The Biosynthesis of Marine Natural Products†

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Contents

Mary Garson was born in Rugby, UK, and graduated (B.A. Hons) from the University of Cambridge in 1974. Her Ph.D. research with Dr. Jim Staunton was concerned with the biosynthesis of polyketide metabolites from soil fungi and in the development of stable isotope methodology (^{13}C, ^{2}H) for studying biosynthetic pathways. She was awarded a Ph.D. in 1977 and then did postdoctoral research at the Universita Cattolica del Sacro Cuore, Rome, with Professor G. B. Marini-Bettolo. From 1978 to 1981, she held a research fellowship at New Hall, Cambridge which allowed her to continue her work in terrestrial biosynthesis. She left academia in 1981 to take up a position as a medicinal chemist at Smith Kline and French Research Ltd (now Smith Kline Beecham Corp.). In 1983, she took the deep plunge into marine research when she went to the Sir George Fisher Centre for Tropical Marine Studies, Townsville, Australia as a Queen Elizabeth II Research Fellow to work on the biosynthesis of sponge and soft coral terpenes. She moved to a lectureship in biological chemistry at the University of Wollongong in 1986 and, in 1990, to the University of Queensland, where she is now a Senior Lecturer in Organic Chemistry. Her research interests span structure, function, and formation of marine metabolites and have involved valuable collaborative work with the laboratories of John Faulkner, Carl Djerassi, Pat Bergquist and Synnove Liaaen-Jensen.

I. Introduction

This article is written as an overview of current marine biosynthetic knowledge and as an extension of previous reviews in which the biosynthetic origins of marine natural products were considered.[1,2] Articles of interest were found by survey of *Biological Abstracts* up to July 1992. Coverage is not restricted solely to secondary metabolites; however, fatty acids, sterols, and other lipids which are usually considered primary metabolites are given less detailed coverage. The majority of the published literature is concerned with intact organism studies, although recent work with algae[3] and on sterol biosynthesis[4] indicate the potential of work with cell-free extracts. The range of experimental techniques together with the practical difficulties inherent in biosynthetic studies on marine organisms have been documented previously.[1] A major difficulty is the low level of incorporation obtained which forces the researcher to use radioisotopes rather than stable isotope methodology. The practical advantages in using stable isotopes over radioisotope work have been documented elsewhere,[5–7] but stable isotopes also provide more detailed biosynthetic information, namely on the specificity of labeling, on the fate of C–H, C–C, C–O, or C–N bonds, and on the operation of multiple biosynthetic pathways.[8,9] A major disadvantage in the use of stable isotopes is that the incorporation of high levels of precursor, required by the inherently lower sensitivity of detection afforded by NMR, also has the potential to alter the metabolic pathway under study.

For convenience, a phylogenetic approach has been adopted in this review. Some of the microorganisms discussed in the article are not strictly marine; freshwater cyanobacteria or microalgae are described which contain natural products extremely similar to those in marine cyanobacteria or microalgae. Some of the bacteria described live in unusual or extreme conditions prevailing at the interface of the terrestrial and marine environments. The origins of marine natural products can be obscured when the host organism, a sponge, coral,

† This review is dedicated to Professor Carl Djerassi, the author's biosynthetic "grandfather", on the occasion of his 70th birthday.

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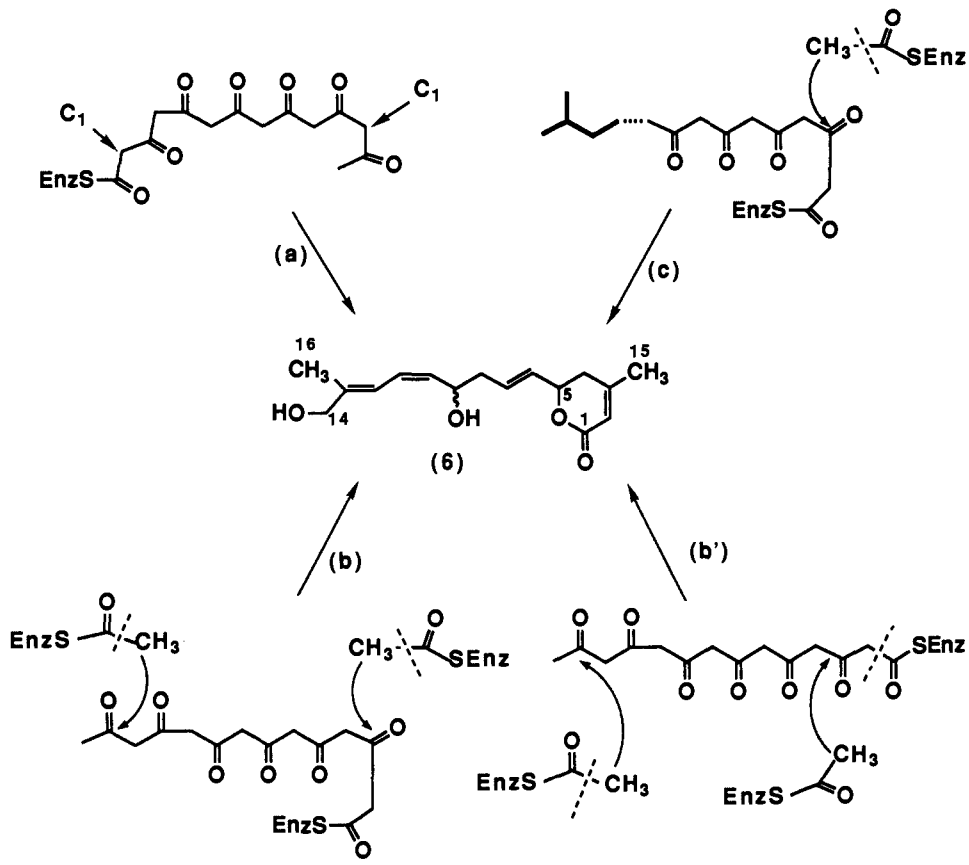
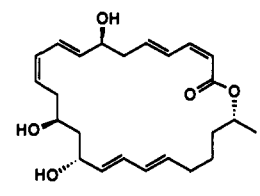
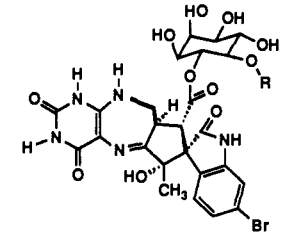
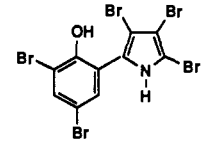
Scheme 1



or mollusk, for example, lives in symbiotic association with a microorganism since the natural product may be a product of symbiont metabolism or of host metabolism or the product of joint metabolism. The possibility of a compound being obtained from dietary sources also exists, thus the complex food webs of the marine environment must be kept in mind when undertaking this type of research. In this review, descriptions of biosynthetic topics are cataloged according to the phylum of the organism used for the biosynthetic experiments, with cross-referencing where appropriate. Finally, a marine natural product may be stored in a different cellular environment to where it was synthesized, and appropriate experiments must be designed to establish whether this is the case or not. These aspects are discussed in more detail in the relevant sections below.


(1)


(2) R = β - D-xyl
(3) R = H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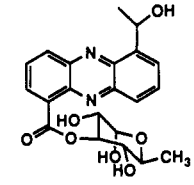
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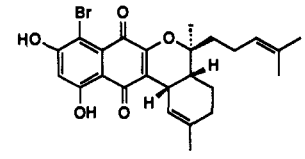
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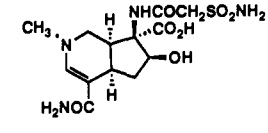
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II. Marine Microorganisms

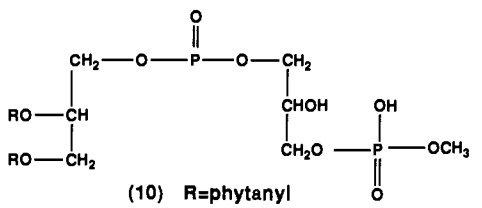
A. Marine Bacteria and Fungi

Marine bacteria represent a relatively unexplored source of unusual, bioactive chemical constituents, and details of their chemistry are reviewed elsewhere in this issue.[10] Products of marine bacterial metabolism isolated to date include macrolactin A (1),[11] neosurugatoxin (2)[12] and prosurugatoxin (3),[13] some brominated pyrroles, e.g. 4,[14] phenazine esters 5,[15] the polyketide oncorhyncolide (6),[16] the naphthoquinone marinone (7),[17] and altemicidin (8);[18] thus it can be seen that a wide range of secondary metabolic pathways appear to operate in the few genera studied.

Biosynthetic studies on terrestrial bacteria, notably actinomycetes, and fungi have contributed enormously to our knowledge of biosynthetic pathways and are of commercial importance.[19,20] Recent work by Andersen *et al.* establishes the feasibility of biosynthetic work with marine bacteria.[21] The biologically-inactive oncorhyncolide (6) can be viewed as an acetate-derived metabolite (Scheme 1), with C-15 and C-16 methyls provided by *S*-adenosyl methionine (SAM) (path a) or by use of propionate instead of acetate. Alternatively, the methyls might derive from addition of a C_2 unit to

carbonyl groups of a heptaketide chain, followed by decarboxylation (paths b or b′) or by use of a non-acetate chain starter unit (path c). Labeling experiments with [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, and [2-^{13}C,2-$^{2}H_3$]acetate have shown that all the carbons of oncorhyncolide are acetate-derived. The presence of seven intact acetate-derived units, plus the incorporation of C-2 of acetate into C-15 and C-16, is consistent with the biosynthesis of the metabolite by paths b or b′. An acetate origin for methyl branchs has been reported before for virginiamycin[22] and myxovirescin[23] and has been suggested for the dinoflagellate metabolites brevetoxins A and B (see section II.C).

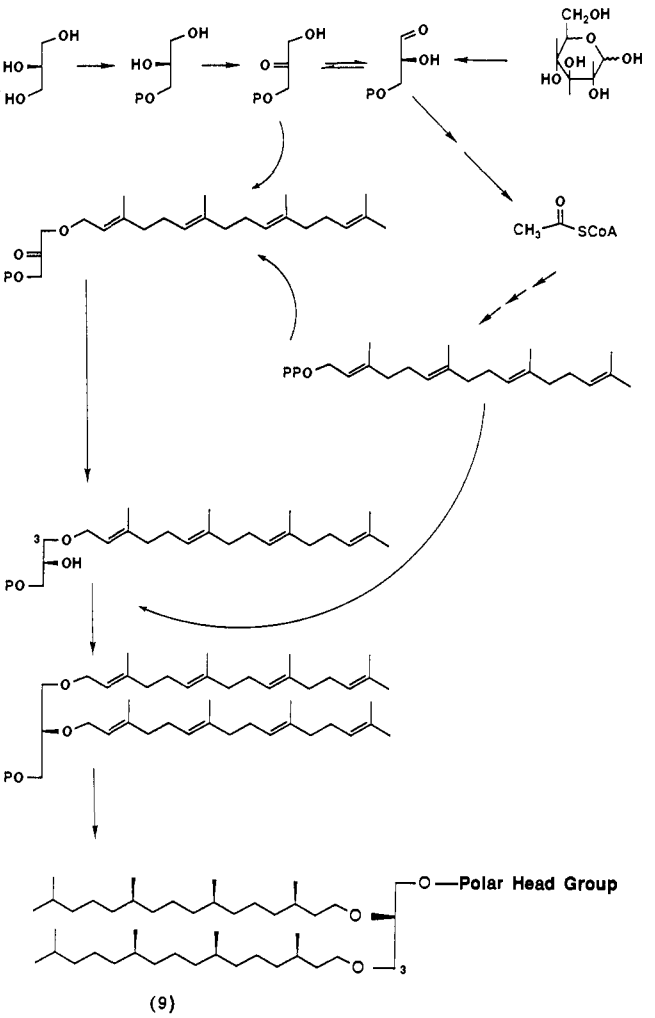
The halophilic bacteria ("extremophiles"),[24] present in hypersaline environments such as salt pans or salt lakes, have unusual cell wall and membrane chemistry and cannot be considered true prokaryotes. They contain ether lipids quite unlike the fatty acid esters found ubiquitously in eukaryotes. Thermophilic bacteria also share this unusual lipid chemistry.[25] Halobacterial lipids are composed of *sn*-2,3-di-*O*-phytanylglyceryl units, with polar groups at the *sn*-C(1) position, which may be biosynthesized by sequential transfer of prenyl groups onto a glycerol acceptor. In early work, Kates established the utilization of glycerol for biosynthesis of the sugar and phytanyl groups as well as the expected incorporation into the glycerol moiety,[26,27] while experiments by Kakinuma *et al.* have involved ^{13}C and ^{2}H NMR analysis of ^{18}O- and ^{2}H-labeled glyceryl ether lipids **9** from *Halobacterium halobium*.[28] The *sn*-C(3) oxygen of glycerol was shown to be retained, in agreement with a nucleophilic displacement of the prenyl group pyrophosphate. Inversion of configuration occurs at C-2 of the glycerol while C-6 of glucose and *sn*-C-3 of glycerol provide the *sn*-C-1 position of the ether lipids. A stepwise alkylation mechanism (Scheme 2) is proposed to explain the results with the diphytanylglyceryl chain originating from isoprenyl intermediates.[27,28] ^{2}H and ^{31}P NMR studies have been used to investigate the conformational and organizational properties of these lipids, and it is believed that the branched phytanyl chains may perform a regulatory function in maintaining membrane fluidity (c.f. marine sponge lipids, section IV).[29] The structure of the major lipid from *Halobacterium halobium* was confirmed to be a methyl ester of phospatidyl glycerophosphate (**10**) following cultivation using [*methyl*-$^{2}H_3$]methionine and mass spectrometric and NMR analysis.[30] Similar results have been obtained for *Halobacterium cutirubrum*,[31] which also utilizes amino acids, notably lysine, for the biosynthesis of lipids.[32] *Halobacterium halobium* is an important source of bacteriorhodopsin; a number of papers report the growth of this archaeobacterium on ^{13}C-labeled amino acids for solid-state NMR studies.[33-36]



(10) R=phytanyl

Pathways of fatty acid synthesis in the marine pathogen *Vibrio* sp. have been investigated; evidence supporting both an anaerobic pathway using a type II fatty acid synthase and a route involving aerobic desaturation has been obtained.[37]

Scheme 2

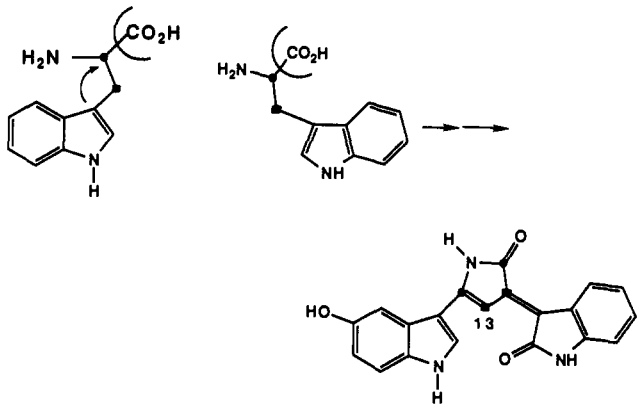


(9)

Bacteria of the genus *Chromobacterium* are characterized by the presence of the blue pigment violacein (**11**); a marine *Chromobacterium* also produces antibacterial brominated pyrroles.[14] The formation of the pyrrolidone ring of violacein has been investigated using ^{13}C isotopes and is known to be constructed from the side chains of two tryptophan units with 1,2-migration of one of the indole units (Scheme 3). The very high incorporation of label (42% from [2-^{13}C]tryptophan) led to couplings between adjacent carbons.[38] H-13 of the pyrrolidone ring derives from the 3-*S* position of tryptophan. The nitrogen of tryptophan is incorporated intact into violacein while all three oxygens derive from molecular oxygen.[39] Labeling studies with 5-hydroxytryptophan established the intermediacy of this compound in violacein biosynthesis, although the timing of decarboxylation remains unknown.[40] It is not yet known whether the same biosynthetic route operates in marine chromobacteria.

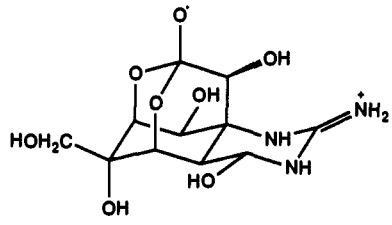
The association of bacterial symbionts with marine organisms is of interest as it may obscure the origin of marine natural products produced by the host. The isolation of the toxin tetrodotoxin (**12**) and related compounds from a range of hosts, including octopus, puffer fish, newts, crabs, and algae[41-50] supports the description of this potent compound as of bacterial

Scheme 3



(11)

origin. Cultures of a *Pseudomonas* sp. isolated from the red alga *Jania* sp. produced tetrodotoxin and anhydrotetrodotoxin (13);[43] since then there have been



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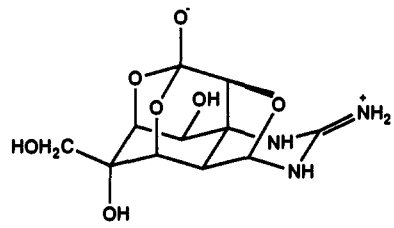
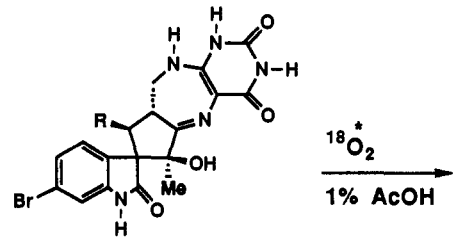


(13)

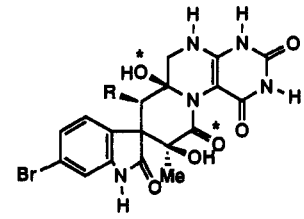
several reports of bacterial sources of tetrodotoxins.[44-48] It has been suggested that the carbocyclic ring in the tetrodotoxin compounds may be produced from an isoprenoid unit.[49,50] Incorporation studies using ^{14}C amino acids, acetate and glucose have given negative results.[42] The surugatoxin family of toxins have been reported to be isolated from bacteria associated with the japanese ivory shell.[51,52] The toxic substances are neosurugatoxin (2) and prosurugatoxin (3) rather than surugatoxin (14) itself. Prosurugatoxin is converted into the nontoxic surugatoxin on treatment with dilute acid in the presence of molecular oxygen. ^{18}O-labeling studies were used to investigate the mechanism of the transformation (Scheme 4).[52] Another family of toxins believed to be of bacterial origin are the saxitoxins; details of their biosynthesis are deferred to section II.B below.

The symbiotic association between sponges and bacteria is of particular interest, given the variety of bioactive metabolites isolated from sponges. Some recent papers describe the isolation of "sponge" metabolites from bacterial cultures. The marine sponge *Tedania ignis* is a rich source of metabolites including tedanolide (15) and the piperazine diones 16–18, all of

Scheme 4

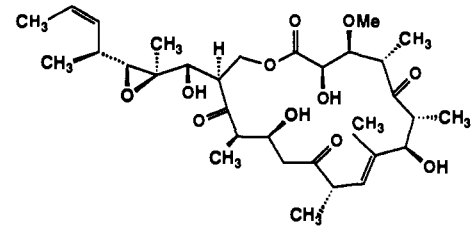


(3) R = CO_2Sugar

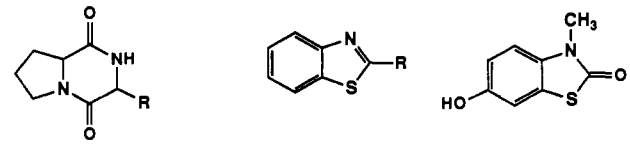


(14) R = CO_2Sugar

which were isolated in trace amounts from the sponge.[53,54] A bacterium tentatively identified as a *Micrococcus* sp. was isolated from *T. ignis* and cultured, providing the piperazine diones 16–18[55] and the four benzothiazoles 19–22;[56] the authors do not indicate whether the same strain of bacteria was responsible for all of these metabolites. Piperazine diones are common products of fungal and bacterial metabolism,[57] but they can be also generated chemically from products derived from the enzymic decomposition of the peptides present in the culture medium. Some biosynthetic evidence in support of the enzymic rather than chemical synthesis of 16–18 would be useful. A bacterial origin for other aromatic secondary compounds from *T. ignis* has been suggested.[58] The isolation of the bacteria presumed responsible for tedanolide production would be of great interest in view of the potent biological activity of tedanolide.[59]



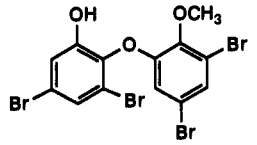
(15)



(16) R= $-CH_2CH(CH_3)_2$
(17) R= $-CH(CH_3)_2$
(18) R= $-CH_3$
(19) R= SH
(20) R=CH_3
(21) R=OH
(22)

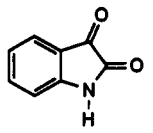
In a second example, Elyakov *et al.* have cultured bacterial isolates from the tropical marine sponge *Dysidea herbacea* which produces bromophenols.[60] Eight pure bacterial cultures were grown on a peptone-based medium and one strain of a *Vibrio* sp. appeared

to contain the brominated phenol (23) by chromatography and mass spectrometry;[60,61] further characterization of the material by NMR is however desirable. Circumstantial support for the role of marine bacteria in the production of brominated compounds is provided by the isolation of marinone (7) by Fenical *et al.* from a marine bacterium[17] and by the isolation of other brominated metabolites from bacterial cultures.[12-14]



(23)

Bacterial isolates cultured from the marine sponge *Ceratoporella nicholsoni* have been compared with those isolated from the surrounding seawater. Sponge-associated bacteria showed greater metabolic diversity.[62,63] A bacterium, *Alteromonas* sp., isolated from the surface of embryos of the shrimp *Palaemon macrodactylus*, produces the antifungal isatin (24) which protects the embryos from fungal infection.[64]



(24)

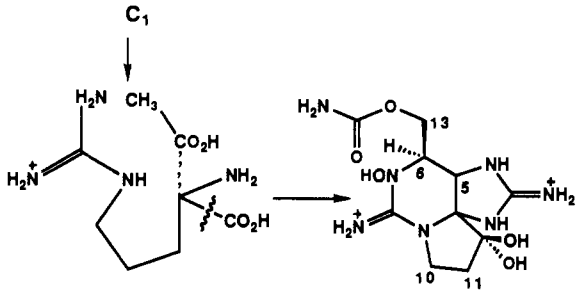
Lipids have frequently been used as markers for bacteria in marine sediments[65] and in marine invertebrates.[66,67] Stable isotope ratio measurements on fatty acids and sterols have been used to investigate the nutrient contribution of bacteria to host metabolism.[67]

B. Cyanobacteria

Cyanobacteria (= blue-green algae) are prokaryotic, photosynthetic organisms which lack cellular organelles and which do not generally contain sterols in their cellular membranes. Toxins from freshwater or brackish water species of cyanobacteria have been responsible for the poisoning of livestock and of humans drinking contaminated waters.[88] Cyanobacterial metabolites show promising cytotoxic, fungicidal, and antiviral activity.[59,69]

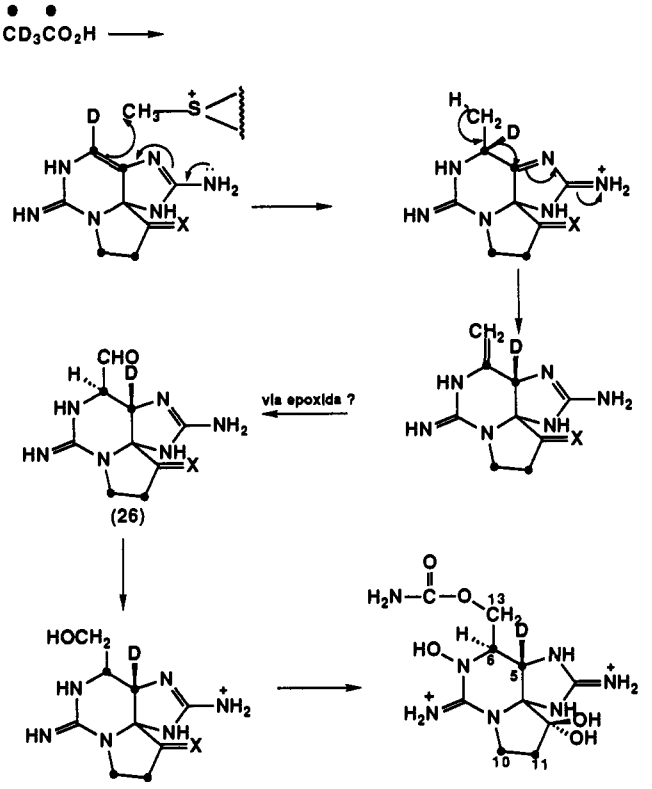
Shimizu *et al.* have studied the biosynthesis of the toxin neosaxitoxin (25) in *Aphanizomenon flos-aquae* and have demonstrated that the tricyclic skeleton is formed from acetate and arginine (Scheme 5).[70,71] Incorporation of [1,2-$^{13}C_2$]3-hydroxypropionate of [^{13}C]-bicarbonate did not result in enrichment of C-13, therefore this carbon did not come from carbon dioxide. The incorporation of [1,2-$^{13}C_2$]glycine did however result in enrichment of the C-13 center, although a labeling experiment with [^{13}C]formate gave a negative result. The feeding of [3-^{13}C]serine and of [*methyl*-^{13}C]methionine confirmed that the C-13 carbon is derived from the C-1 pool; presumably the use of formate as a C_1 donor in this organism is inefficient. The high incorporation of methionine enabled further

Scheme 5



(25)

Scheme 6

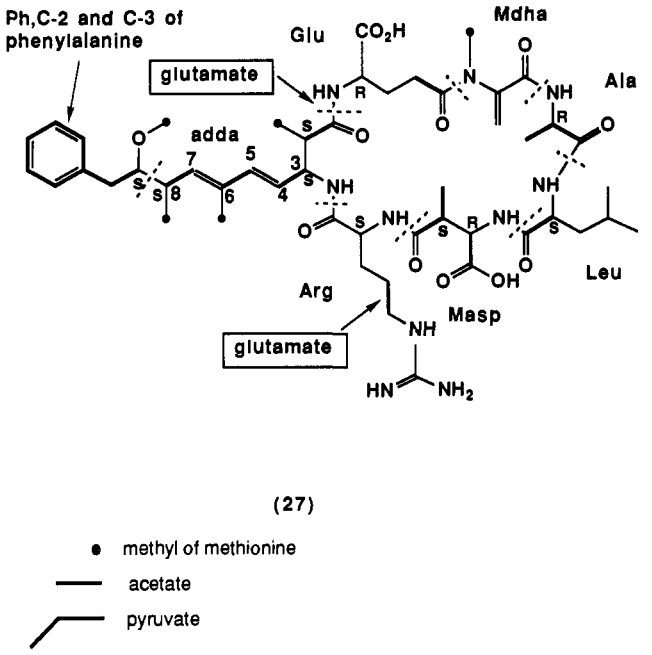


(26)

labeling studies to be carried out. [*methyl*-$^2H_3$,^{13}C]-Methionine gave labeled neosaxitoxin (25) in which the C-13 methylene contained only one deuterium, rather than the expected two deuteriums. Feeding of [2-$^2H_3$,1,2-$^{13}C_2$]acetate provided evidence for the retention of one deuterium at the carboxyl-derived C-5 and which must therefore have originated from C-6 by a hydride shift. A mechanism, involving aldehyde 26, is suggested for the incorporation of methionine, as shown in Scheme 6.[71,72] The origin of the C-13 oxygen atom is thus of interest. Additional peaks at the base of both the C-10 and C-11 signals are visible in the spectrum of the [2-$^2H_3$,2-^{13}C]acetate-enriched material. The C-11 position derives from the 2-position of acetate and may be expected to have retained some deuterium from acetate. The bacterial origin of the saxitoxin metabolites has been established;[44,45,73] these toxins also occur in dinoflagellates as discussed in section II.C.

The microcystins are cyclic heptapeptides responsible for the potent hepatoxicity of certain cyanobacteria, notably *Microcystis aeruginosa* (Kutzing).[74-76] The

Scheme 7

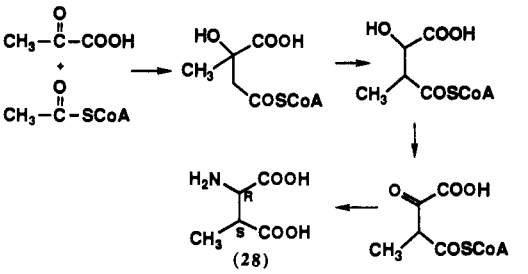


(27)

toxins are characterized by the presence of unusual amino acid units and are thus of biosynthetic interest.[77,78] Incorporation of sodium [1,2-$^{13}C_2$]acetate into microcystin-LR (**27**) revealed that C-1 through C-8 of the (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) unit were polyketide derived (Scheme 7). Although L-[U-^{13}C]-phenylalanine also gave labeled Adda, it was not possible to confirm that the chain starter unit of the Adda unit was phenylacetate. L-[*methyl*-^{13}C]Methionine gave microcystin-LR in which the C-2, C-6, and C-8 methyls were all labeled; however, enrichment of the C-2 methyl was small unless the labeled methionine was provided together with unlabeled acetate. Longer term incorporation of the alga with labeled acetate gave lower incorporations into the C-1/C-2 unit compared with the C-3 to C-8 portion of Adda. These results were explained by the possible involvement of propionate for C-1/C-2 and attached methyl as the acetate supply diminishes; unfortunately propionate and succinate could not be assimilated by the organism to confirm this. The biosynthetic origins of the methyl groups of the fungal polyketide asteltoxin provide a precedent for this biosynthetic alternative.[79] Incorporations of acetate into the γ-glutamate, arginine, and leucine residues were as expected; C-1/C-2 of the iso-linked (2*R*,3*S*)-3-methylaspartate residue (**28**, Masp) were also labeled by [1,2-$^{13}C_2$]acetate, however [1,2-$^{13}C_2$]glutamate failed to label the Masp unit although it did label the arginine and γ-glutamate residues. These results were interpreted in terms of formation of and rearrangement of 2-hydroxy-2-methylsuccinic acid (Scheme 8) and are supported by the incorporation of pyruvate into the Masp unit. Related work on the biosynthesis of the amino acid Masp is reported in section III.C. Preliminary biosynthetic studies on tolytoxin (**29**) have been reported and suggest a polyketide chain is built onto a glycine starter unit (Scheme 9).[78]

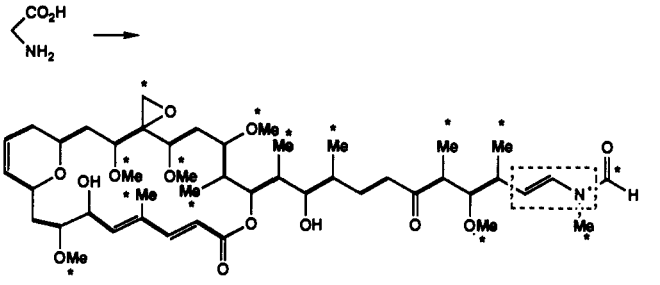
Calophycin (**30**) is a cyclopeptide isolated from the freshwater cyanobacterium *Calothrix fusca* (Kutzing) Bornet and Flahaut and has broad spectrum fungicidal

Scheme 8



(28)

Scheme 9

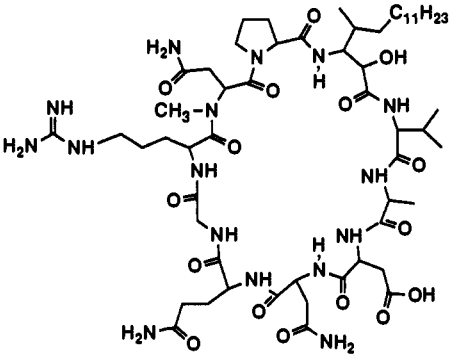


(29)

* = [*methyl*-^{13}C] methionine

$^{13}CH_3$—$^{13}CO_2Na$

activity. The alga was grown on sodium [^{13}C]carbonate and on sodium [^{15}N]nitrate to identify the location of a free carboxyl group as belonging to an aspartate residue rather than a glutamate or a methylaspartate unit. ^{15}N-Coupled carbonyl signals led to the identification of methylasparaginyl, asparaginyl, and glutaminyl units as their amides rather than the free acids.[80] Other cyanobacterial metabolites whose structure elucidation was facilitated by growth of the culture on sodium [^{13}C]bicarbonate or sodium [^{15}N]nitrate include the mirabimides (*Scytonema mirabile*),[81] anatoxin-a(s) (**31**) from *Anabaena flos-aquae*),[82] some polymethoxy-1-alkenes (*Scytonema ocellatum*),[83] and cylindrospermopsin (*Cylindrospermopsis raciborski*).[84]



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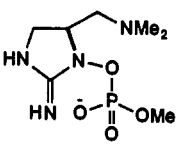
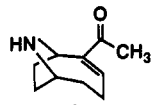


(31)

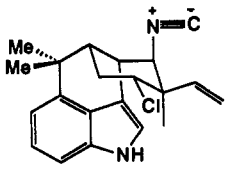
The novel toxin anatoxin-a (**32**) has the same pyrrolidine ring system as the plant tropane alkaloids, but differs by a C_4 rather than C_3 bridging unit. Preliminary biosynthetic studies on **32** have been reported.[85] So-



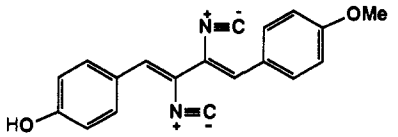
(32)

dium [^{14}C]carbonate, L-[U-^{14}C]proline, L-[U-^{14}C]ornithine, [1,4-$^{14}C_2$]putrescine, and [U-^{14}C]acetate all gave low (<0.02%) incorporations after 64-h incubations. A higher incorporation of [U-^{14}C]ornithine was obtained if the culture was made nitrogen-deficient before incubation. Replacement of sodium nitrate by ammonium chloride increased the yield of anatoxin-a, as did reducing the K^+/Ca^{2+} ratio. Incorporation of labeled ornithine in the presence of unlabeled putrescine resulted in an increased yield of anatoxin-a, but with lower radioactivity, therefore putrescine was an intermediate on the pathway between ornithine and **32**. The enzyme ornithine decarboxylase (EC 4.1.1.17) was partially purified on DEAE-cellulose. Some evidence was obtained that the genetic material encoding toxin production was plasmid-derived. In particular, DNA from a toxic strain of *Anabaena flos-aquae* transformed a nontoxic strain into a toxic strain.

Work on the biosynthesis of cyanobacterial isonitriles[88] has been reviewed previously.[1] The isonitrile carbon of the terrestrial cyanophyte metabolite hapalindole A (**33**) derives from the C_1-THF pool (c.f. hazimycins[87]), in contrast to marine-sponge isonitriles in which the isonitrile substituent is derived from cyanide.[88-90] A recent report by Herbert *et al.* shows that the isonitrile carbons of the terrestrial fungal metabolite xanthocillin-X monomethyl ether (**34**) derive from glucose; [U-^{13}C]glucose, but not [1-^{13}C]glucose, labeled the two isonitrile carbons.[91] The origin of isonitriles is thus a clear example of differences in marine and terrestrial metabolism. Further members of the fungicidal hapalindole alkaloids have been reported from *Fischerella ambigua*, *Hapalosiphon hibernicus*, and *Westiellopsis prolifica* (Stigonemataceae)[92] and an aerial form of *Scytonema mirabile* (Scytonemataceae) produces isotactic poymethoxy-substituted alkene isonitriles[93] which represent a biosynthetic modification of the polymethoxy alkenes from *Scytonema ocellatum*.[83] *H. hibernicus* also produces microcystin-LA.[94]

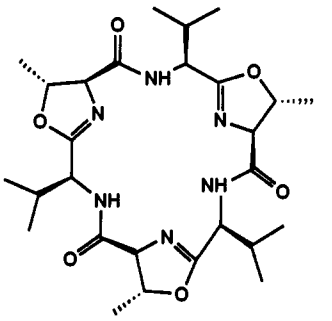


(33)



(34)

The isolation of a bistratamide analogue, the cyclic peptide **35** from the terrestrial cyanobacteria *Westiellopsis prolifica*,[95] provides circumstantial evidence for the algal production of these cytotoxic peptides in ascidians.[96] Further details are described in section VII.



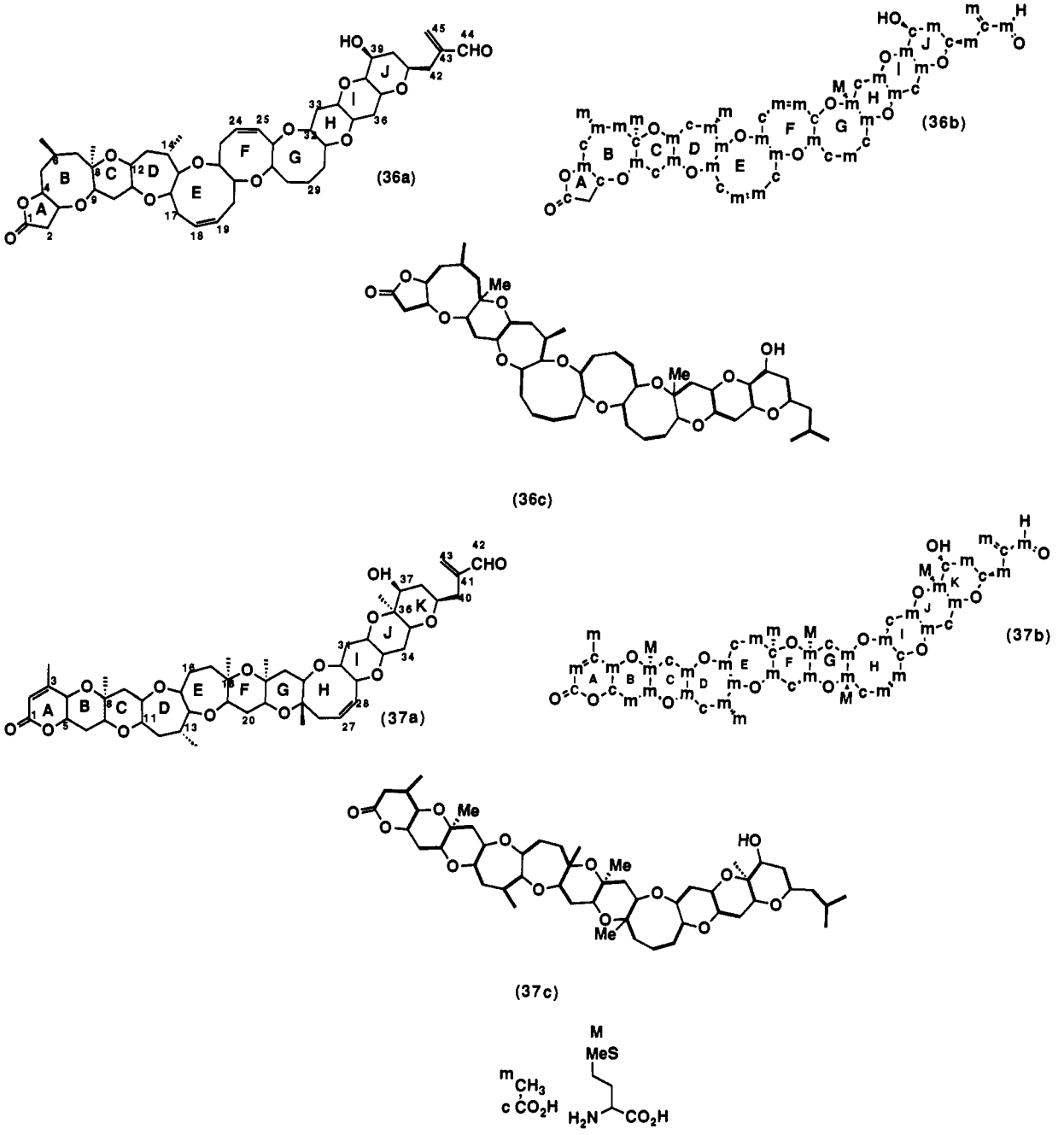
(35)

C. Dinoflagellates and Diatoms

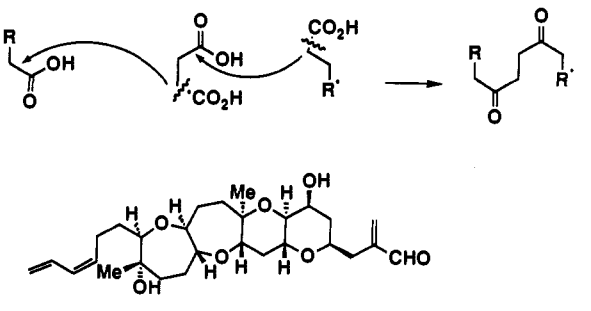
Dinoflagellates are primitive unicellular microalgae which are an important component of phytoplankton. The presence of nitrogenous toxins in dinoflagellates is a serious environmental problem of commercial importance; there are numerous reports of the toxic, diarrhetic, or paralytic effects of shellfish contaminated with the microorganisms.[72] Early attempts to study the biosynthesis of the toxins were hampered by the slow growth of the dinoflagellates and by their inability to take up exogenous organic materials. Shimizu *et al.* have studied the biosynthesis of neosaxitoxin (**25**) in the dinoflagellate *Gonyaulax* (= *Alexandrium*[97]) *tamarensis*,[70] but more recent work utilized the freshwater cyanobacterium *Aphanizomenon flos-aquae* (see section II.B above for details).

The dinoflagellate *Gymnodinium breve* (*Ptychodiscus breve*) is the "red tide" organism responsible for fish kills and for human poisoning through eating contaminated shellfish. The two major toxins are brevetoxin A (**36a**) and B (**37a**), of which A is the most toxic (Chart 1); both exert their biological effect by acting on sodium channels. Both toxins possess a polycyclic *trans*-fused ether ring system and are of biosynthetic interest given their structural resemblance to terrestrial polyether antibiotics such as monensin-A and salinomycin; feeding studies in these organisms are complicated by the semiheterotrophic nature of the dinoflagellates.[78] Initially, the toxins were believed to originate from a single polyketide chain with the methyl groups deriving either from the use of propionate as an alternative to acetate or by methylation of an acetate-derived skeleton. Experimental work by the Nakanishi[98] and Shimizu[99,100] groups using labeled acetate and methionine established the labeling pattern of brevetoxin B (see **37b**) and necessitated the modification of earlier biosynthetic proposals. The data suggested that the brevetoxins were mixed polyketides whose biosynthesis involved the utilization of dicarboxylic acids generated by passage of acetate in the citric acid cycle (Scheme 10). Further evidence for the involvement of the citric acid cycle is the presence of extraneous ^{13}C–^{13}C couplings after extended feeding experiments with [1-^{13}C]acetate, consistent with the involvement of [^{13}C]CO_2. Experiments with [2-^{13}C]acetate gave additional couplings explained by the involvement of [2,3-$^{13}C_2$]oxaloacetate. The 13 Me and C-13 and C-14 carbons are all derived from the methyl group (m) of acetate and this was explained by the involvement of propionate in which each carbon derives from C-2 of acetate, i.e., labeled (m–m–m) as in **37b**. Methylation

Chart 1



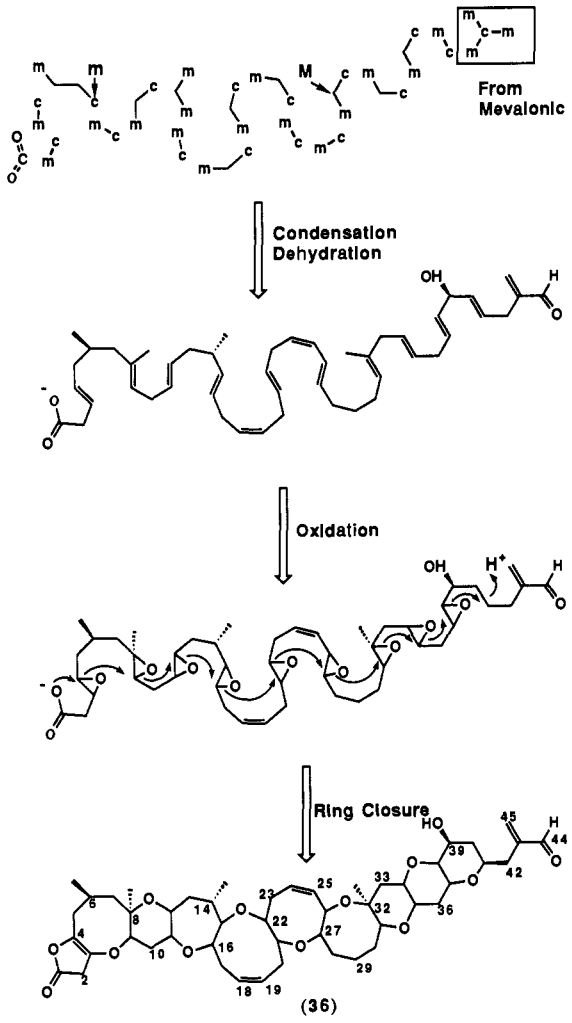
Scheme 10



(38)

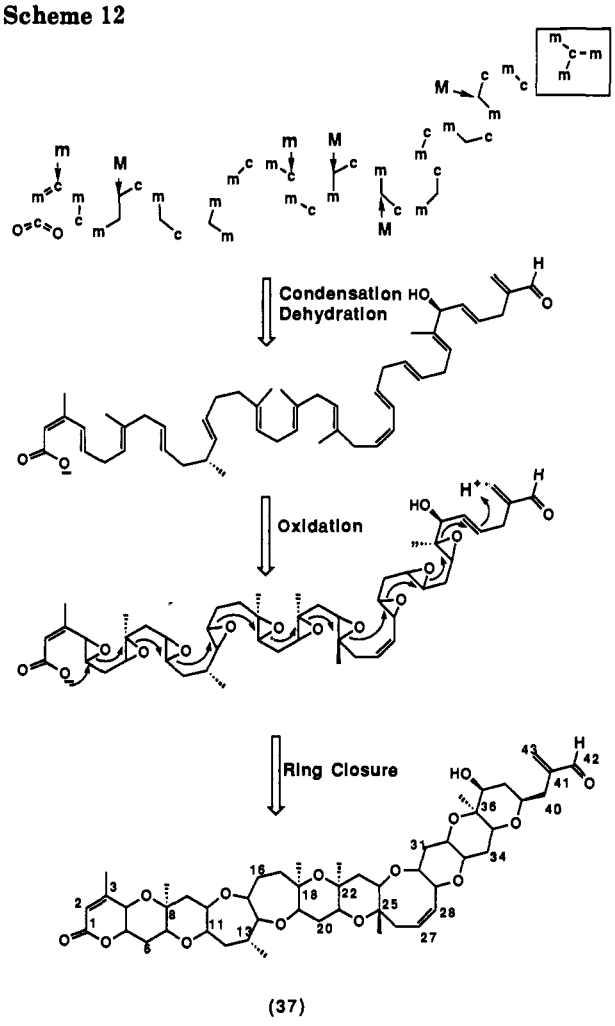
of C-8, C-22, C-25, and C-36 represent standard modifications of polyketide chains using methionine, while the methylations at C-3 and C-18 represent addition of an acetate methyl carbon to a carboxyl-derived carbon and may result from the involvement of 3-hydroxy-3-methylglutarate and of an isoprenoid unit respectively.[99] Alternatively an acetate unit adds to C-3 and C-18 and the carboxyl group is lost by decarboxylation,[101] as has been shown for virginiamycin,[22] myxovirescin,[23] and more recently the marine bacterial metabolite oncorhyncolide (**6**).[21] The detection of a weak ^{13}C–^{13}C coupling between C-2 and C-3 of brevetoxin B, but not between C-1 and C-2, was suggestive that the latter explanation might be correct and raised questions about the mode of introduction of C-1. The labeling pattern of C-40 through C-42 and the C-41 methylene is consistent with the use of an C_4 unit derived from decarboxylation of an isoprenoid unit. The pattern of incorporation of acetate and methionine into brevetoxin A has been determined[101] and the overall labeling pattern of **36a** is similar to that of brevetoxin B. Of the four methyls, that at C-6 represents part of a C-2 of acetate-derived propionate unit (m–m–m),

Scheme 11



(36)

Scheme 12



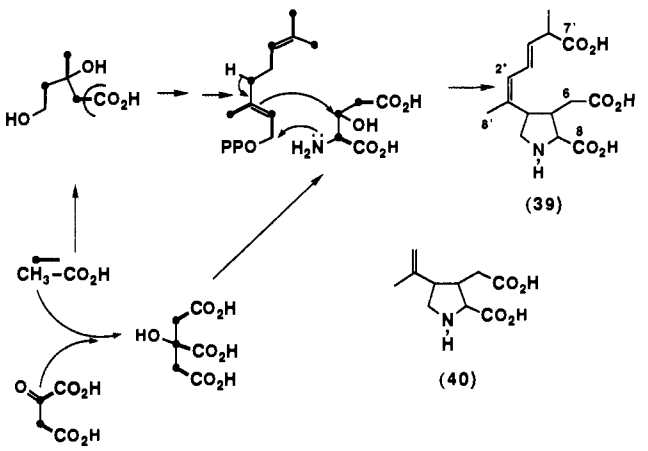
(37)

and that at C-14 presents part of a four carbon unit derived entirely from C-2 of acetate (m–m–m–m). The C-8 methyl may come from an acetate unit which has lost its carboxyl partner while the C-32 methyl is methionine-derived. As in brevetoxin B, the side chain is probably isoprenoid. Another similarity to **37b** is the C-1 carbon which appears to derive from the carboxyl group of acetate. The involvement of a malonyl unit in the formation of C-1 to C-3 in each metabolite was therefore suggested and implied a key role for carbon dioxide in providing C-1 or C-3. Feeding of sodium [^{13}C]bicarbonate resulted in extensive scrambling of label because of the photosynthetic capability of *G. breve*, but an enhancement of the signal for C-1 over that of other quaternary centers was supportive of the proposed role for carbon dioxide.[101] It would be interesting, but experimentally difficult, to test the presumptive role of malonate through precursor incorporation.

Neither the Nakanishi or Shimizu group were able to obtain useful incorporation of succinate, highlighting the difficulties in incorporation work with dicarboxylic acids and with nonheterotrophic organisms. However Nakanishi has reported preliminary data on the utilization of propionate and mevalonate.[101] A low incorporation of [1-^{14}C]propionate was detected by radioactive scanning of HPLC fractions, but the incorporation level was too low to enable use of [^{13}C]-propionate or of its *N*-acetylcysteamine ester. Incorporation of (*RS*)-[2-^{14}C]mevalonolactone was indicated by radioactive scanning of HPLC fractions, but, like the propionate experiment, cannot be taken to represent a genuine incorporation until the metabolites of interest have been purified to constant radioactivity. A ^{13}C experiment with mevalonate was unsuccessful. Suggested outlines of the biosynthetic pathways leading to brevetoxin A and B are shown in Schemes 11 and 12, respectively. The epoxide cascades indicated are of interest given the current knowledge concerning monensin biosynthesis[102] and the proposal of Townsend concerning oxidative cyclization and polyene stereochemistry.[103] The structure of the recently-isolated hemibrevetoxin-B (**38**) supports the above biosynthetic proposals and confirms the availability of polyene intermediates in *G. breve*.[104] Recent synthetic studies by Martín *et al.* provide models for the stereochemical outcome of polyepoxide cyclizations.[105] Further discussion of the biosynthesis of branched chain polyketides is found in section VII.B, while another article in this issue also discusses the biosynthesis of microalgal toxins.[106]

Domoic acid (**39**) is a neuroexcitatory amino acid isolated from the diatom *Nitzschia pungens* and was the causative agent of an outbreak of shellfish poisoning on the east coast of Canada in 1987.[107] It has also been implicated in bird poisoning. The toxin shows structural resemblance to kainic acid (**40**), a metabolite of several marine macroalgae,[108] and may share a common biosynthetic pathway in which an isoprenoid unit (C_{10}

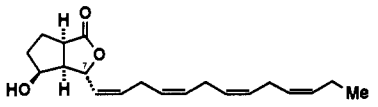
Scheme 13



(39)

(40)

for domoic acid or C_5 for kainic acid) condenses with an activated C_5 unit, possibly 3-hydroxyglutamate, obtained from operation of the citric acid cycle (Scheme 13). Incorporation of [1-^{13}C]acetate under pulse feeding conditions gave domoic acid labelled at C-7 and to a lesser extent C-8. The small amount of material available precluded determination of the labeling pattern of the isoprenoid portion. Incorporation of [1,2-$^{13}C_2$]acetate gave rise to labeled acetate units at C-2/C-8, at C-6/C-7, and at the expected positions of the isoprenoid chain (Scheme 13). Additionally, C-3, C-2′, and C-7′ were enriched. A highly incorporation into the C-6/C-7 unit over the C-2/C-8 unit and over the geranyl unit was interpreted in terms of the use of two distinct pools of acetate, one for the TCA cycle and one for the isoprenoid pathway.[109] Other metabolites isolated from *N. pungens* include the arachidonic acid derivatives bacillariolides I (**41**) and II (**42**). It is not

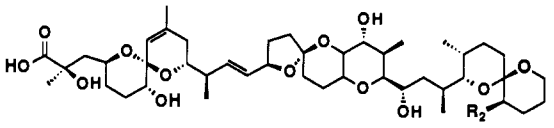


(41)

(42) epimer at C-7

yet clear whether the presence of these metabolites is related to the toxic effect of domoic acid. There is evidence that domoic acid alone may not be responsible for the observed toxic effects.[110] Analysis of the lipid composition of *N. pungens* reveals an unusually high level of the fatty acid C16:4n1 which therefore represents a useful marker for the presence of this toxic diatom in plankton blooms.[111] Flow cytometry is a useful method for the analysis of phytoplankton blooms[112-114] which has been used in the isolation of *N. pungens* gametes.[112]

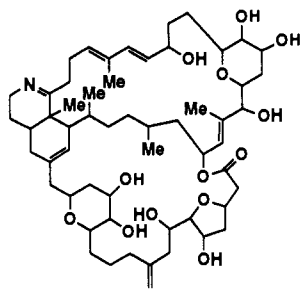
Okadaic acid (**43**) is a potent inhibitor of protein phosphatase-1 and -2a *in vitro* and also has tumour-promoting activity, but does not activate protein kinase C *in vitro*. Okadaic acid and related compounds such as the 35-methyl derivative dinophysistoxin-1 (**44**) have been isolated from a number of marine sponges,[115-117] but the real source of these compounds appears to be from dinoflagellates such as *Prorocentrum lima*, *Dinophysis fortii*, and *Dinophysis acuminata*.[118,119] Differences in the toxicity of mussels on a worldwide basis



(43) R=H

(44) R=CH$_3$

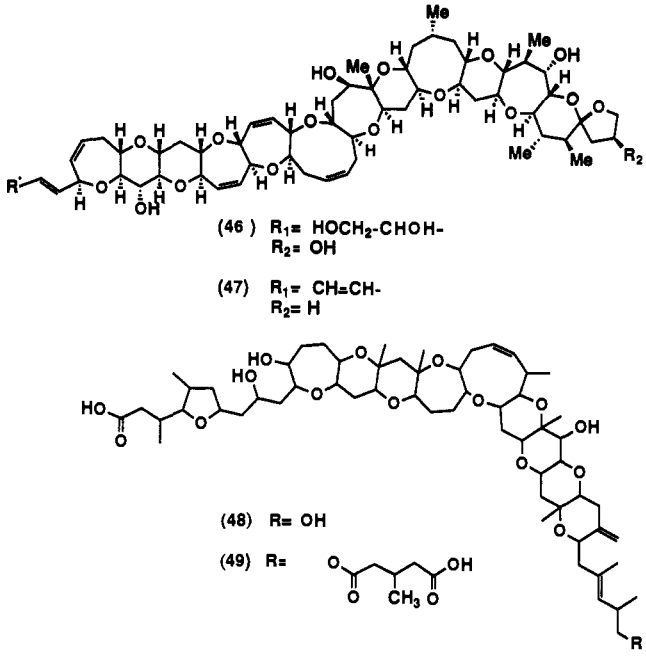
appear to result from different dinoflagellate strains being present and in the differing toxicities of the individual toxins.[120] *Prorocentrum lima* also produces the unusual macrocycle prorocentrolide (**45**), revealing the interesting biosynthetic capability of this organism.[121] Okadiac acid is responsible for diarrhetic shellfish poisoning and possesses a polyether structure as in brevetoxins, ciguatoxin and the terrestrial polyethers monensin, lasalocid, and related compounds. Details of the ^{1}H and ^{13}C assignments of okadaic acid have appeared.[122] The biosyntheses of okadaic acid (**43**) and prorocentrolide (**45**) have been studied in *P. lima* using stable isotopes; preliminary results support the involvement of acetate, hydroxymethyl glutarate and dicarboxylic acids such as citrate or succinate.[78]



(45)

Ciguatoxin (**46**)[123] and maitotoxin[124] are the toxic principles of the dinoflagellate *Gambierdiscus toxicus* obtained from the moray eel *Gymnothorax javanicus* and are the causative agents of ciguateria poisoning from ingestion of fish from tropical waters. They resemble the brevetoxins in having a ladder-shaped polyether skeleton; ciguatoxin has a similar mechanism of action to the brevetoxins, although it is 2 orders of magnitude more potent. A second toxin gambiertoxin-4*b* (**47**), which is less oxidized than ciguatoxin, may represent a biosynthetic precursor.[123,125] A strain of *Gambierdiscus toxicus* produces the potent antifungal compounds, the gambieric acids **48** and **49**.[126,127]

The sterols of dinoflagellates have been recently reviewed[128] and are of biosynthetic interest since many filter feeders obtain sterols from their planktonic diet.[1,2,128,129] All sterol-producing organisms can be divided into two groups, depending on whether they cyclize squalene oxide (**50**) to lanosterol (**51**) or cycloartenol (**52**) (Scheme 14). Cell-free preparations from five different dinoflagellates convert squalene to lanosterol, in contrast to preparations from other microalgal or macroalgal species which gave cycloartenol.[130] Therefore photosynthetic organisms do not always produce cycloartenol, as had been proposed earlier.[131] Details of sterol side-chain biosynthesis, in particular the roles of sterol methyltransferases, in cell-free extracts of the dinoflagellates *Cryptothecodinium cohnii* and *Peridinium foliaceum* have been evaluated by the Djerassi



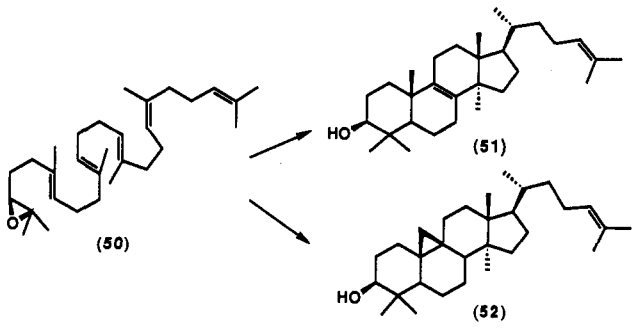
(46) R_1= $HOCH_2$-CHOH-
R_2= OH

(47) R_1= CH≡CH-
R_2= H

(48) R= OH

(49) R=

Scheme 14

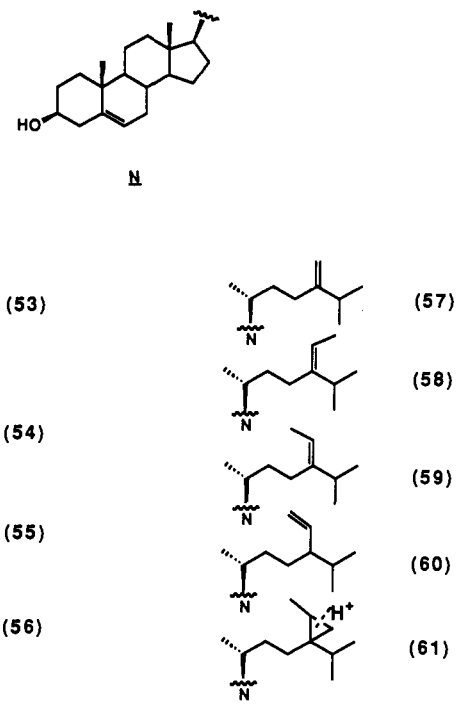


group.[4,132] Incorporation of [^{14}C]acetate and the [^{14}C]-labeled fatty acids, C18:0 and C18:1(9), into the lipids of *C. cohnii* has been accomplished. Both preformed exogenous acids and those synthesized *de novo* from acetate were incorporated into polar lipids rather than into triacylglycerols.[133]

D. Miscellaneous Microorganisms

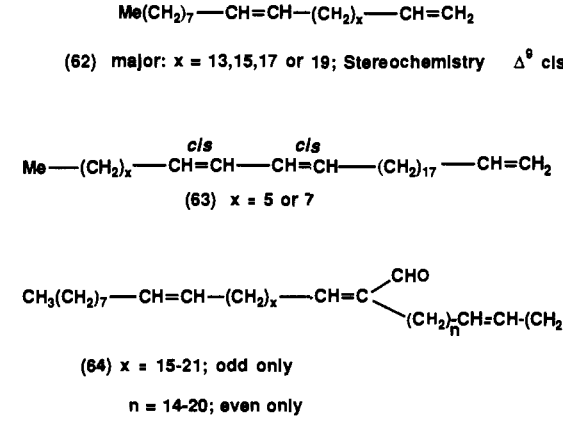
Sterols of the crysophyte alga *Chrysoderma mucosa* have been the subject of two biosynthetic investigations. In common with other microalgae, and in contrast to dinoflagellates, cell-free extracts from this microalga produce cycloartenol as their primary squalene cyclization product.[130] The common marine sterol 24-propylidenecholesterol (**53**, Chart 2) is also produced by *C. mucosa* as are the cyclopropane sterols **54** and **55**; details of the biosynthetic transformations which occur during the formation of **53** have now been published.[134] Desmosterol (**56**), 24-methylenecholesterol (**57**), and isofucosterol (**58**) were all identified as intermediates, while fucosterol (**59**) and 24-vinylcholesterol (**60**) were not incorporated. Evidence for the involvement of the protonated cyclopropane intermediate **61** is presented. Details of the cell-free preparation are provided; the microscale incubation experiments necessitated the use of high specific activity tritiated precursors over ^{14}C precursors. Also noteworthy was the fact that the enzyme preparation gave low incorporations of advanced sterol precursors but useful incorporations of

Chart 2



S-adenosylmethionine. Further details are to be found elsewhere in this issue.[4] The incorporation of radiolabeled carbon dioxide, acetate, and oleic acid into fatty acids of the marine cryptophyte *Chroomonas salina* has been reported.[135] Changes to the fatty acid and sterol composition of microalgae in the presence of hydrocarbons have been reported.[136]

The freshwater, unicellular chlorophyte *Botryococcus braunii*, also found in brackish and saline environments,[137] is a hydrocarbon-rich algae with potential use as a fuel source. Three chemically-distinct strains of algae have been isolated, the A, B, and L races, although all three are morphologically similar. The A race produces C_{23}–C_{31} dienes **62**, with terminal unsaturation and with a *cis*-Δ^9 double bond, which have been shown to derive from oleic acid (Δ^9-18:1) via an elongation–decarboxylation pathway. Some strains convert oleic acid to elaidic acid and thereby produce alkadienes in which the Δ^9 double bond is *trans* rather than *cis* together with *trans* monoenoic acids.[138] Another strain of the A race produces non-isoprenoid methyl-branched fatty acids and the corresponding aldehydes which the authors feel may reflect the involvement of methyl malonate in their biosynthesis.[139] Substantial amounts



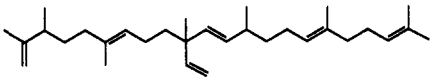
(62) major: x = 13,15,17 or 19; Stereochemistry Δ^9 cis

(63) x = 5 or 7

(64) x = 15-21; odd only

n = 14-20; even only

of C_{29} and C_{31} alkatrienes **63** also occur in the A race and have double bonds in the terminal, Δ^7 and Δ^9 positions. Incorporation experiments have shown that linoleic acid $\Delta^{6,9}$-18:2, a major dienoic acid in plants, but present only in trace amounts in the alga, is not the alkatriene precursor under normal growth conditions; rather, the trienes originate from oleic acid with the additional desaturation occurring during the elongation–decarboxylation process rather than at the alkadiene stage.[140] Investigations of higher molecular weight materials in the algae have led to the characterization of C_{52}–C_{64} α-branched, α,β-unsaturated aldehydes, the botryals (**64**), and their epoxy derivatives,[141,142] possibly formed by head-to-head aldol condensation of Δ^9-unsaturated aldehydes, followed by dehydration. Incorporation of [1,2-$^{13}C_2$]acetate gave labeled botryals,[141] and their biosynthesis from alkadiene precursors has also been investigated.[143] An abnormal biosynthetic pathway resulting in the formation of alkadienones was detected when alkadienes were added to the culture medium. Other hydrocarbons isolated from the A race include botryococcoid ether lipids,[144] biosynthesized from epoxy precursors,[145,146] and *n*-alkylphenols.[147] The decarbonylase activity of *B. braunii* has been investigated.[148] The B race produces branched C_{30}–C_{37} hydrocarbons, the botryococcenes (**65**) which result from the dimerization of farnesol with methylation of the resulting triterpene by *S*-adenosylmethionine. The mechanism of the dimerization process is analogous to squalene biosynthesis and has been investigated by incorporation of tritiated and deuteriated farnesols.[149] The isolation of braunicene (**66**) and related methylenecyclohexanes are of mechanistic interest[150–152] while botryococcenone (**67**), the first oxygenated botryococcene, has been isolated from an Australian strain.[153] Race L produces the tetraterpene lycopadiene (**68**).[154] The carotenoids of races B and L have been investigated.[155] [^{14}C]Acetate is incorporated into lipids and hydrocarbons of *B. braunii* at a low rate compared to [^{14}C]CO_2 incorporation.[156]



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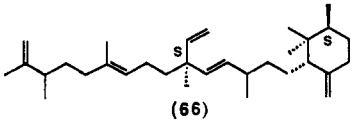

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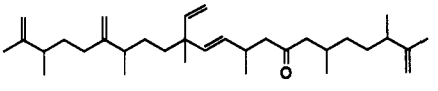

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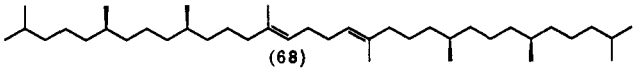
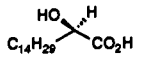


(68)

III. Macroalgae

A. Chlorophyta

Chlorophyta are underrepresented as candidates for biosynthetic studies, but this is not surprising in view of the very few secondary metabolites isolated from the phylum.[157] The isolation of 2(*R*)-hydroxyhexadecanoic acid (**69**) and 2-oxohexadecanoic acid (**70**) from



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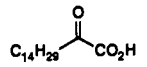

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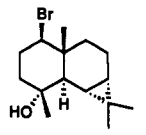
the green alga *Ulva perfusa* is of interest as long-chain aldehydes exemplified by (8*Z*,11*Z*,14*Z*)-heptatrienal[158] has been implicated as contributors to the flavor of this alga which is consumed commercially in Japan. Incubation of an algal homogenate with palmitic acid led to increased amounts of oxygenated fatty acids, while addition of [1-^{13}C]palmitate gave ^{13}C-labeled acids.[159] ^{31}P and ^{14}N NMR studies on phosphorus and nitrogen metabolism in *Ulva lactuca* have been reported.[160,161]

Halogenated sesquiterpenes **71–73** have been isolated from the calcareous green alga *Neomeris annulata*,[162] and are of biosynthetic interest since 1(*R*)-bromo-*ent*-maaliol (**71**) has been proposed as a biosynthetic precursor of heterocladol (**74**) and oppositol (**75**).[163] Neomanerol (**72**) has an unusual substitution pattern.[162] Previously, only halogenated phenols and quinones had been isolated from the chlorophyta.[156]



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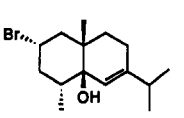

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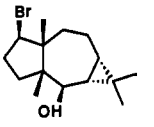

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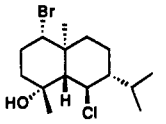

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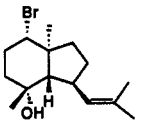

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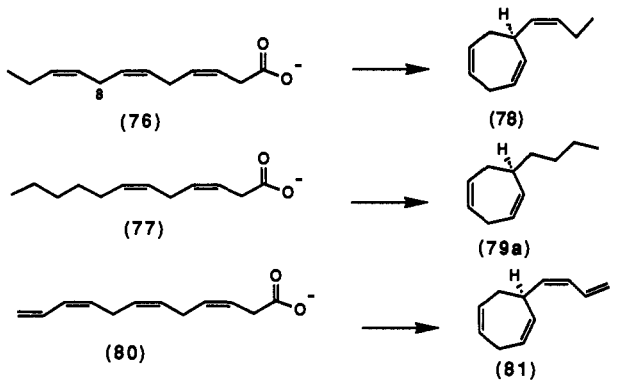
B. Phaeophyta

Brown macrophytic algae, notably those from temperate regions, are rich in phenolic metabolites while tropical species contain characteristic nonpolar metabolites, notably diterpenes.[157] Physical and biological factors responsible for the striking chemical differences between temperate and tropical brown algae have been discussed by Steinberg *et al.*[164] The diterpenes reported from the common brown alga *Dictyota dichotoma* fall into three distinct groups, but the chemical differences reported for populations of this alga on a worldwide basis suggest that taxonomic revision is needed.[165]

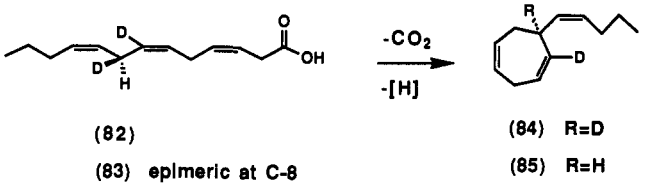
The pheremonal role of volatile hydrocarbons in sexual reproduction of brown algae has led to an interest in their biosynthesis[1] and to model studies with plants of the family *Compositae* which fortuitously produce the same metabolites.[166] (3*Z*,6*Z*,9*Z*)-Dodeca-3,6,9-trienoic acid (**76**) and (3*Z*,6*Z*)-dodeca-3,6-dienoic acid (**77**) have been shown experimentally to serve as precursors of $C_{11}H_{16}$ and $C_{11}H_{18}$ plant hydrocarbons such as ectocarpene (**78**) and dictyotene B (**79a**) isolated from *Senecio isatideus* (Scheme 15),[166] while (3*Z*,6*Z*,9*Z*)-dodeca-3,6,9,11-tetraenoic acid (**80**) may be a likely precursor for the $C_{11}H_{14}$ hydrocarbons such as **81** isolated from this plant. The precursor C_{12} acids **76**, **7**, and **80** have been assumed to derive from oxidation

Scheme 15



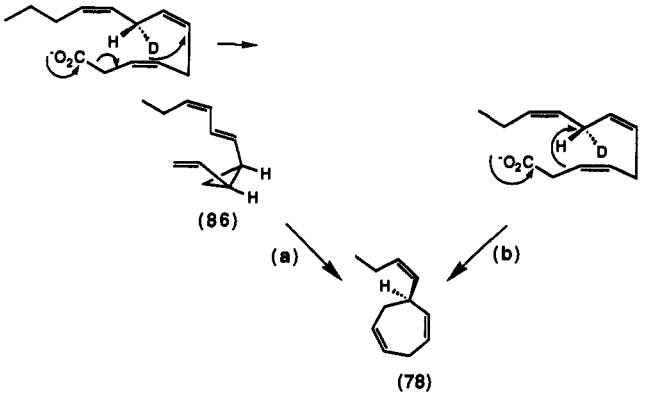
of C_{18} fatty acids; however, experiments were unable to substantiate a biosynthetic connection between the C_{18} acids and the plant-derived hydrocarbons.[166] Experiments with C_{11} and C_{13} deuterium-labeled fatty acids have given rise to ectocarpene analogues and established that the biosynthetic path to ectocarpene (**78**) in *Senecio isatideus* involved removal of the C-8 hydrogen from the fatty acid precursor. Fragmentation to a cyclopropane and carbon dioxide followed by a homo-Cope rearrangement may then generate ectocarpene.[166] In recent work on the biosynthesis of ectocarpene, (8*S*,3*Z*,6*Z*,9*Z*)-[7,8-$^2H_2$]trideca-3,6,9-trienoic acid (**82**) and its 8*R* isomer (**83**) were supplied to *Senecio isatideus* with loss of protium or deuterium, respectively, from C-8 of the trienoic acids (Scheme 16);

Scheme 16

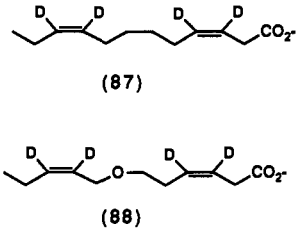


although **82** and **83** are unnatural fatty acids, they were metabolized in exactly the same way as the natural fatty acid precursors and thus yield the ectocarpene analogues **84** and **85**, respectively.[167,168] The biosynthesis of **84** and **85** and, by analogy, that of ectocarpene (**78**) from dodeca-3,6,9-trienoic acid (**76**), involves exclusive cleavage of the C(8)–H_{re} bond. In addition to the cyclopropane route to ectocarpene (Scheme 17,

Scheme 17



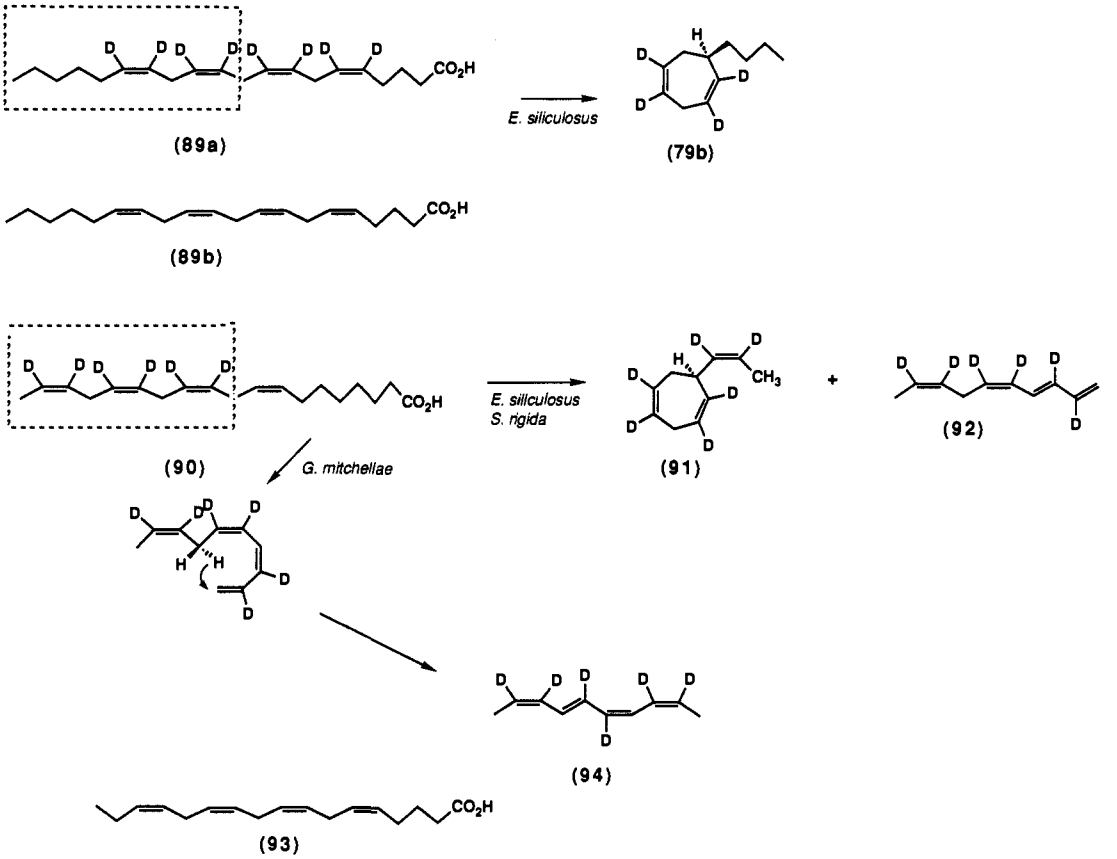
path a), a direct cyclization of C-2 onto C-8 can be envisaged (Scheme 17, path b). The known absolute configuration, 6*S*, of ectocarpene requires that either of these cyclizations occur on the *si* face of the precursor unsaturated acid. The timing of the cleavage of the C_8–H bond is in fact unclear; for convenience it is shown as the initial step in the utilization of the fatty acids in Scheme 17. To distinguish between the cyclization modes represented by paths a and b, incorporation of [3,4,9,10-$^2H_4$]undeca-3(*Z*),9(*Z*)-dienoic acid (**87**) and of [3,4,9,10-$^2H_4$]7-oxaundeca-3(*Z*),9(*Z*)-dienoic acid (**88**),



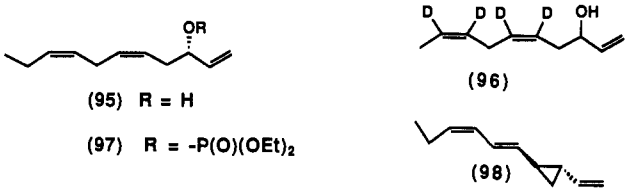
both analogues of dodecatrienoic acid but lacking the central 6*Z* double bond, was carried out. Under conditions in which the plant's enzyme system was shown to be functioning normally, neither of these artificial substrates was converted to product, thereby demonstrating the important role of the central C-6 double bond in the biosynthesis of ectocarpene; the data support the involvement of cyclopropane intermediate **86** formed by overlap of the C-3 and C-6 double bonds, as in path a, rather than direct cyclization of C-2 onto the C-8 double bond, as in path b of Scheme 17.[168] Syntheses of **82**, **83**, and related decadienoic acids from [2,3-$^2H_2$]-*trans*-2,3-epoxyalk-4-yn-1-ols, prepared by assymetric epoxidation, have been described.[167]

By analogy with the plant work, the biosynthesis of algal hydrocarbon pheremones was also assumed to be initiated by the oxidation of unsaturated C_{18} acids to precursor C_{12} acids, but recent experiments have shown that in algae C_{20} rather than C_{18} acids are the precursors. Incorporation of [$^2H_8$]arachidonic acid (**89a**) by female gametes of *Ectocarpus siliculosus* provides [$^2H_4$]-dictyotene B (**79b**), implying the utilization of C-10 through C-20 of (**89a**), while the [$^2H_6$]C_{19} analogue of eicosapentaenoic acid (**90**) provides hydrocarbons **91** and **92** (Scheme 18); in contrast to the plant work, incubation with dodeca-3,6,9-trienoic acid (**76**) or with deuteriated homologues did not result in the formation of ectocarpene or analogues in *E. siliculosus*. Therefore the carbon skeleton of the eicosanoids **89b** or **93** is require for algal pheremone synthesis.[169] Similar results were obtained with the brown algae *Spacelaria rigidula*, while *Giffordia mitchellae* transformed **90** into a giffordene analogue **94**,[169] thus providing experimental evidence for an antarafacial [1,7] hydrogen shift similar to that involved in the biosynthesis of vitamin D.[170] (3*S*,5*Z*,8*Z*)-Undeca-1,5,8-trien-3-ol (**95**) has been proposed as the link between fatty acids and the $C_{11}H_{16}$ algal pheremones;[171] however, incorporation experiments with the C_{10} analogue **96** were unsuccessful. The authors concluded that 9-hydroxy or hydroperoxyeicosanoids must be the key intermediates and therefore that the C_{11} hydrocarbons arise in algae from fragmentation of a C_{20} acid to give C_{11} and C_9 hydrocarbons; in contrast, in plants, C_{12} acids (formed from either C_{18} or C_{20} acids) break down to give C_{11} and C_1 fragments. These interesting results highlight the need for marine

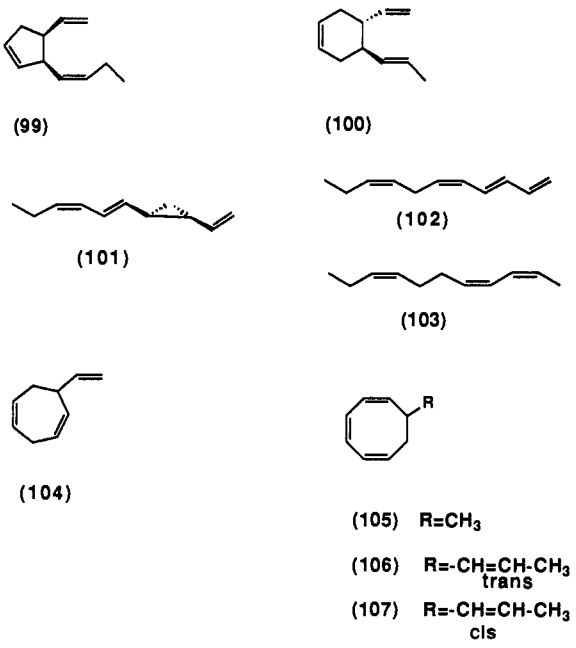
Scheme 18



biosynthetic experimentation rather than reliance on experimental data from terrestrial systems.



Stereospecific synthesis of hormosirene (= dictyopterene B) (**98**) from **95** has been reported, involving [1,2,(3),5] elimination from phosphate ester **97**;[172] in view of the biosynthetic work described above, this synthesis of **79** can no longer be considered biomimetic. Algal pheremones are usually isolated in optically-pure form. The separation of synthetic samples of racemic multifidene (**99**) and aucantene (**100**) by gas–liquid chromatography using modified cyclodextrins as chiral stationary phases has been reported;[173] the method provides confirmation of the enantiomeric excess of some naturally-occurring pheremones. The brown alga *Cutleria multifida* produces a range of C_{11} hydrocarbons, **77** and **99–103** and two novel C_9 hydrocarbons **104** and **105**; on the basis of plant model studies,[166] the C_{11} hydrocarbons were suggested to be biosynthesized from the C_{12} acid **76**. By analogy with the biosynthesis of ectocarpene (**78**), hydrocarbon **105** was considered to derive from (3*Z*,6*Z*)-deca-3,6,9-trienoic acid, which may also serve as precursor for **104** by analogy with the biosynthesis of giffordene (**103**), which involves an antarafacial 1,7-sigmatropic hydrogen shift. This proposal needs to be modified in the light of the incorporation of arachidonic acid into *Ectocarpus siliculosus*



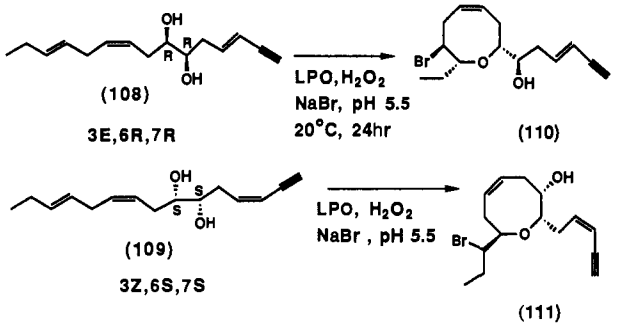
described above. Two new C_{11} hydrocarbons **106** and **107** were isolated and provide circumstantial evidence that decarboxylation may be an initiating step in the biosynthetic pathway.[174] The structures isolated from *Cutleria multifida* provide a fascinating example of the range of sigmatropic and electrocyclic processes in brown algae. The essential oils of two species of the genus *Dictyopteris* show much variation in chemical composition,[175] providing a further example of geographic variation in this genus to that reported previously.[176]

Marine organisms, including the microorganisms associated with macroalgae, appear to detoxify inorganic arsenic by converting it to methylated arsenates and arsenolipids. The arsenic content of edible brown seaweeds, and of shellfish, is of concern. The arsenic compounds found in algae are not however the same as those in the animals feeding on the algae. A number of studies on the incorporation of ^{74}As into marine plants and animals have been reported, and the reader is referred to recent comprehensive articles for full details of biosynthetic proposals relating to marine organoarsenic chemistry.[177,178]

C. Rhodophyta

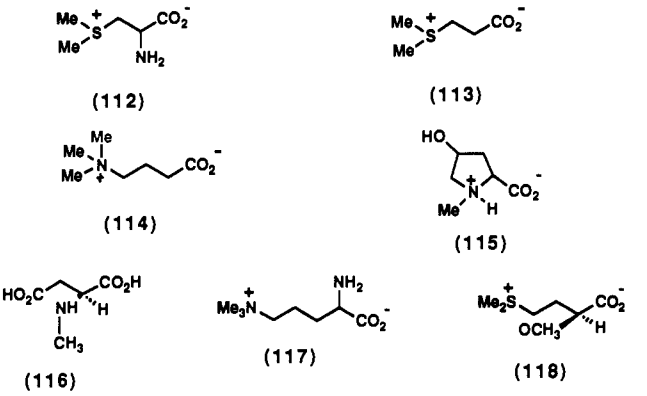
Algae of the genus *Laurencia* possess a wide range of secondary metabolites, mainly terpenes and acetogenins.[157] The chemical studies on a number of collections of *Laurencia implicata* from the Great Barrier Reef provides a good illustration of this variation.[179] The metabolic and genetic costs associated with this range of enzymic transformations must be high. Cyclic bromo ethers are among the characteristic metabolites of many *Laurencia* species and may derive from bromonium ion-induced cyclization of acyclic polyene precursors. Fukuzawa *et al.* have reported the biomimetic cyclization of alkene diols **108** and **109** to cyclic bromo ethers **110** and **111** in the presence of lactoperoxidase (Scheme 19).[180,181]

Scheme 19



The first biosynthetic studies on macroalgae involved classical incorporation experiments and enzymological studies on halogenated terpenes in *Plocamium cartilagineum*.[182] One of the difficulties of this study was the inability of cell-free extracts to brominate monochlorodimedone, the traditional assay for chloro- or bromoperoxidase activity, and suggests that there may be a high degree of substrate specificity in haloperoxidase reactions involving terpenes. Marine bromoperoxidase chemistry is reviewed in a companion article in this issue.[183]

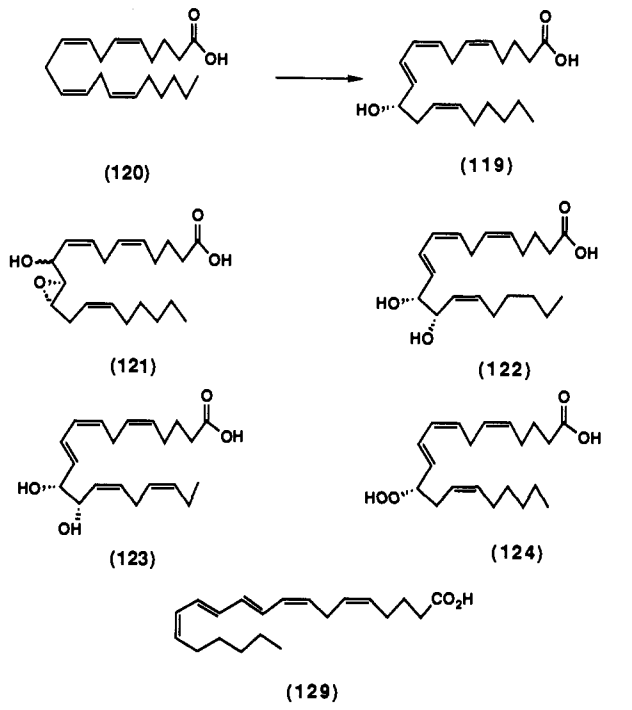
Sulfonium and quaternary ammonium compounds occur in a wide range of red algae,[184-187] and it has been suggested that the sulfonium salts act as methyl donors in the biosynthesis of the *N*-methylated compounds. In feeding experiments using a specially-made incubation apparatus, [*methyl*-^{14}C]methionine, [*methyl*-^{14}C]-*S*-methylmethionine (**112**), [*methyl*-^{14}C]dimethyl-β-propietin (**113**), and [*methyl*-^{14}C]-γ-aminobutyric acid betaine (**114**) were supplied to the red alga *Chondria coerulescens*.[188] After 24-h incubation, **112**–**114** and 4-hydroxy-*N*-methylproline (**115**) were isolated and checked for radiochemical purity for 2D autoradiography, counted, and then subjected to chemical degradation to establish sites of labeling. As expected,



methionine acted as a methyl source for all the *S*- and *N*-methylated compounds, while the sulfonium salt **112** and the betaine **114** were taken up, but did not act as methyl donors. Label from dimethylpropietin (**113**) was located in the methyl of **115**. Control experiments in the absence of algae confirmed the enzymatic nature of the transformations. The data reinforce previous work by Greene[189] on the biosynthesis of dimethylpropietin (**113**) in green algae and show that **113** functions as a methyl donor in transmethylation reactions. The red alga *Vidalia volubilis* contains the two *N*-methylated compounds *N*-methylaspartic acid (**116**) (c.f. microcystin-LR, section II.B) and 2-amino-5-(trimethylammonio)pentanoate (**117**) and the *S*-methyl compound (*S*)-4-(dimethylsulfonio)-2-methoxybutanoate (**118**). Incorporation experiments using 4-([*methyl*-^{14}C]dimethyl sulfonio)-2-methoxybutanoate gave labeled (**116**) but not the pentanoate (**117**); therefore, in this alga also, selective transmethylation of organic substrates by methylsulfonium salts can occur.[190] The decomposition of sulfonium compounds such as dimethylpropietin (**113**) in the marine environment is of concern as it leads to dimethyl sulfide, important in atmospheric sulfate production.[191]

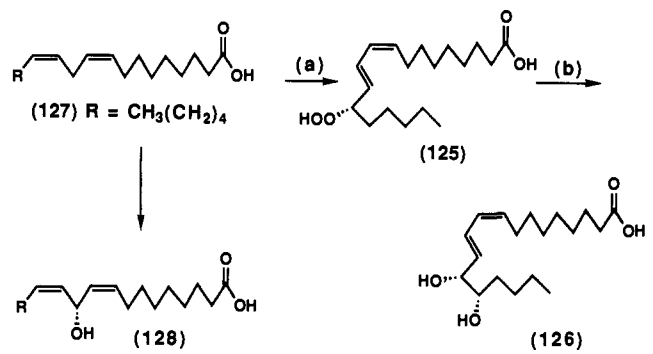
Details of the isolation and biosynthesis of marine oxylipins from red alga have been reviewed by Gerwick.[192] 12-HETE, [12(*S*)-hydroxyeicosatetraenoic acid (**119**)] and other biomedically-important products of 12-lipoxygenase activity on arachidonic acid (**120**) have been isolated from a range of red algae[193-196] as has the mammalian insulin release modulator hepoxilin B_3 (**121**).[195] The temperate red alga *Gracilariopsis lemaneiformis*, which is rich in hydroxyicosanoids, galactoliipids,[197] and pyrroles,[198] has been used to study eicosanoid biosynthesis. Incubation of arachidonic acid with an acetone powder preparation from *G. lemaneiformis* gave 12-HETE (Scheme 20).[199] Other lipids recovered included 12,13-diHETE (**122**) and 12,13-diHEPE (**123**). Incubation in the presence of $^{18}O_2$ gas gave **122** which was determined by ^{13}C NMR and mass spectrometry to be labeled with ^{18}O at both C-12 and C-13. This result excludes 12,13-epoxide formation followed by attack of water as the biosynthetic route from **119** to **122**.[200] Further work with labeled hydroperoxyoctadecadienoic acids has led to the definition of a novel hydroperoxide isomerase which catalyzes the conversion of fatty acid hydroperoxides into vicinal diol fatty acids. The intermediate 12*S*-HPETE (**124**),

Scheme 20



supplied in ^{14}C-labeled form, was converted to ^{14}C-12,-13-diHETE (**122**) while incubation of mixtures of unlabeled and $^{18}O_2$-labeled 13(*S*)-hydroperoxyoctadecadienoic acid (**125**) gave 13,14-dihydroxyoctadecadienoic acid (**126**) (Scheme 21) which contained either

Scheme 21



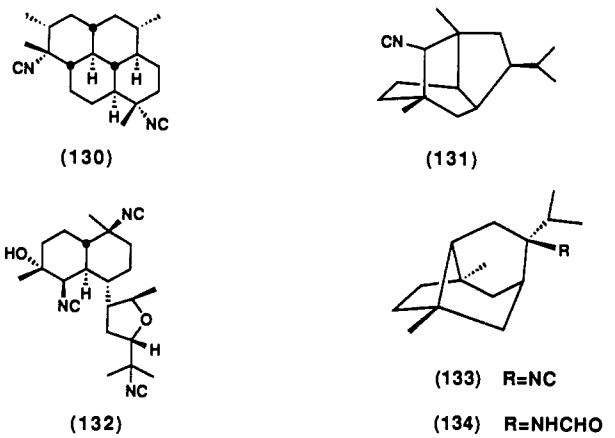
none or two ^{18}O atoms at C-13 and C-14. Thus the diol fatty acids are generated by intramolecular rearrangement of fatty acid hydroperoxides rather than intermolecular hydroxylation.[201] The enzymic conversion of linoleic acid (**127**) into hydroxyoctadecadienoic acids has also been studied in *Lithothamnion coralloides* (Scheme 21). The hydroxyl oxygen of the product 11-(*R*)-hydroxy-9(*Z*),12(*Z*)-octadienoic acid (**128**) was shown unexpectedly to derive from water, thus neither lipoxygenase nor cytochrome-P450 activity is involved in this transformation.[202] The role of lipoxygenase or other oxidase enzymes in the metabolism of polyunsaturated acids requires evaluation in other algae. The coralline red alga *Bossiella orbigniana* converts arachidonic acid to 5(*Z*),8(*Z*),10(*E*),12(*E*),14(*Z*)-eicosapentaenoic acid (**129**).[203]

The effect of temperature[204] and of light[205] on uptake and distribution of radiolabel from [1-^{14}C]acetate into *Chondrus crispus* and *Polysiphonia lanosa* has been evaluated. Marine algae, like sponges (section IV) may respond to differences in environmental temperature by adjusting their lipid content so as to regulate membrane fluidity.

IV. Sponges

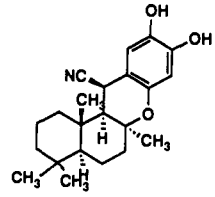
The wide range of novel, bioactive compounds isolated from marine sponges has made them a favored target for biochemical and ecological investigations.[157,206] Biosynthetic studies with intact sponges are complicated by the slow rates of growth of these sessile filter feeders and by the presence of symbiotic microorganisms.[1,2,128,129,207] Several experimental techniques have been used in sponge biosynthetic studies, including aquarium incubation,[208-210] short-term aquarium incorporation followed by *in situ* incubation,[88,211-213] or *in situ* gelatin capsule[212-214] or liposome[91,209,212] incorporation.

The biosynthesis of the isonitrile group, frequently a metabolic substituent in marine sponges, has been an active area of experimental study.[1,88-90,207,215] Initial work established the role of inorganic cyanide in the biosynthesis of diisocyanoadociane (**130**)[88-89] and more recently for 2-isocyanopupukeanane (**131**) and kalihinol-F (**132**);[90] in contrast in terrestrial isonitriles, cyanide is not involved in isonitrile biosynthesis,[87,91,216] the sole exception being the freshwater cyanophyte metabolite hapalindole A.[88] It has now been demonstrated by use of [^{13}C,^{15}N]cyanide and ^{13}C NMR that the C–N bond of 9-isocyanoneopupukeanane (**133**) is incorporated intact,[90] as is the case for the terrestrial cyanophyte metabolite hapalindole A.[88] The evidence for the doublet ($J_{^{15}N-^{13}C}$ 6 Hz) nature of the labeled isonitrile carbon is rather unconvincing, and it might have been beneficial to hydrolyze **132** to the corresponding formamide **134** in which the relevant coupling ($J_{^{15}N-^{13}C}$ 13 Hz) is more easily detected. Cyanide



incorporation has been shown to be an enzymic rather than chemical process.[89,90,215] Evidence for an amino acid origin of the isonitrile group is still lacking;[1,215] [2-^{14}C]histidine is not incorporated into the isonitrile group of diisocyanoadociane (**130**).[217] Other recent developments in the isonitrile story include the isolation of terpene isocyanates[218] and thiocyanates,[219,220] previously isonitriles had frequently been isolated with isothiocyanate and formamide analogues. The biological conversion of isonitriles to formamides and isothiocyanates has been documented,[214] but formamides may sometimes be products of hydrolysis during chroma-

tography;[221] an isocyanate derivative can reasonably derive from an isonitrile, but it seems unlikely that an isonitrile could be the biological precursor to a thiocyanate. The role of thiocyanate ion in the biosynthesis of isothio- and thiocyanatoterpenes requires evaluation,[219] and it would be extremely interesting if cyanates were found in marine sponges; there is currently no evidence for the presence of cyanate derivatives in sponges, although the nitrile **135** has been reported.[222]



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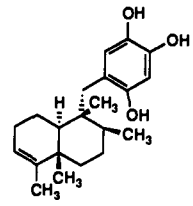
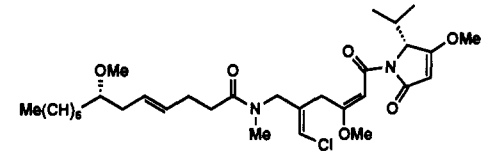
The role of symbionts in sponge terpene chemistry is of interest. [1-^{14}C]Acetate labels tetraterpene pigments in *Amphimedon* sp.,[223] a result which implies a precursor uptake role for the cyanobacterial (= blue-green algal) symbionts found in this sponge.[223,224] The possible role of symbionts in cyanide uptake in this sponge requires evaluation.[88,89] *Amphimedon* sp. also contains bacterial symbionts, mostly concentrated in the mesohyl, whereas the cyanobacteria are located in the pinacodermal tissue. The terpene diisocyanoadociane (**130**) is distributed in both tissue types, consistent with its location in sponge cells and where it may play a structural role. The absence of unusual sterols[223] combined with the metabolic stability and low turnover[88] of **130** provide circumstantial evidence for the proposed structural role, which has recently been evaluated by cell and membrane fractionation studies with *Amphimedon* sp.[224] Diisocyanoadociane was found to be associated with sponge cell membranes,[224] but not to be an integral component of lipid bilayers prepared using conventional phospholipids.[225] *Amphimedon* sp. contains unusual brominated fatty acids as components of its phospholipids,[226] but membrane modeling studies with these unconventional phospholipids have not yet been carried out. In the marine sponge *Dysidea avara*, the terpene hydroquinone avarol (**136**) is located in one sponge cell type alone; the potent



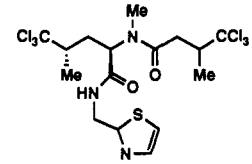
(136)

pharmacological behavior of avarol has been discussed in terms of a regulatory role.[227] Further, defensive roles have been ascribed to bromotyrosine metabolites located in spherulous cells of *Aplysina fistularis*.[228] *Amphimedon* sp. represents a contrasting situation to *D. avara* and *A. fistularis*; its bioactive secondary products are present in more than one cell type and appear to play a structural rather than defensive role. Isonitriles however have been implicated in the chemical defence of sponges and nudibranchs.[207] A range of antibacterial and cytotoxic effects have been demonstrated for marine isonitriles[59] so possibly diisocyanoadociane also plays a similar ecological role in *Amphimedon* sp.[224]

In sponges of the genus *Dysidea*, terpene production has been attributed to the animal host.[1,229] The tropical species *Dysidea herbacea* produces a range of polychlorinated alkaloids such as **137** or brominated diphenyl ethers, e.g. **23**, in addition to sesquiterpenes,[157,230] and it had been proposed that the halogenated compounds represent symbiotic contributions to the sponges' chemistry.[229,231] Bromophenyl ether (**23**) has been isolated from bacterial isolates of *D. herbacea*[60,61] and other similarly brominated phenols have been obtained from macroalgae[232,233] and worms.[234] The chlorinated metabolites of *D. herbacea*, e.g. **137**, have a structural resemblance to cyanobacterial metabolites such as malyngamide A (**138**). The cyanobacterial symbiont



(138)

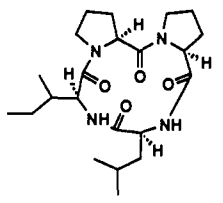


(137)

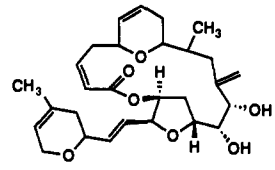
Oscillatoria spongeliae present in *D. herbacea* has been demonstrated by flow cytometry to be the true source of 13-demethylisodysidenin (**137**).[235] An amino acid origin for **137** has been confirmed by incorporation of radiolabeled leucine, valine, and alanine into intact sponge tissue,[236] providing further evidence of the experimental accessibility of amino acid-based pathways in sponges.[209] The biosynthesis of these chlorinated alkaloids is presumed, but not yet experimentally proven, to occur in the symbiont. Other sponge metabolites (Chart 3) believed to be products of microbial metabolism include the fenestins, e.g. **139**,[237] the fijianolides, e.g. fijianolide A (**140**),[238] tedanolide (**15**),[53] the bromotryptophan metabolites of *Fascaplysinopsis reticulata*[239,240] and other sponges,[241,242] swinholide (**141**) and related compounds,[243,244] and the bengazole metabolites, e.g. **142**, produced by an undescribed Jaspidae sponge.[245] Theonellamide F (**143**)[246] contains the unusual amino acid 3-amino-4-hydroxy-6-methyl-8-(*p*-bromophenyl)-5,7-octadienoic acid (**144**) which is structurally related to the aminophenyldecanoic acids isolated from cyanobacteria.[74-77,247] The pronounced odor associated with specimens of the halichondrid sponge *Halichondria panicea* is caused by the presence of sulfides such as dimethyl disulfide, possibly produced by symbionts within the sponge.[188,246]

Brominated compounds isolated from sponges are frequently amino acid-derived (Chart 4); however, several bromoterpenes have been reported from sponges in recent years. The encrusting dictyoceratid sponge *Cacospongia* sp. produces novel brominated meroterpenoids such as tribromocacoxanthene (**145**)[249] and the

Chart 3



(139)



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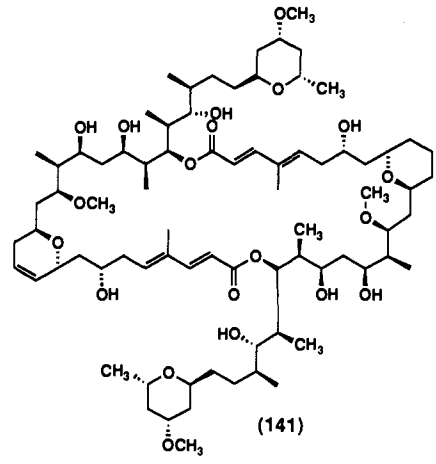

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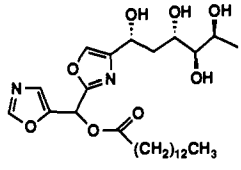

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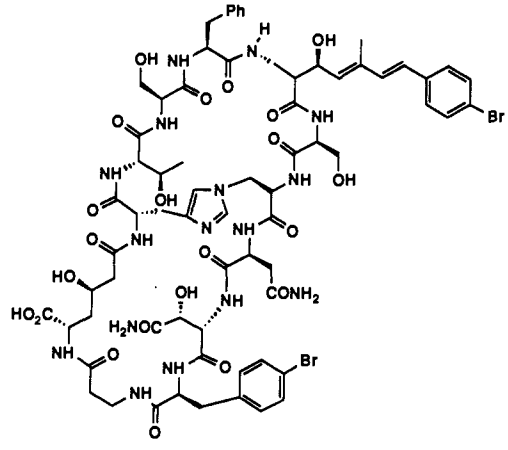

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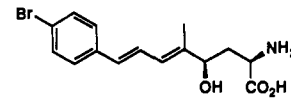
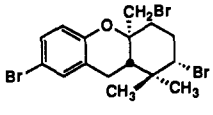
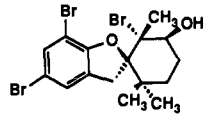


(144)

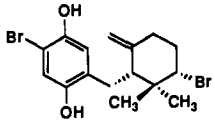
Chart 4



(145)



(146)



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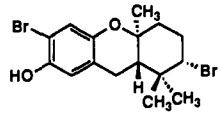
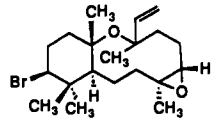
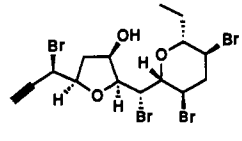


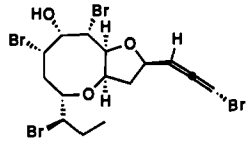
(148)



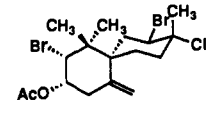
(149)



(150)



(151)

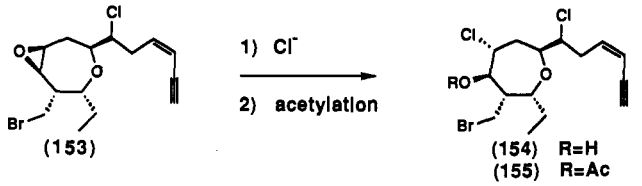


(152)

spiro-fused cyclohexanol derivative **146**,[250] structures which are related to the prenyl hydroquinone metabolites of the tropical green alga *Cymopolia barbata* typified by cyclocymopol (**147**)[251,252] and 4-isocymobarbatol (**148**).[253] The sponge *Cacospongia* sp. was surface fouled by red alga and growing in close association with a brown alga, but neither of these was found to contain brominated terpenes. Electron microscopic studies have revealed the presence of large amounts of bacteria, but no cyanobacteria, in *Cacospongia* sp., but cell fractionation studies have been unable to demonstrate conclusively a sponge or symbiont location for the brominated terpenes.[254] Other reports of brominated terpenes from sponges include rotalin B (**149**) from *Mycale rotalis*,[255] which also contains brominated acetogenins, e.g. **150**,[256] and a polybromo ether **151**.[257] The brominated chamigranes, for example rogiolol acetate (**152**), isolated from the sponge *Spongia zimocca* have been traced to *Laurencia microcladia* growing nearby,[258-260] although the precise nature of the sponge–macroalgal interaction is unclear.[259] Rogiolenyne A (**153**), obtained from *L. microcladia*, was not found in the sponge, and the rogiolenynes B (**154**) and C (**155**) isolated from the sponge were not found in the alga. This suggests sponge modification of algal-derived compounds as shown in Scheme 22.

The potent antitumor activity present in a Californian collection of *Lissodendoryx isodictyalis* has been traced to the presence of antineoplastic bryostatins from the fouling organism *Bugula neritina* present within the sponge. This arrangement constitutes an informal symbiosis since the sponge was found to flourish in the presence of the bryozoan contaminant; furthermore the bryostatin metabolites differed from those found in *B.*

Scheme 22

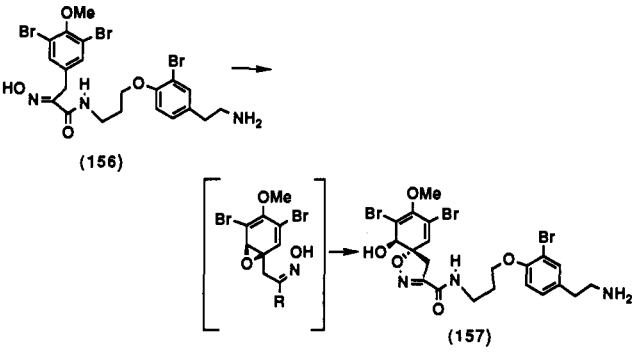


(153) (154) R=H (155) R=Ac

neritina and may represent metabolic transformations performed by the sponge.[261] It should be noted however that the chemistry of *B. neritina* shows geographic variation. Curiously, in view of the above interaction, ethyl acetate extracts of *Lissodendoryx isodictyalis* are documented as having antifoulant activity.[262]

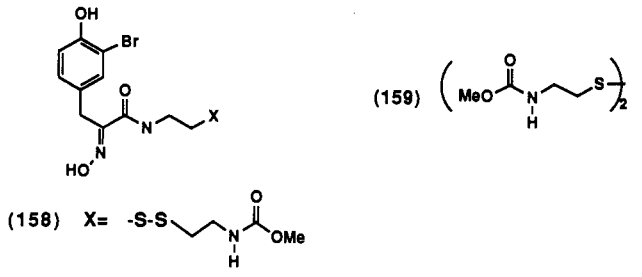
Sponges of the order Verongida are characterized by their ability to synthesize bromotyrosine metabolites; biosynthetic work in this area[208,209] has been reviewed previously.[1] Evidence that the biosynthesis of the isoxazoline ring present in many of these metabolites[157] is generated by cyclization of an oxime hydroxyl onto an unstable arene oxide (Scheme 23) is provided by the

Scheme 23



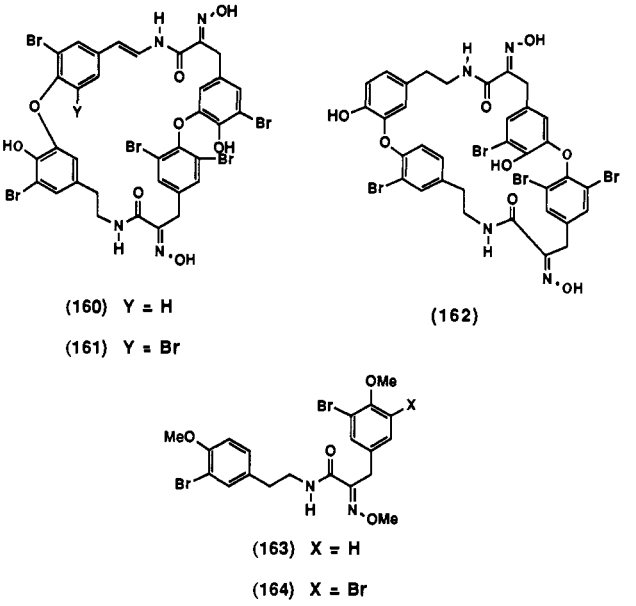
(156) (157)

isolation of **156** and **157** from *Druinella* (= *Psammaplysilla*) *purpurea*.[263] Many bromotyrosine metabolites contain a cysteine residue, as in psammaplin D (**158**) also from *D. purpurea*; however, the isolation of a cysteine dimer **159** from this sponge shows that the chemistry is not exclusively bromotyrosine-based.[264]



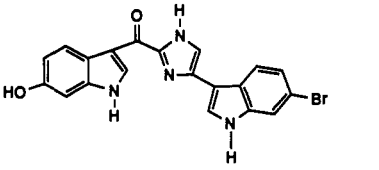
(158) X= -S-S- (159)

The most unusual verongiid metabolites are the bastadins, exemplified by bastadins 4 (**160**) and 6 (**161**).[157,265] The majority of the bastadins are based on a 13,32-dioxa-4,22-diazabastarane ring system, but the recent isolation of bastadin-12 (**162**) possessing a 13,32-dioxa-4,22-diazaisobastarane skeleton demonstrates the biosynthetic versatility of this sponge. Hemibastadins 1 (**163**) and 2 (**164**), also isolated from *Ianthella basta*, may possibly be bastadin precursors.[265] *I. basta* also contains unusual carotenoid sulfates[266,267] which may represent sponge-derived modifications of phytoplankton carotenoids. The apodid holothuroid *Synaptula lamperti* lives preferentially on the surface of *I. basta*

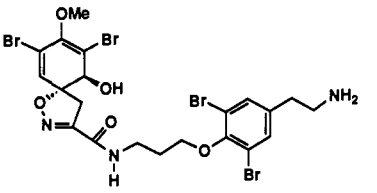


(160) Y = H
(161) Y = Br
(162)
(163) X = H
(164) X = Br

and is reported to be toxic; radiolabeling experiments have shown that *S. lamperti* takes up organic material from the sponge,[268] but this does not correspond to known bastadin compounds.[265] The deep-water *Hexadella* sp. (order Verongida) was found growing on skeletons of dead hexactinellid sponges at depths of −40 to −200 m. Samples collected at −100 to −200 m gave the bromotryptophan metabolite topsentin B2 (**162**) as the only detectable secondary metabolite while samples collected at −40 m gave two bromotyrosine metabolites, hexadellin A (**166**) and B (**167**); although the sponge may have shown a depth-related variation in its chemical composition, an alternative possibility raised by the authors was that the deep-water sample contained traces of the sponge *Topsentia* sp.[269]



(165)



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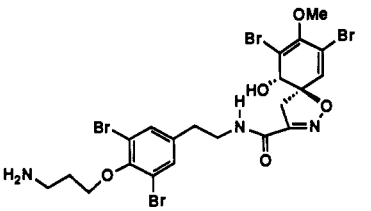
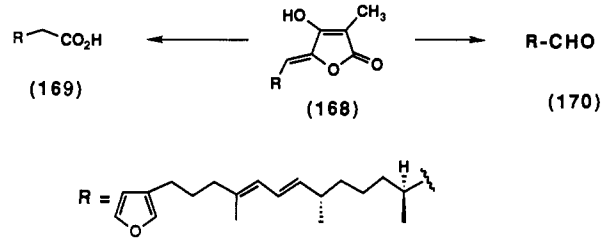


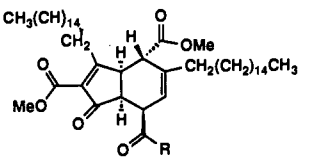
(167)

The dictyoceratid sponge *Hyatella intestinalis* produces spongian diterpenes or sesterterpenes depending on depth or locality of collection.[270] Sponges of the orders Spongiida or Thorecta contain sesterpene tetron-

Scheme 24



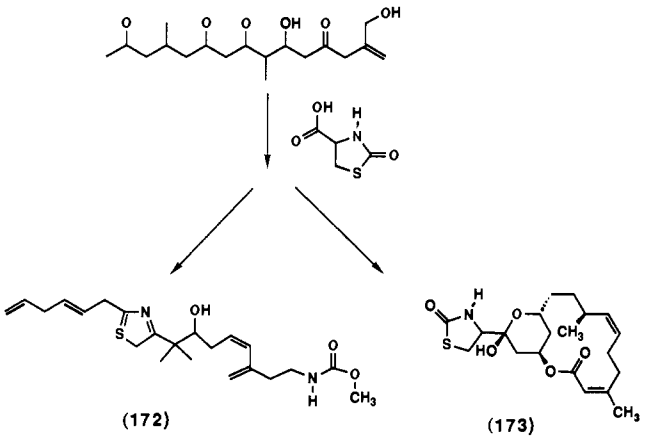
ic acids **168** and the biogenetically-related C_{21} furanoterpenes **169** together with C_{20} aldehydes **170** (Scheme 24); a biomimetic oxidative sequence leading to either the C_{21} or C_{20} product has been reported.[271] Marine sponges of the genus *Plakortis* contain a rich variety of bioactive compounds including alkaloids, peroxy compounds and the fatty acid derivatives, the manzamenones (e.g. A, **171**) which represent the biosynthetic condensation of two fatty acyl-derived components by a [4 + 2] cyclization.[272]



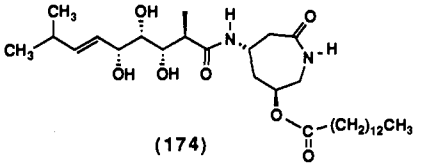
(171)

Mycothiazole (**172**) is an unusual thiazole with anthelmintic activity from *Spongia mycofijiensis* whose biosynthesis can be envisaged to follow from condensation of two polycarbonyl chains to 2-oxo-4-thiazolidinecarboxylic acid generated from cysteine (Scheme 25). Combination of the two polyacyl chains prior to

Scheme 25



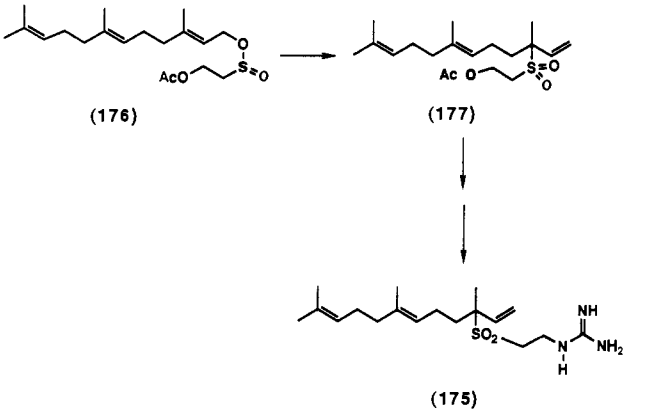
attachment of the amino acid residue leads to known sponge compound latrunculin B (**173**) which was isolated as a cometabolite.[273] The bengazoles, e.g. **142**, and bengamides, e.g. **174**, also appear to be mixed poyketide amino acid metabolites with the added involvement of a fatty acid unit. Thus three distinct



(174)

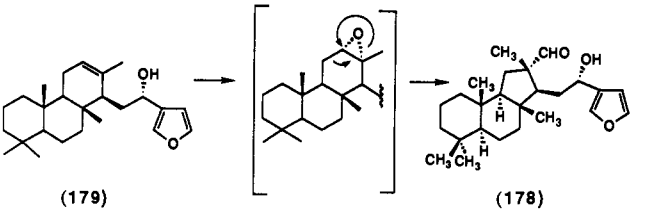
biosynthetic pathways appear to act in concert in this sponge; the involvement of microorganisms in the biosynthetic pathways has been proposed.[245] A biomimetic synthesis (Scheme 26) of agelasidine A (**175**)

Scheme 26



has been reported in which the [2,3] sigmatropic rearrangement of an allylic sulfenate **176** to the sulfoxide **177** produces the unusual quaternary carbon–sulfur linkage.[274] Hyrtiosal (**178**), a sesterterpene from *Hyrtios erecta* may derive from the more common cheilanthane skeleton **179** via rearrangement of an epoxy intermediate as shown in Scheme 27.[275] The biosynthetic

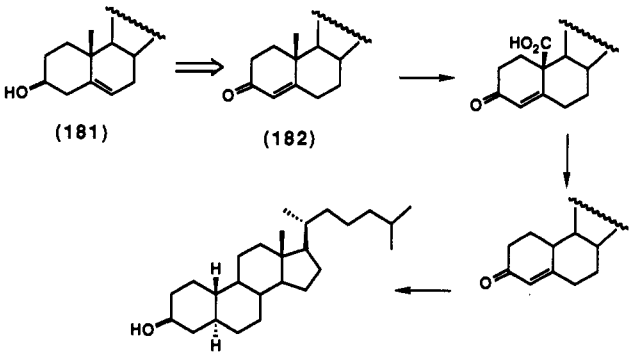
Scheme 27



diversity of all of these sponge metabolites represents an unexplored and inviting challenge to the experimentalist.

Although it is not the role of this article to cover the chemical ecology of marine sponges, the diterpene chemistry of dendroceratid sponges requires comment because of its biosynthetic relevance. A high degree of chemical variability has been detected in collections of *Aplysilla glacialis* from British Columbia[276,277] and the Caribbean,[278] with some collections, but not others, being rich in diterpenes or sterol peroxides. Similarly, collections of *Chelonaplysilla* sp. from Palau[279,280] and Pohnpei[281] differed in their diterpene chemistry; additionally, the Palau collection contained unusual aromatic alkaloids suggested to derive biosynthetically from tryptophan and tyrosine; some, but not all, of these alkaloids were brominated.[282] The question arises as to whether these variations are caused by geographic, environmental or seasonal factors, or whether the species collected are all the same or instead are chemically-distinct subspecies. The possible involvement of symbiotic microorganisms is one explanation of the chemical variation. In any case, the metabolite variation detected implies a high metabolic cost which may be compensated for by an ecological benefit to the sponge; antifouling or antifeedant roles have been hypothesized for these compounds.[278]

Scheme 28

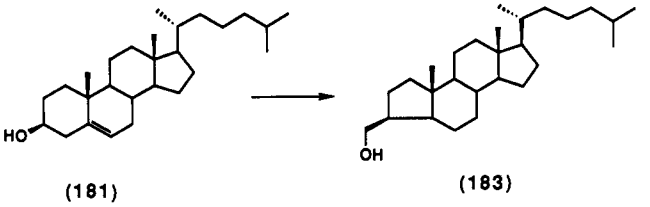


Although sterols and fatty acids are usually classified as primary metabolites, and therefore beyond the scope of this review, mention must be made of the substantial progress made in our understanding of marine sterol and fatty acid biosynthesis, and the biological roles of marine sterols. Much of this work has been reviewed in depth.[1,4,128,129,283-285] Structural highlights include the isolation of sterols with novel cyclopropane substituents,[285-288] alkylation patterns,[210,213,289,290] or other structural features[291] which have been of value in chemotaxonomic analysis.[292-294] The *de novo* biosynthesis of sterols from mevalonate is inefficient[212,295] owing to the water-soluble nature of the precursor[212] and, likely, the efficiency of the tetraterpene pathway.[223] *De novo* sterol biosynthesis from squalene has been demonstrated in intact sponges[212,213,288,296] (including some sponges previously believed[297,298] incapable of *de novo* synthesis) and by using cell-free extracts.[212,299] The inefficient incorporation of farnesol, despite its poor water solubility, suggests that the sponge enzymes can only efficiently handle precursors which do not need to be phosphorylated and which have no other metabolic fate. The incorporation and transformation of cycloartenol and lanosterol in a range of marine and freshwater sponges, including those lacking symbionts, is taken as evidence that the symbionts do not contribute to either *de novo* sterol synthesis or dietary sterol modification.[300] The major product of cyclization of squalene is lanosterol,[212] but sponges can also utilize cycloartenol by converting it to lanosterol.[284]

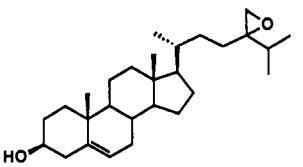
The Mediterranean sponge *Axinella polypoides* is an important source of the nuclear-modified 19-norstanols (**180**); their investigation by Sodano *et al.* constitutes one of the first successful biosynthetic studies on sponges.[1,2,298,301] The pathway (Scheme 28) has now been delineated by precursor incorporation study with $^{14}C/^{3}H$-labeled precursors. Cholesterol (**181**) is oxidatively converted to a Δ^4-3-ketone **182** prior to oxidation and decarboxylation at C-19.[302] Other modified sterols include the *A*-norstanols (**183**) which are formed by ring contraction of a cholesterol precursor (Scheme 29);[297,303-305] further modification of the sterol nucleus occurs after ring contraction.[306] Sponges can also convert Δ^5 sterols into $\Delta^{5,7}$ sterols.[223,307,308]

Although the biosynthesis of the multiply alkylated side chains present in many sterols appears to involve successive *S*-adenosylmethionine additions to double bonds,[1,4,128,284] the role of methionine is difficult to test experimentally in intact sponges,[212,309,310] owing to the water-soluble nature of the precursors. The role of

Scheme 29



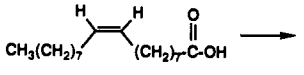
methionine in sponge cell-free extract[299] and microalgal[1,132,134] sterol synthesis has however been demonstrated. Stereochemical features of the formation of the ethylidene group of fucosterol and isofucosterol have been evaluated.[311] Among the most interesting sterols have been those with cyclopropane- and cyclopropene-containing side chains; these are produced by both sponges[285-288] and microalgae.[134] Full mechanistic details of these transformations have been reported.[287,312-315] One other interesting, and ironic, feature of sponge metabolism is that sponges are capable of dealkylation as well as alkylation processes; the dealkylation proceeds through the intermediacy of a 24,28-epoxide **184**,[316] as



has been shown for insects. One sponge investigated, *Tethya aurantia*, was found to have a choice of three different sterol-producing pathways, namely *de novo* biosynthesis, side-chain alkylation, and dealkylation processes. Simultaneous operation of the dealkylation reaction, which has also been demonstrated in cell-free extracts,[317] and of *S*-adenosylmethionine-mediated alkylation was evaluated by double-labeling experiments.[310]

Novel fatty acids from marine sponges have been the subject of investigations in the Djerassi and Carballeira laboratories, with over 50 novel phospholipids identified.[283,318] Eicosanoid fatty acids more typical of marine algae have also begun to be isolated from sponges.[319] Morales and Litchfield investigated the biosynthesis of fatty acids from acetate and proposed that the very long chain fatty acids (VLFA's) were formed by a chain-elongation mechanism from precursors such as palmitic and palmitoleic acids; by comparison, the *de novo* biosynthesis from acetate was inefficient.[320] Experiments with four marine[211,321-323] and one freshwater[324] sponge have confirmed homologation from short-chain precursors as the major pathway, with precursor fatty acids supplied by the diet or from symbionts.[211,321] Desaturation occurs after chain homologation and can occur first at either the Δ^5 or Δ^9 position,[322,323] in contrast to the accepted sequence in animal fatty acid biosynthesis whereby the first double bond is always introduced at the Δ^9 position, with the second and subsequent double bonds inserted between the first bond and the carboxyl terminus. The ability of sponges to desaturate at the Δ^9 position of a Δ^5-monoenoic acid is more reminiscent of plants. The synthesis of trienoic acids, e.g. $\Delta^{5,9,19}$26:3 **185**, requires the availability of a

Scheme 30



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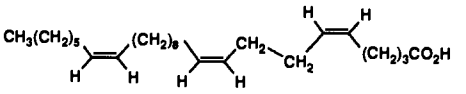
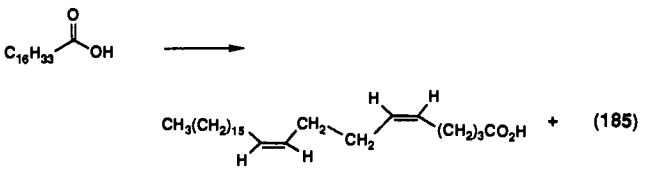


(185)

short-chain monoenoic acid **186** which is chain extended, and then desaturated at the Δ^5 and Δ^9 positions (Scheme 30).[322] Freshwater sponges, unusually, possess additional desaturase activity; *Ephydatia fluviatilis* converts palmitic acid to both the $\Delta^{5,9}$26:2 **187** and $\Delta^{5,9,19}$26:3 **185** (Scheme 31) fatty acids.[324] Additional function-

Scheme 31



(187)

alization, e.g. bromination, appears to occur after homologation and desaturation.[323] The incorporation of fatty acyl residues into phospholipids occurs by the well-known cytidine pathway;[325] however, interconversion of phospholipid headgroups can be appreciable. Sponge VLFA's are frequently incorporated into the amino phospholipids phosphatidylethanolamine (PE) and phosphatidylserine (PS);[283,323,325,326] indeed sponges contain a higher percentage of PE and PS phospholipids than the phosphocholine (PC)-containing phospholipids which predominate in animals.[327] The lipid composition of sponge cellular membranes has been investigated in detail.[327-331] VLFA's,[328-330] together with unconventional sterols,[327,330] have been identified as phospholipid components of membranes. The physiological function and structural details of sponge cell membranes are not well defined. Membrane modeling studies have demonstrated that conventional phospholipid bilayers exclude cholesterol and other common marine sterols and point to the importance of hydrophobic membrane proteins in stabilizing membrane interactions.[331,332] In the sole report on proteins and sponge membrane structure reported to date, it was found that conventional fatty acids (C_{16}–C_{18}) rather than VLFA's were bound to membrane proteins.[332]

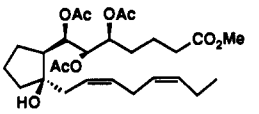
The biosynthetic studies described above highlight the role of sponge symbionts in providing short-chain precursors for VLFA biosynthesis. Qualitative and quantitative assessment of the fatty acids in marine sponges can provide valuable information about symbiont types.[66,226] The differing sponge cells can be separated from each other and from symbionts by biochemical techniques such as density gradient centrifugation using Ficoll or Percoll or by fluorescence-activated cell separation. In a study on *Pseudaxinyssa* sp. the sponge host was found to contain VLFA's and unconventional sterols;[333] these lipids were not present in cultured microbial symbionts. Three studies[330,333-334] demonstrate the variability of lipid composition according to sponge cell type and suggest that the lipid content may relate to the function of individual cell types. Clearly, the next step for analysis is the biosynthetic capability of individual sponge or symbiont cell types.[283]

V. Coelenterates

The chemistry of the octocorals is predominantly, but not exclusively, that of diterpenes[157,335] not unlike those found in the tobacco plant. Both soft corals and gorgonians possess the symbiotic dinoflagellate *Symbiodinium microadriaticum* and the role of this microorganism in biosynthetic processes in coelenterates is therefore of interest. The controversy as to whether the terpene metabolites are uniquely of animal or plant origin is unresolved. While $^{13}C/^{12}C$ ratios have been used to provide evidence for the animal origin of octocoral sterols[336] and it is also clear from the chemical composition of some octocorals that the symbionts alone cannot be responsible for the observed chemistry of coelenterates,[337,338] evidence discussed below supports an important biosynthetic role of symbionts in terpene formation in some, if not all, coelenterates.

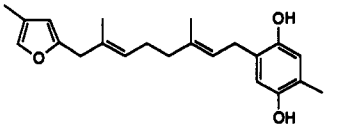
A. Soft Corals

Although the dinoflagellate symbionts contribute substantially to the nutritional processes in soft corals, they are not essential for secondary metabolite synthesis. The punaglandin metabolites exemplified by **188** are produced by *Telesto riisei* which does not contain symbionts.[337]



(188)

Studies on the alcyonacean soft coral *Sinularia capillosa* by Coll *et al.* have demonstrated the *de novo* formation of the sesquiterpene hydroquinone metabolite **189** from mevalonate and established that the quinone moiety of this metabolite was not derived from mevalonate.[339] A more searching, comparative biosynthetic study was carried out on the alcyonacean soft coral *Alcyonium molle* and the xeniid soft coral *Heteroxenia* sp. (previously *Cespitularia* sp.).[340] In this



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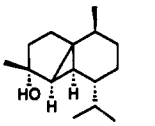

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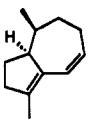
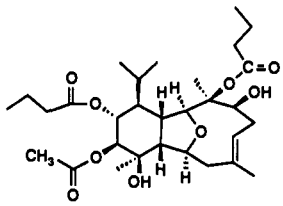


(191)

work, the *de novo* biosynthesis of the sesquiterpene metabolite cubebol (**190**) and the norsesquiterpene clavukerin A (**191**) from acetate was confirmed by

chemical degradation; the utilization of [2-^{14}C]mevalonate was consistent with prior degradation of this precursor to [2-^{14}C]acetate, a common problem in terpene biosynthetic studies. It was therefore surprising to discover an alternative biosynthetic fate for the radiolabeled acetate supplied to *A. molle*. This precursor labeled the butyrate ester functions, but neither the acetate ester substituents nor the terpenoid skeleton of the major diterpene **192** was labeled. *De novo* terpene synthesis was therefore not demonstrated; however, significant incorporation of acetate into the major lipid component cetyl palmitate (**193**) supported the efficient



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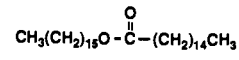


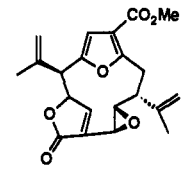
(193)

operation of the fatty acid pathway during the duration of this experiment. The complication presented by the efficient operation of competing pathways has been noted previously in biosynthetic studies on marine sponges;[88,89,223] the *A. molle* result is still very surprising in view of the nonincorporation of acetate into precisely those parts of the molecule which were expected to be acetate-derived. Lack of material precluded chemical degradation of the butyrate side chains to determine the specificity of labelling therefore the biosynthetic mechanism by which they were labelled exclusively remained unexplored. Butyrate was not utilized as a precursor, most likely because of its toxicity, while mevalonate was also poorly utilized. Soft corals appear to take up water-soluble small-molecule precursors without difficulty; however, biosynthetic studies with intact animals appear restricted by the difficulty with which these precursors reach the correct cellular location for metabolism. This experimental problem is resolvable through the development of cell-free extracts, as have been investigated successfully for gorgonians (see section V.B), and which would allow research workers in addition to those in tropical marine locations to study biosynthesis. Biosynthetic processes in soft corals appear to be an attractive area for future research.

B. Gorgonian Corals and Other Coelenterates

As in soft corals, the symbiotic microalgae present in gorgonians contribute to the overall nutrition in these organisms, but they are not always essential partners in the formation of secondary metabolites. *Pseudopterogorgia acerosa* produces the antiinflammatory pseudopterolides, e.g. **194**, despite the absence of symbionts in this gorgonian.[338] The gorgonian *Briareum asbestinum* produces diterpenes in all stages of its life cycle, but does not contain algae in its developmental phase, therefore the animal cells are the likely source of diterpene synthesis in this symbiosis.[341]

Studies on the biosynthesis of crassin acetate (**195**) in *Pseudoplexaura porosa* were carried out by Anderson *et al.* in 1970 and demonstrated the acetate origin of this metabolite; these workers also obtain histological



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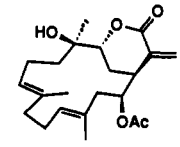
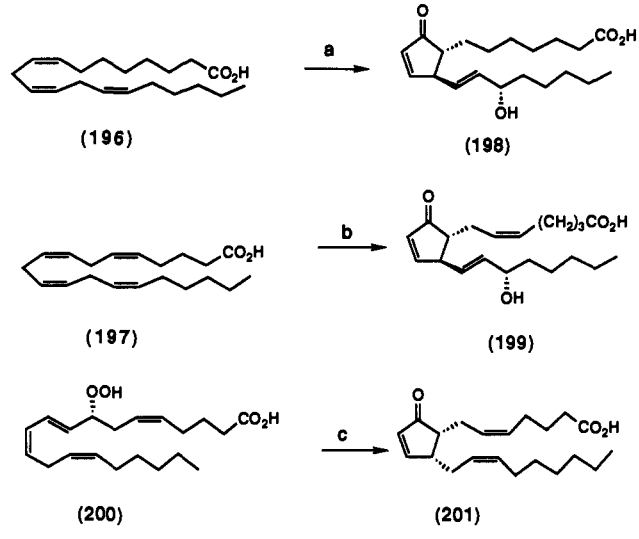


(195)

evidence to support the production of crassin acetate by zooxanthellae.[342] In more recent research, Anderson has demonstrated the incorporation of mevalonate and other terpene precursors into (**195**) by a cell-free preparation of zooxanthellae.[343] Attempts to demonstrate cell-free synthesis of sterols in zooxanthellae were however less successful, although it was possible to demonstrate squalene biosynthesis. It was therefore suggested that the direction of sterol synthesis is controlled by the animal host.[344,345] An alternative explanation is that the viability of the appropriate enzymes had not been retained in the cell-free preparation. In claiming biosynthesis by only one component of a symbiotic partnership it is vital to demonstrate the complete absence of contaminants from the other component of the partnership.[345]

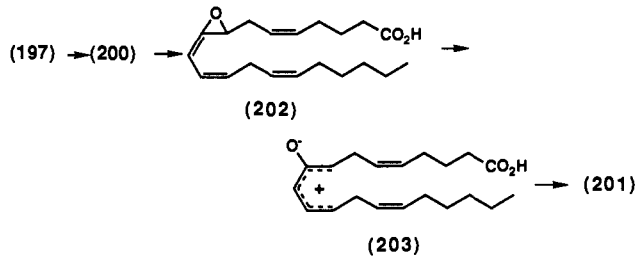
Biosynthetic attention has focused on the origin of prostaglandins in octocorals because of the commercial and biomedical importance of these metabolites; much of the work has used the gorgonians *P. porosa* and *P. homomalla* as a source of the prostaglandins. Initial work in this area has been reviewed by Barrow.[2] Cell homogenates from *P. homomalla* converted 8,11,14-eicosatrienoic acid (**196**) or arachidonic acid (**197**) into PGA_1 (**198**) or PGA_2 (**199**), respectively (Scheme 32,

Scheme 32

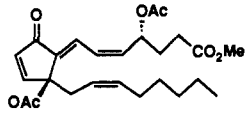


paths a and b),[346] although a cell-free preparation from a purified algal preparation did not produce prostag-

Scheme 33



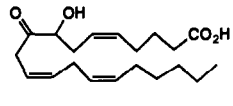
landins, but did contain arachidonic acid.[347] This suggested that the algal symbionts generate arachidonic acid which is then utilized by the coral for prostaglandin synthesis. It will be recalled from section III that macroalgae can synthesize and metabolize arachidonic acid using a lipoxygenase pathway distinct from the mammalian cyclooxygenase/endoperoxidase route. The biosynthetic pathway to prostanoids in corals also differs from the mammalian process[348,349] and involves an 8-lipoxygenase since incubation of acetone powders from *P. homomalla*, *P. porosa*, or *Clavularia viridis* with arachidonic acid gave 8(*R*)-HPETE (**200**)[350,351] or preclavulone A (**201**);[349,352] further the conversion of 8(*R*)-HPETE to preclavulone A (**201**) was also demonstrated in *C. viridis*[350] and *P. porosa* (Scheme 32, path c).[352] The conversion of **200** to **201** may involve allene oxide **202** and oxidopentadienyl cation **203** as intermediates (Scheme 33), the feasibility of which is demonstrated by a biomimetic chemical synthesis.[353] Preclavulone A (**201**) has now been suggested to be a key biosynthetic precursor in the formation of the two types of marine prostanoid exemplified by prostaglandin A_2 (**199**) and clavulone I (**204**);[354] however, the



(204)

conversion of preclavulone A or of 8(*R*)-HPETE into naturally-occurring prostanoids has yet to be demonstrated experimentally,[354] while the isolation of racemic rather than optically-active **201** from the biosynthetic experiments casts doubt on its proposed intermediacy.[354-356] Other stereochemical and structural features of preclavulone A are inconsistent with it being a prostaglandin precursor.[355,357]

The isolation of allene oxide **202** from an acetone powder of *P. homomalla* supplied with 8(*R*)-HPETE has been reported, together with the α-ketol **205**[355,356] and the cyclopropyl derivative **206**,[358] both of which



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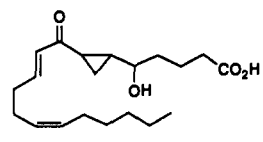
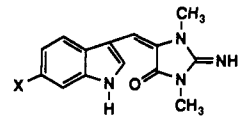


(206)

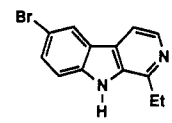
are potentially solvolysis products of the allene oxide. The presence of **206**, which was only formed in the presence of the coral acetone powder, may explain the formation of prostaglandin double bond isomers in corals.[358] The lack of utilization of 8,15-diHPETE isomers[359] or of 15-oxygenated substrates[357] by the *P. homalla* enzyme preparation provides information about the timing of introduction of the 15-oxygen functionality.[359] Work with hydroxyhydroperoxyeicosanoids and a enzyme preparation from flaxseed has recently provided the first experimental evidence in support of cyclization to prostanoid-type products via allene oxide intermediates.[359] Undoubtedly, marine prostaglandin biosynthesis will continue to be an active area of research. The biological role of the marine prostaglandins is of interest, and the proposal that they function as antifeedant compounds[360] has been challenged.[361]

Tryptophan-derived metabolites, such as aplysinopsin (**207**) and 6-bromoaplysinopsin (**208**), identical with those reported from marine sponges,[157,362-365] have been isolated from scleractinian corals *Tubastraea aurea*,[366,367] *Astroides calycularis*,[366,368] and *Leptopsammia pruvoti*[366] suggestive of a bacterial origin for these compounds. The hydroid *Aglaophenia pluma* contains brominated alkaloids, e.g. **209**,[369] that are reminiscent of the tunicate eudistomin metabolites.



(207) X=H

(208) X=Br

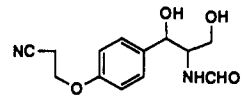


(209)

VI. Marine Mollusks

A. Sea Hares

Sea hares (order Anaspidea) are herbivorous opistobranch mollusks which sequester secondary metabolites from seaweeds and other algae and store them in digestive glands. A number of literature reports document the dietary origins of sea hare metabolites.[157,370-372] Metabolites isolated from sea hares which are unlikely to be macroalgal in origin include bursatellin (**210**)[373] and the dolastatins (which resemble the cyclic peptides of tunicates).[157,374,375] The capabil-



(210)

ities of sea hares for *de novo* synthesis of chemicals is unexplored and there are as yet no reports of biosynthetic studies on secondary metabolites from sea hares, although the ability of sea hares to chemically modify dietary metabolites has been demonstrated.[371,376,377] The sequestered metabolites appear to deter predators from eating the sea hare by their bitter taste or form part of the mucus secretions of the sea hare, but there is no current evidence that biosynthetic modification of dietary metabolites enhances their bioactivity.[371]

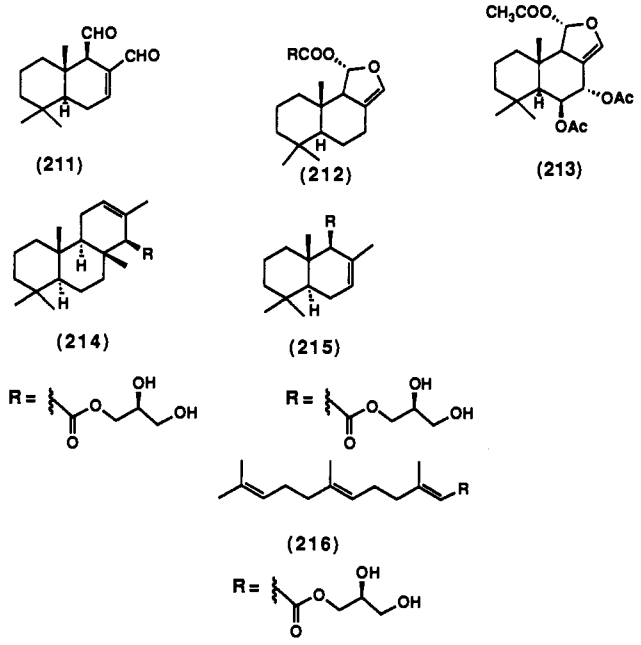
The lipoxygenase products of arachidonic acid, specifically 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE, **124**), may act as chemical messengers in neurons of the marine mollusk *Aplysia californica*. Conversion of [^{3}H]arachidonic acid to 12-HETE (**119**) and to the biologically-active 12-keto derivative has

been reported;[378] however, the operation of the 12-lipoxygenase pathway in their algal diet (section III.C) may provide an important alternative source of these mollusk neurochemicals.

B. Nudibranchs

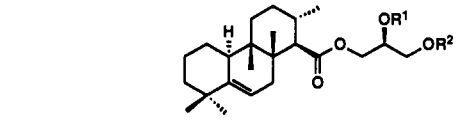
Nudibranchs represent one of the largest orders of the subclass Ophisthobranchia and have provided a wide range of secondary metabolites of both *de novo* and dietary origin.[157] Dorid nudibranchs feed on sponges, ascidians, and bryozoans, sequestering (and possibly modifying[379-382]) secondary metabolites for defensive purposes. Drimane sesquiterpene metabolites **211–213** isolated from the Mediterranean nudibranchs *Dendrodoris limbata* and *Dendrodoris grandiflora* have been shown to derive *de novo* from mevalonate.[383-387] Details of this work has been reviewed recently.[1,387] A low rate of *de novo* synthesis has been detected for the terpene glycerides **214–216** (Chart 5) in the Canadian nudibranchs *Archidoris*

Chart 5

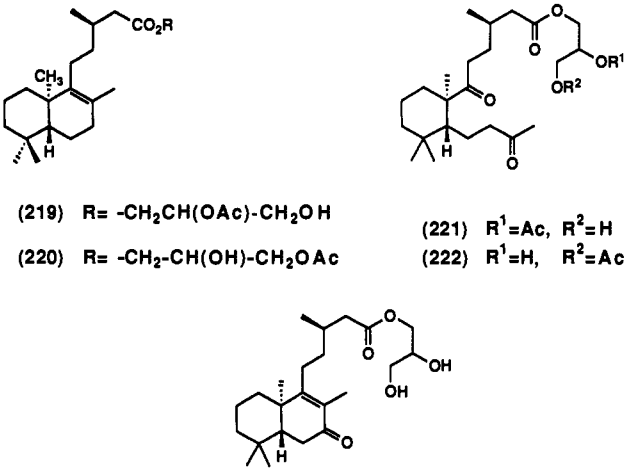


montereyensis and *A. odhneri*.[388,389] The Mediterranean nudibranch *Doris verrucosa* produces the toxic verrucosins A (**217**) and B (**218**); despite their structural similarity to the *A. montereyensis* and *A. odhneri* products, it was not possible to demonstrate efficient *de novo* biosynthesis of these terpenes from either mevalonate or glycerol under incorporation conditions in which the nudibranch was shown to be metabolically active.[387,390] Since the verrucosins are stored in the mantle while the precursor was injected into the hepatopancreas,[390] the radioactive precursors may have been unable to reach the site of synthesis of the verrucosins.

The fish antifeedant polygodial (**211**) is toxic to *D. limbata*[385] and is therefore stored in the nudibranch's mantle in protected form.[387,391,392] Careful dissection of animals has provided information about the storage sites and biological roles of drimane terpenes in this highly organized animal.[387,392] The sesquiterpene **212** in which the fatty acyl substituent is acetyl, which was isolated from the gills of *D. limbata*,[392] has recently also been isolated from the temperate sponge *Dysidea* sp.,[393] therefore a dietary origin should not be excluded as an alternative source of this compound. Through their analysis of six different nudibranchs collected at a range of geographic locations, Faulkner and Andersen have proposed that those nudibranchs capable of *de novo* biosynthesis will show no chemical variation; in contrast, nudibranchs that obtain their chemicals from dietary sources will show variation in their chemical constituents depending on the collection site.[394] On this basis, the Mediterranean *D. verrucosa* would have been expected to be capable of synthesizing terpenes since the verrucosins **217** and **218** have been found from nudibranchs collected in a range of locations. Furthermore the verrucosins have not been isolated from the known sponge diet of *D. verrucosa*.[387,390] The Antarctic nudibranch *Austrodoris kerguelensis* contains diterpenoid glycerides **219–223** which resemble those isolated from *A. montereyensis*, *A. odhneri*, and *D. verrucosa* and it has been proposed that they may also be biosynthesized *de novo*.[395]



(**217**) R^1 = H, R^2 = Ac
(**218**) R^1 = Ac, R^2 = H

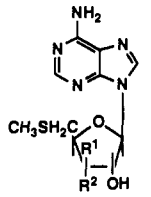


(**219**) R= -$CH_2CH(OAc)$-CH_2OH
(**220**) R= -CH_2-$CH(OH)$-CH_2OAc
(**221**) R^1=Ac, R^2=H
(**222**) R^1=H, R^2=Ac

(**223**)

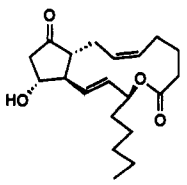
D. verrucosa also produces the sulfur-containing nucleoside 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine, xylosyl-MTA, (**224**) which is concentrated in hermaphroditic glands and also present in egg masses of the nudibranch.[396] Incorporation of [3′-^{3}H,*methyl*-^{14}C]-5′-deoxy-5′-methylthioadenosine (MTA, **225**), a bioactive nucleoside which is a cometabolite of (**224**), although present in smaller amounts than **224**, demonstrates that **224** is produced by oxidation–reduction of **225** since the ^{3}H label is lost during the conversion.[387,397] Both adenine and methionine were also identified as biological precursors.[397] Xylosyl-MTA is not a substrate for the enzymes which modify MTA, nor does it isomerize back to MTA, and it therefore accumulates in the tissue of the nudibranch.[397] The

biological function of this unusual, nontoxic secondary metabolite has not yet been established.[387,398]



(224) R^1=OH; R^2=H
(225) R^1=H; R^2=OH

Tethys fimbria is a large nudibranch which utilizes a novel defense strategy known as autonomy. It lives hidden in the sand, until, when molested, it detachs one or more of its cerata which distracts the predator while the nudibranch swims away. Prostaglandin 1,-15-lactones, exemplified by those of the PGE_2 (**226**), PGA_2 (**227**), and $PGF_{2\alpha}$ (**228**) series, together with prostaglandin free acids such as PGE_3 (**229**) and PGE_2 (**230**) have now been isolated from the cerata of *T. fimbria*;[399,400] it has been suggested that the lactones



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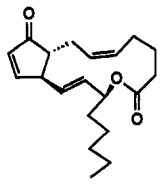

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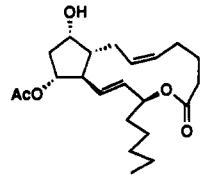

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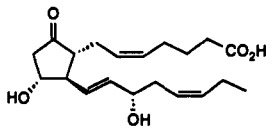

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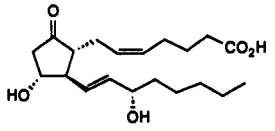
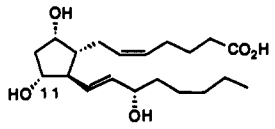


(230)

function as precursors of the prostanoids which control the detachment and contraction process. Injection of [^{3}H]PGE_2 (**230**) into mantle tissue gave labeled (**226**) while [^{3}H]$PGF_{2\alpha}$ (**231**) gave labeled $PGF_{2\alpha}$ 11-acetate together with **226** and **230**. The mollusk was unable to introduce a Δ^{17} double bond into any of the precursors tested, therefore prostanoids of the $PGF_{3\alpha}$, PGE_3, or PGA_3 series were unlabeled. The conversion of labeled $PGF_{2\alpha}$ (**231**) to PGE_2 (**230**) demonstrated unusual

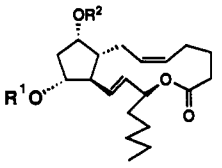


(231)

oxidative activity. The conversion of PGE_2 (**230**) into its lactone in mollusks lacking cerata, but not in isolated cerata themselves, showed that lactonization occurs primarily in the mantle tissue. Injection of radiolabeled PGE_2 1,15-lactone (**226**) into mantle tissue confirmed that this metabolite is transported to the cerata for storage purposes.[401,402] Arachidonic acid also served as a precursor of prostaglandins of the E series, probably following initial incorporation into membrane phospholipids, and the radioactive lactone products were again transferred to the cerata for storage. It has not yet been demonstrated experimentally whether the *T. fimbria* prostaglandins derive via the mammalian endoperoxide route, or the lipoxygenase pathway present in soft corals, or by some other route.

The lactones were shown to be converted back to free prostaglandins, e.g. PGE_2 (**230**) and PGE_3 (**229**), in detached cerata, and this transformation did not occur in heat-inactivated ceratal homogenates and was therefore enzymic in nature. Defensive secretions from *T. fimbria* were found to contain toxic prostaglandin 1,15-lactones rather than free prostaglandins. Furthermore, the free prostaglandins were nontoxic; by analogy with their documented effect on smooth muscle, it was suggested that they might play a role in inducing contractions of the cerata once detached.[401,402]

Another class of prostaglandin derivatives, the 9- and 11-fatty acyl esters of $PGF_{2\alpha}$ (**232**) and $PGF_{3\alpha}$ (**233**),

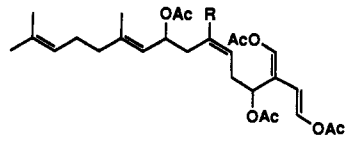


(232) R^1 =H, R^2=fatty acid or R^1=fatty acid, R^2=H
(233) Δ^{17}Z

abundant in egg masses and reproductive glands, but biosynthesized from $PGF_{2\alpha}$ in the mantle tissue with transesterification using phosphoglycerids as acyl donors, were proposed to play a biological role in oocyte production and fertilization.[402,403] The detailed distribution of prostanoids in the various tissues of *T. fimbria* has been studied.[400,402] During the processes of sexual maturation, the prostaglandin lactones are transferred to the reproductive glands as fatty acyl esters; changes in prostaglandin lactone levels were detected during the development of fertilized eggs and after hatching.[403] The detection of multiple biological roles for a single biosynthetic product demonstrates the metabolic efficiency of *T. fimbria* and suggests that the prostaglandin pathway might be worthy of investigation in other mollusks.

C. Other Mollusks

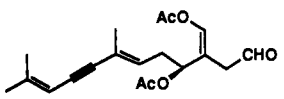
A number of other opistobranch mollusks utilize multiple defensive mechanisms to avoid predation. The animals generally live camouflagued on their specific diet of algae or sponges or buried in the sand, and when molested they secrete a mucus containing toxic chemicals. *Elysia halimedae* feeds specifically on the green algal *Halimeda macroloba* and can take up algal chloroplasts, utilizing primary photosynthetic products for its own nutrition. In addition, the mollusk modifies the ingested algal diterpenoid halimedatetraacetate **234** to the alcohol **235** then secretes it in its mucus when irritated by predators. High concentrations of **235** were also found in egg masses.[404] Finally some mollusks, c.f. *T. fimbria* above, utilize a strategy in which their tail spontaneously detaches and distracts the predator. The ascoglossan *Oxynoe olivacea*, which utilizes such a



(234) R=CHO

(235) R=CH_2OH

strategy, contains the ichthyotoxic metabolites oxytoxin 1 (**236**) and 2 (**237**),[387,405] structurally related to sesquiterpenes such as **238** isolated from *Caulerpa prolifera*, the specific algal diet of this mollusk.[406] The algal compounds are not found directly in the mollusk. Most likely, the animal stores **236** as a protected form of **237**[405] in the same way that olepupuane (**239**) represents a protected form of polygodial (**211**) in *D. limbata*.[391]



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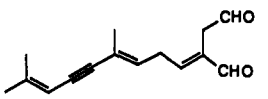

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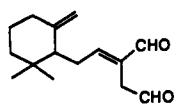

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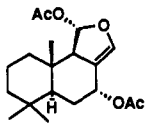
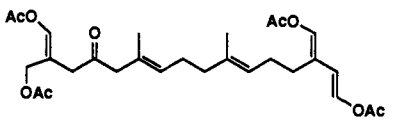


(239)

Cyerce nigricans is a brightly-colored ascoglossan which feeds on the green alga *Chlorodesmis fastigiata* and sequesters chlorodesmin (**240**) from this source. Chemical studies have shown that the mollusk also contains polypropionate metabolites exemplified by **241** and **242**, but these were not feeding deterrents.[407] The



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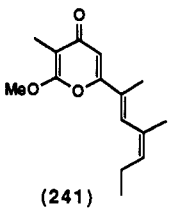

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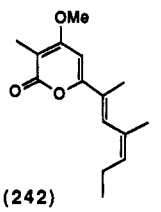
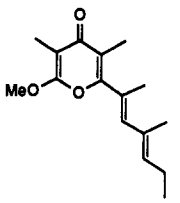


(242)

related mollusk *Cyerce cristallina* from the Mediterranean produces seven other pyrones, the cyercenes, **243–249**.[408] Of the nine compounds, only **243** is derived uniquely from propionate; all of the other pyrones contain at least one, and usually two "acetate-derived" units as part of their carbon skeleton. ^{14}C-labeling experiments with propionate have been undertaken, and provided radioactive cyercenes.[408,409] It would be of value to determine experimentally whether the nonmethylated sites of **241** and **242** and of the cyercenes **244–249** are derived from acetate (c.f. siphonarin A) or whether these sites derive from demethylation of polypropionate-derived precursors. The cyercenes are



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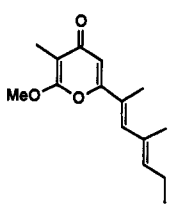

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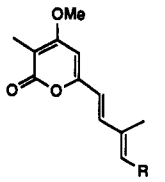
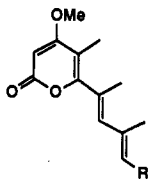


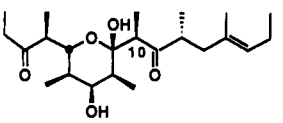
(245) R=Me
(246) R=Et
(247) R=$CHMe_2$



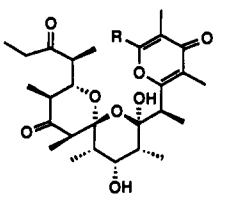
(248) R=Me
(249) R=Et

distributed in the mucous secretion, mantle tissue, and cerata of the animals and are not present in the digestive tract; this chemical distribution is consistent with a *de novo* origin for the cyercenes.[408,409] Cyercene A (**243**) may assist in regrowth of the cerata in the mollusk.[409] *Placida dendritica* also produces polypropionate-like pyrones.[410]

Pulmonates of the genus *Siphonaria* are intertidal, air-breathing mollusks which contain highly methylated polyketide metabolites, consistent with biosynthesis *via* condensation of propionate units. Typical examples include the denticulatins A (**250**) and B (**251**)[411] and the siphonarins A (**252**) and B (**253**).[412] The polypro-

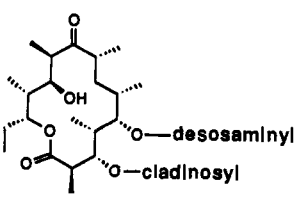


(250)
(251) epimer at C-10



(252) R=Me
(253) R=Et

pionate origin of these metabolites has been demonstrated by incorporation of [1-^{14}C]propionate.[413,414] A low incorporation of acetate was detected,[413] and this was most likely *via* conversion of acetate to succinate, since succinate is also efficiently incorporated into **250–253**.[414] Siphonarin A (**252**) contains a pyrone methyl substituent consistent with the utilization of acetate as a chain starter unit in its biosynthesis; preliminary biosynthetic experiments are in agreement with this proposed direction of polyketide chain building.[414] The demonstration that **250–253** share a common biosynthetic origin to actinomycete metabolites such as erythromycin (**254**)[415] raises the question as to whether



(254)

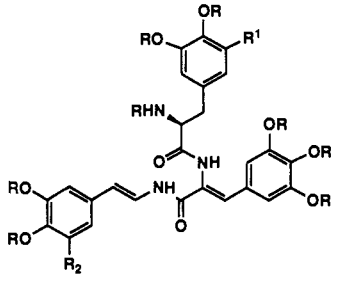
these mollusk metabolites are products of bacterial metabolism. Microbiological examination has not revealed the presence of actinomycetes in siphonariid tissue.[416] The siphonariid polypropionate metabolites are located in mantle tissue and also excreted in mucus produced by the animals when irritated, but do not appear to serve a defensive purpose since siphonariids are commonly eaten by starfish and other predators.[413] Both biosynthetic[416] and synthetic[417] evidence suggests that **250–253** are not genuine mollusk metabolites and may be artifacts produced by facile cyclization of an acyclic metabolite during isolation. The carnivorous mollusks, *Philinopsis* (= *Aglaja*) *depicta* and *Philinopsis speciosa*, which feed on other mollusks, have been shown to contain polypropionate compounds.[418,419] In the case of *P. depicta*, the polypropionates have been traced to its prey, the herbivorous mollusk *Bulla striata*,[420] but the capability of *Bulla* spp. to synthesize polypropionates *de novo* has yet to be evaluated. The dietary origins of pyridine metabolites isolated from the carnivorous mollusk *Navanax inermis* have been discussed,[421] although experimental work has also demonstrated their *in vivo* synthesis.[422]

The capability of mollusks to synthesize sterols or sequester them from dietary sources has been recently reviewed by Kerr and Baker.[128]

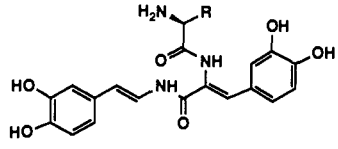
VII. Tunicates

Interest in the tunicates (ascidians) stems from the numerous reports of bioactive, nitrogenous metabolites isolated from this phylogenetic class.[59,157,206,423]

The tunichromes **255–259** are a series of unstable, reducing blood pigments isolated from *Ascidia nigra*, *Ascidia Ceratodes*, and *Molgula manhattensis* which have been implicated in vanadium or iron sequestration.[424,425] Tunichrome production may be seasonally-

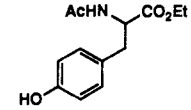


(255) An-1 $R_1=R_2=OH$, R = H
(256) An-2 $R_1=H$, $R_2=OH$, R = H
(257) An-3 $R_1=R_2=H$, R = H
(260) R=Ac, $R^1=R_2=OAc$



(258) Mm-1 R = H
(259) Mm-2 R = I-Bu

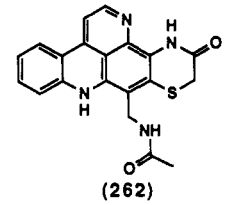
dependant since September collections produced higher yields than February collections; alternatively, the variation may simply reflect the instability and experimental difficulties associated with isolation of these compounds.[424,426] The tunichromes are hydroxy-Dopa tripeptides which structurally resemble the celenamides[427] and other sponge[428] or molluskal[429] metabolites. The incorporation of [^{14}C]tyrosine and [^{14}C]phenylalanine into tunichrome An-1 (**255**) from *A. ceratodes* has been reported.[425,430] To the best of the reviewer's knowledge, this represents the first report of a biosynthetic experiment on a tunicate secondary metabolite. Animals were kept in aquaria to which the radiolabeled material, diluted with unlabelled precursor, had been added for 25 h, then transferred to clean aquaria for 20 days. The seawater was constantly aerated and kept at 12–14 °C. Over 50% of the radioactivity was taken up by the animals during the first 3 h of incubation. An alternative incorporation procedure was direct injection into the animal maintained in an aquarium. Tunichrome An-1 was isolated as its acetate derivative **260** by TLC and purified by reverse-phase HPLC. Radiolabel was detected in a number of TLC bands in addition to the band identified as **260**, indicating the presence of tunichrome precursors or degradation products. The amount of radioactivity associated with the tunichromes increased over the duration of the experiment. The authors concluded that tunichrome synthesis occurs in fully-differentiated blood cells. Studies on the oxidation of *N*-acetyl-3,4-dihydroxyphenylalanine ethyl ester (**261**) provide an



(261)

alternative biosynthetic model for the formation of tunichromes, by enzymic hydrolysis and oxidative tautomerization of dopa-containing peptides.[431] It would therefore have been of interest to monitor the incorporation of phenylalanine into protein in the biosynthetic experiments described above for *A. ceratodes*. Free tunichromes have been identified in the morula cells of *A. ceratodes*[432] while vanadium complexes of tunichrome are presumed to be present in signet ring cells.[424,430] The differing cell types were separated by fluorescence-activated cell sorting;[424,425] a similar picture for the vanadium distribution pattern has been detected using X-ray microanalysis[433] and staining techniques.[434] An improved method for the isolation of tunichromes has recently been reported.[435] Direct proof in the *in vivo* interaction between vanadium and the tunichromes remains elusive.

A number of other tunicate metabolites have structural similarities to sponge metabolites.[436] Among them are the biologically-active shermilamine alkaloids, represented here by shermilamine B (**262**).[437] A brief report on the incorporation of [^{13}C]tryptophan into (**262**) has appeared, but full experimental details are not yet available.[438]



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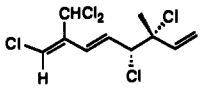
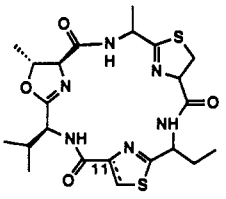
In addition to alkaloids, the tunicate *Clavelina lepadiformis* contains a pentachlorooctatriene **263**

which is more typically an algal compound.[439] Algae of



(263)

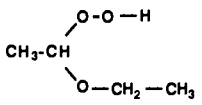
the genus *Prochloron* have been identified as symbionts in tunicates[440] and have been implicated in the biosynthesis of the cytotoxic cyclic peptides of *Lissoclinum patella*,[96,441] although further investigation is warranted.[442] The isolation of the same cyclic peptide, westelliamide (**35**), from cultures of the cyanobacterium *Westellopsis prolifica*[95] and from *L. bistratum*[443] further supports an algal role in the formation of these compounds. In the original report,[96] algal cells were separated from animal cells by gentle and mechanical dissociation; the cyclic peptides bistratamide A (**264**) and B (**265**) were located in the algal cells by NMR. It



(264)

(265) Δ^{11}

is known that ascidian symbionts can transfer amino acids to their hosts.[441,444] The controversy over the origin of these peptides now appears experimentally-resolvable following the successful separation of sponge[235] and tunicate[424,425] cells by fluorescence-activated sorting. Chemical interest in ascidians of the genus *Lissoclinum* may be expected to continue since there is pronounced variation in the chemistry of *L. patella* collections from different sites.[441,445] The hydroperoxide **266**, which has been isolated from ascidians[446,447] and algae,[447] may represent another example of an algal–ascidian symbiosis, although the lack of optical activity associated with **266** raises the question of it being a solvent-derived artifact.[447]



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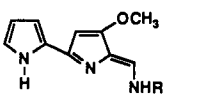
Interest in the chemical ecology of ascidians and the role of secondary metabolites in their protection has developed; recent literature includes work from Paul,[379] Davis,[448] and others.[449] The tambjamines, e.g. tambjamine C (**267**), are pyrrolic pigments in ascidians, bryozoans,[379,450–452] and mollusks that feed on these invertebrates;[379,451,452] the tambjamines resemble bacterial pigments[453,454] and other bacterial metabolites,[14,38–40] and therefore represent an interesting biosynthetic target. Microscopic investigation of the ascidian *Atapozoa* sp. suggests that the animal may be capable of the *de novo* biosynthesis of tambjamine metabolites,[452] but this has yet to be evaluated experimentally.

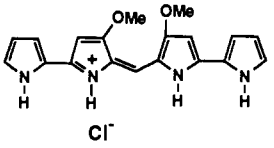


(267) R=

VIII. Other Marine Organisms

A. Bryozoa

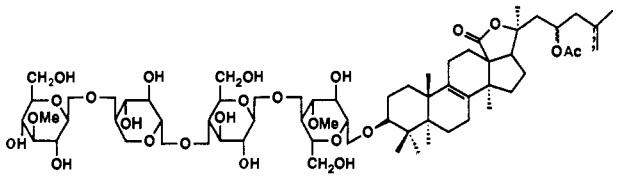
The marine natural products chemistry of bryozoa[157] is currently dominated by the biomedically-important bryostatins.[59,455] More unusual secondary metabolites identified in bryozoans include dimethyl disulfide, dimethyl sulfide, and methanethiol,[456] analogous to the isolation of these and related compounds from sponges.[248] A microbial source of the compounds has been discussed[457] and suggested for other bryozoan secondary compounds.[454,455] The same blue tetrapyrrolic pigment **268** has been isolated from the bryozoan *Bugula dentata*[459] and an ascidian.[460] Related compounds have been isolated from marine bacteria, therefore a microbial origin for the pigments seems possible.



(268)

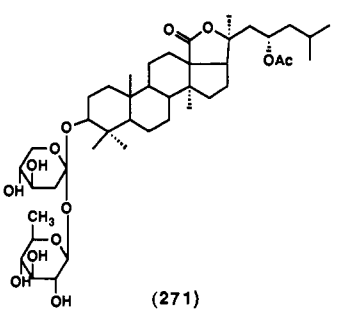
B. Echinoderms

Echinoderms contain physiologically-active saponins together with sterol sulfates and glycosides as their predominant metabolites.[59,157,461,462] Biosynthetic experiments suggest that echinoderms are capable of limited *de novo* sterol and triterpene synthesis, but that they can modify dietary precursors. The low incorporation of acetate into the aglycon moiety of the thelothurins **269** and **270** in the holothurians *Thelonata ananas*[463] and stichopogenin A_4 (**271**) from *Stichopus japonicus*[464] has been claimed, but *Stichopus califor-*



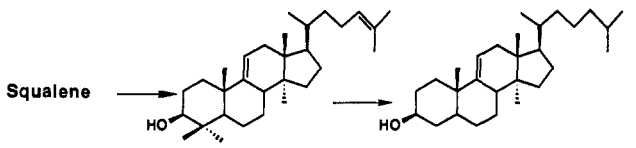
(269)

(270) Δ^{25}



(271)

Scheme 34



(272)

nicus utilizes lanosterol in preference to acetate for stichopogenin A_4 synthesis.[465] Squalene is transformed by the sea cucumbers *Bohadschia argus*, *Holothuria mexicana*, *Holothuria arenicola*, and *S. californicus* into $\Delta^{9(11)}$ sterols but not into saponins.[466] Δ^7-Sterols in *S. californicus* derive from dietary Δ^5-sterols,[466] although an earlier report had suggested the possibility of *de novo* synthesis.[467] Sterol glycoside biosynthesis from cholesterol has been demonstrated,[468-470] as has the conversion of lanost-9(11),24-dien-3β-ol (**272**) to the $\Delta^{9(11)}$-14α methyl sterols of *H. arenicola* (Scheme 34).[466,471] The possibility exists that the toxic sterol and triterpene constituents of starfish and holothurians may only be produced in response to developmental or other needs; this may provide an explanation for the conflicting biosynthetic literature in this area.

A number of triterpene glycosides possess antifungal activity which may be linked to their binding with membrane sterols, a similar mechanism of action to that evaluated for the potent antifungal agent amphotericin. The significance of membrane sterol composition and the likely membrane disruption caused by the cytotoxic echinoderm compounds has been discussed.[472,473] Elyakov has suggested that the amphiphilic nature of sterol sulfates and glycosides in echinoderms may have evolved in response to the surrounding marine habitat.[474]

A number of echinoderms contain bacterial symbionts in their digestive systems and these may play an important role in providing nutrients such as fatty acids for the animal host.[475] Compounds formed from the oxidation of arachidonic acid and other polyenoic acids occur in starfishes and may be involved in developmental processes;[476] it will be interesting to determine whether the biosynthesis of these starfish eicosanoids follows the lipoxygenase pathway detected in other marine organisms. Holothurians also contains high concentrations of arachidonic acid[477] which suggests a search for eicosanoids in them may be of value.

IX. Concluding Remarks

Progress in our understanding of the paths of marine-based biosynthetic processes has progressed significantly in recent years. There are now experimental examples in all of the major phyla which have been investigated for their secondary metabolite composition. Highlights for the reviewer included the diverse biosynthetic paths to isonitriles in marine and terrestrial systems and for C_{11} hydrocarbons such as ectocarpene. The continued development of marine eicosanoid biosynthetic study is encouraging, given the biological and biomedical importance of prostaglandins. The major areas of progress in marine biosynthetic study have all been with organisms which are amenable to culture or from which cell-free preparations can been developed. The interaction between a host, commonly an invertebrate, with a bacterial, cyanobacterial, or dinoflagellate symbiont provides an extra level of interest and complexity to the biosynthetic investigation.

The origins of marine natural products, in addition to their structures and biological profiles, are highly relevant to the development of these secondary compounds as biomedically-useful agents or as agrochemicals. Biosynthetic knowledge may enable the identificatioin of trace intermediates which may have a preferred therapeutic profile or provide information about the optimization of metabolite production. Many of the complex, biomedically-interesting marine natural products isolated to date do not present facile targets for organic synthesis. Synthetic modification of compounds to provide semisynthetic analogues requires access to reasonable quantities of pure compound from natural sources. It is no longer acceptable to harvest bulk quantities of marine organisms using SCUBA on the basis of environmental and ecological grounds, therefore a clearer picture of the biochemical strategies of marine organisms and the enzymes that they use to put together complex secondary metabolites may enable us to move toward a more acceptable goal of "farming" marine organisms on a commercial scale.

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XI. References

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