2 synthetase" were unable to convert [1-^{14}C]-PGH_2 (**59**) or PGG_2 (**60**, endoperoxide intermediates in the mammalian route) to prostaglandin products [80]. The conclusion of these early efforts was that *P. homomalla* uses a non-cyclooxygenase pathway to form prostanoids in what is a remarkable example of convergent biochemical evolution.

The question of whether the coral or symbiotic algae effect the biosynthesis of prostaglandins in this system was investigated using a non-clonal culture of algal cells isolated from the coral [81]. Arachidonic acid was found to comprise only 0.71% of the fatty acids in these cultured cells. Furthermore, no appreciable levels of prostaglandins could be detected from isolated cells, and a lysed cell preparation was ineffective at metabolizing exogenous eicosatrienoic acid to PGA_1. From these experiments it was concluded that the animal cells must be the site of prostaglandin biosynthesis. Further, it was speculated that if the zooxanthellae are the ultimate source of arachidonic acid for prostaglandin biosynthesis, then they must pass most of it on to the animal cells since little resides in the algae.

Further clues concerning the *P. homomalla* prostaglandin pathway were obtained several years later in work with another coral species, *Pseudoplexaura porosa* [82]. A 30% ammonium sulfate precipitate of the crude *P. porosa* buffer homogenate was resuspended, dialyzed and found to be fairly active (154 ng hydroperoxide $min^{-1} mg^{-1}$; $K_m = 3.8$ μM; $V_{max} = 163$ nmol $min^{-1} mg^{-1}$), although unstable, in converting exogenous arachidonic acid to a hydroperoxide product. TLC of this hydroperoxide versus standard indicated its identity as 15-hydroperoxyeicosatetraenoic acid (15-HPETE, **61**, stereochemistry not determined). This was confirmed by conversion to the corresponding alcohol and GC-MS analysis of the TMS ether, methyl ester in comparison with authentic materials. The lipoxygenase showed a high degree of specificity for arachidonic acid over linoleic, α-linolenic and γ-linolenic acids. However, no production of prostaglandins was detected in these experiments. This was the first report of a lipoxygenase from an invertebrate species.

Lipoxygenase metabolism in *P. porosa* was reinvestigated several years later with very different results [83]. In this case, either acetone powder preparations or buffer homogenates were used without any further purification and found to give 8*R*-hydroperoxyeicosatetraenoic acid (8*R*-HPETE, **29**) as the major product of incubation with arachidonic acid. It was speculated that partial purification of the lipoxygenase in the earlier work may have decomposed this unstable putative 8-lipoxygenase. The structure of the 8-HPETE product was assigned based on various spectral features, including ^{1}H NMR analysis of methyl 8-HETE (**62**), formed by $NaBH_4$ reduction and methylation (the reported $J_{9,10} = 6.41$ Hz must be a typographical error as it appears from included spectra to be ca. 16 Hz). Absolute stereochemistry of the 8-OH group was shown to be *R* by the production of several derivatives, including the menthyl carbonate, followed by ozonolysis and HPLC or GC analysis. Further, arachidonic



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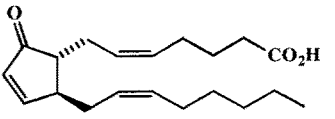

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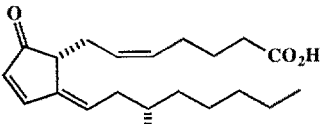

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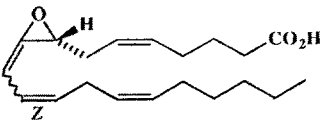

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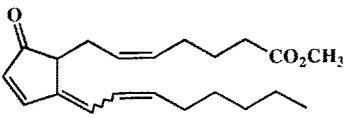
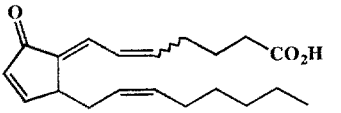


66 12*Z*
67 12*E*

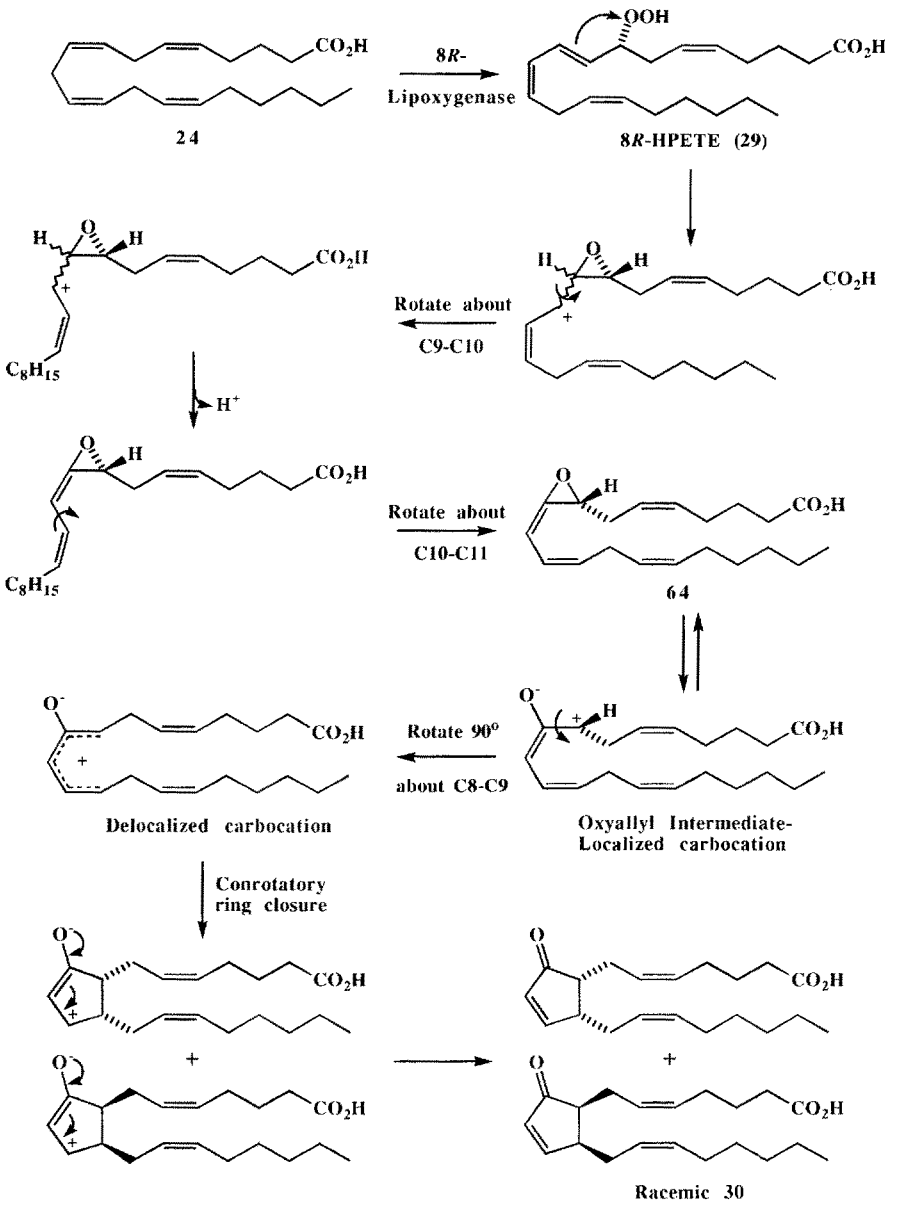


69 5*Z*
70 5*E*

acid was shown to be a major constituent of the total fatty acids in *P. porosa*. However, no endogenous oxylipins were observed in lipid extracts of *P. porosa*.

This pathway was further studied and parallels were observed between the biosynthetic capabilities of *P. porosa* [84], *Clavularia viridis* and *P. homomalla* (see below). An acetone powder preparation of *P. porosa* was moderately active in transforming exogenous arachidonic acid to a cyclopentenone product which had previously been obtained from similar biosynthetic experiments with *C. viridis* [85]. This product, **30**, which had been termed "preclavulone A" in the earlier work, was produced in several milligram quantity from incubations with *P. porosa* and characterized by spectroscopic methods as well as by comparison with authentic standards. Unfortunately, the absolute stereochemistry of **30** was not determined. Separate incubations of 8*R*-HPETE with the *P. porosa* acetone powder were also reported to produce preclavulone A (**30**), although experimental details were not given. It was proposed, based on the biochemical precedent provided by this work with *P. porosa*, that **30** could be an intermediate in the formation of PGA_2 via an oxidopentadienyl cation route in other corals, such as *Plexaura homomalla*.

Shortly after this report, a similar 8*R*-lipoxygenase pathway was described from *Plexaura homomalla* [86]. Again, both whole tissue homogenates and acetone powders were highly active in the metabolism of exogenous arachidonic acid to three principal products. The planar structure of the least polar of these was shown to be 8-HPETE (**29**) by comparison of the reduction product, 8-HETE, with an authentic standard using HPLC and by separate GC-MS analysis. An 8*R* assignment was deduced by reaction of the reduction product, 8-HETE, with soybean lipoxygenase followed by reduction and RP-HPLC analysis versus 8*R*,15*S*-diHETE and 8*S*,15*S*-diHETE standards (96% 8*R*). Two more polar compounds, shown by separate incubations to derive from 8*R*-HPETE, were also isolated and identified by a combination of GC-MS, ^1H NMR, and chemical derivatizations. The most abundant of these was characterized as a 70:30 mixture of 8*S*- (**58**) and 8*R*-hydroxy-9-oxo-eicosa-5*Z*,11*Z*,14*Z*-trienoic acid (**63**, steric analysis by conversion to a hydrogenated *threo*-8,9-diol, derivatized to the bis-(−)-menthoxycarbonyl (MC) derivative and compared with standards by HPLC). By performing the biosynthesis in $H_2{}^{18}O$, the C8 hydroxyl in this α-ketol was shown to come from water, a fact consistent with its derivation from non-enzymatic hydrolysis of an allene oxide. Formation of α-ketols **58** and **63** via hydrolysis of allene oxide intermediate **64** was additionally supported by showing the incorporation of one deuterium atom in **58** and **63** when the incubation was conducted in D_2O. This arises by direct attack at C8 in allene oxide **64** by water followed by proton addition at C10 during the tautomerization of the C9-10 enol. Similar α-ketol products have been shown to be the "signature" of allene oxide formation in work with higher plants [87, 88]. The structure of the most minor product of the incubation was determined to be a racemic mixture of 9-oxo-(8,12-*cis*)-prosta-5*Z*,10,14*Z*-trienoic acid (**30**), the same product recently isolated in biosynthetic experiments with *Clavularia viridis*, and also deriving from 8*R*-HPETE (**29**, see below) [89].



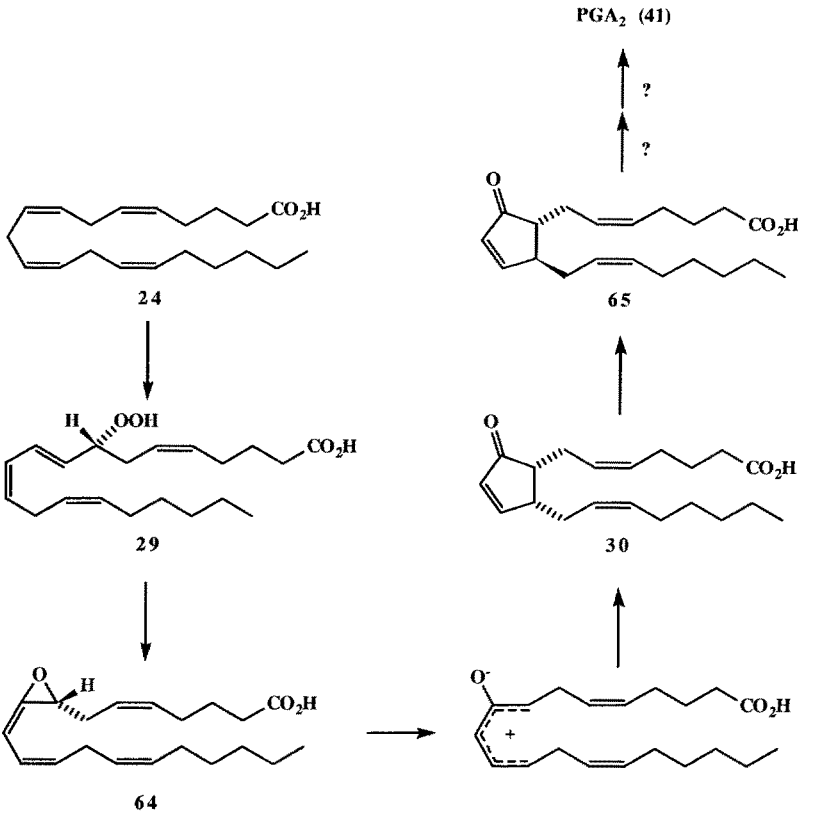
Scheme 5. Proposed biosynthesis of allene oxide **64** from 8*R*-lipoxygenase initiated metabolism of arachidonic acid and subsequent non-enzymatic transformations to racemic cyclopenteone **30** [86]

A reasonable route for the formation of this racemic cyclopentenone (**30**) was proposed from 8*R*-HPETE (**29**), which involves the non-enzymatic hydrolysis of an allene oxide (**64**) intermediate (Scheme 5) [86]. Biochemical precedence for these transformations was provided by earlier work with plants [90] and *C.*

viridis (see below) [89]. However, in contrast with earlier reports [79], no fully formed PGA_2 products were obtained in these experiments with *P. homomalla.* The racemic nature of the cyclopentenone **30** obtained in this work, despite its close structural similarity to PGA_2, raises questions concerning the relevance of these findings to the occurrence of chiral PGA_2 in *P. homomalla.*

Subsequently, 8*R*-HPETE (**29**), 8,12-*cis*-preclavulone A (PC-A, **30**) and 8,12-*trans*-preclavulone A (**65**) were characterized as products of biosynthetic experiments involving six species of Caribbean gorgonians [57]. These studies employed both homogenates of fresh-frozen coral tissue as well as acetone powders. Product identifications utilized ^{1}H NMR, FT-IR, UV, MS and comparison with synthetic standards. *P. homomalla* acetone powder was capable of transforming exogenous AA into 8*R*-HPETE (**29**), while the fresh-frozen tissue homogenate converted exogenous AA or 8*R*-HPETE to a 3:1 mixture of racemic, 8,12-*cis*-preclavulone A (**30**) and racemic 8,12-*trans*-preclavulone A (**65**). Heat-denatured homogenates were unable to transform 8*R*-HPETE into PC-A, a result which was interpreted to signify that formation of PC-A from 8*R*-HPETE was enzyme-mediated. An alternative explanation, taking into account that more than one step is required for the transformation of 8*R*-HPETE into PC-A (1. 8*R*-HPETE to allene oxide **64**, 2. allene oxide to PC-A **30**), is that the first of these steps is enzyme-mediated, while the second is not. This would be consistent with the racemic nature of the PC-A product, as well as with subsequent findings with transformations of purified allene oxide [91] and other biosynthetic investigations with *P. homomalla* [86]. While none of the above biosynthetically produced products were detected in extracts of the coral, two new eicosanoids were obtained and characterized as **66** and **67**, although experimental details were not provided. It is suggested that these could represent intermediates between preclavulone A and PGA_2 (Scheme 6) [57]. Acetone powders or tissue homogenates of two other species of *Plexaura*, *P. nina* and *P. flexuosa*, were also found capable of converting exogenous AA to 8*R*-HPETE and a mixture of 8,12-*cis* and *trans*-preclavulone A. Additionally, a new 12,13-dehydro-PGA_2 eicosanoid (**68**) was reported from *P. nina* based on ^{1}H NMR, IR, and mass spectra. However, the data were not presented and the absolute stereochemistry appears not to have been investigated. From AA incubation experiments involving *P. flexuosa*, another pair of new eicosanoids (**69** and **70**) of the clavulone-type (see below) was reported. Again, neither of these isomers was detected in extracts of *P. flexuosa.* Homogenates of three additional species of gorgonians, *Pseudopterogorgia americana*, *Muriceopsis flavida*, and *Eunicea asperula*, were also found to metabolize exogenous AA to 8*R*-HPETE and racemic mixtures of 8,12-*cis* and *trans*-preclavulone A (**30**, **65**).

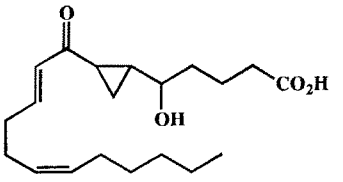
An acetone powder of *P. homomalla* was subsequently used to generate allene oxide **64** from exogenous 8*R*-HPETE [91]. This highly unstable compound ($t_{1/2}$ = ca. 15 s at 0 °C, pH 7.4) was obtained by performing the biosynthesis at low temperature (0 °C) for 2 min in a vortexed emulsion of pH 6 buffer and pentane. Under these conditions, the allene oxide partitioned into the pentane where it was relatively protected from hydrolysis. HPLC analysis of the



Scheme 6. Proposed biosynthesis of preclavulone A (**30**) and prostaglandin A_2 (**41**) from arachidonic acid, modified from Corey and coworkers [57]

pentane extract showed **64** to be the major non-polar product. Allene oxide **64** was characterized by ^{1}H NMR and the 8*R* configuration established by reduction to 8*S*-hydroxyeicosanoate (reversal of priorities at C8) followed by chiral HPLC analysis. It was also shown to convert rapidly to the cyclopentenone **30** and α-ketols **58** and **63** as previously described (see above).

Subsequent investigation of the minor products resulting from incubations of arachidonic acid with the coral acetone powder led to the isolation of a novel cyclopropyl containing product (**71**) [92]. The structure of this minor constituent was fully characterized by ^{1}H NMR (< 150 μg), mass spectroscopy, UV, and examination of several derivatives. The spontaneously forming lactone derivative **72** was useful in defining the structure of **71** by ^{1}H NMR. Relative stereochemistry in **72** was deduced based on ^{1}H NMR data, and a featureless CD spectrum suggested **71** was a racemate. This new product was isolated in very low yield from arachidonic acid (ca. 0.2%), although it was apparently obtained in higher yield from incubations of allene oxide **64** with either the



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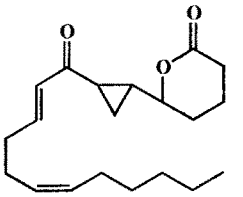

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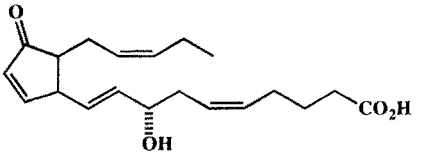
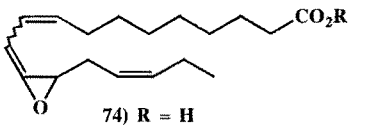
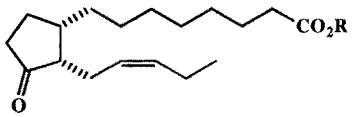


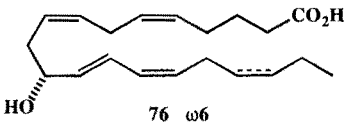
73



74) R = H
111) R = CH_3



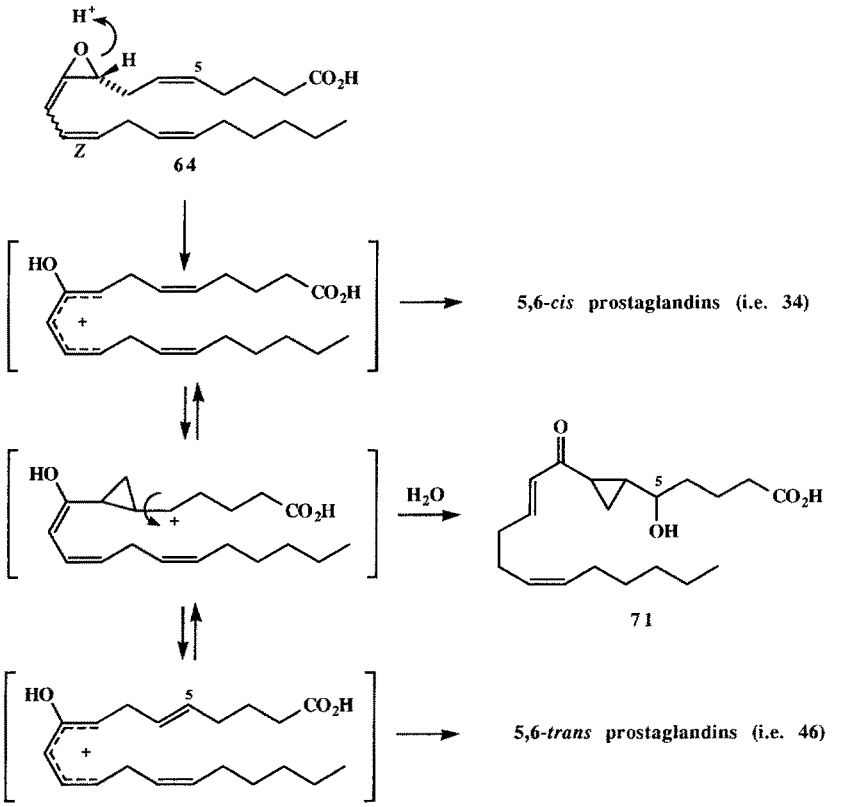
75) R = H
114) R = CH_3



76 ω6
148 ω3

intact or boiled acetone powder (but not buffer alone). Hence, while cyclopropyl metabolite **71** was shown to be a solvolytic product of allene oxide **64**, it was also demonstrated to be only produced in the presence of constituents of the coral. The importance of these findings lies in the implication of a mechanism, by which the coral is able to produce 5,6-*trans* PGA_2 (**46**) which is found as about 10% of the PGA_2 content of the coral (the coral does not contain appreciable amounts of 5-*trans* arachidonic, acid, see above). Specifically, the C8 carbocation formed by solvolytic opening of the allene oxide could rearrange to a C5 carbocation with the cyclopropyl group bridging C6–C8. This intermediate could be trapped to form the racemic cyclopropyl-containing metabolite **71** or revert to the C8–C12 delocalized carbocation. If rotation occurs around the C5–C6 bond before this latter process, then a C5–C6 *trans* olefin would result, which could then be further transformed by additional coral enzymes to 5-*trans* PGA_2 (Scheme 7). Structurally related cyclopropyl oxylipins have recently been reported from a variety of marine animals (sponges, gastropod molluscs; see above and below) and algae [41].

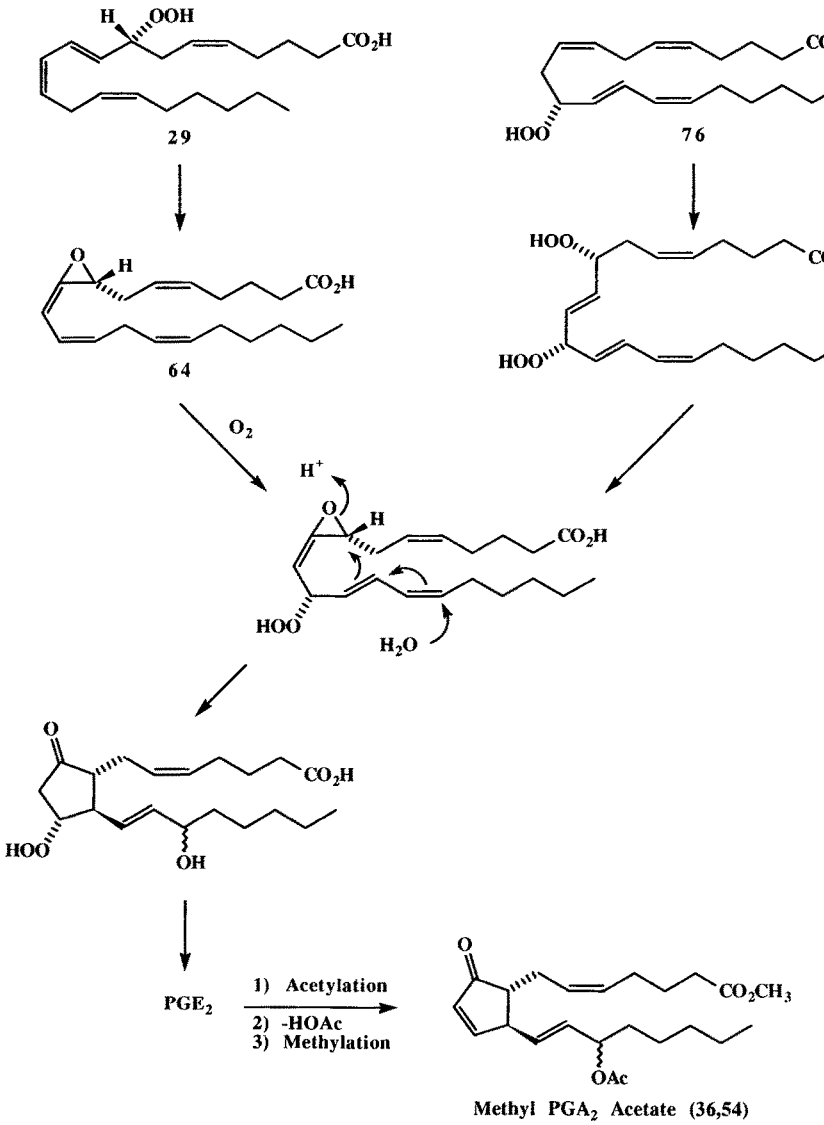
Recently [77] model work with flaxseed, which apparently uses highly analogous reactions to transform eicosapentaenoic acid to C11–C15 cyclopentanoids (i.e. **73**), has proven that allene oxides can be transformed into PGA_2 analogs. It was shown that 8*S*-hydroxy-15*S*-hydroperoxy-eicosapentaenoic acid could be transformed by flaxseed into the corresponding PGA_2 analog (**73**); however, all possible ring juncture stereoisomers were formed in these apparently non-enzymatic reactions. It has previously been shown with pure α-linolenate-derived allene oxide (**74**) that even brief storage in neutral buffer can form



Scheme 7. Proposed biosynthesis of cyclopropyl metabolite **71**, modified from Brash and coworkers [92]

reasonable yields of α-ketols and racemic *cis*-cyclopentanoids such as **75** [93]. Incubation of comparable substrates with the *P. homomalla* enzymes had earlier failed to result in formation of PGA_2 derivatives [86] (however see below) [94], thus indicating that another pathway, or a variant of this pathway, is responsible for PGA_2 formation in the coral.

Additional hypotheses concerning prostaglandin biosynthesis in *P. homomalla* resulted from isolation of 11*R*-HETE (**76**) from the polar lipid fraction [95]. Apparently, 11*R*-HETE (**76**) is also a minor product of incubations of arachidonic acid with acetone powder preparations of *P. homomalla* [95]. In this alternate hypothesis (Scheme 8), an 11-hydroxy or 11-hydroperoxy-8,9-allene oxide intermediate is formed from a sequence of oxidations at C8 and C11. Opening of the allene oxide to a transient C8 carbocation induces cyclization with a consequent addition of water to C15. This proposed pathway leads initially to formation of PGE_2 (**16** or **38**), which following acetylation, elimination of acetic acid from C11–12, and esterification, forms the observed major natural product in the coral, 15-acetoxy methyl PGA_2 (**36** or **54**). Notably, if

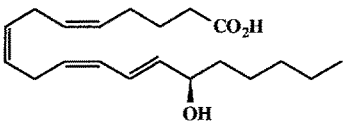


Scheme 8. Proposed biogenesis of prostaglandin A_2 acetate (**35**) via 11*R*-lipoxygenase initiated route [95]

PGA_2 biosynthesis could be accomplished with this acetone powder preparation, this hypothesis could readily be tested, since it predicts that the C15 hydroxyl would derive from water rather than molecular oxygen. However, no further experimental substantiation of this intriguing hypothesis has appeared.

The most recent chapter (but certainly not the last!) in this perplexing saga evaluates a biosynthetic hypothesis raised earlier [7, 86] and suggested from

parallel studies with flaxseed [77]. Specifically, it was thought that if 15-HETE could act as substrate for the *P. homomalla* 8-lipoxygenase and subsequent conversion to allene oxide, then this could provide a mechanism for "directing" the cyclization of the allene oxide to a chiral *trans*-prostaglandin as well as solve the dilemma of oxidation at C15 [94]. Addition of racemic 15- HETE to a dilute buffer homogenate of the coral led to preferential utilization of 15*R*-HETE (**77**) and a corresponding ca. 20 fold greater production of 8*S*,15*R*-diHETE (**78**) over 8*S*,15*S*-diHETE (**79**). Interestingly, this process was found to be enhanced about 5-fold in the presence of 1 M NaCl. A more concentrated coral homogenate metabolized pure 15*R*-HETE (**77**) to a complex mixture of compounds, following triphenylphosphine reduction: α-ketols (**80–82**, two *cis*-PGA_2 diastereomers **83**, **84**), and a new epoxy-diol (**85**). Comparison of the spectrum of products formed from synthetic 8*S*-hydroperoxy-15*S*-hydroxyeicosatetraenoic acid (**86**) with those formed above was ingeniously used to discriminate between enzymatic and non-enzymatic products. A scheme incorporating both biosynthetic transformations and non-enzymatic oxidations was developed to explain the occurrence of this complex suite of products (Scheme 9). It seems likely that the



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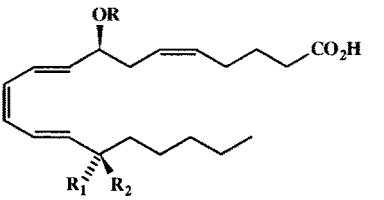
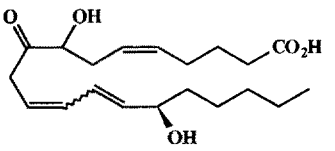
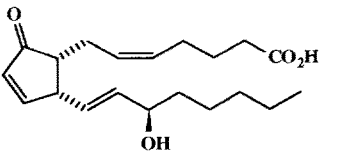


78) R=H, R_1=H, R_2=OH
79) R=H, R_1=OH, R_2=H
86) R=OH, R_1=OH, R_2=H
87) R=OH, R_1=H, R_2=OH



80 11*Z*
81 11*E*



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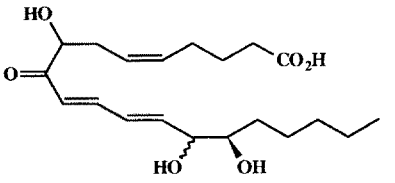

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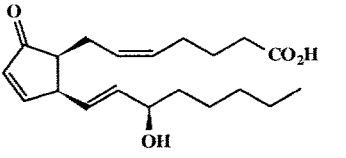

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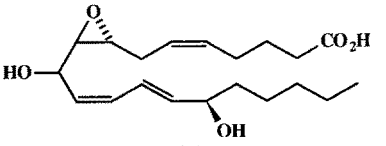

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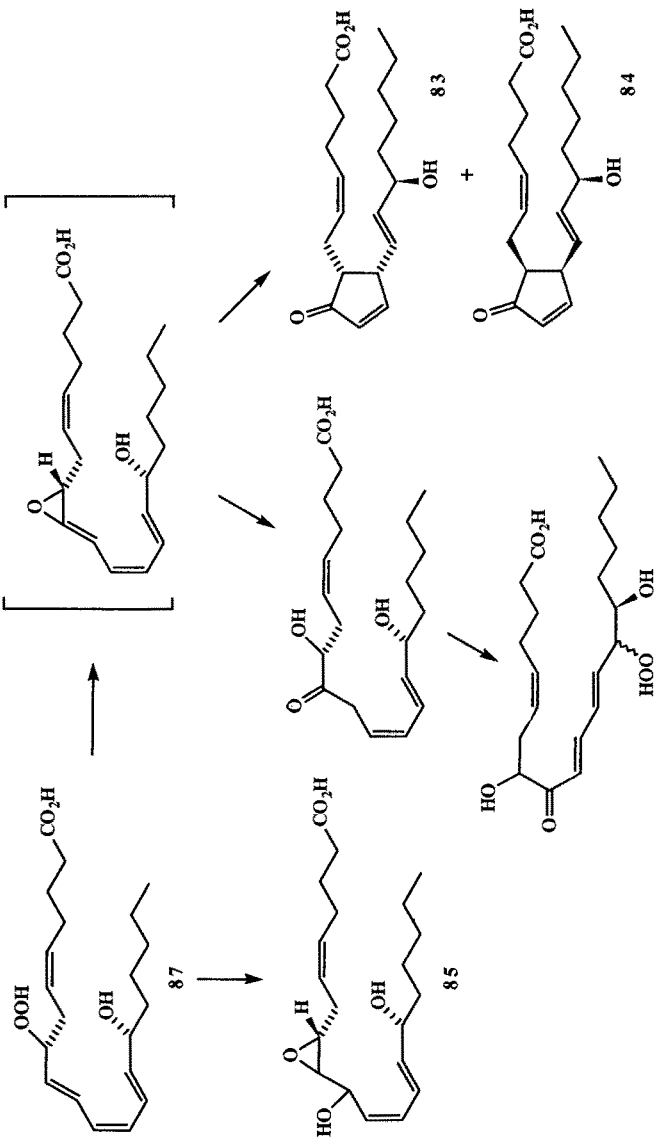


Scheme 9. Biosynthetic and non-biosynthetic transformations of 8*S*-hydroperoxy-15*R*-hydroxyeicosatetraenoic acid (**87**) by *Plexaura homomalla* preparations [94]

8*S*-hydroperoxy-15*R*-hydroxyeicosatetraenoic acid (**87**) product of the coral 8-lipoxygenase is indeed a substrate for the coral allene oxide synthase [96]. The allene oxide so formed then undergoes non-enzymatic hydrolysis to a mixture of α-ketols and PGA_2-isomers. It was suggested that the lack of stereocontrol in the formation of these PGA_2 isomers, and their low yield, signifies that this is not the natural route of prostaglandin biosynthesis in *P. homomalla*. Epoxy-diol **85** likely derives directly from the intermediate 8*S*-hydroxyperoxy-15*R*-HETE (**87**).

Future progress towards understanding prostaglandin biosynthesis in *P. homomalla* will likely require improvements in the biosynthetic system itself. It is largely the inability of acetone powders or cell-free homogenates to convert exogenous substrates to chiral prostaglandins that has made understanding of the mechanism of prostaglandin biosynthesis in this coral such an intractable problem.

3.3.3 Chemical Ecology

The role of prostaglandin production in the ecology of *P. homomalla* has been examined in several systems [62, 97–99] and reviewed to different degrees [60, 100, 101]. Initially, only an anti-herbivory role for 15*R*- (**34**) and 15*S*-PGA_2 (**41**) was considered [97]. A crude aqueous extract of the gorgonian tissue was toxic to goldfish. Pure 15*R*- and 15*S*-PGA_2 were obtained from Cayman Islands and Florida Keys populations of *P. homomalla*, respectively, and evaluated for fish antifeedant properties when incorporated into agar gels or pieces of cooked fish. It should be noted that this study utilized the unesterified prostaglandins rather than the acetate-methyl ester which had previously been shown to be the stored forms (and see below) [53]. Two types of assay fish were used: killifish (*Fundulus heteroclitus*) in laboratory-based experiments and yellowhead wrasses (*Halichoeres garnoti*) for in situ experiments. In both systems it was observed that fish would initially feed on the pellets, but then would become ill and vomit. After several attempts to feed on these prostaglandin-containing pellets, fish developed an aversion to the prostaglandins and refused further adulterated pellets. The levels of PGA_2 incorporated into these pellets were comparable with levels in the corals themselves. As fish were shown to feed freely on control food pellets lacking prostaglandins at the end of the experiment, it was demonstrated that the antifeedant activity was not an artifact caused by hunger in the fish. Oral administration of prostaglandins is well known to produce nausea, vomitting and diarrhea in mammalian systems. The in situ experiments are particularly convincing of the antifeedant role of these prostaglandins, and it is insightful that both PGA_2 C15 epimers elicited this response.

It was subsequently shown that not all fish are deterred from feeding on *P. homomalla*. The butterflyfish, *Chaetodon capistratus*, avidly consumes the extended polyps of large colonies of *P. homomalla* during its summer spawning cycle (during or shortly after full moon) [68]. The nature of any adaptations or

signalling clues which allow this predator to preferentially feed on *P. homomalla* during these periods is unknown.

A second study by Gerhart examined the possibility that the coral prostaglandins could also inhibit the colonization and growth of fouling organisms [62]. Using the air dried axial skeleton as substrate, prostaglandins of both C15 stereochemistries were administered from submerged reservoirs so as to bathe portions of the skeleton. After 3 weeks, the axial skeletons were collected and oven-dried and the epibiota removed and weighed. Using this methodology, no significant difference was observed in the levels of epibiota in sections adjacent to the pump outlets versus those at a distance from the outlets. Additionally, analysis of water from nearby coral colonies failed to show detectable levels (25 ppb) of prostaglandins. Hence, it appears unlikely that the coral prostaglandins possess allelopathic or antifoulant properties in addition to the above described antifeedant properties.

Concern that the above work had utilized unesterified forms of PGA_2 (**34**, **41**), and the finding that the diethyl ether extract of a *Plexaura* sp. was only partially unpalatable to a carnivorous wrasse [102], prompted an additional evaluation of the ichthyodeterrent roles of prostaglandins in *P. homomalla* [103]. The antifeedant activity of several of the possible mono- (**35**, **37**) and bis-esters of PGA_2 (**36**), as well as unesterified 15*R*-PGA_2 (**34**, stereochemistry determined by ^1H NMR), was evaluated in the field by their incorporation into carrageenan strips attached to ropes. Unesterified PGA_2 and both monoesters were nearly equally effective as antifeedants at the $P < 0.0005$ level. However, based on a poor recovery of test strips, only a $P > 0.25$ confidence level could be assigned to experiments with the fully esterified form which showed no antifeedant activity. Based on these results, and the relatively slow rate of hydrolysis of these esters by coral homogenates (however, it is nearly quantitative after 24 h) [53], it was concluded that it is unlikely that fully esterified PGA_2 could effectively function as an ichthyodeterrent. In a counter-argument to the above concerns, it is uncertain what relationship observations on the esterase activity in homogenates have to the responses of the living, albeit presumably damaged, tissues in nature. Furthermore, the nature of the antifeedant assay used by Pawlik and Fenical [103] differed enough from the learned aversion assay of Gerhart [97] that it remains unknown if fully esterified PGA_2 possesses emetic properties in these fish.

A repetition of in situ experiments with the yellowhead wrasse, partially in response to the above criticisms, again showed that fish developed a learned aversion to otherwise palatable food that had been adulterated with 15*R*-PGA_2 [99]. This was presumably due to the emetic properties of PGA_2. It was pointed out that methyl esters of prostaglandins are also known to possess emetic properties [104], and reasoned that if the 15-acetoxy ester inhibits the emetic properties of PGA_2, then it is likely to be quickly lost because of its known lability, especially in the presence of coral esterase. Further, the acidic environment in the stomachs of predatory fish would promote hydrolysis of these labile esters.

Based on the above investigations, it appears likely that prostaglandins in *P. homomalla* function to inhibit feeding by predatory fish. However, determination of the mechanism of their release and the degree of esterification in the ecologically active substance await further investigation.

Another dimension of the chemical ecology of *P. homomalla*, examined principally by Gerhart [98, 105], concerns the gastropod *Cymphoma gibbosum* (flamingo tongue snail) which aggregates and feeds on gorgonian corals, including *P. homomalla*. In these studies, it was first shown that the gregarious clumping of *C. gibbosum* on particular colonies of *P. homomalla* probably reflects a behavior pattern of the gastropod rather than a greater palatability of individual colonies to predation [105]. Further, it appears that slime trails of the gastropod facilitate this clumping behavior. Feeding occurs principally on the lower portions of the colonies, and the newly exposed axial skeleton is rapidly colonized by fouling organisms, including algae [98]. While it is unknown what role, if any, the prostaglandins play in this predator-prey relationship, it has been shown that the gastropod does contain prostaglandins, principally as 15*S*-PGB_2 (**45**), although some 15*S*-PGA_2 (**41**) was also detected [106]. As PGB_2 is generally less toxic than PGA_2, this finding has been suggested to represent a detoxification of the coral defensive chemistry by *C. gibbosum*. Additional interpretations of these experiments have been provided by Coll [60].

A survey of the ability of various marine invertebrates and their isolated tissues to accumulate prostaglandins from surrounding fluids has led to the speculation that some marine invertebrates may rely on this mechanism to obtain their physiological requirement of prostaglandins [107]. It is further speculated that *P. homomalla*, with its wealth of prostaglandins, may be the ultimate provider of these substances in the Caribbean through release and subsequent uptake by dependent species.

3.4 *Euplexaura erecta*

A guinea pig ileum bioassay was used to detect and aid in the isolation of a smooth-muscle-contracting substance from the Japanese gorgonian coral *Euplexaura erecta* [108]. This process led to the isolation of prostaglandin $F_{2\alpha}$ (**17**) from the MeOH extract of the coral. Its identification was based on comparison with authentic $PGF_{2\alpha}$ and its methyl ester by TLC, and comparison with authentic methyl $PGF_{2\alpha}$ trimethylsilyl ether by mass spectrometry. Because of the nature of the techniques employed, some aspects of the stereochemistry in this isolate of $PGF_{2\alpha}$ remain uncertain.

3.5 *Gersemia rubiformis*, *Bebryce indica* and *Mopcella aurantia*

The soft coral *Gersemia rubiformis* was analyzed for its seasonal variation in fatty acids [109]. In April, C_{18} acids predominated (18:1, 18:2ω6, 18:3ω3,

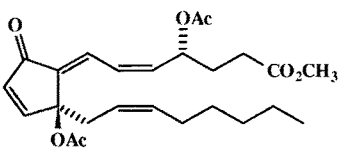
18:4ω3), while in November and July there were higher levels of C_{20} acids (20:4ω6, 20:5ω3). Apparently, these changes in fatty acid composition correlate to gonad maturity in the coral. Prostaglandin A_2 was also apparently characterized from *G. rubiformis*. In a similar study of the fatty acids of three corals from the coast of Vietnam, two, *Bebryce indica* and *Mopcella aurantia*, were found to contain high levels of arachidonic acid (29.1% and 40.9%) and eicosapentaenoic acid (4.1% and 7.6%) [110].

3.6 *Clavularia viridis*

3.6.1 Natural Products

Nearly coinciding communications in 1982 [111, 112] reported on the occurrence of several novel prostaglandins in a tropical Pacific soft coral, *Clavularia viridis*. The first of these [111] described three prostanoids which were termed clavulones I (**88**), II (**89**) and III (**90**). Their planar structures were deduced on the basis of spectroscopic features and some limited degradations. These new compounds were isolated as naturally occurring bis-acetate methyl esters and were double bond stereoisomers of one another in the C5–C8 diene portion. Curiously, while these structures possess oxidations at C4, C9 and C12, they do not possess oxygen at C15, indicating again that they must be synthesized by routes different than those in operation in mammalian systems. The clavulones were reported to possess antiinflammatory activity at 30 $\mu g\,ml^{-1}$ in a fertilized chicken egg assay.

The report by Kitagawa and coworkers [112] examined *C. viridis* and found the same three compounds (**88–90**) plus the expected fourth C5–C8 diene geometrical isomer (**91**). In this and a subsequent report by this group, the new PG-analogs were called the "claviridenones A-D", a name which has not been generally accepted. Their structures were determined principally by NMR



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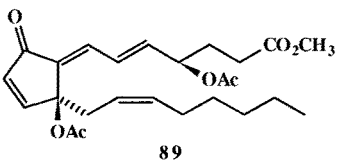

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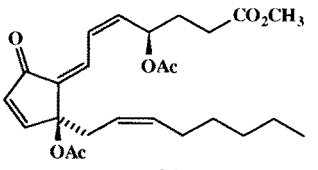

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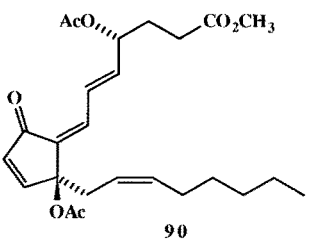


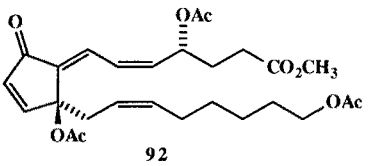
90

investigation of the natural products and derivatives. It was further shown that irradiation of any of the isolated products, **88–91**, with a fluorescent lamp (15 W × 2, 40 h, benzene), gave the full complement of four geometrical isomers in roughly the same proportions as originally isolated.

The following year both groups reported on the absolute stereochemistries of these *C. viridis* prostaglandins. The Kitagawa group produced a series of chromophore-containing esters at C4 and C12 (as well as C9) in claviridenone C (**89**) and D (**88**) which was then evaluated by circular dichroism [113]. This defined the stereochemistry at C4 as '*R*' and C12 as '*S*' (unfortunately, while the CD analysis and structural formula appear correct, the incorrect stereodescriptor '*R*' was used in describing the stereochemistry at C12). Based on earlier interconversions of the claviridenones by irradiation, the absolute stereochemistry in A (**91**) and B (**90**) was also inferred. The Yamada group determined the stereochemistry at C4 in clavulone I (**88**) by ozonolysis and formation of a δ-lactone derivative which was then compared by optical rotation with the same lactone derivative formed by chiral synthesis, thus defining a 4*R* stereochemistry [114]. The stereochemistry at C12 in clavulone I (**88**) was determined by reduction of the C9 ketone to both the α- and β-alcohols, determination of the relative stereochemistry at C7 and C9 in these two derivatives by ^{1}H NMR chemical shifts, and then determination of the C9 absolute stereochemistry by formation of both C9-monobenzoates and CD analysis. Parallel to the results obtained above, 12*S* stereochemistry was deduced and correctly pictured; however, once again, the incorrect stereodescriptor '12*R*' was applied [115]. Absolute stereochemistries in clavulones II (**89**) and III (**90**) were inferred by conversion of clavulone I (**88**) into clavulone II (**89**) and III (**90**) by prolonged treatment with oxalic acid in methanol (presumably, clavulones II and III formed via this route were compared by optical rotation with the originally isolated compounds). Subsequently, the complete structures of clavulones II (**89**) and III (**90**) were conclusively proven by their stereospecific synthesis beginning with the Corey lactone and D-glutamic acid [116]. Additional total syntheses of the clavulones have been accomplished in both racemic [117] and chiral forms [118, 119].

The 20-acetoxy derivatives of clavulones I, II and III (**92–94**) were reported shortly afterward with their structures being determined by spectroscopic methods [120]. A 4*R* and 12*S* (again, the incorrect 12*R* stereodescriptor was applied) stereochemistry in these three new compounds was deduced by the close comparability of their CD spectra with those obtained for clavulones I, II, and III (**88–90**).

Patterned after findings with another group of marine-derived oxylipins, the punaglandins (**95–98**, see below), which contain chlorine at C10 and enhanced cytotoxic activity, a halogenated analog (**99**) of the clavulones and punaglandins was stereospecifically synthesized [121]. Chlorination of a partially formed clavulone/punaglandin analogue utilized Cl_2. As predicted, analog **99** also displayed enhanced cytotoxic properties to B16 melanoma cells (IC_{50} = 0.03 $\mu g\,ml^{-1}$) relative to non-halogenated clavulones.



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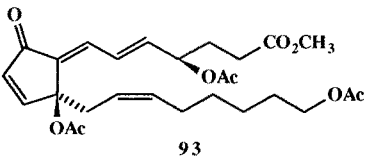

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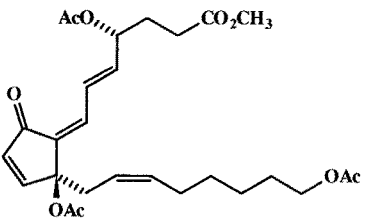

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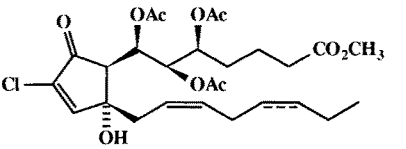
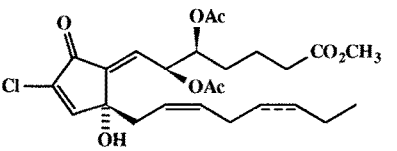
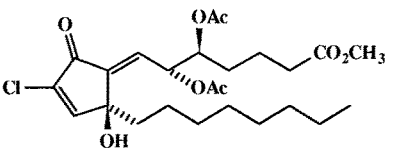


95 ω6
96 ω3



97 ω6
98 ω3



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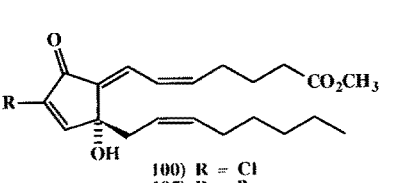
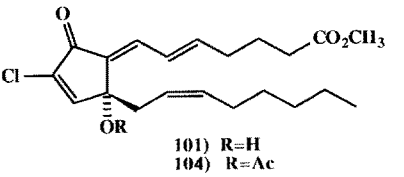
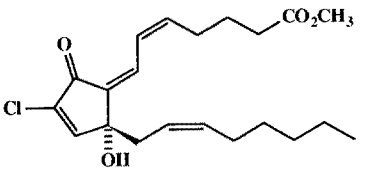
Interestingly, the above synthetic work was reported only shortly before the isolation of the first naturally occurring halogenated oxylipin from *C. viridis* by the same group [122]. These were termed the chlorovulones and represent chlorine-containing analogs of clavulone I–III (**88–90**) and claviridenone A (**91**), though they also lack the C4 acetoxyl function. The structures of the four compounds (**100–103**) were based on spectral features and close comparability to data obtained for the clavulones. The chlorine atom present in each, as shown by mass spectrometry, was located at C10 based on the absence of this proton signal and a change in chemical shift for nearby proton and carbon atoms when compared with the clavulones. Only the planar structures were established in this initial report. Double bond geometries were determined by ^1H–^1H coupling constants and chemical shifts. Interrelation of these various double bond isomers was affected by photoisomerization of chlorovulone I (**100**) into a mixture of the four compounds (**100–103**). One of the new compounds, chlorovulone I (**100**) was evaluated for cytotoxic properties and shown to possess



100) R = Cl
105) R = Br
106) R = I



101) R=H
104) R=Ac



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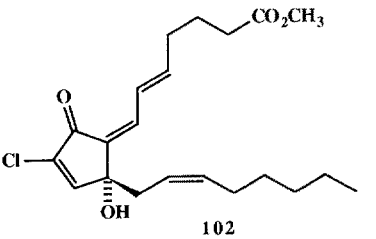

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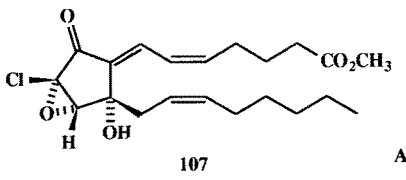
a 10–15 fold increased potency to HL-60 promyelocytic leukemia cells relative to clavulone I (**88**).

The absolute stereochemistry at C12 in the clorovulones was demonstrated in two ways [115]. The C9 ketone of chlorovulone II acetate (**104**) was reduced to a mixture of epimers which were separated, converted to their corresponding *p*-bromobenzoates, and analyzed by CD to assign the 9*R* and 9*S* stereochemistries. The 9*R* alcohol, prior to benzoate formation, showed an intramolecular hydrogen bond by high dilution IR analysis, and thus, defined the configuration at C12 as *R* as well. Enantioselective total synthesis of (–)-chlorovulone II (**101**) confirmed these results [115]. It is a truly remarkable finding that the chlorovulones (**100**–**103**) contain the opposite absolute stereochemistry at C12 as compared to the clavulones (**88**–**90**), as both occur in the same organism.

Soon afterwards [123] the bromo- (**105**) and iodo- (**106**) analogs of chlorovulone I (**100**) were also isolated from *C. viridis* in exceptionally low yield (ca. 0.01% of lipid extract). Their structures were established principally by spectroscopic means in comparison with chlorovulone I. Both of the new compounds possess the same olefin geometries as found in clavulone I and chlorovulone I. *R* Stereochemistry at C12 was established in **105** by comparisons of CD spectra with those of chlorovulone I (**100**). These new halogenated clavulones showed levels of antiproliferative activity and cytotoxicity comparable to those of chlorovulone I (**100**).

The 10,11-epoxide of chlorovulone I (**107**) was also obtained in low yield (0.05%) from the hexane extract of *C. viridis* [124]. Its structure was assembled from spectroscopic data which showed a high degree of similarity to that obtained for chlorovulone I (**100**) except for UV and ^{1}H NMR features due to the C10,11-olefin. Confirmation of structure came from synthesis of **107** by epoxidation of chlorovulone I. Epoxy-chlorovulone I (**107**) was found to possess the same 12*R* hydroxyl stereochemistry. The *cis* relationship of the epoxide and hydroxyl group was indicated by an intramolecular hydrogen bond as revealed by characteristic IR absorptions at high dilution. This 10,11-epoxide derivative



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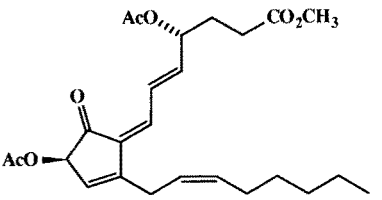

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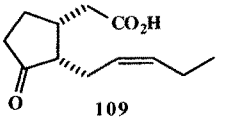

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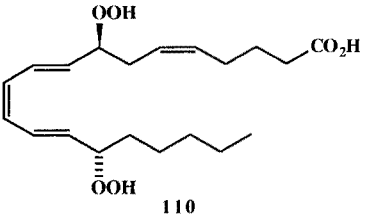

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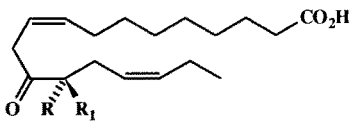


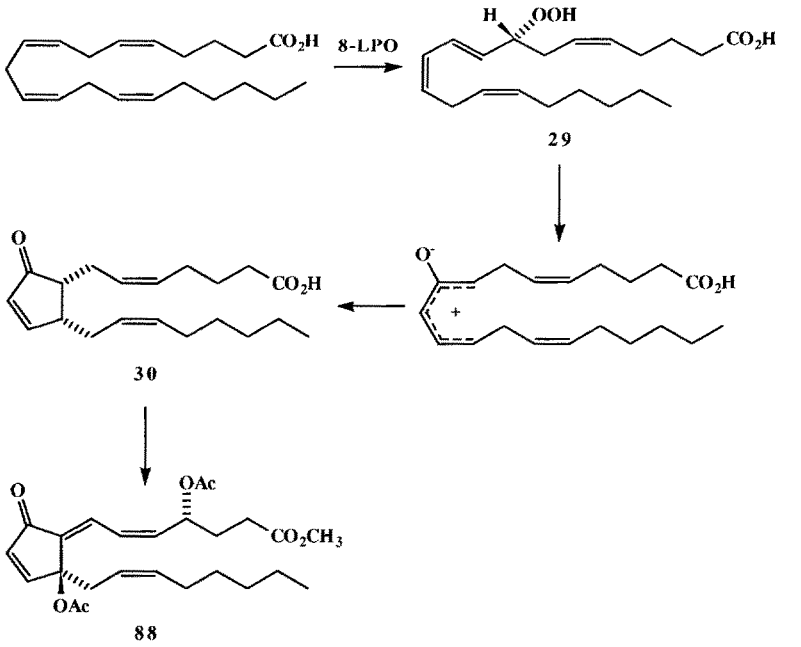
112) R=H, R_1=OH
113) R=OH, R_1=H

(**107**) showed antiproliferative activity in HL60 cells comparable to chlorovulone I (**100**) indicating that the 10,11-olefin is not required for activity as had been thought previously [124].

Recently, a thermally induced (190 °C, *o*-dichlorobenzene) [3,3]-sigmatropic rearrangement of either clavulones I (**88**) or II (**89**) was reported [125]. The product, a conjugated triene with 10*R*-acetoxyl group (**108**), was structurally described by a combination of ^{1}H NMR data, chemical derivatization, and CD analysis of the C10-benzoate derivative. Thermal isomerization was suggested as the reason why both clavulones I (**88**, 5*E*,7*Z*) and clavulone II (**89**, 5*E*,7*E*) give the same 5*E*,7*Z* product (**108**).

3.6.2 Biosynthesis

The biosynthesis of the clavulones was first hypothesized to be a variant of the endoperoxide pathway found in mammalian systems and involving a number of radical intermediates [126]; however, this was subsequently found not to be the case. Rather, biosynthetic experiments with crude buffer homogenates of *C. viridis* resulted in the transformation of exogenous arachidonic acid to a cyclopentenone-containing product, termed "preclavulone A" (PC-A, **30**). Its planar structure and relative stereochemistry were determined through an elaborate process involving chemical transformations, synthesis, and chromatographic comparisons [85]. However, no PC-A has been detected in extracts of *C. viridis* [84]. The structural similarity of PC-A to *cis*-jasmonic acid (**109**) suggested that it might derive via analogous transformations [90], namely



Scheme 10. Proposed biosynthesis of clavulone I (**88**) in *Clavularia viridis* [85]

lipoxygenation to 8-HPETE (**29**) followed by formation of a 2-hydroxypentadienyl cation intermediate which cyclizes to PC-A (**30**, Scheme 10). It was envisioned, but not experimentally evaluated, that PC-A could be enzymatically oxidized at C4, C7, and C12 to yield the clavulone metabolites. A biomimetic synthesis has been accomplished of two preclavulone model compounds which possess a *cis* ring but lack some of the sites of unsaturation found in PC-A [127].

The intermediacy of 8*R*-HPETE (**29**) in the production of preclavulone (**30**) from arachidonic acid was subsequently shown in cell-free biosynthetic work with *P. porosa* (see above) [84] and *C. viridis* [89]. The acetone powder of frozen *C. viridis* converted exogenous arachidonic acid to 8*R*-HPETE (**29**) in up to 19% yield. The planar structure of this product was determined by comparison with authentic material and its absolute stereochemistry by reduction to 8*R*-HETE followed by esterification with (−)menthyl chloroformate, ozonolysis to the C6 to C9 fragment and analysis of the methylated product by GC versus standards. Further, it was shown that a buffer homogenate of *C. viridis* would convert ^{3}H-labeled 8*R*-HPETE (**29**) to preclavulone A (**30**) in up to 13% yield. Based on precedents provided by the formation of *cis*-jasmonic acid in higher plants, it was suggested that 8*R*-HPETE is converted to preclavulone A via allene oxide (**64**) and oxidopentadienyl cation intermediates. Furthermore, it was surmised that PGA_2 biosynthesis in *P. homomalla* may involve a similar pathway, although involving an 8,15-diHPETE (**110**) intermediate. However, it was subsequently shown that homogenates of *P. homomalla* were incapable of transforming 8,15-diHPETE (**110**) into PGA_2 [86]. Further, cell-free homo-

genates of *P. homomalla* were also found to produce PC-A (**30**); however, it was shown to be racemic and probably represents a non-enzymatic decomposition product of allene oxide **64**. Work with a pure α-linolenate-derived allene oxide methyl ester (**111**) showed that even brief storage in pH 7.4 phosphate buffer gives good yields of α-ketols (**112**, **113**) and the racemic *cis*-cyclopentanoid **114** (*cis*-12-oxo-phytodienoic acid) [91, 93]. Hence, the relevance of these findings utilizing coral acetone powders or homogenates to coral prostaglandin biosynthesis has been questioned.

3.6.3 Biological Activities

The antiproliferative properties of clavulone I (**88**) were studied in greater detail using the HL60 leukemia cell line [128]. Application of the natural product at 0.4 μM (0.2 μg ml^{-1}) irreversibly decreased the growth rate of these cells to half that of controls. Complete growth inhibition was observed at about 1.0 μM and cytotoxicity was observed at higher concentrations.

Further testing showed the HL60 cell line slightly more sensitive (2–3 fold) than other transformed and normal cell lines. Incubation of HL60 cells in media containing clavulone I (**88**) at concentrations between 0.5–4.0 μM and ^{3}H-thymidine showed it to be strongly inhibitory to DNA synthesis. Flow cytometry showed that cells in growth inhibiting concentrations of the agent accumulate in G_1 phase. These results with clavulone I are consistent with an inhibition of DNA synthesis in S-phase, as seen for other prostaglandins [129]. Comparable studies were later reported for the halogenated clavulones in which the order of antiproliferative and cytotoxicity potency [chlorovulone I (**100**) > bromovulone I (**105**) = iodovulone I (**106**) > clavulone I (**88**) or II (**89**)] was established in HL60 cells [130]. At low concentrations, the antiproliferative effect of chlorovulone I was reversible, in contrast to the results obtained with clavulone I. This reversibility was also seen in the arrest of HL60 cells in G_1 upon treatment with non-toxic levels of chlorovulone I (**100**) or II (**101**, 0.03–0.09 μM). Based on evaluation of analogs of the clavulones and PGA_2 in these assays, the following structure-activity (antiproliferative and cytotoxic) relationships were deduced: 1) the introduction of a halogen atom at C10 increases the activity of the clavulones (Cl = F > Br = I > H), 2) the C10–11 olefin is essential for activity, 3) the C5–8 diene potentiates these activities, and 4) the stereochemistries of the C12 and C15 hydroxyls are not important to the activity. It has also been observed that both the C10 chlorine and C12 hydroxyl are required to potentiate the cytotoxicity of PGA analogs unsaturated at C7-8 (alkylidenecyclopentenones) [131].

In L1210 leukemia cells, clavulone II (**89**) again showed good cytotoxicity (IC_{50} = 0.3 μg ml^{-1}) [132]. In Ehrlich ascites tumor bearing mice, application of clavulone II at 10 mg kg^{-1} day^{-1} for five days gave a T/C = 151 [131] or at 20 mg kg^{-1} day^{-1} gave a T/C of 160 [132].

At about the same time, however, observation of strong antiproliferative activity in the C10–11 epoxy analog of clavulone I (**88**) showed that the C10–11

olefin was not strictly required [124]. An expanded set of natural and synthetic compounds was subsequently tested in this same system and essentially the same structure-activity results obtained as above [133]. It was additionally found that changes in length of the α-chain decrease the antiproliferative and cytotoxic activity.

More recently, the clavulones (not specified which ones) [134] were described to have potent effects on the spontaneous beating rate of cultured myocardial cells from fetal mouse hearts. At a concentration of 0.45 μM, clavulone had positive chronotropic action on these cells; however, the character of this effect clearly differed from the positive ionotropic and negative chronotropic effects of the steroid glycoside ouabain or the drug Bay K 8644. Hence, these results suggest that the clavulones may possess a new mechanism of action in this assay system.

3.7 *Telesto riisei*

3.7.1 Natural Products

Hawaiian collections of the octocoral *Telesto riisei* were reported in 1985 to be a source of four biologically active oxylipins that closely resembled those previously isolated from *C. viridis* [135]. However, at the time of their isolation, they were the first known to contain halogen atoms. They were obtained in moderate yield (0.08–0.8%) from the hexane extract by reverse phased HPLC and their planar structures determined by spectroscopic methods, principally, high field ^{1}H NMR. Interconversion of punaglandin 1 (**95**) and 3 (**97**) and punaglandin 2 (**96**) and 4 (**98**) was effected by elimination of the elements of acetic acid upon treatment with pyridine at room temperature. Substantial amounts of the *Z*-7,8-isomers of punaglandins 3 (**97**) and 4 (**98**) were also produced in this reaction. The *trans* ring juncture was suggested in punaglandins 1 and 2 from nOe experiments (H_2-13 ⇒ H-8) and the relative stereochemistry of acetoxyl substituents deduced by coupling constants. The *Z* geometry of the major HOAc elimination product supported the relative stereochemistry at C7 and C8 in punaglandins 1 and 2, and a subsequent report indicated a likely 8*R* configuration [136]. Further, this latter report indicated, without experimental elaboration, that both the 7*E* and 7*Z* isomers of punaglandins 3 and 4 are natural products [136]. Absolute stereochemistry was suggested in the original report to be the same as found in the clavulones [135]. Punaglandin 3 (**97**) was highly active in inhibiting L1210 leukemia cell proliferation ($IC_{50} = 0.02\ \mu g\,ml^{-1}$).

However, soon afterward the original authors [137] as well as one other group, simultaneously revised the structures of punaglandins 3 (**97**) and 4 (**98**) based on synthesis of all possible stereoisomers [138]. These synthetic efforts not only established that the stereochemistry at C12 was opposite to that proposed, they also defined the complete absolute stereochemistry in punaglan-

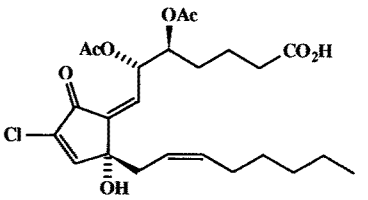
dins 3 and 4 as 5*S*,6*S*,12*R*. Apparently, the nature of the original data set as well as consideration of the probable mechanism of elimination of acetic acid from punaglandins 1 (**95**) and 2 (**96**) did not unequivocally establish this element of the structure. A racemic synthesis of punaglandin 4 (**98**) was also accomplished [139]. Additional stereospecific syntheses of both 7*E*- and 7*Z*-punaglandin 4 confirmed the revision in the relative stereochemistry at C12 as well as assignment of absolute stereochemistry [136, 140]. Further, based on analogy to these results, similar stereochemistry was assigned for 7*E*- (**97**) and 7*Z*-punaglandin 3 (**115**). Little difference in the potency of the antiproliferative effect in L1210 cells was observed between these and several other stereoisomers [136].

3.7.2 Biological Activities

In a separate study, punaglandin (which one is not specified) was shown to be exceptionally cytotoxic to L1210 leukemia cells ($IC_{50} = 0.02\ \mu g\ ml^{-1}$) and caused a significant life extension in Ehrlich ascites tumor bearing mice (T/C = 180 at 5 mg kg^{-1} day^{-1} for 5 days) [132]. Another report on the in vivo activity of the punaglandins yielded comparable results (punaglandin 3 (**97**) at 5 mg kg^{-1} day^{-1} gave T/C = 151; punaglandin 4 (**98**) at 5 mg kg^{-1} day^{-1} gave T/C 179) [131].

3.8 *Lobophyton/Lobophytum*

Investigations of the natural products chemistry of the Red Sea soft coral *Lobophyton depressum* (Alcyonacea) led to the isolation of a crystalline prostanoid derivative [70]. On the basis of ^1H NMR data, the planar structure was developed as methyl 11-acetoxy-$PGF_{2\alpha}$ (**56**). Stereochemistry at C15 was established as *S* by comparison of the biological and NMR features of the hydrolyzed



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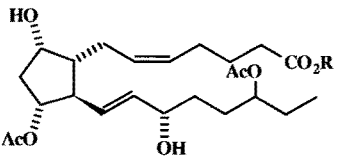
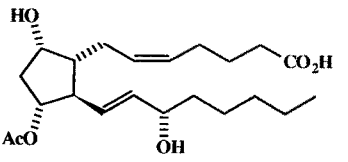


116) R = CH_3
118) R = H



117

product ($PGF_{2\alpha}$, **17**) and by chromatographic comparison of the synthetically produced mixture of epimers at C15. Subsequently, three additional prostanoids were isolated from the soft coral extract. The structure of a second methyl ester derivative was established as 11α,18-diacetoxy $PGF_{2\alpha}$ (**116**) by a close comparability of NMR data with **56** and by theoretical consideration of the ^{13}C NMR shifts for C16 to C20. Two more polar compounds (**117**, **118**) were isolated and shown to be the free acids of the above methyl esters since methylation with CH_2N_2 gave mono-acetate **56** and diacetate **116**.

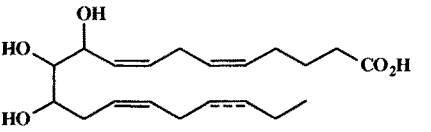
More recently, *Lobophytum carnatum* from the coast of Vietnam was investigated for both its fatty acid and prostaglandin content [141]. A remarkably high level of polyunsaturates was found in this coral (63%) with γ-linolenic (10.7%), eicosa-8,11,14-trienoic (10.7%), arachidonic (18.9%), and eicosapentaenoic (4.1%) acids predominating. Both methyl and ethyl esters of PGA_2 (**42**, **119**) and its degradation product PGB_2 (**52**, **120**), see Sect. 3.2.1, were reported; however, the ethyl esters were likely artifacts of "fixation" in ethanol. The prostaglandins were identified by comparison of HPLC and GC retention times versus standards as well as by mass spectrometry. Stereochemical features were not examined.

4 Crustacea

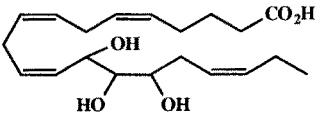
4.1 Barnacles

4.1.1 Natural Products

The majority of the oxylipins isolated from Crustacea have been from barnacles. Early studies in the search for the "barnacle hatching factor", a substance released into the mantle cavity that stimulates hatching, suggested that a prostaglandin-like compound might be responsible [142]. Subsequent research elucidated the structure of the hatching factor from *Balanus balanoides* as 10,11,12-trihydroxyeicosa-5,8,14,17-tetraenoic acid (**121**) [143]. The structure was determined by GC-MS of the methyl esters of the tris-TMS derivative and of the hydrogenated tris-TMS derivative. No stereochemical information was given, although the structure was depicted with all-*Z* double bonds, based on its presumed formation from all-*cis* EPA. Several other oxylipins were discovered in *B. balanoides* during further investigations [144]. In addition to the previously defined hatching factor, 10,11,12-trihydroxyeicosa-5,8,14-trienoic acid (**122**, trioxilin B_3), 13,14,15-trihydroxyeicosa-5,8,11,17-tetraenoic acid (**123**), and 12,13,14-trihydroxydocosa-4,7,10,16,19-pentaenoic acid (**124**) were also characterized by GC-MS of their TMS, methyl ester derivatives and of the hydrogenated TMS, methyl ester derivatives. Two other compounds were identified as chlorine containing dihydroxy oxylipins. Their tentative structures were assigned as 9-chloro-8,12-dihydroxyeicosa-5,11,14,17-tetraenoic acid (**125**) and



121 ω3
122 ω6



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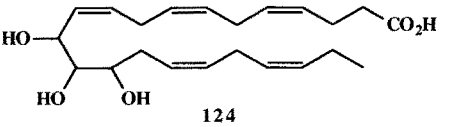

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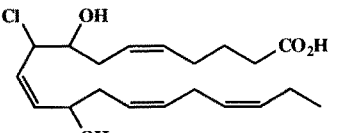

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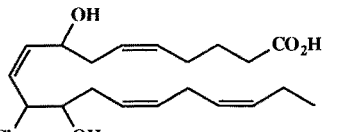

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11-chloro-8,12-dihydroxyeicosa-5,9,14,17-tetraenoic acid (**126**). However, due to the almost identical mass spectral fragmentation patterns reported for these two compounds, it is possible that the two are actually diastereomers, such as 11-chloro-8*S*,12-dihydroxyeicosa-5,9,14,17-tetraenoic acid and 11-chloro-8*R*,12-dihydroxyeicosa-5,9,14,17-tetraenoic acid, not positional isomers as suggested in the paper. No stereochemical details were provided for the compounds characterized in this article and the double bond geometries were based on putative precursor configurations. Trioxilin B_3 has been synthesized [145].

Another barnacle species, *Elminius modestus*, was found to produce mono and trihydroxy fatty acids [146]. Analysis of the extract of whole animal homogenates by TLC provided two "hatching factor active" bands. The more polar band was tentatively identified as a trihydroxy fatty acid (THFA) band. The less polar band had an R_f-value similar to a 5-HETE standard. The compounds from this latter band were eluted from the TLC plate, methylated, and trimethylsilylated. GC-MS analysis detected several HEPE's and small amounts of monohydroxy derivatives of $C_{18:1}$, $C_{18:2}$, and C_{22} fatty acids. Hydrogenation and subsequent GC-MS analysis allowed identification of the major compound as 8-HEPE (ca. 70%). Five to ten percent of 9-, 11-, and 15-HEPE and minor amounts of 5-, 6-, 12-, and 13-HEPE were also detected. No stereochemical features of these oxylipins were determined.

Ten barnacle species, including the two above, were assayed for hatching factor activity and the presence of monohydroxy fatty acids (MHFA) and THFA bands [147]. The other eight species were: *Balanus crenatus*, *B. balanus*, *B. hameri*, *B. amphitrite*, *B. perforatus*, *Chthalamus montagui*, *Pollicipes pollicipes*, and *Lepas anatifera*. All ten showed both MHFA and THFA bands.

4.1.2 Biosynthesis

Holland and coworkers mentioned the TLC detection of a trihydroxy compound related to the hatching factor when homogenates of *B. balanoides* were incubated with ^{14}C-AA, but no experimental details were given [143]. By using ammonium sulfate cuts from rat lung homogenates, it has been shown that trioxilin B_3 can arise from 12-HPETE via a 10-hydroxy-11,12-epoxy intermediate [148].

4.1.3 Function

A factor that promotes hatching of barnacle eggs was first detected in 1956 [149]. It was almost 30 years later before the first substance identified as a hatching factor, 10,11,12-trihydroxyeicosatetraenoic acid (**121**), was characterized. Several reviews cover the early results in this area [9, 100, 150, 151]. Although both MHFA and THFA bands contained hatching factor activity in vitro, the trihydroxy fatty acid component seems to be the active hatching factor in vivo, as it is the only fraction isolated from seawater surrounding adult barnacles [147]. The seawater surrounding adult specimens of *B. balanoides* and *B. hameri* that had been maintained in small glass containers was extracted and the residue purified by TLC. Only a band corresponding to the THFA band showed activity to *E. modestus* egg masses. For the other species examined, the exclusive role of THFA's as hatching factors needs to be confirmed. Since all of the barnacles contained MHFAs in their homogenates, a separate role of these compounds may be possible. In *E. modestus* homogenates, the MHFA band had stronger hatching factor activity than the THFA band and commercially obtained 8*S*-HEPE was active at concentrations as low as 10^{-8} M [146]. However, other positional isomers of monohydroxyeicosapentaenoic acid and 8*R*-HEPE were not tested. A mixture of various HEPE methyl esters, prepared by autoxidation, was extremely active in this assay. Since 8*S*-HEPE was active at such low levels, the determination of the stereochemistry of the naturally occurring 8-HEPE is of great interest. If the stereochemistry is *S*, this would be opposite of the known 8*R* stereochemistry seen in other marine invertebrates (*Plexaura homomalla* and starfish).

4.2 Arthropods

Tissue accumulation of exogenously added tritiated PGs was shown for the spider crab *Libinia emarginata* [107]. The earliest report of possible oxylipin production in marine arthropods was from gill and stomach tissues of the lobster *Homarus* sp. [15]. These were tested for their ability to produce prostaglandins in incubations with all-*cis*-[8,9,11,12,14,15-$^3H_6$]eicosatrienoic acid. Carrier PGE_1 was added and the PGE_1-type compounds from the extract were purified by TLC. Quantitation was done by UV absorption at 278 nm after

alkali treatment and by liquid scintillation counting. A 4% conversion of substrate to PGE_1 was determined for gill tissue and a 1% conversion for stomach tissue. Gas chromatography with electron capture detection was used to determine PG biosynthesis from endogenous substrates. Low levels ($0.8\ \mu g\,g^{-1}$ tissue) of a compound designated as PGE_2 were found in lobster tissue; however, the structural assignments were not definitive. Nomura and Ogata detected the presence of PG-like compounds in the gill tissue of the blue crab *Portunus trituberculatus* ($15\ ng\,g^{-1}$ wet tissue) using a rat stomach fundus bioassay [17]. The rockcrab *Pachygrapsus crassipes* and the mantis-shrimp *Squilla oratoria* did not show PGs by this method. Ogata and coworkers tested the ability of gill, heart, and liver tissues of the crab *Erimacrus isenbeckii* to produce PG-like products after incubation with ^{14}C-labeled eicosa-8,11,14-trienoic acid [19]. All three tissues showed very low levels of conversion of substrate to PG-like products. The gill tissue (0.5%) produced slightly more conversion to a PGE fraction than heart (0.2%) or liver (0.1%). Fever induction in marine arthropods by PGs has been examined [150]. Further, exogenously supplied $PGF_{2\alpha}$ and PGE_2 can induce abdominal flexion in the estuarine crab *Rhithropanopeus harrisii* at levels of 10^{-12} M or below [100].

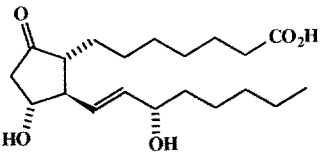
5 Mollusca

Marine molluscs are a rich source of new oxylipins. Perhaps even more importantly, marine molluscs have provided several clues which aid our understanding of oxylipin function in both invertebrate and mammalian systems. The biological significance of molluscan oxylipins appears to go far beyond that of acting as defensive allomones as in the opisthobranch *T. fimbria*. It appears that these highly functionalized oxylipins are more than secondary metabolites; they may also be vital for the initiation of neurochemical communication, maintaining normal biochemical and physiological balances, and controlling reproductive responses.

Several reviews have discussed the role of oxylipins in molluscan osmotic regulation [150, 151]. Bioassay and radioimmunoassay techniques were used to demonstrate the production and release of prostaglandins from the bivalve *Modiolus demissus* [152]. PGE and PGA analogs were treated with dilute methanolic KOH and determined by reaction with anti-PGB. Both hyposmotic stress (salinity of 8.6%) and magnesium-free sea water induced significant PG release from intact animals into sea water. Prostaglandin release was significantly decreased by addition of aspirin or indomethacin, and increased by addition of arachidonic acid. Subsequently, homogenized *M. demissus* gill tissues were shown to contain specific low-affinity PGA_2 binding sites, the first proof of an eicosanoid receptor site in an invertebrate [153].

5.1 Mussels and Scallops

Prostaglandin synthetase activity was first observed in the gills and other tissues of mussels (*Mytilis* sp.) over twenty years ago [15]. A 3% conversion of eicosa-8*Z*,11*Z*,14*Z*-trienoic acid to PGE_1 (**127**) was measured by electron capture gas chromatography. While it has been suggested that some marine molluscs, such as scallops, accumulate prostaglandins which are secreted into the water by other animals (i.e. *Plexaura homomalla*), more recent studies have indicated that this phenomenon is probably insignificant. Japanese workers, using an isolated rat stomach fundus bioassay, were able to demonstrate prostaglandin activity in the tissues of mussels and scallops [17]. A semiquantitative determination of prostaglandins in isolated mollusc tissue was achieved using PGE_2 as a standard. Samples, principally composed of mantles and adductor muscles from the mussel *Mytilis edulis*, contained prostaglandin activity equivalent to 32 ng of PGE_2 (**16**) per gram of wet tissue. The gills and "feet" of the scallop *Patinopecten yessoensis* had activities equivalent to PGE_2 concentrations of only 2 to 4 $ng\,g^{-1}$ wet tissue. However, no chemical evidence was provided as to which specific prostanoids were present.



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Egg release in the scallop *Patinopecten yessoensis* has been shown to be induced apparently via a serotonin (5-hydroxytryptamine, 5-HT) receptor-mediated process [154–157]. An investigation of the in vitro effects of prostaglandins on ovarian egg release in *P. yessoensis* has produced several interesting findings [158]. Both aspirin and indomethacin, known cyclooxygenase inhibitors, inhibited 5-HT-induced egg release. In the absence of 5-HT neither PGE_2 (**16**) nor $PGF_{2\alpha}$ (**17**) stimulated egg release. The addition of 10^{-6} M PGE_2 to the media reestablished the egg releasing effect of 5-HT even in the presence of aspirin or indomethacin. PGE_2 was shown to augment the 5-HT-stimulated release of eggs, while a significant dose-dependent reduction in egg release was demonstrated with $PGF_{2\alpha}$. While little is known concerning endogenous prostaglandin levels in molluscs, it appears that $PGF_{2\alpha}$ concentrations in the ovary of both *P. yessoensis* and the oyster *Crassostrea gigas* increase during the sexual maturation process [159]. It has been suggested that in *P. yessoensis* the 5-HT-induced egg release may be under a form of prostaglandin regulation; however, further examination of in vivo prostaglandin production and release during this process is required.

5.2 *Crassostrea gigas*

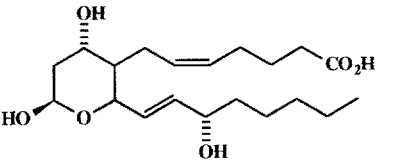
Gonadal $PGF_{2\alpha}$ levels were measured throughout the sexual maturation of the Japanese oyster, *Crassostrea gigas* [159]. Radioimmunoassay (RIA) analysis of $PGF_{2\alpha}$ concentrations was correlated with histological observations of gonadal tissues. A three and one-half fold increase in PG levels was observed in ovarian tissues extracted immediately prior to spawning (25 ng g^{-1} fresh wt. in August versus 7 ng g^{-1} in June and July), thereby suggesting that they may play a role in the oyster's reproduction. It is important to note that no chemical confirmation was presented for the identity of the PG structure other than its reactivity with $PGF_{2\alpha}$ antiserum.

5.3 *Haliotis rufescens*

The addition of hydrogen peroxide to seawater induces a reproducible spawning in both gravid males and females of the red abalone *Haliotis rufescens* [160]. Pretreatment with aspirin, a well-characterized cyclooxygenase inhibitor, blocked the H_2O_2-induced synchronous spawning. Morse and coworkers suggested that H_2O_2 serves as a source of an activated oxygen species, which accelerates the production of prostaglandin endoperoxides, since H_2O_2, sodium peroxide (Na_2O_2), and potassium superoxide (KO_2) all stimulate fatty acid cyclooxygenase in vitro. However, the mechanistic role of prostaglandin endoperoxide synthetase and its metabolic products in the induction of abalone spawning remains unclear.

5.4 *Aplysia californica*

Both acyclic hydroxy-acids and prostanoids were detected in the mollusc *Aplysia californica* by radioimmunoassay (RIA) [35]. RIA analysis of aqueous extracts of *A. californica* confirmed the presence of all major oxylipin classes tested including the prostaglandins PGE_2 (**16**), $PGF_{2\alpha}$ (**17**), 6-keto-$PGF_{1\alpha}$ (**18**), thromboxane B_2 (**128**), and the hydroxyeicosanoid 12-HETE (**132**). These assays were not confirmed by HPLC, nor were the products subjected to mass spectral analysis. Therefore, the detected metabolites represent compounds only structurally similar to known eicosanoids. Greater than 90% of the binding energy of these antigen-antibody complexes is reported to result primarily from the highly functionalized C5 through C15 portion of twenty carbon-derived prostanoids. Major factors determining antigenic character include the spatial orientation of the cyclopentane ring, the presence of unsaturation, and relative patterns of oxidation. Further chemical analysis is necessary since oxylipins deriving from unusual fatty acid precursors may react with serological assays designed for arachidonic acid metabolites and still represent structurally different compounds.



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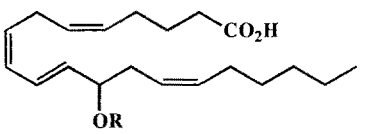
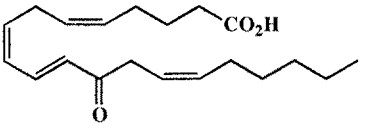


129) R=OH
132) R=H



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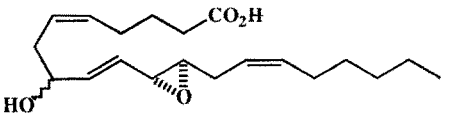

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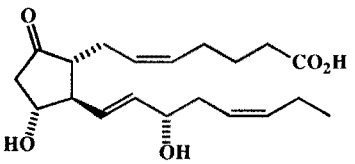
The relatively simple nervous system of the marine mollusc *Aplysia californica* has proven to be an invaluable model for neurochemical investigation. The large and readily isolated neurons of *Aplysia* have provided a wealth of biochemical information concerning the function of oxylipins, especially arachidonic acid metabolites, in neurochemical signal transduction. The role of arachidonic acid metabolism in molluscan signal transduction has been the subject of several reviews and will be summarized only briefly [151, 161, 162]. Both active lipoxygenase and cyclooxygenase systems appear to be present in *Aplysia* neurons as evidenced by the conversion of arachidonic acid (AA) to 5- and 12-HETE and PGE_2 and $PGF_{2\alpha}$ in nerve cell bodies and synaptosomes [163]. Synaptic stimulation with histamine was shown to induce the conversion of labeled arachidonic acid to ^{3}H-12-HETE in cerebral ganglia. It was suggested that lipoxygenase metabolites may act as second messengers for presynaptic inhibition of sensory neurons of the abdominal and pleural ganglia [164]. It appears that LPO products may impart a second messenger-type regulation of the responses produced by the molluscan inhibitory tetrapeptide FMRF amide (Phe-Met-Arg-Phe amide) which opens S-channels (specialized 5-HT sensitive K^+ channels). AA mimics the effect of FMRF amide and is converted to the hydroperoxide 12-HPETE (**129**) following FMRF amide stimulation [164, 165]. In addition, the lipoxygenase inhibitor NDGA blocks the effect of both FMRF amide and AA, while the cyclooxygenase inhibitor indomethacin has no effect. Additionally, 12-HPETE mimics the actions produced by both AA and FMRF amide, although it remains unclear, whether 12-HPETE or one of its many

metabolites actually acts as a second messenger [166]. Several 12-HPETE-derived compounds, including 12-KETE (12-ketoeicosatetraenoic acid) (**130**) and hepoxilin A_3 (8-hydroxy-11,12-epoxyeicosa-5,9,14-trienoic acid, **131**), appear to modulate the K^+ S-channels in *Aplysia* neurons, while another 12-HPETE metabolite, 12-HETE (**132**), produces no response [167–170]. It was suggested that unlike other second messengers, eicosanoids may also function as first messengers, facilitating direct communication between adjacent cells. Further, it is hypothesized that this first messenger-type activity may relate to "long-term potentiation" phenomena, as observed in mammalian hippocampus-derived NMDA-type glutamate receptors, where prolonged postsynaptic responses are observed following presynaptic stimulation [164]. It appears that the study of *Aplysia* neurochemistry may eventually lead to a better understanding of neuropharmacological processes in higher animals.

5.5 *Tethys fimbira*

5.5.1 Natural Products

Lipid extracts from the mantles of the Mediterranean nudibranch *Tethys fimbria* were shown to contain small quantities of compounds not found in similar extracts of hepatopancreas tissues from the same animals [171]. A combination of NMR analysis and comparison with synthetically produced analogs established these structures as naturally occurring prostaglandin lactones. The three compounds identified were PGE_2-1,15-lactone (**133**), PGE_3-1,15-lactone (**134**), and PGE_3-1,15-lactone-11-acetate (**135**). While PG-1,15-lactones were unknown in nature, they had previously been synthesized [172] and characterized by high-field ^1H NMR spectroscopy [173]. Further examination of *T. fimbria* mantle and cerata extracts revealed the presence of PGE_2 (**16**), PGE_3 (**136**), PGA_2-1,15-lactone (**137**), PGA_3-1,15-lactone (**138**), PGF_2-1,15-lactone-11-acetate (**139**), as well as prostaglandins of the F series including



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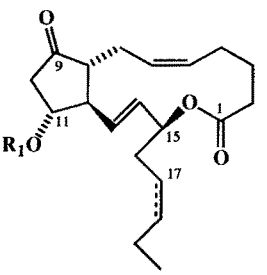
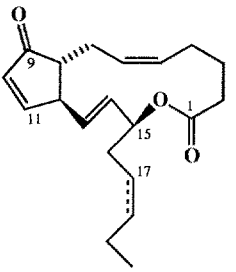
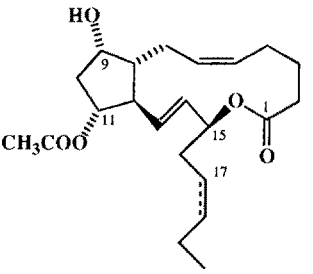


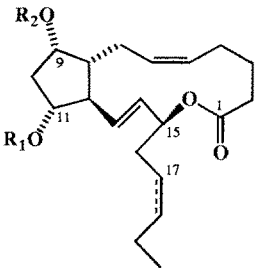
133) R=H; ω7
134) R=H; ω3
135) R=Ac, ω3
139) R=Ac, ω7



137 ω7
138 ω3



140 ω7
141 ω3



142 ω7
143 ω3
(R_1=H, R_2=fatty acid) or (R_1=fatty acid, R_2=H)
R_1 or R_2 = $C_{20:4}$, $C_{20:5}$, $C_{18:0}$, $C_{18:1}$, $C_{14:0}$

$PGF_{2\alpha}$-1,15-lactone-11-acetate (**140**) and $PGF_{3\alpha}$-1,15-lactone-11-acetate (**141**) [174–176]. Further, *T. fimbria* reproductive tissues and egg masses contained a relatively high concentration of saturated and unsaturated long-chain fatty acid esters of $PGF_{2\alpha}$ and $PGF_{3\alpha}$ (**142, 143**) [177, 178].

5.5.2 Biosynthesis

The origin of the prostaglandin lactones, isolated from both the mantles and detached cerata of *Tethys fimbria*, has been the subject of detailed biosynthetic investigation [179]. The metabolism of radiolabelled arachidonic acid to both PGE_2 (**16**) and PGE_2-1,15-lactone (**133**) was demonstrated in vivo. Radiolabelled PGE_2, when subcutaneously injected into the mantles of animals without cerata, is converted to PGE_2-1,15-lactone. PGE_2-1,15-lactone injected into the mantle of intact animals is transferred to the cerata over time. While ^{3}H-labeled PGE_2 injected into the mantle is lactonized and redistributed to the cerata, ^{3}H-labeled PGE_2 injected into detached cerata is not converted to PGE_2-1,15-lactone. A rapid acetylation of labelled $PGF_{2\alpha}$-1,15-lactone at the

C11 position appears to explain a failure to isolate any $PGF_{2\alpha}$-1,15-lactone from *T. fimbria*. From these experiments, a complex enzymatically driven system of lactonization and hydrolysis, linked to an intricate redistribution system begins to emerge in *Tethys*. PGE_2 and $PGF_{2\alpha}$ are converted, in the mantle, to PGE_2-1,15-lactone and then shuttled to the dorsal appendices (cerata). Following "autotomy," a process whereby the cerata are spontaneously detached when the mollusc is attacked by predators, PG-1,15-lactones are enzymatically re-opened to their corresponding free acids. Labelled $PGF_{2\alpha}$ is converted by the ovotestis and egg masses to $PGF_{2\alpha}$-1,15-lactone 9- and 11-fatty acyl esters (**142**). The significance of this esterification of PG-lactones in reproductive tissues is still unknown.

A recent investigation of the role of prostaglandin-lactone metabolism throughout *T. fimbria*'s reproductive process has provided several interesting findings [178]. PG-lactone levels were determined for the ovotestis and compared to levels in the mantle and cerata at three stages in the mollusc's sexual development. Results suggested that PG-lactones and PG-lactone-11-acetates are synthesized in the mantle during sexual maturation. A *trans*-esterification process, utilizing phosphoglyceride pools of fatty acids in the mantle, forms the PG-lactone 9- and 11-acyl fatty acid esters which are then redistributed to the ovotestis. These PG-lactone fatty acyl esters are transported to the egg masses immediately before they are released. Following egg laying, PG-lactone synthesis is resumed in the mantle. During the late stages of egg development and immediately preceding hatching, significant levels of PGE-lactone-11-acetates (**135, 139**) are produced within the egg. It is suspected that these metabolites may be involved in oocyte maturation or hatching, since they are not detected in the veligera, once hatched and therefore are unlikely to play a role in veligera development or chemical defense.

5.5.3 Chemical Ecology

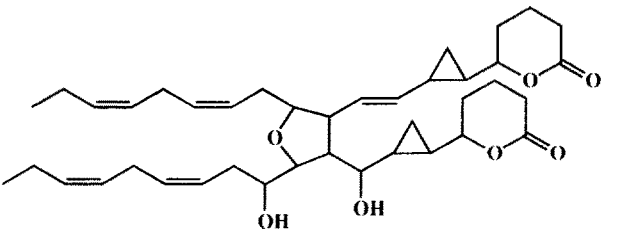
Research into the chemical ecology of Mediterranean opisthobranchs has yielded a great deal of information concerning the sophisticated, often chemically mediated, defensive strategies that these shell-less molluscs use in order to avoid predation [180–184] When handled, the dorsal cerata of *T. fimbria* are autotomized. These appendages continue to contract for up to 8 hours by way of epithelial smooth muscle fibers [185]. PG-lactones in the cerata are converted to their free acid form. PG free acids are well-characterized inducers of smooth-muscle contraction and relaxation in vertebrates. Simultaneously, a mucous slime containing high levels of PG-lactones is secreted from epithelial glands in the cerata. Experiments have shown PG-1,15-lactones of the E and A series are toxic to mosquito fish at concentrations of 1 and 10 $\mu g\,ml^{-1}$, respectively [185]. The authors suggest a dual role for PGE-lactones in *T. fimbria*: (1) as direct acting ichthyotoxic defense allomones; (2) as a source of PG free acids which contract the cerata and may facilitate release of mucous. It must be noted that the mosquito fish, *Gambusia affinis*, is a freshwater species and therefore may be

of little actual ecological relevance. Further study is required in order to establish the significance of these findings under natural conditions.

5.6 *Aplysia kurodai*

5.6.1 Natural Products

Cyclopropane and lactone-containing eicosanoids have been isolated from several marine organisms including a sponge [38], a soft coral [92], and two species of red algae [41, 186]. Similarly, an unsymmetrical eicosanoid dimer (**144**) has been isolated from lipid extracts of the Japanese sea hare *Aplysia kurodai* [187, 188]. The structure of (**144**) was established by spectroscopic analysis of the natural product, a diacetate derivative, and several degradative fragments. By this methodology, the relative stereochemistry of the *trans*-cyclopropyl group and the double bond were determined. This unusual metabolite, aplydilactone (**144**), produces a two-fold activation of phospholipase A_2 in vitro, however, only at very high concentrations (50 mM).



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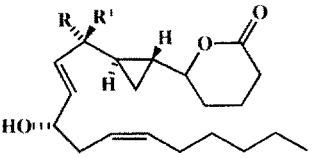
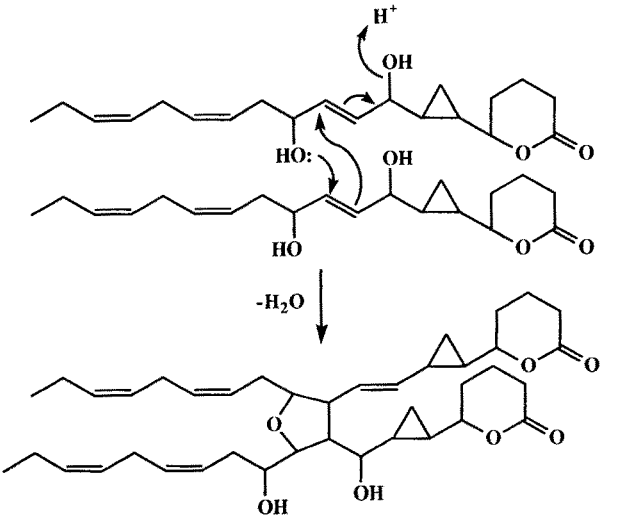


145) R = H, R' = OH
146) R = OH, R' = H

5.6.2 Biosynthesis

Structural similarities between the monomeric units of aplydilactone (**144**) and the red algal metabolites constanolactone A (**145**) and B (**146**), suggest an analogous biosynthetic origin. Dehydration of two $\omega 3$ unsaturated constanolactone-type units could result in the formation of the unsymmetrical dimer (Scheme 11). Since sea hares are known to feed extensively on red algae and sequester natural products from this source, it remains possible that aplydilactone (**144**) derives, at least in part, from a dietary source.



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Scheme 11. Proposed biogenesis of aplydilactone (**144**) from monomer units

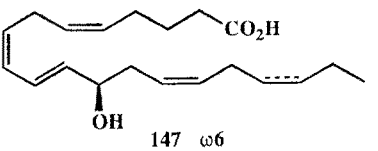
6 Echinodermata

6.1 Sea Urchins

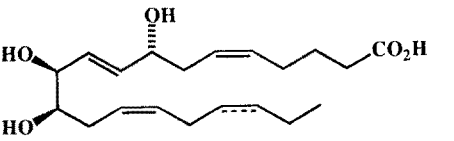
The sea urchin *Arbacia punctulata* could be shown to accumulate in its gut or stomach tritiated prostaglandins from the surrounding water. Further, both fertilized and unfertilized eggs were able to accumulate PGA_1, although fertilized eggs concentrated larger amounts than unfertilized ones [107]. Korotchenko and coworkers detected PG-like activity, as determined by a rat uterine muscle contraction assay, in the inner organs of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius* [189]. TLC indicated that the activity migrated to zones with R_f values similar to PGE_2 and $PGF_{2\alpha}$ standards. Levine and Kobayashi used radioimmunoassays to show that *Arbacia* sp. possessed compounds immunologically related to PGs and HETE's [35]. Due to the cross-reactivity of the antibodies to related species, exact identification of the compounds was not possible.

Perry and Epel discovered a Ca^{2+}-stimulated lipid peroxidizing system using egg homogenates of *Strongylocentrotus purpuratus* [190]. When AA was incubated with the egg homogenates, the major oxidation product was a HETE as determined by co-chromatography in several TLC systems. Based on these results, the authors suggested the presence of a lipoxygenase system in *S. purpuratus*. In the same research, *Arbacia punctulata* homogenates were

shown to oxidize AA, but *Lytechinus pictus*, a member of the same order as *S. purpuratus*, did not possess the lipid-peroxidizing ability under these conditions. Further work showed that fertilization stimulates the lipid peroxidation in vivo and this activity coincides with an increase in intracellular calcium [191]. Based on these results, four hydroxyeicosanoids were prepared by incubating egg homogenates with either AA or EPA [192] and characterized by UV and GC-MS analysis of TMS, methyl ester derivatives: 11*R*- (**76**) and 12*R*-HETE (**147**) and 11*R*- (**148**) and 12*R*-HEPE (**149**). The absolute stereochemistries were determined by HPLC of menthoxycarbonyl derivatives and by comparison with racemic standards. No specific activities have been determined for the oxylipins found in sea urchins, but the evidence is building that they play a key role in preventing polyspermic fertilization. Stanley-Samuelson [151] and Schuel [193] cover this topic in their reviews.



147 ω6
149 ω3



150 ω3
151 ω6

Hawkins and Brash investigated some aspects of the biosynthesis of 11*R*- and 12*R*-HETE in eggs of *S. purpuratus* [192, 194]. Based on experiments using octadeuterio-AA they showed that the 11- or 12-keto intermediate is not involved. Isolation and characterization of both 11*R*- and 12*R*- hydroperoxyeicosatetraenoic acids from incubations of desalted ammonium sulfate fractions of the egg homogenate with arachidonic acid support involvement of a lipoxygenase.

6.2 Starfish

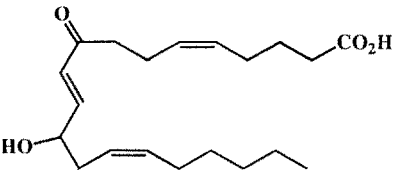
6.2.1 Natural Products

Korotchenko and coworkers detected PG-like compounds in five starfish from the Sea of Japan: *Asterias amurensis*, *Distolasterias nippon*, *Evasterias r. tabulata*, *Lysastrosoma anthosticta*, and *Patiria pectinifera* [189]. Perry and Epel showed that *Pisaster ochraceous* homogenates were able to oxidize AA [190]. Early studies on oocyte maturation in starfish suggested that HETE's might play a role [195]. Two compounds, 12- and 15-HETE, were able to induce oocyte maturation. Subsequent work showed that the activity was actually due to 8-HETE (**33**), which was a minor contaminant of both HETE preparations [196]. The 8*R* enantiomer of this compound (**33**) and 8*R*-HEPE (**32**) were also isolated from the aqueous extracts of *Patiria miniata* from the Gulf of California [197].

The absolute stereochemistries for both compounds were determined by CD analysis of the *p*-bromobenzoate derivatives. Two other eicosanoids were also isolated from whole animals [198]. The trihydroxylated oxylipin 8*R*,11*S*,12*R*-trihydroxyeicosa-5*Z*,9*E*,14*Z*,17*Z*-tetraenoic acid (trioxilin A_4, **150**) was detected in, or isolated from, five starfish species: *P. miniata*, *Dermasterias imbricata*, *Pycnopodia helianthoides*, *Culcita novaeguinea*, and *Nardoa tubercolata*. Its structure was determined by ^1H and ^{13}C NMR and FAB-MS analyses of the natural product and of an acetonide derivative. The $\omega 6$ analog of this compound (**151**) was isolated only from *P. helianthoides*. The relative stereochemistry in these metabolites (**150**, **151**) was established from a comparison of NMR shifts with malyngic acid, a trihydroxylated C_{18} compound isolated from *Lyngbya majuscula*, while the absolute stereochemistry was proposed on the basis of the earlier isolation of 8*R*-HETE from *P. miniata*.

6.2.2 Biosynthesis

Meijer and coworkers investigated the production of 8*R*-HETE in starfish by incubating oocytes of *Orthasterias koehleri* and *Evasterias troschelii* with ^{14}C-labelled AA [199]. In addition to the formation of 8-HETE, several other HETE's were produced including 9-, 12-, and 15-HETE. The stereochemistry of the radiolabelled 8-HETE produced by *E. troschelii* oocytes was determined to be *R* by converting the 8-HETE to 8,15-diHETE with soybean lipoxygenase. The resulting two diastereomers, 8*R*,15*S*-diHETE and 8*S*,15*S*-diHETE, are readily separable by HPLC. This method is useful for analyzing small samples. Further investigations into the enzymatic system involved in 8-HETE production provided some unexpected results [200]. The intermediacy of 8*R*-HPETE in the pathway to 8*R*-HETE was shown, but in addition HPETE underwent some unusual enzymatic events. Allene oxide synthase and hydroperoxide lyase activities were also present in the oocytes as determined by structural elucidation of products characteristic of these pathways. Two α-ketols, 8-hydroxy-9-ketoeicosa-5*Z*,11*Z*,14*Z*-trienoic acids (**58**, **63**), were isolated and characterized by ^1H NMR and mass spectrometry. These compounds were identical to the previously identified α-ketols from allene oxide hydrolysis in the *Plexaura homomalla* system. Incubations with $H_2{}^{18}O$ showed that the oxygen at C8 came almost exclusively from water. The configuration at C8 was determined to be 71% *S*. A cyclopentenone (**30**) and a γ-ketol (**152**) were also detectable in minor amounts from the incubation of AA with ovary microsomes of starfish. These products strongly suggest the intermediacy of the allene oxide 8,9-epoxyeicosa-5*Z*,9,11*Z*,14*Z*-tetraenoic acid (**64**). Also isolated from the incubation were 7-hydroxyhept-5*Z*-enoic acid (**153**) and 2*E*,4*Z*,7*Z*-tridecenal (**154**) and its 4*E* isomer (**155**). The methyl ester of hydroxy acid **153** was characterized by ^1H NMR and the methyl ester TMS derivative was examined by GC-MS. Isolation of these products indicate hydroperoxide lyase activity, an activity not seen previously in animals. These enzyme systems specifically use 8*R*-HPETE as a substrate; 8*S*-HPETE was not transformed into products.



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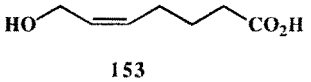

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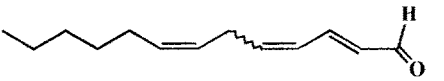


154 4*Z*
155 4*E*

6.2.3 Ecology

8*R*-HETE is a very potent and selective inducer of oocyte maturation [196, 199]. 8*S*-HETE does not show this activity. The allene oxide hydrolysis products and the hydroperoxide lyase products were not active in promoting oocyte maturation [200].

6.3 Sea Cucumbers

Korotchenko and coworkers detected PGs by TLC and bioassay in three sea cucumbers (Holothuroidea): *Stichopus japonicus*, *Cucumaria fraudatrix*, and *C. japonica* [189].

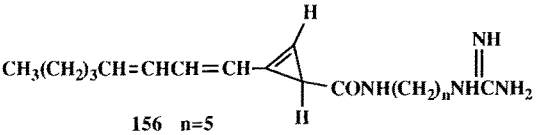
7 Urochordata

7.1 Natural Products

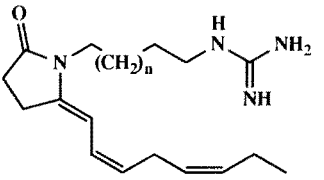
Nomura and Ogata provided the first evidence that tunicates can produce PGs [17]. Using a rat stomach fundus bioassay, *Halocynthia roretzi* tissues were shown to possess low levels of PGs. The testes showed higher levels (9 ng g^{-1} wet tissue) than ovary and muscle tissue. The sea-squirt *Styela clava* did not show PGs by this method. No structures were determined in this work. Re-examination of the ability of *H. roretzi* to produce PGs was carried out by Ogata and coworkers [19]. Incubation of selected tissues with ^{14}C-labeled eicosa-8,11,14-trienoic acid and subsequent isolation of PGE and PGF fractions after addition of carrier showed the branchial tissue to have the highest conversion levels. Quantitation was done by LSC. Using a TLC radioscanner, the authors determined that fractions with metabolites similar to PGE and PGF

were present when compared with standards. Again, no structural work was carried out. Subsequently, it was shown that an *Ascidia* sp. was able to produce oxidized AA products, both in the presence or absence of calcium, when incubated with ^{14}C-labelled AA [190].

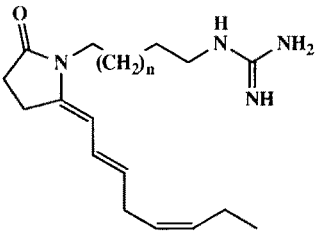
Cheng and Rinehart first proposed cyclopropene-containing structures for polyandrocarpidines I (**156**) and II (**157**) from *Polyandrocarpa* sp. [201]. Carte and Faulkner published revised structures for polyandrocarpidines A–D (**158–161**) in 1982 [202]. The structural revisions were based on NMR studies and ozonolysis to *N*-alkyl succinimides. A polyunsaturated C_{12} fatty acid oxidized at C4 and a decarboxylated arginine (or an analog biosynthesized from lysine) appear to be the components that form these *N*-alkyl-γ-alkylidene-γ-lactams. In addition to possible biogenetic similarities to other oxylipins, the polyandrocarpidines bear a spatial resemblance to the prostaglandins. Hexahydropolyandrocarpidine I (**162**) was synthesized [203], converted into its 4,6-dimethylpyrimidyl derivative and compared by GC and MS with the DMP derivative of authentic hexahydropolyandrocarpidine I. This synthesis supported the structural revisions.



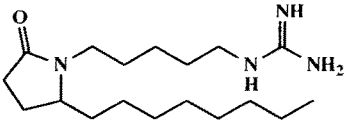
156 n=5
157 n=4



158) A n=2
160) C n=1



159) B n=2
161) D n=1



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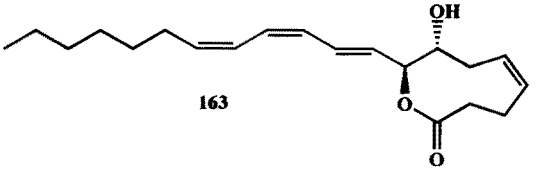

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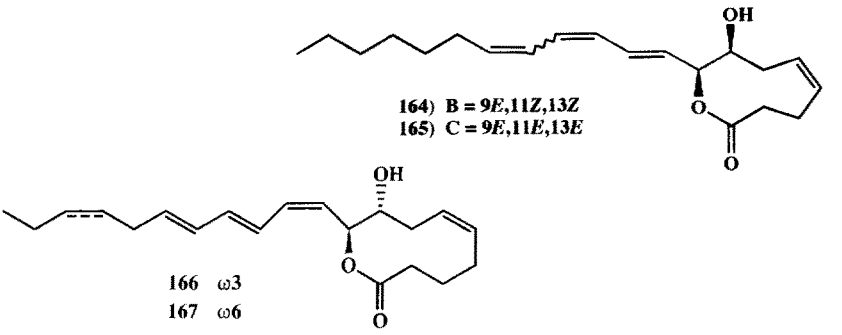


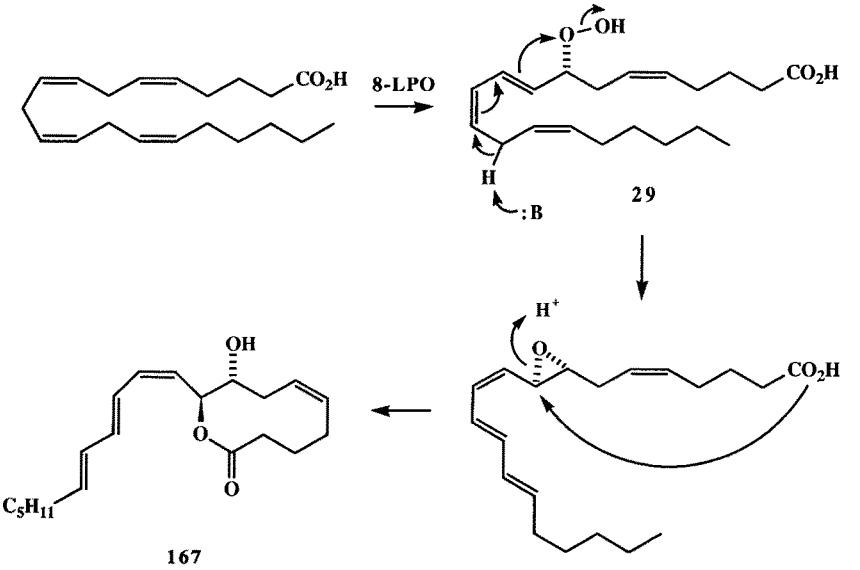
Three novel oxylipins, ascidiatrienolides A–C (**163–165**), were isolated from the colonial marine ascidian *Didemnum candidum* collected in Florida [204]. The structures were determined using 1D ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, and UV data. The relative stereochemistry at C7 and C8 was determined through coupling constant data and molecular modeling. The proposed structures contain a 9-membered ring lactone and a conjugated triene. Two closely related compounds, didemnilactone (**166**) and neodidemnilactone (**167**), were subsequently isolated from a Japanese collection of *Didemnum mosleyi* [205]. The structures were elucidated using ^{1}H and ^{13}C NMR, mass, UV, and IR spectra. These compounds possess a 10-membered lactone and a conjugated triene. The absolute stereochemistry of these molecules was determined by synthesis of neodidemnilactone (**167**) from 3,4-*O*-isopropylidene-2-deoxy-D-ribose. Synthetic 8*S*,9*R* **167** had opposite rotation to the natural product. Thus, neodidemnilactone has 8*R*,9*S* stereochemistry as depicted. Didemnilactone (**166**) was assigned the same absolute stereochemistry based on similarities of optical rotation. The occurrence of both 9- and 10-ring lactones in closely related organisms suggests a possible misassignment in one or the other set of compounds. The neodidemnilactone structure seems solid based on the confirmation by synthesis. The constitution of the ascidiatrienolides is less secure. Several lines of evidence make structures **163–165** potentially suspect: 1) the assigned chemical shifts for the C2 protons in all three ascidiatrienolides (**163–165**) are upfield of 2 ppm, whereas the C2 protons in a number of model compounds (the halicholactones (**25**, **26**) [38], hybridalactone [186], methyl 12-HEPE [206]) are all at or downfield of 2 ppm in benzene-d_6; 2) a ^{1}H-^{13}C correlation spectrum was not recorded and without this information the reported assignments cannot be fully evaluated; 3) the putative precursor for the ascidiatrienolides would be an unusual Δ^4, $\omega 7$ C_{20} fatty acid; and 4) oxidation at C7 in eicosanoids is rare. Spectral assignments for the ^{1}H and ^{13}C NMR shifts of the didemnilactones are not given, which prevents direct comparison [205].

7.2 Biosynthesis

Little work has been reported on the biosynthesis of oxylipins in ascidians. One report describes the production of numerous oxidized AA metabolites by eggs

from *Ascidia ceratodes* [207]. Homogenates and intact eggs were incubated with ^{14}C-AA and then the products were analyzed by TLC and autoradiography. Quantitation was performed by removing the radioactive bands and liquid scintillation counting. While the oxidation of AA is stimulated by Ca^{2+} in tunicates, it is not strictly a Ca^{2+} dependent process as in sea urchin eggs. Unfertilized eggs, zygotes, and cleaving embryos had similar profiles of oxidized compounds as shown by TLC. One of the minor bands in unfertilized egg incubations was lost upon fertilization.

A proposed biogenesis for neodidemnilactone (**167**) would involve formation of 8*R*-HPETE, conversion to an 8,9-leukotriene A_4-like intermediate, and internal epoxide opening by the terminal carboxylate (Scheme 12).



Scheme 12. Proposed biogenesis of neodidemnilactone (**167**) from arachidonic acid

7.3 Biological Activity

The didemnilactones (**166, 167**) exhibit weak binding to leukotriene B_4 receptors in human polymorphonuclear leukocyte membranes [205]. The polyandrocarpidines (**158–161**) exhibit antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes,* and *Mycobacterium avium,* are cytotoxic to L1210 leukemia cells (ID_{50} 4.8 $\mu g\,ml^{-1}$), and have slight antiviral activity against the *Herpes* type I virus [201].

Perry and Lambert suggest that the natural role of oxylipins in tunicates may be in the control of metabolic processes [207].

8 Conclusion

As this review has shown, marine invertebrates provide a rich source of structurally unique oxylipins. Study of the pharmacological and biological properties of these unique substances has provided new insights into such diverse topics as structure/activity relationships in antiproliferative drugs and the nature of second messengers involved in neurochemical communication. Marine oxylipins displaying such potent and unique pharmacological properties in initial studies have a high likelihood of developing into research biochemicals and new pharmaceutical agents. Inherent in these novel structures are biosynthetic events without close parallel in terrestrial fatty acid metabolism. Remarkably, even oxylipins which are the same or similar to those of terrestrial occurrence, such as prostaglandin A_2 or prostaglandin $F_{2\alpha}$, are synthesized in some marine invertebrates by completely different pathways. Hence, the study of the pathways of oxylipin metabolism in marine invertebrates is uncovering fundamentally new biosynthetic reactions. Knowledge of these pathways, and of the stimuli which activate them, will undoubtably be of tremendous value to a deeper understanding of the biochemistry, physiology, and ecology of these life forms. Furthermore, discovery and description of these metabolic pathways in these primitive biota provides the basic knowledge for their future discovery in other more highly evolved organisms, including perhaps *Homo sapiens.*

9 References

1. Gerwick WH, Moghaddam MF, Hamberg M (1991) Arch Biochem Biophys 290:436
2. Weinheimer AJ, Spraggins RL (1969) Tetrahedron Lett 5185
3. Rychnovsky SD, Skalitsky DJ, Pathirana C, Jensen PR, Fenical W (1992) J Am Chem Soc 114:671
4. Gerwick WH, Bernart MW, Moghaddam MF, Jiang ZD, Solem ML, Nagle DG (1990) Hydrobiologia 204/205:621
5. Gerwick WH, Proteau PJ, Nagle DG, Wise ML, Jiang ZD, Bernart MW, Hamberg M (1992) Hydrobiologia (in press)
6. Munro MHG, Luibrand RT, Blunt JW (1987) The search for antiviral and anticancer compounds from marine organisms. In: Scheuer PJ (ed) Bioorganic marine chemistry, vol 1, Springer, Berlin Heidelberg New York, p 93
7. Corey EJ (1987) Pure Appl Chem 59:269
8. Gerwick WH, Bernart MW (1992) Eicosanoids and related compounds from marine algae. In: Attaway DH, Zaborsky OR (eds) Marine biotechnology, vol I: Pharmaceutical and bioactive natural products. Plenum, New York, p 101
9. Ruggieri B, Thoroughgood CA (1985) Mar Ecol Prog Ser 23:310
10. Brown JA, Gray CJ, Hattersley G, Robinson J (1991) Gen Comp Endocrinol 84:328
11. Faulkner DJ (1991) Nat Prod Rep 8:97
12. Ravi BN, Wells RJ (1982) Aust J Chem 35:105
13. Lai SMF, Manley PW (1984) Nat Prod Rep 1:409
14. Bundy GL (1985) Adv. Prostaglandin Thromboxane Leukotriene Res 14:229
15. Christ EJ, Van Dorp DA (1972) Biochim Biophys Acta 270:537

16. Miyares Cao CM, Menendez Cepero E, Ruiz Rodríguez I (1974) Rev Cubana Farm 8:97
17. Nomura T, Ogata H (1976) Biochim Biophys Acta 431:127
18. Morse DE, Kayne M, Tidyman M, Anderson S (1978) Biol Bull 154:440
19. Ogata H, Nomura T, Hata M (1978) Bull Jpn Soc Sci Fish 44:1367
20. Bergman W, Swift AN (1951) J Org Chem 16:1206
21. Morales RW, Litchfield C (1977) Lipids 12:570
22. Ayanoglu E, Kurtz K, Kornprobst JM, Djerassi C (1985) Lipids 20:141
23. Nagle DG, McClatchey WC, Gerwick WH (1992) J Nat Prod 55:1013
24. Wells RJ (1976) Tetrahedron Lett. 30:2637
25. Quiñoà E, Kho E, Manes LV, Crews P, Bakus GJ (1986) J Org Chem 51:4260
26. Snider BB, Shi Z (1990) J Org Chem 55:5669
27. Sakemi S, Higa T, Anthoni U, Christophersen C (1987) Tetrahedron 43:263
28. Murayama T, Ohizumi Y, Nakamura H, Sasaki T, Kobayashi J (1989) Experientia 45:898
29. Tsukamoto S, Takeuchi S, Ishibashi M, Kobayashi J (1992) J Org Chem 57:5255
30. Ciminiello P, Fattorusso E, Mangoni A, Ialenti A, Di Rosa M (1991) Experientia 47:739
31. Gunstone FD, Wijesundera RC, Love RM, Ross D (1976) J Chem Soc Chem Commun 630
32. Sand DM, Glass RL, Olson DL, Pike HM, Schlenk H (1984) Biochim Biophys Acta 793:429
33. Corbett MD, Chipko BR (1979) Experientia 35:718
34. Baden DG, Corbett MD (1979) Comp Biochem Physiol 64B:279
35. Levine L, Kobayashi T (1983) Prostaglandins Leukotrienes Med 12:357
36. Guerriero A, D'Ambrosia M, Pietra F, Ribes O, Duhet D (1990) J Nat Prod 53:57
37. Gardner HW (1991) Biochim Biophys Acta 1084:221
38. Niwa H, Wakamatsu K, Yamada K (1989) Tetrahedron Lett 30:4543
39. Kigoshi H, Niwa H, Yamada K, Stout TJ, Clardy J (1991) Tetrahedron Lett 32:2427
40. Olson ES (1977) J Am Oil Chem Soc 54:51
41. Nagle DG, Gerwick WH (1990) Tetrahedron Lett 31:2995
42. Wilson HV (1907) J Exp Zool 5:245
43. Wilson HV (1910) J Exp Zool 9:537
44. Wilson HV, Penney JT (1930) J Exp Zool 56:73
45. Galtsoff PS (1925) J Exp Zool 42:183
46. Curtis ASG (1962) Nature (*London*) 196:245
47. Spiegel M (1955) Ann NY Acad Sci 60:1056
48. Serhan CN, Korchak HM, Weissmann G (1980) J Immunol 125:2020
49. Dunham P, Anderson C, Rich AM, Weissmann G (1983) Proc Natl Acad Sci USA 80:4756
50. Serhan CN, Fridovich J, Goetzl EJ, Dunham PB, Weissmann G (1982) J Biol Chem 257:4746
51. Rich AM, Weissmann G, Anderson C, Vosshall L, Haines KA, Humphreys T, Dunham P (1984) Biochem Biophys Res Commun 121:863
52. Schneider WP, Morge RA (1971) Upjohn Technical Report 3102/71/7231/002, Upjohn Co., Kalamazoo, Michigan.
53. Schneider WP, Bundy GL, Lincoln FH, Daniels EG, Pike JE (1977) J Am Chem Soc 99:1222
54. Schneider WP, Morge RA, Henson BE (1977) J Am Chem Soc 99:6062
55. Bayer FM, Weinheimer AJ (eds) (1974) Prostaglandins from *Plexaura homomalla*: Ecology, utilization and conservation of a major medical marine resource. A symposium. University of Miami Press, Florida
56. Miyares Cao CM, Menendez Cepero E, Ruiz Rodriquez I (1975) Chem Abstr 83:15540p
57. Corey EJ, Matsuda SPT, Nagata R, Cleaver MB (1988) Tetrahedron Lett 29:2555
58. Doerge DR, Corbett MD (1982) Chem Biol Interact 42:117
59. Guerriero A, D'Ambrosio M, Pietra F (1988) Helv Chim Acta 71:1094
60. Coll JC (1992) Chem Rev 92:613
61. Dominguez JN, Adams DR, Flamerich J (1980) Rev Latinoam Quim 11:56
62. Gerhart DJ (1986) Biochem Syst Ecol 14:417
63. Ciereszko LS, Gopichand Y, Schmitz FJ, Schneider WP, Bundy GL (1985) Experientia 41:37
64. Schneider WP, Hamilton RD, Rhuland LE (1972) J Am Chem Soc 94:2122
65. Light RJ, Samuelsson B (1972) Eur J Biochem 28:232
66. Light RJ (1973) Biochim Biophys Acta 296:461
67. Bundy GL, Daniels EG, Lincoln FH, Pike JE (1972) J Am Chem Soc 94:2124
68. Lasker HR (1985) Mar Ecol Prog Ser 21:213
69. Groweiss A, Fenical W (1990) J Nat Prod 53:222
70. Carmely S, Kashman Y (1980) Tetrahedron Lett 21:875
71. Spraggins RL (1972) Tetrahedron Lett 42:4343

72. Bundy GL, Schneider WP, Lincoln FH, Pike JE (1972) J Am Chem Soc 94:2123
73. Chen C-T, Shih Y-E (1973) Bull Inst Chem, Acad Sin 22:101
74. Chen C-T, Linn E-C (1973) Bull Inst Chem, Acad Sin 22:105
75. Chen C-T, Chang K-L (1973) Bull Inst Chem, Acad Sin 22:109
76. Chen C-T, Chen L-H (1973) Bull Inst Chem, Acad Sin 22:112
77. Brash AR, Baertschi SW, Harris TM (1990) J Biol Chem 265:6705
78. Brash AR, Baertschi SW, Harris TM (1990) Non-cyclooxygenase prostaglandin biosynthesis: formation of PGA_3 isomers via an allene oxide. In: Reddy CC, Hamilton GA, Madyastha KM (eds) Biological oxidation systems, vol 2. Academic, San Diego, p 683
79. Corey EJ, Washburn WN, Chen JC (1973) J Am Chem Soc 95:2054
80. Corey EJ, Ensley HE, Hamberg M, Samuelsson B (1975) J Chem Soc Chem Commun 277
81. Corey EJ, Washburn WN (1974) J Am Chem Soc 96:934
82. Doerge DR, Corbett MD (1982) Experientia 38:901
83. Bundy GL, Nidy EG, Epps DE, Mizsak SA, Wnuk RJ (1986) J Biol Chem 261:747
84. Corey EJ, Matsuda SPT (1987) Tetrahedron Lett 28:4247
85. Corey EJ, Lansbury PT Jr, Yamada Y (1985) Tetrahedron Lett 26:4171
86. Brash AR, Baertschi SW, Ingram CD, Harris TM (1987) J Biol Chem 262:15829
87. Zimmerman DC (1966) Biochem Biophys Res Commun 23:398
88. Hamberg M (1988) Biochem Biophys Res Commun 156:543
89. Corey EJ, D'Alarcao M, Matsuda SPT, Lansbury PT Jr, Yamada Y (1987) J Am Chem Soc 109:289
90. Vick BA, Zimmerman DC (1983) Biochem Biophys Res Commun 111:470
91. Brash AR (1989) J Am Chem Soc 111:1891
92. Baertschi SW, Brash AR, Harris TM (1989) J Am Chem Soc 111:5003
93. Brash AR, Baertschi SW, Ingram CD, Harris TM (1988) Proc Natl Acad Sci USA 85:3382
94. Song W-C, Brash AR (1991) Arch Biochem Biophys 290:427
95. Brash AR, Baertschi SW, Ingram CD, Harris TM (1989) Adv Prostaglandin Thromboxane Leukotriene Res 19:70
96. Song W-C, Brash AR (1991) Science 253:781
97. Gerhart DJ (1984) Mar Ecol Prog Ser 19:181
98. Gerhart DJ (1990) Mar Ecol Prog Ser 62:103
99. Gerhart DJ (1991) Am J Physiol 260: R839
100. Gerhart DJ, Clare AS, Eisenman K, Rittschof D, Forward RB Jr (1990) Prog Comp Endocrinol 83:598
101. Sammarco PW, Coll JC (1988) The chemical ecology of alcyonarian corals (Coelenterata: Octocorallia). In: Scheuer PJ (ed) Bioorganic marine chemistry, vol 2, Springer, Berlin Heidelberg New York, p 87
102. Pawlik JR, Burch MT, Fenical W (1987) J Exp Mar Biol Ecol 108:55
103. Pawlik JR, Fenical W (1989) Mar Ecol Prog Ser 52:95
104. Robert A, Yankee EW (1975) Proc Soc Exp Biol Med 148:1155
105. Gerhart DJ (1986) Mar Ecol Prog Ser 31:255
106. Cieresko LS, Schneider WP (1987) Bull Mar Sci 41:634
107. Bito LZ (1972) Comp Biochem Physiol 43A:65
108. Komoda Y, Kanayasu T, Ishikawa M (1979) Chem Pharm Bull 27:2491
109. Imbs AB, Dautov SS, Latyshev NA (1989) Chem Abstr 111:191783a
110. Cham LN, Hung NK, Stekhov VB, Svetashev VI (1982) Comp Biochem 366
111. Kikuchi H, Tsukitani Y, Iguchi K, Yamada Y (1982) Tetrahedron Lett 23:5171
112. Kobayashi M, Yasuzawa T, Yoshihara M, Akutsu H, Kyogoku Y, Kitagawa I (1982) Tetrahedron Lett 23:5331
113. Kobayashi M, Yasuzawa T, Yoshihara M, Son BW, Kyogoku Y, Kitagawa I (1983) Chem Pharm Bull 31:1440
114. Kikuchi H, Tsukitani Y, Iguchi K, Yamada Y (1983) Tetrahedron Lett 24:1549
115. Nagaoka H, Iguchi K, Miyakoshi T, Yamada N, Yamada Y (1986) Tetrahedron Lett 27:223
116. Hashimoto S, Arai Y, Hamanaka N (1985) Tetrahedron Lett 26:2679
117. Corey EJ, Mehrotra MM (1984) J Am Chem Soc 106:3384
118. Nagaoka H, Miyakoshi T, Yamada Y (1984) Tetrahedron Lett 25:3621
119. Shibasaki N, Ogawa Y (1985) Tetrahedron Lett 26:3841
120. Iguchi K, Yamada Y, Kikuchi H, Tsukitani Y (1983) Tetrahedron Lett 24:4433
121. Nagaoka H, Miyakoshi T, Kasuga J-i, Yamada Y (1985) Tetrahedron Lett 26:5053
122. Iguchi K, Kaneta S, Mori K, Yamada Y, Honda A, Mori Y (1985) Tetrahedron Lett 26:5787

123. Iguchi K, Kaneta S, Mori K, Yamada Y, Honda A, Mori Y (1986) J Chem Soc Chem Commun 981
124. Iguchi K, Kaneta S, Mori K, Yamada Y (1987) Chem Pharm Bull 35:4375
125. Iguchi K, Kaneta S, Yamada Y (1992) Chem Pharm Bull 40:1923
126. Corey EJ (1983) Experientia 39:1084
127. Corey EJ, Ritter K, Yus M, Nájera C (1987) Tetrahedron Lett 28:3547
128. Honda A, Yamamoto Y, Mori Y, Yamada Y, Kikuchi H (1985) Biochem Biophys Res Commun 130:515
129. Wiley MH, Feingold KR, Grunfeld C, Quesney-Huneeus V, Wu JM (1983) J Biol Chem 258:491
130. Honda A, Mori Y, Iguchi K, Yamada Y (1987) Mol Pharmacol 32:530
131. Fukushima M, Kato T (1985) Adv. Prostaglandin Thromboxane Leukotriene Res 15:415
132. Fukushima M, Kato T, Yamada Y, Kitagawa I, Kurozumi S, Scheuer PJ (1985) Proc Am Assoc Cancer Res 26:980
133. Honda A, Mori Y, Iguchi K, Yamada Y (1988) Prostaglandins 36:621
134. Honda A, Hong S, Yamada Y, Mori Y (1991) Res Commun Chem Pathol Pharmacol 72:363
135. Baker BJ, Okuda RK, Yu PTK, Scheuer PJ (1985) J Am Chem Soc 107:2976
136. Suzuki M, Morita Y, Yanagisawa A, Baker BJ, Scheuer PJ, Noyori R (1988) J Org Chem 53:286
137. Suzuki M, Morita Y, Yanagisawa A, Noyori R, Baker BJ, Scheuer PJ (1986) J Am Chem Soc 108:5021
138. Nagaoka H, Miyaoka H, Miyakoshi T, Yamada Y (1986) J Am Chem Soc 108:5019
139. Sasai H, Shibasaki M (1987) Tetrahedron Lett 28:333
140. Mori K, Takeuchi T (1988) Tetrahedron 44:333
141. Latyshev NA, Bezuglov VV, Kogtev LS, Hung NK, Sadovskaya VL, Rozynov BV, Bergel'son LD (1986) Sov J Mar Biol Engl Transl 12:116
142. Clare AS, Walker G, Holland DL, Crisp DJ (1982) Mar Biol Lett 3:113
143. Holland DL, East J, Gibson KH, Clayton E, Oldfield A (1985) Prostaglandins 29:1021
144. Song W-C, Holland DL, Gibson KH, Clayton E, Oldfield A (1990) Biochim Biophys Acta 1047:239
145. Lumin S, Yadagiri P, Falck JR (1988) Tetrahedron Lett 29:4237
146. Hill EM, Holland DL, Gibson KH, Clayton E, Oldfield A (1988) Proc R Soc Lond B 234:455
147. Song W-C, Holland DL, Hill EM (1990) Proc R Soc Lond B 241:9
148. Pace-Asciak CR, Granstrom E, Samuelsson B (1983) J Biol Chem 258:6835
149. Crisp DJ (1956) Nature (London) 178:263
150. Stanley-Samuelson DW (1987) Biol Bull 173:92
151. Stanley-Samuelson DW (1991) Am J Physiol 260:R849
152. Freas W, Grollman S (1980) J Exp Biol 84:169
153. Freas W, Grollman S (1981) J Exp Zool 216:225
154. Matsutani T, Nomura T (1982) Mar Biol Lett 3:353
155. Matsutani T, Nomura T (1984) Bull Jpn Soc Sci Fish 50:425
156. Matsutani T, Nomura T (1986) Cell Tissue Res 244:515
157. Matsutani T, Nomura T (1986) Bull Jpn Soc Sci Fish 52:1589
158. Matsutani T, Nomura T (1987) Gen Comp Endocrin 67:111
159. Ono K, Osada M, Matsutani T, Mori K, Nomura T (1982) Mar Biol Lett 3:223
160. Morse DE, Duncan H, Hooker N, Morse A (1977) Science 196:298
161. Shimizu T, Wolfe LS (1990) J Neurochem 55:1
162. Piomelli D (1991) Am J Physiol R849
163. Piomelli D, Shapiro E, Feinmark ST, Schwartz JH (1987) J Neurosci 7:3675
164. Piomelli D, Volterra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH, Belardetti F (1987) Nature (London) 328:38
165. Sweatt D, Volterra A, Siegelbaum SA, Kandel ER (1988) Cold Spring Harbor Symp Quant Biol 53:395
166. Belardetti F, Campell WB, Falck JR, Demontis G, Rosolowsky M (1989) Neuron 3:497
167. Piomelli D, Feinmark ST, Shapiro E, Schwartz JH (1988) J Biol Chem 263:16591
168. Piomelli D, Shapiro E, Zipkin R, Schwartz JH, Feinmark ST (1989) Proc Natl Acad Sci USA 86:1721
169. Piomelli D, Feinmark ST, Shapiro E, Schwartz JH (1989) Ann NY Acad Sci 559:208
170. Feinmark ST, Piomelli D, Shapiro E, Schwartz JH (1989) Ann NY Acad Sci 559:121
171. Cimino G, Spinella A, Sodano G (1989) Tetrahedron Lett 30:3589

172. Bundy GL, Peterson DC, Cornette JC, Miller WL, Spilman CH, Wilks JW (1983) J Med Chem 26:1099
173. Andersen NH, Lin B (1985) Biochemistry 24:2338
174. Di Marzo V, Cimino G, Sodano G, Spinella A, Villani G (1990) Adv Prostaglandin Thromboxane Leukotriene Res 21:129
175. Di Marzo V, Cimino G, Sodano G, Spinella A, Villani G, A novel prostaglandin metabolic pathway from a marine mollusc: prostaglandin 1,15-lactones. 7th Internation Conf, on Prostaglandins and related compounds, 28 May–1 June 1990, Florence, Italy, p 13
176. Cimino G, Crispino A, Di Marzo V, Sodano G, Spinella A, Villani G (1991) Experientia 47:56
177. Cimino G, Crispino A, Di Marzo V, Spinella A, Sodano G (1991) J Org Chem 56:2907
178. Di Marzo V, Minardi C, Vardaro RR, Mollo E, Cimino G (1992) Comp Biochem Physiol 101B:99
179. Di Marzo V, Cimino G, Crispino A, Minardi C, Sodano G, Spinella A (1991) Biochem J 273:593
180. Cimino G, Sodano G (1989) Chem Scr 29:389
181. Karuso P (1987) Chemical ecology of the nudibranchs. In: Scheuer PJ (ed) Bioorganic marine chemistry, vol 1, Springer, Berlin Heidelberg New York, p 31
182. Faulkner DJ (1988) Feeding deterrents in molluscs. In: Fautin DG (ed) Biomedical importance of marine organisms. California Academy of Sciences, San Francisco, p 29
183. Faulkner DJ, Ghiselin MT (1983) Mar Ecol Prog Ser 13:295
184. Schulte GR, Scheuer PJ (1982) Tetrahedron 38:1857
185. Marin A, Di Marzo V, Cimino G (1991) Mar Biol 111:353
186. Higgs MD, Mulheirn LJ (1981) Tetrahedron 37:4259
187. Ojika M, Yoshida Y, Nakayama Y, Yamada K (1989) Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 31:664
188. Ojika M, Yoshida Y, Nakayama Y, Yamada K (1990) Tetrahedron Lett 31:4907
189. Korotchenko OD, Mishchenko TY, Isay SV (1983) Comp Biochem Physiol 74C:85
190. Perry G, Epel D (1985) Dev Biol 107:47
191. Perry G, Epel D (1985) Dev Biol 107:58
192. Hawkins DJ, Brash AR (1987) J Biol Chem 262:7629
193. Schuel H (1984) Biol Bull 167:271
194. Hawkins DJ, Brash AR (1989) FEBS Lett 247:9
195. Meijer L, Buerrier P, Maclouf J (1984) Dev Biol 106:368
196. Meijer L, Maclouf J, Bryant RW (1986) Dev Biol 114:22
197. D'Auria MV, Minale L, Riccio R, Uriarte E (1988) Experientia 44:719
198. Bruno I, D'Auria MV, Iorizzi M, Minale L, Riccio R (1992) Experientia 48:114
199. Meijer L, Brash AR, Bryant RW, Ng K, Maclouf J, Sprecher H (1986) J Biol Chem 261:17040
200. Brash AR, Hughes MA, Hawkins DJ, Boeglin WE, Song W-C, Meijer L (1991) J Biol Chem 266:22926
201. Cheng MT, Rinehart KL (1978) J Am Chem Soc 100:7409
202. Carte B, Faulkner DJ (1982) Tetrahedron Lett 23:3863
203. Rinehart KL, Harbour GC, Graves MD, Cheng MT (1983) Tetrahedron Lett 24:1593
204. Lindquist N, Fenical W (1989) Tetrahedron Lett 30:2735
205. Niwa H, Inagaki H, Yamada K (1991) Tetrahedron Lett 32:5127
206. Bernart MW, Gerwick WH (1988) Tetrahedron Lett 29:2015
207. Perry G, Lambert C (1988) Comp Biochem Physiol 90B:785

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Klenze, R., see Kim, J. I.: *157*, 129–180 (1990).
Knauer, M., see Bley, K.: *166*, 199–233 (1993).
Knops, P., Sendhoff, N., Mekelburger, H.-B., Vögtle, F.: High Dilution Reactions – New Synthetic Applications *161*, 1–36 (1991).
Koca, J., see Hladka, E.: *166*, 121–197 (1993).
Koepp, E., see Ostrowicky, A.: *161*, 37–68 (1991).
Kohnke, F. H., Mathias, J. P., and Stoddart, J. F.: Substrate-Directed Synthesis: The Rapid Assembly of Novel Macropolycyclic Structures *via* Stereoregular Diels-Alder Oligomerizations. *165*, 1–69 (1993).
Kostikov, R. R., Molchanov, A. P., and Hopf, H.: Gem-Dihalocyclopropanos in Organic Synthesis. *155*, 41–80 (1990).
Kratochvil, M., see Hladka, E.: *166*, 121–197 (1993).
Krogh, E., and Wan, P.: Photoinduced Electron Transfer of Carbanions and Carbacations. *156*, 93–116 (1990).
Kunkeley, H., see Vogler, A.: *158*, 1–30 (1990).

Kuwajima, I. and Nakamura, E.: Metal Homoenolates from Siloxycyclopropanes. *155*, 1–39 (1990).
Kvasnicka, V., see Hladka, E.: *166*, 121–197 (1993).

Lange, F., see Mandelkow, E.: *151*, 9–29 (1989).
Lefort, D., see Fossey, J.: *164*, 99–113 (1993).
Lopez, L.: Photoinduced Electron Transfer Oxygenations. *156*, 117–166 (1990).
Lozach, B., see Collet, A.: *165*, 103–129 (1993).
Lymar, S. V., Parmon, V. N., and Zamarev, K. I.: Photoinduced Electron Transfer Across Membranes. *159*, 1–66 (1991).

Mandelkow, E., Lange, G., Mandelkow, E.-M.: Applications of Synchrotron Radiation to the Study of Biopolymers in Solution: Time-Resolved X-Ray Scattering of Microtubule Self-Assembly and Oscillations. *151*, 9–29 (1989).
Mandelkow, E.-M., see Mandelkow, E.: *151*, 9–29 (1989).
Mathias, J. P., see Kohnke, F. H.: *165*, 1–69 (1993).
Mattay, J., and Vondenhof, M.: Contact and Solvent-Separated Radical Ion Pairs in Organic Photochemistry. *159*, 219–255 (1991).
Matyska, L., see Hladka, E.: *166*, 121–197 (1993).
Mekelburger, H.-B., see Knops, P.: *161*, 1–36 (1991).
Meng, Q., Hesse, M.: Ring Closure Methods in the Synthesis of Macrocyclic Natural Products. *161*, 107–176 (1991).
Merz, A.: Chemically Modified Electrodes, *152*, 49–90 (1989).
Meyer, B.: Conformational Aspects of Oligosaccharides. *154*, 141–208 (1990).
Misumi, S.: Recognitory Coloration of Cations with Chromoacerands. *165*, 163–192 (1993).
Moffat, J. K., Helliwell, J.: The Laue Method and its Use in Time-Resolved Crystallography. *151*, 61–74 (1989).
Molchanov, A. P., see Kostikov, R. R.: *155*, 41–80 (1990).
Moore, T. A., see Gust, D.: *159*, 103–152 (1991).

Nagle, D. G., see Gerwick, W. H.: *167*, 117–180 (1993).
Nakamura, E., see Kuwajima, I.: *155*, 1–39 (1990).
Nordahl, A., see Carlson, R.: *166*, 1–64 (1993).

Okuda, J.: Transition Metal Complexes of Sterically Demanding Cyclopentadienyl Ligands. *160*, 97–146 (1991).
Ostrowicky, A., Koepp, E., Vögtle, F.: The "Vesium Effect": Syntheses of Medio- and Macrocyclic Compounds. *161*, 37–68 (1991).

Parmon, V. N., see Lymar, S. V.: *159*, 1–66 (1991).
Polian, A., see Fontaine, A.: *151*, 179–203 (1989).
Pospichal, J., see Hladka, E.: *166*, 121–197 (1993).
Potucek, V., see Hladka, E.: *166*, 121–197 (1993).
Proteau, P. J., see Gerwick, W. H.: *167*, 117–180 (1993).

Raimondi, M., see Copper, D. L.: *153*, 41–56 (1990).
Riekel, C.: Experimental Possibilities in Small Angle Scattering at the European Synchrotron Radiation Facility. *151*, 205–229 (1989).
Roth, H. D.: A Brief History of Photoinduced Electron Transfer and Related Reactions. *156*, 1–20 (1990).
Roth, H. D.: Structure and Reactivity of Organic Radical Cations. *163*, 131–245 (1992).
Rüsch, M., see Warwel, S.: *164*, 79–98 (1993).

Sachs, H., see John, P.: *153*, 145–180 (1990).
Saeva, F. D.: Photoinduced Electron Transfer (PET) Bond Cleavage Reactions. *156*, 59–92 (1990).

Sauvage, J.-P., see Chambron, J.-C.: *165*, 131–162 (1993).
Sendhoff, N., see Knops, P.: *161*, 1–36 (1991).
Sessler, J. L., Burrell, A. K.: Expanded Porphyrins *161*, 177–274 (1991).
Sheldon, R.: Homogeneous and Heterogeneous Catalytic Oxidations with Peroxide Reagents. *164*, 21–43 (1993).
Sheng, R.: Rapid Ways of Recognize Kekuléan Benzenoid Systems. *153*, 211–226 (1990).
Schäfer, H.-J.: Recent Contributions of Kolbe Electrolysis to Organic Synthesis. *152*, 91–151 (1989).
Scheuer, P. J., see Chang, C. W. J.: *167*, 33–76 (1993).
Sodano, G., see Cimino, G.: *167*, 77–116 (1993).
Sojka, M., see Warwel, S.: *164*, 79–98 (1993).
Sorba, J., see Fossey, J.: *164*, 99–113 (1993).
Stanek, Jr., J.: Preparation of Selectively Alkylated Saccharides as Synthetic Intermediates. *154*, 209–256 (1990).
Stein, N., see Bley, K.: *166*, 199–233 (1993).
Stoddart, J. F., see Kohnke, F. H.: *165*, 1–69 (1993).
Stumpe, R., see Kim, J. I.: *157*, 129–180 (1990).
Suami, T.: Chemistry of Pseudo-sugars. *154*, 257–283 (1990).
Suppan, P.: The Marcus Inverted Region. *163*, 95–130 (1992).
Suzuki, N.: Radiometric Determination of Trace Elements. *157*, 35–56 (1990).

Thiem, J., and Klaffke, W.: Syntheses of Deoxy Oligosaccharides. *154*, 285–332 (1990).
Timpe, H.-J.: Photoinduced Electron Transfer Polymerization. *156*, 167–198 (1990).
Tolentino, H., see Fontaine, A.: *151*, 179–203 (1989).
Tomalia, D. A.: Genealogically Directed Synthesis: Starburst/Cascade Dendrimers and Hyperbranched Structures. *165* (1993).
Tourillon, G., see Fontaine, A.: *151*, 179–203 (1989).

Ugi, I., see Bley, K.: *166*, 199–233 (1993).

Vögtle, F., see Dohm, J.: *161*, 69–106 (1991).
Vögtle, F., see Knops, P.: *161*, 1–36 (1991).
Vögtle, F., see Ostrowicky. A.: *161*, 37–68 (1991).
Vogler, A., Kunkeley, H.: Photochemistry of Transition Metal Complexes Induced by Outer-Sphere Charge Transfer Excitation. *158*, 1–30 (1990).
Vondenhof, M., see Mattay, J.: *159*, 219–255 (1991).

Wan, P., see Krogh, E.: *156*, 93–116 (1990).
Warwel, S., Sojka, M., and Rüsch, M.: Synthesis of Dicarboxylic Acids by Transition-Metal Catalyzed Oxidative Cleavage of Terminal-Unsaturated Fatty Acids. *164*, 79–98 (1993).
Willner, I., and Willner, B.: Artificial Photosynthetic Model Systems Using Light-Induced Electron Transfer Reactions in Catalytic and Biocatalytic Assemblies. *159*, 153–218 (1991).

Yoshihara, K.: Chemical Nuclear Probes Using Photon Intensity Ratios. *157*, 1–34 (1990).

Zamarev, K. I., see Lymar, S. V.: *159*, 1–66 (1991).
Zamarev, K. I., Kairutdinov, R. F.: Photoinduced Electron Tunneling Reactions in Chemistry and Biology. *163*, 1–94 (1992).
Zander, M: Molecular Topology and Chemical Reactivity of Polynuclear Benzenoid Hydrocarbons. *153*, 101–122 (1990).
Zhang, F. J., Guo, X. F., and Chen, R. S.: The Existence of Kekulé Structures in a Benzenoid System. *153*, 181–194 (1990).
Zimmermann, S. C.: Rigid Molecular Tweezers as Hosts for the Complexation of Neutral Guests. *165*, 71–102 (1993).
Zybill, Ch.: The Coordination Chemistry of Low Valent Silicon. *160*, 1–46 (1991).