Carbocyclic Oxylipins of Marine Origin

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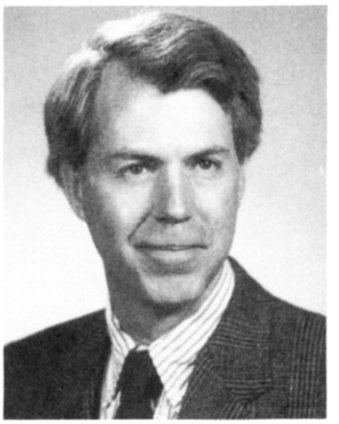

A native of Oakland, CA, Bill Gerwick developed an early interest in marine life from the California coast. While earning his undergraduate B.S. degree in Biochemistry (1976) at the University of California at Davis, he participated in phycological research with Norma Lang in the Botany Department. This led him to join William Fenical for Ph.D. studies at the Scripps Institution of Oceanography, University of California at San Diego where he investigated the terpenoid natural products of tropical brown algae. After completing his degree in 1981, he joined Steve Gould's group at the University of Connecticut for postdoctoral studies on the mechanism of biosynthesis of the antitumor antibiotic streptonigrin. This was followed by a brief appointment in the Department of Chemistry at the University of Puerto Rico, Rio Piedras, where he initiated independent studies of marine algal chemistry. In 1984 he joined the faculty at the College of Pharmacy at Oregon State University where he now holds the position of Professor of Medicinal Chemistry and Natural Products. During the last 8 years at Oregon State, he and his research group have discovered many new natural products from marine algae, focusing, however, on the oxylipins of cold water macroalgae and the anticancer-type natural products of collected and cultured cyanobacteria. In recent years, his research has also explored the mechanism of biosynthesis of several algal-derived oxylipins, work performed in part through sabbatical collaboration with Mats Hamberg at the Karolinska Institute, Stockholm (1990–91). He holds affiliate positions in the Department of Biochemistry and Biophysics and the Department of Chemistry at Oregon State University, and the Department of Marine Sciences at the University of Puerto Rico.

I. Introduction and General Remarks

Discovery in 1969 that the marine coral *Plexaura homomalla* produces enormous quantities of prostaglandins initiated a fascinating era in the field of marine natural products chemistry. Clearly, it is the relationship of these marine-derived prostanoids to the mammalian substances that provides the primary interest in this structure class. In mammalian systems, these arachidonic acid-derived substances (Figure 1) are of fundamental importance in maintaining normal physiological conditions, a process known as homeostasis. A few representative functions of these mainly arachidonic acid-derived compounds, as well as several disease states with underlying etiologies involving this pathway, are listed in Table I.

Since this initial discovery in the marine environment, related substances of varied structure have been isolated from the full spectrum of marine life. Because marine organisms, as well as higher plants, commonly utilize 18-carbon fatty acids as well as 20-carbon fatty acids in these pathways, a new term, "oxylipin", has been required to collectively describe these compounds

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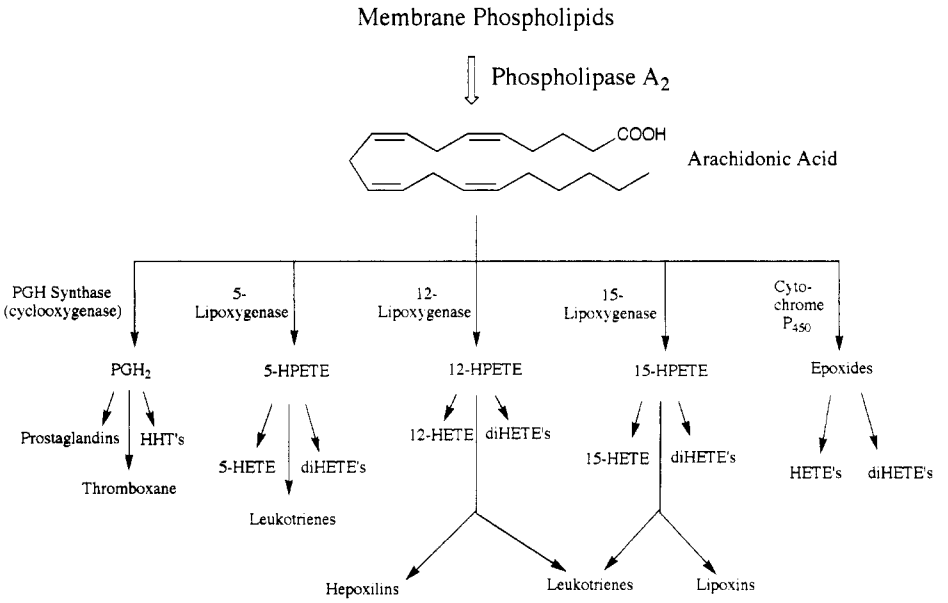


Figure 1. Overview of the arachidonic acid cascade in mammalian tissues.

Table I. Oxylipins (Eicosanoids) in Human Physiology and Disease

representative functions of oxylipins	representative diseases involving oxylipins
platelet aggregation	inflammation
smooth muscle contraction	psoriasis
bronchial and vascular constriction	asthma
chemotaxis and adherence	atherosclerosis
immune responses	heart disease
ion secretion (stomach acidity)	stomach ulcers
reproductive phenomena	cancer
neuro-endocrine roles	

because "eicosanoid" refers only to those of 20 carbons. Oxylipin has been defined as an encompassing term for oxidized compounds which are formed from fatty acids by reactions involving at least one step of mono- or dioxygenase-dependent oxidation.[1,2] Thus, this term includes eicosanoids as well as biosynthetically related compounds of longer and shorter chain length. It is important to note that this definition does not require the incorporation of molecular oxygen into the structure of an oxylipin; however, utilization of molecular oxygen is required at some stage of the biosynthetic pathway. Recently, there has been recognition that mammalian species also utilize 18-carbon polyunsaturated fatty acids in some of these biosynthetic manifolds.

Two recent reviews have provided a comprehensive treatment of the oxylipin chemistry of marine algae[3] and invertebrates.[2] Consequently, the intent of this review is to examine and update that subset of marine-derived oxylipins which possess the most "intriguing" structures. Intriguing has been interpreted in this context as a mixture of structural sophistication and relatedness to substances of mammalian importance. This has defined the scope of this review to those marine oxylipins containing at least one carbocyclic ring. Inherent in these carbocyclic structures are biosynthetic events which match or exceed the complexity and elegance of biosynthetic events leading to the prostanoids of mammalian occurrence. It is truly magnificent to contemplate the forces which have impelled nature to generate at least give distinct biosynthetic routes to a skeleton which is recognizably prostaglandin-like: mammalian prostaglandin H synthase,[4] prostaglandin synthesis in the coral *Plexaura homomalla*,[5,6] algal carbocyclic oxylipin biosynthesis (hybridalactone,[7] ecklonialactone,[8] cymathere ether[9]), brefeldin biosynthesis by *Penicillium brefeldianum*,[10] and jasmonic acid and phytodienoic acid production by higher plants.[19] However, for most of the marine cases it must be cautioned that the proposed pathways are presented only as hypotheses at this point.

The material covered in this review is arranged by degree of ring complexity, starting with metabolites containing monocarbocyclic rings (cyclopropyl, cyclopentyl) and ending with bicarbocyclic oxylipins (Table II). Not covered in this review are the fatty acid-derived pheromones of brown algae, some of which also contain carbocyclic rings. These substances are intimately involved in the normal sexual cycle of marine brown algae. Recently, at least some of these have been shown to derive from arachidonic and eicosapentaenoic acids, possibly through a 9-lipoxygenase-initiated metabolic route.[11]

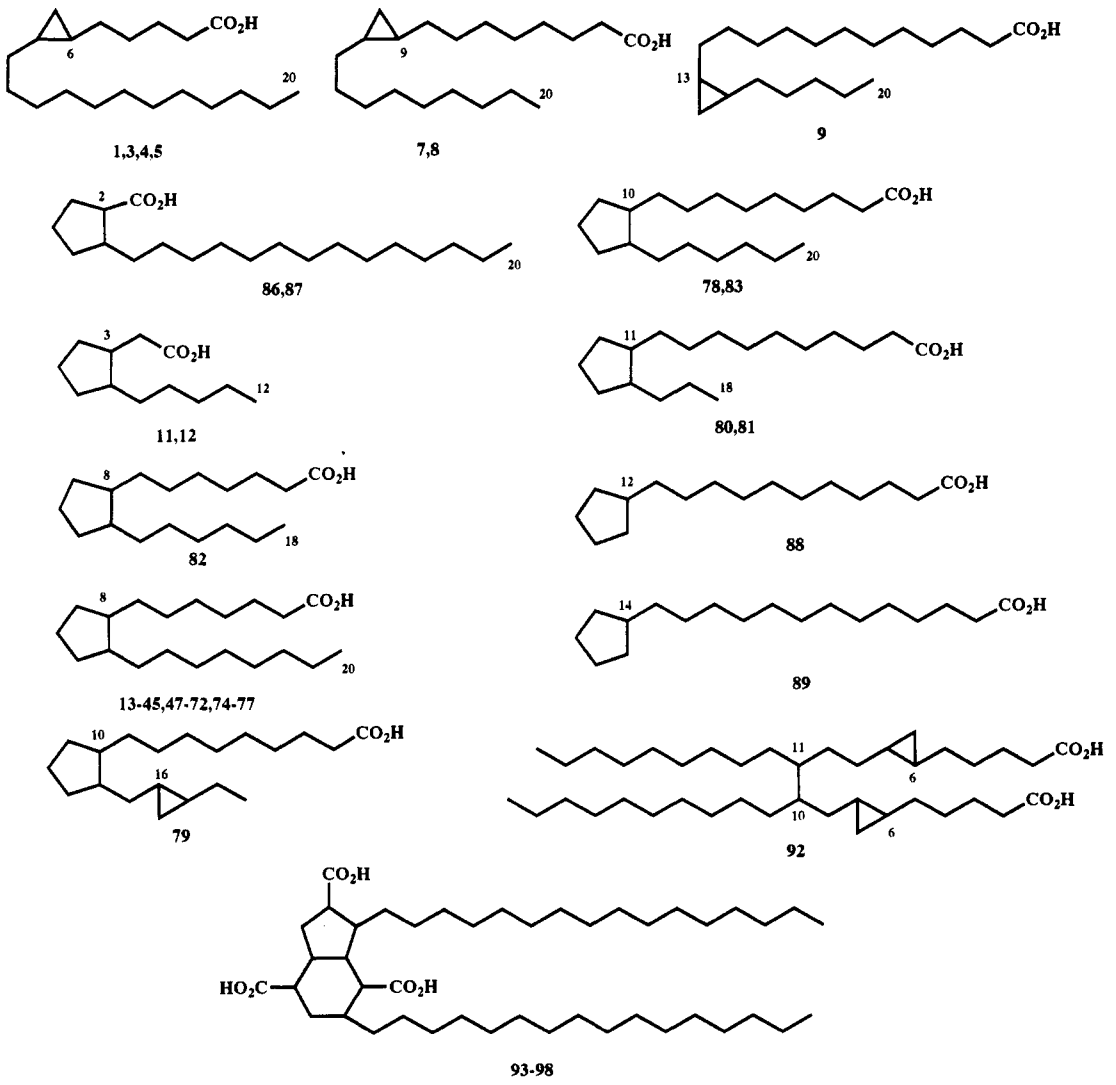
II. Monocarbocyclic Oxylipins

A. Oxylipins Containing a Cyclopropyl Ring

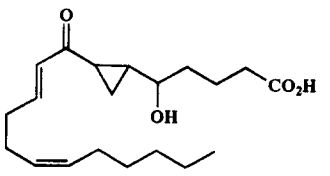
1. Cyclopropyl Product from the Gorgonian Coral Plexaura homomalla

The first monocarbocyclic cyclopropyl- and lactone-containing oxylipin from a marine organism came as a result of studies on the mechanism of prostaglandin biosynthesis by the Caribbean gorgonian *Plexaura homomalla*.[12] In this cell-free study, exogenously supplied arachidonic acid was converted in a very low yield to a product, **1**, containing a cyclopropyl ring, a ketone, and an alcohol. Cyclopropyl metabolite **1** was also obtained when allene oxide **2** was provided to the enzyme preparation. While it was subsequently shown with heat-denatured controls that this product formed nonenzymatically from allene oxide **2**, this occurred only in the presence of the coral homogenate (heated or unheated); perhaps some inorganic constituent is

Table II. Carbocyclic Oxylipin Skeletal Types in Marine Organisms



critical for this rearrangement. Compound 1 spontaneously lactonized to 3 which was amenable to detailed ^{1}H NMR analysis. The structure was assembled from spectroscopic analyses of product 1 and its lactone 3, and a featureless CD curve was interpreted to indicate that it was racemic. The significance of this work was that it suggested a mechanism for the formation of 5-*trans*-prostaglandins in *P. homomalla* (Figure 2), products which had long been known in the coral but whose origins were an enigma.[13]



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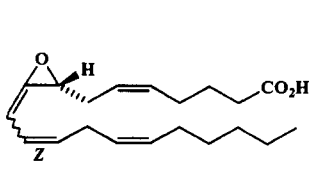

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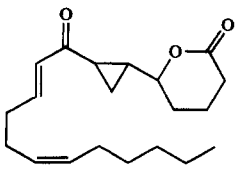

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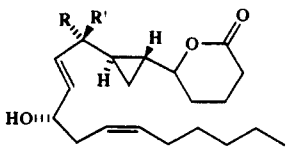
2. Constanolactones from the Red Alga Constantinea simplex

The Oregon (United States) coast intertidal red alga, *Constantinea simplex*, has also been a source of cyclopropyl containing oxylipins[14] of structures remarkably similar to that obtained in the above coral biosynthetic experiments. However, the algal metabolites (i.e. 4 and 5) are natural products present in abundance (1–2% of the lipids). They were initially



4) R = H, R' = OH
5) R = OH, R' = H

obtained in pure form as protected peracetate esters and characterized as to planar structure by various spectroscopic information, principally that derived from multinuclear NMR. Isolation of 12(*S*)-hydroxyeicosatetraenoic acid (12(*S*)-HETE), 12(*S*)-hydroxyeicosapentaenoic acid (12(*S*)-HEPE), and 12-oxo-dodeca-

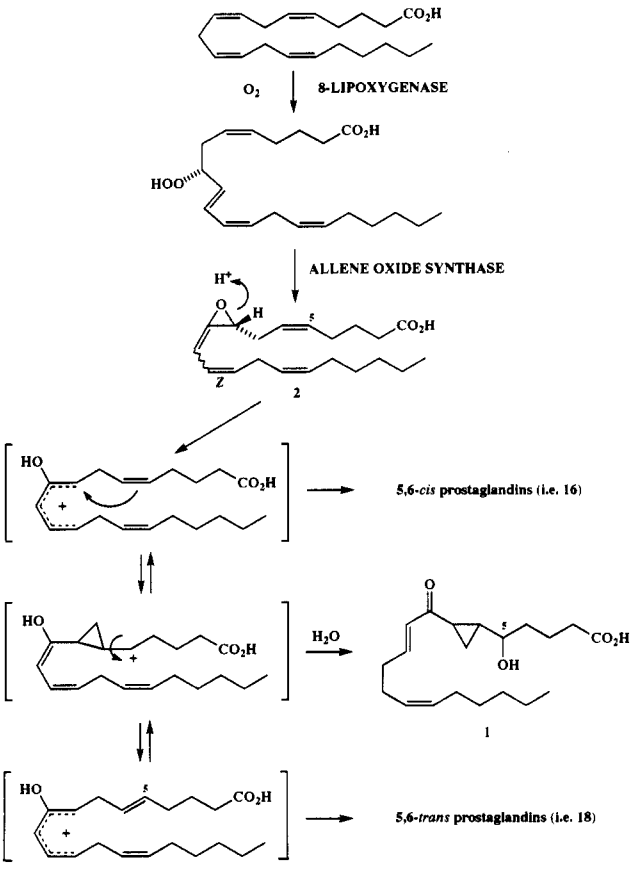
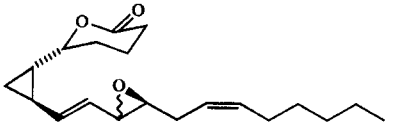


Figure 2. Proposed biosynthesis of cyclopropyl metabolite 1 and 5-*trans*-PGA_2 by *Plexaura homomalla* (from ref 12).

5(*Z*),8(*E*),10(*E*)-trienoic acid as cometabolites in the alga supported the contention of an active (12*S*)-lipoxygenase in the alga. The absolute stereochemistry of the constanolactones has recently been solved via degradation to chiral fragments, CD analysis of the separately formed C9 and C12 monobenzoates, and ^1H NMR studies (Nagle and Gerwick, work in progress). A 12-lipoxygenase-initiated metabolism of constanolactones A (**4**) and B (**5**) was hypothesized which explained the occurrence of both epimers at C9 (Figure 3). Subsequently, additional constanolactones with C9–10 olefins and C11–12 vicinal diols have been isolated (Nagle and Gerwick, work in progress). As these were also isolated as a mixture of epimers (at C11), this finding suggested that epoxyconstanolactone **6** was the product actually formed from enzymatic metabolism. This unstable allylic epoxide could then be subject to nonenzymatic hydrolysis, both inside and outside of the cellular system. Occurrence of metabolites **1** in coral and **4** and **5** in algae are yet further striking examples of how nature employs disparate metabolic pathways to construct an identical ring skeleton.



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3. Halicholactones from the Sponge Halichondria okadai

The sponge *Halichondria okadai* was the third source of cyclopropyl-containing oxylipins (**7** and **8**),[15] these showing an identical relationship of functional groups within the fatty acid chain as seen for constanolactones **4** and **5**. However, the positions of these groups were shifted to C8–15 versus C5–12 in the constanolactones. The planar structure of halicholactone (**7**) and neohalicholactone (**8**) were originally formulated on the basis of spectroscopic data and partial degradation,[15] and relative stereochemistry provided by X-ray diffraction analysis of neohalicholactone (**8**).[16] While the relative stereochemistry is identical in these metabolites to that found in the constanolactones **4** and **5**, the absolute stereochemistry at C15 was shown to be *R* by degradation to 1,2(*R*)-diacetoxyheptane and comparison with authentic materials.[15] It is likely that the halicholactones originate from transformations analogous to those represented for the constanolactones, albeit initiated through a 15-lipoxygenase introduced hydroperoxide (Figure 4).

Isolation of these cyclopropyl-containing oxylipins from such diverse marine life as a coral homogenate

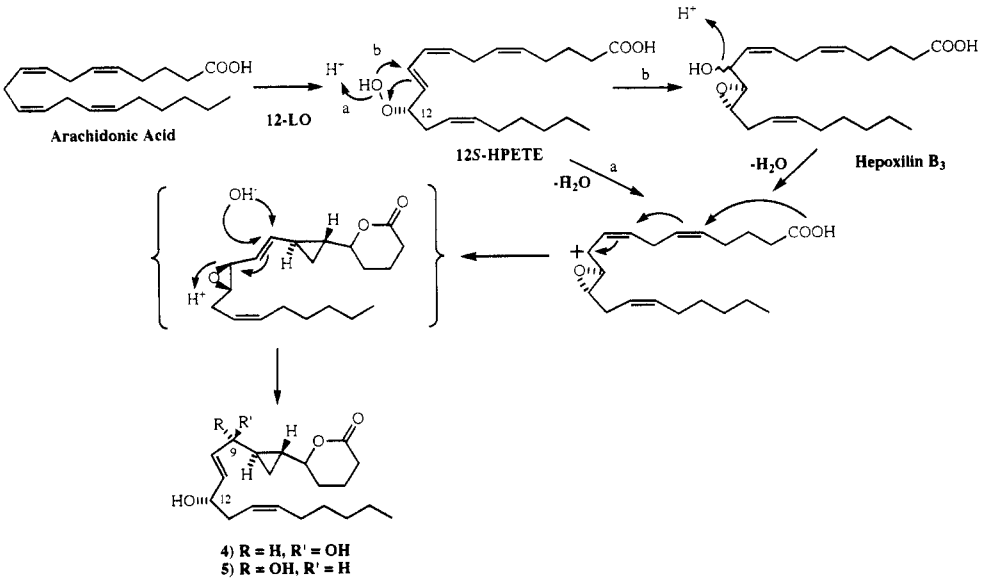


Figure 3. Proposed biosynthesis of constanolactones A (**4**) and B (**5**) by the red alga *Constantinea simplex* (from ref 14).

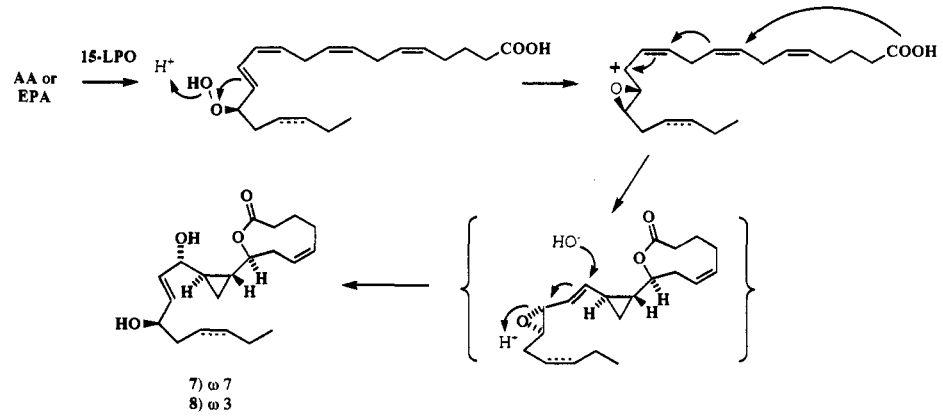
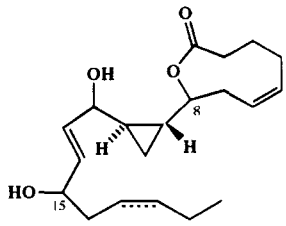


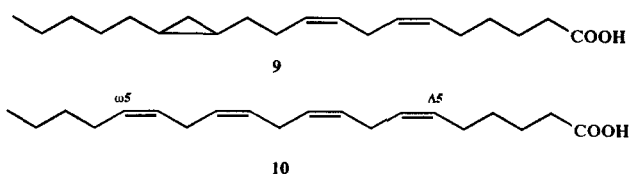
Figure 4. Biogenesis of sponge metabolites halicholactone (7) and neohalicholactone (8) from arachidonic and eicosapentaenoic acids, respectively (from ref 2).



incubated with arachidonic acid, a red alga, and a sponge, and with oxygen and olefin functional groups at highly analogous positions in all three, suggests that this is a new theme of oxylipin metabolism with broad distribution and, likely, important function.

4. Cyclopropyl Fatty Acid from the Sea Hare Bursatella leachii

A study of the digestive gland chemistry from the sea hare *Bursatella leachii* collected from the coast of Texas led to the isolation of a new cyclopropane-containing fatty acid **9**.[17] Accounting for 75% of the fatty acid content of the digestive gland, its structure was solved by ^{1}H NMR analysis of both the natural product and a C11 fragment formed by ozonolysis. Stereochemical aspects of the structure of **9** were apparently not considered, although a *cis* ring juncture was pictured in the original report. Report of this fatty acid metabolite in *B. leachii* was unique in that most sea hares accumulate terpenoid natural products in their digestive gland originating from their algal diet. Since neither the cyclopropane fatty acid **9** nor the putative ω5-eicosatetraenoic acid precursor to **10** (Figure 5) have been reported from algae, it is uncertain where this metabolite originates. However, as this work was reported without experimental details,[17] some elements of the unsaturation pattern in **9** remain uncertain.



B. Oxylipins Containing a Cyclopentyl Ring

1. Jasmonic Acid from the Red Alga Gelidium latifolium

The marine cyclopentanoids with the least complex structures, (−)-jasmonic acid (11, 0.7 μg/g fresh weight)

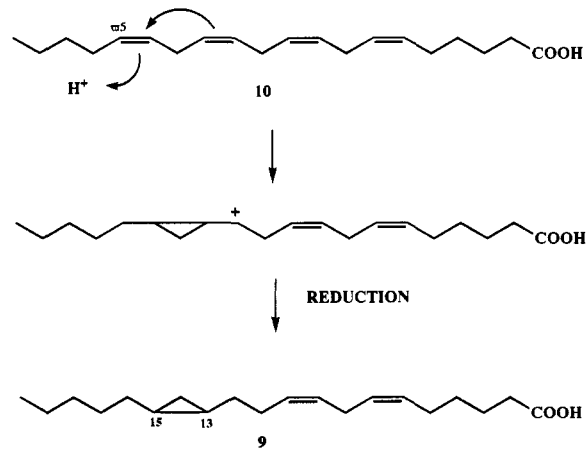


Figure 5. Possible biogenesis of cyclopropane fatty acid **9** from the sea hare *Bursatella leachii* (from ref 14).

and 7-(−)-isojasmonic acid (12, 0.04 μg/g fresh weight) were recently reported as constituents of the Black Sea red alga, *Gelidium latifolium*.[18] In higher plants,

jasmonic acid is an important plant growth hormone, the biosynthesis of which has been the subject of intense investigation and shown to involve lipoxygenase initiation to an allene oxide which subsequently cyclizes to a cyclopentanoid.[19] β-Oxidation is responsible for shortening of the α-chain (Figure 6). A MeOH extract of field-collected *G. latifolium* was fractionated over DEAE-Sephadex A-25, methylated, and then fractionated over Adsorbex RP 18. The jasmonic acid fractions were analyzed by GC and GC–MS in comparison with authentic standards. The approximate 93:7 ratio of jasmonic acid to 7-isojasmonic acid (12) was determined in two ways. Curiously, in most terrestrial plant species, more 7-isojasmonic acid (12) than jasmonic acid (11) is usually found.[19] Additionally, 7-isojasmonic acid is reported to be considerably more bioactive than jasmonic acid itself and is probably the substance with true physiological relevance in most species.[19] However, the ratios of these isomers in *G. latifolium* might suggest a different relative importance of these two compounds, although no other experimental evidence exists to support this contention.[18]

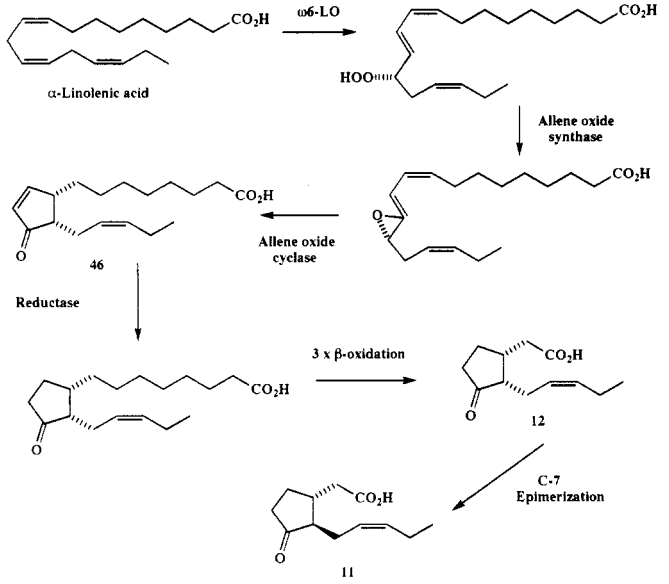
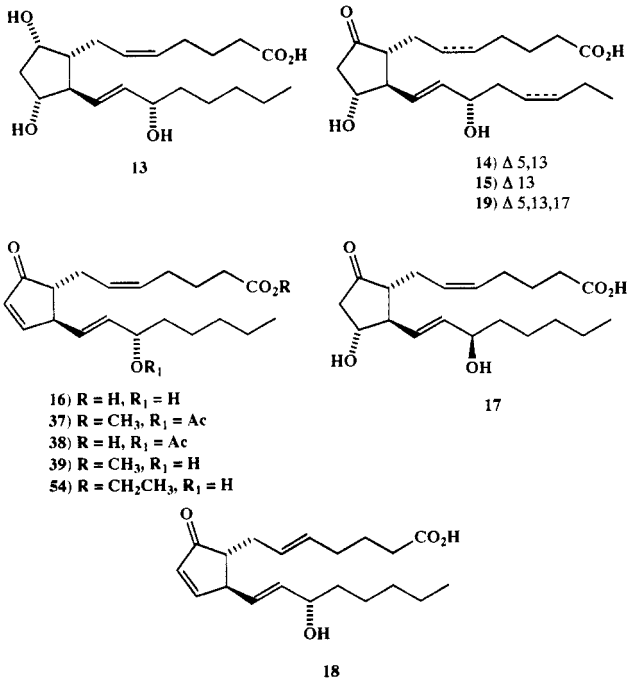


Figure 6. Biosynthesis of jasmonic acid (from ref 19).

2. Marine Organisms Containing Prostaglandin E and Its Derivatives

Intriguingly, the mammalian oxylipins prostaglandin $F_{2\alpha}$ (**13**) and prostaglandin E_2 (**14**) have been the most commonly encountered carbocyclic oxylipins in marine algae and invertebrates (Table III). This is particularly noteworthy given the biosynthetic studies to date which have shown that some marine organisms utilize pathways completely different than those of mammals for the formation of these structures. Specifically, it appears that at least in corals, and likely in algae, that these prostaglandins are formed via lipoxygenase metabolism, in contrast to the prostaglandin H synthase (cyclooxygenase) pathway found in mammals.[4]

Homogenates of a number of marine invertebrate species were found to have a modest ability to convert exogenous eicosatrienoic acid to PGE_1 (**15**) including the coral *Anthoplexaura* sp., the mussel *Mytilis*, and the lobster *Homarus*.[20] *Mytilis edulis* was subsequently shown to contain endogenous compounds with PGE biological activity.[21] The Caribbean gorgonian coral *Plexaura homomalla*, renowned for its abundance of prostanoids, most notably PGA_2 (**16**), was also found to contain both (15*R*)- and (15*S*)-PGE_2 (**17** and **14**).[22,23] Structural assignments were made as the result of spectroscopic, mass spectrometric, and chemical derivatization procedures. Subsequent studies of the prostaglandins of this gorgonian have confirmed the presence of PGE_2.[13] PGE_1 (**15**), as well as $PGF_{1\alpha}$, was tentatively identified from incubations of radiolabeled eicosatrienoic acid with isolated tissues of the tunicate *Halocynthis roretzi* using TLC methodology.[24]



Following a rat antihypertensive activity assay, PGE_2 (**14**) was isolated from the Australian red alga *Gracilaria lichenoides* as 0.05–0.07% of the dry weight of the alga.[25] Structure elucidation of this isolate of PGE_2 (**14**) was performed on the synthetic methyl ester, formation of which was necessary for final purification. PGE_2 methyl

Table III. Occurrence of the Mammalian-Type Carbocyclic Oxylipins (PGF, PGE, PGA, PGB) in Marine Organisms (See Text for Discussion and References)

organism	type organism	PGF_α derivatives	PGE derivatives	PGA/PGB derivatives
Plexaura homomalla	soft coral	13,22	14,17	16,18,33–34,51
Euplexaura erecta	soft coral	13		
Lobophytum depressum	soft coral	23–26		
Lobophytum carnatum	soft coral			39,54–56
Anthoplexaura sp.	soft coral		14,15	
Terpios zeteki	sponge	13,27	14	
Cymphoma gibbosum	mollusc			16,45
Modiolus demissus	mollusc		PGE derivatives	PGA derivatives
Aplysia californica	mollusc	13,27	14	
Tethys fimbria	mollusc	29–32	14,19–21	57,58
Mytilis sp.	mussel		14,15	
Homarus sp.	lobster		14,15	
Strongylocentrotus intermedius	sea urchin	13	14	
Strongylocentrotus nudus	sea urchin	13	14	
Crassostrea gigas	oyster	13		
Pactinopectin yessoensis	scallop		14	
Halocynthia roretzi	tunicate	PGF_1	14	
Euglena gracilis	protist	13	14	
Gracilaria lichenoides	red alga	13	14	
Gracilaria verrucosa	red alga		14	16

ester was identified from a combination of spectroscopic data, including ^1H NMR and optical rotation. It was speculated that PGE_2 (14) and $PGF_{2\alpha}$ (13, see below) might be responsible for the minimal epiphytization upon this alga by other algae. Furthermore, it was hypothesized that the algae may use a lipoxygenase-initiated pathway to synthesize these prostanoids,[25] in similarity to the pathway now believed to occur in *Plexaura homomalla* (see below)[5,6] and in contrast to the route in mammalian systems.[4]

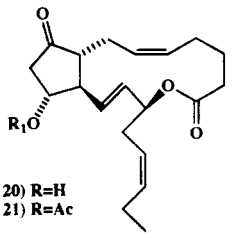
While the related Japanese red alga *Gracilaria verrucosa* is commonly eaten, its ingestion occasionally gives rise to a severe gastrointestinal syndrome known as "ogonori poisoning". The diarrhea and nausea producing components were subsequently isolated using a mouse bioassay and the principal agent shown to be identical to PGE_2 (14) by spectroscopic methods and partial degradations; however, the absolute stereochemistry was not investigated.[26] Authentic PGE_2 elicited the same symptoms in the mouse bioassay. Prostaglandins are known emetic agents in many animal systems,[27] and it is for this property that corals apparently produce large quantities of various prostaglandins as an antipredatory defense.[28,29] Soaking of *G. verrucosa* in fresh water is required to produce PGE_2 (14). It has been speculated that water-soluble inhibitors of PGE_2 synthesis are removed in this process, although it is also possible that the release of bound (e.g. to glycerol)[30] PGE_2 is enhanced by this treatment. A patented process for the isolation of PGE_2 (14) from *G. verrucosa* has appeared.[31]

An organism with both animal- and plant-like biochemical characteristics, the unicell *Euglena gracilis* (Kingdom = Protista), was found to contain PGE_2 using serological and immunochromatographic methods.[32] As production of PGE_2 (14) as well as other eicosanoids in *E. gracilis* (i.e. $PGF_{2\alpha}$, 13) is greater in cells grown in the dark than those grown in the light, it has been suggested that their production may be the result of this organism's animal-type metabolic capacities.

Bioassay and radioimmunoassay were used to demonstrate production and release of PGE-type prostaglandins from the mollusc *Modiolus demissus*.[33] Subjecting the animals to hypoosmotic stress was shown to induce significant PG release into sea water. This was inhibitable by treatment with aspirin or indomethacin, suggesting that their production arises from prostaglandin H synthase metabolism.

Aqueous extracts of the sponge *Terpios zeteki* were found to contain PGE_2 (14), however, only immunochromatographic methods were used in this analysis.[34] Radioimmunoassay techniques were also used to detect PGE_2-like substances, as well as other eicosanoids, in aqueous extracts of the mollusc *Aplysia californica*.[34] More recently, *Aplysia* neurons have been found able to transform exogenous arachidonic acid into PGE_2 (14).[35] Prostaglandin-like biological activity, tentatively identified as PGE_2 (14) by TLC, was localized to the inner organs of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*.[36] Recently, it has been shown that PGE_2 may have an endogenous role in modulating the release of eggs from the scallop *Patinopecten yessoensis*.[37]

The mantles of the nudibranch *Tethys fimbria* were shown to contain a rich diversity of prostanoids, including PGE_2 (14) and PGE_3 (19) as well as the corresponding lactones, PGE_3 1,15-lactone (20) and PGE_3 1,15-lactone 11-acetate (21). These were identified on the basis of spectroscopic analysis and comparison with synthetically produced derivatives.[38] Similar 1,15-lactones in the PGF-series were also characterized.[39,40] It seems that these PGE derivatives serve several roles in the nudibranch; they appear to (1) be involved in oocyte maturation or hatching, (2) function as ichthyotoxic agents in defense of fish predation, and (3) mediate contraction of the dorsal appendage and induce mucous secretion.[41] Biosynthetic studies of prostaglandin production in *T. fimbria* have painted a complicated picture in which the original site of production is the dorsal mantle, particularly during sexual maturation. The prostanoids are then transported to the ovotestis, the site of ultimate storage and utilization.[42,43] However, it is unknown whether cyclooxygenase or lipoxygenase metabolism is responsible for construction of the prostanoid skeleton in *T. fimbria*.

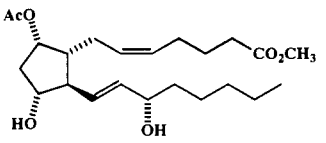


20) R=H
21) R=Ac

3. Marine Organisms Containing Prostaglandin F and Its Derivatives

Detailed investigations of the prostaglandins of the Caribbean gorgonian *Plexaura homomalla* have led to the isolation of numerous known as well as new prostaglandins. In the PGF structure class, these include $PGF_{2\alpha}$ itself (13)[13] and the 9-*O*-acetate of methyl $PGF_{2\alpha}$ 22.[44] The structure of $PGF_{2\alpha}$ was established by comparison with authentic materials while that of its 9-*O*-acetate by spectroscopic means in comparison with model compounds. Interestingly, and in contrast to other classes of prostanoids in *P. homomalla*, all of the PGF analogs reported from this gorgonian are of the mammalian-type stereochemistry at C15 (*S*). A Japanese coral, *Euplexaura erecta*, was found using a guinea pig ileum assay to also be a source of $PGF_{2\alpha}$ (13).[25] It was identified by comparison with authentic $PGF_{2\alpha}$ and several derivatives using TLC and GC–MS. Hence, some dimensions of the stereochemistry of this isolate of $PGF_{2\alpha}$ remain unproven. Methyl 11-acetoxy-$PGF_{2\alpha}$ (23) was isolated from the Red Sea soft coral *Lobophyton depressum* and its structure assembled from spectroscopic data obtained on the natural product, principally from ^1H NMR.[46] The stereochemistry at C15 was established as *S* using a combination of biological and chemical properties of the hydrolysis product, $PGF_{2\alpha}$ (13). Three additional prostanoids were isolated and characterized, using spectroscopic methods and conversion to known compounds; methyl 11,18-diacetoxy-$PGF_{2\alpha}$ (24), 11-acetoxy-$PGF_{2\alpha}$ (25), and 11,-18-diacetoxy-$PGF_{2\alpha}$ (26).

As detailed above, $PGF_{2\alpha}$ (13) was also obtained along with PGE_2 from the Australian red alga, *Gracilaria*



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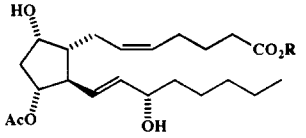
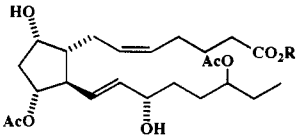


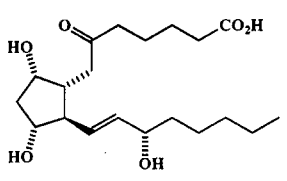
23) R = CH_3
25) R = H



24) R = CH_3
26) R = H

lichenoides and identified by detailed spectroscopic analysis of its methyl ester derivative.[25]

In addition to PGE_2 (14), aqueous extracts of the sponge *Terpios zeteki* were found to contain $PGF_{2\alpha}$ (13) and 6-keto-$PGF_{1\alpha}$ (27) by immunochromatographic analysis.[34] These same two prostanoids were detected using similar methodology in aqueous extracts of the mollusc *Aplysia californica*.[35] Isolation of 6-keto-$PGF_{1\alpha}$ (27) suggests the presence of prostacyclin I_2 (PGI_2, 28) in these two sources. $PGF_{2\alpha}$ (13) was also detected by immunological techniques in extracts of the unicellular protist *Euglena gracilis*[32] and in gonadal tissues of the Japanese oyster, *Crassostrea gigas*.[47] In this latter organism, $PGF_{2\alpha}$ levels were observed to increase almost 4-fold during the maturation process. Using TLC methodology in combination with a bioassay, the inner organs of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius* were shown to contain $PGF_{2\alpha}$ (13).[36] Branchial tissues of the tunicate *Halocynthia roretzi* were shown by chromatographic and bioassay methods to metabolize exogenous ^{14}C-labeled eicosa-8,11,14-trienoic acid to PGF_1-like compounds.[24]



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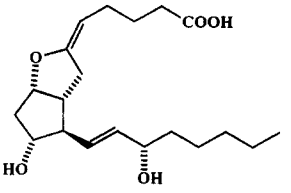
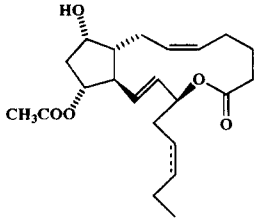
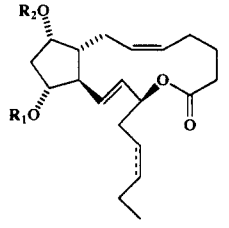


28

The nudibranch *Tethys fimbria* contains, in addition to prostaglandin-1,15-lactones of the A and E series, those of the F series as well, including $PGF_{2\alpha}$ 1,15-lactone 11-acetate (29) and $PGF_{3\alpha}$ 1,15-lactone 11-acetate (30).[39,40] In addition, both the reproductive tissues and eggs of this nudibranch were shown to contain high concentrations of long-chain fatty acid esters of $PGF_{2\alpha}$ and $PGF_{3\alpha}$ (31 and 32).[42,48]



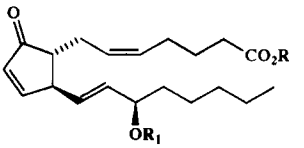
29) ω7
30) ω3



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

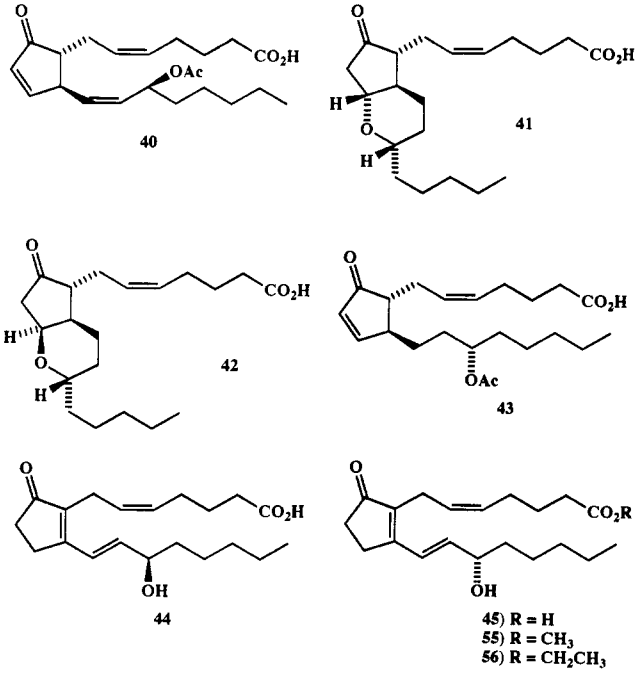
4. Marine Organisms Containing Prostaglandins A and B and Their Derivatives

The discovery that prostaglandins are produced by corals was initiated by the finding of (15*R*)-PGA_2 (33) and methyl 15(*R*)-acetoxy-PGA_2 (34) in the Caribbean gorgonian *Plexaura homomalla* forma *homomalla*.[49] The C15 hydroxyl group of these prostaglandins is epimeric with that found in mammalian systems, and the quantities of prostanoids found in this coral far surpass those found in higher animals (a variable yield of 1–3.5% of the wet weight of the coral has been reported).[49,50] The planar structures of metabolites 33 and 34 were characterized by extensive chemical degradation to structurally informative fragments, although stereochemical aspects were less rigorously established. Subsequent studies confirmed the original structural hypothesis, including absolute stereochemistry.[22,23]



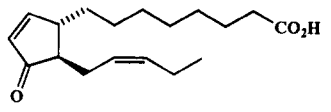
33) R = H, R_1 = H
34) R = CH_3, R_1 = Ac
35) R = H, R_1 = Ac
36) R = CH_3, R_1 = H

Following this initial discovery of large quantities of PGA_2 (33) in *P. homomalla*, this coral became the subject of intense chemical,[51] biological,[52] biochemical,[2] and ecological[29] investigation as it was considered a potential industrial source of these bioactive molecules.[13,53,54] As a result, a number of analogs of PGA and PGB, both of 15*R* and 15*S* stereochemistry (as well as derivatives of PGE and PGF, see above), have been isolated from the coral and structurally characterized (18 and 35–45).[22,55] While the propensity of the coral to make 15*R* or 15*S* prostanoids appears to correlate with geographical region (15*R* from Florida, 15*S* from Cayman Islands),[13] it is premature to conclude from this that the two stereoisomers are produced in different subspecies.[29] In fact, there are reports of individual colonies that produce both C15 stereoisomers [(15*R*)-PGB_2 (44) and (15*S*)-PGB_2 (45)].[13,22] It was also shown that in the coral these prostanoids largely exist in esterified form (methyl or acetoxyl). The hydrolysis of these relatively labile esters is accelerated in the presence of a coral esterase.[13] Hence, depending upon the conditions employed between collection and workup of *P. homomalla* samples, a variable yield of free prostaglandins, mono-esters, and bis-esters have been reported.[22] The biosynthesis of these prostaglandin derivatives by *P. homomalla* has similarly been the subject of intense investigation. As these corals contain an appreciable biomass of symbiotic microalgae in their tissues, it was questioned whether the animal or plant cells were responsible for this metabolism. Isolation of the zooxanthellae and demonstration of their inability to metabolize exogenous labeled substrate in any regard, as well as the absence of any endogenous prostanoids in these cells, was taken as evidence that they are not the site of biosynthesis or storage of prostaglandins.[56] However, it was proposed that the algae could contribute polyunsaturated fatty acid precursor (arachi-



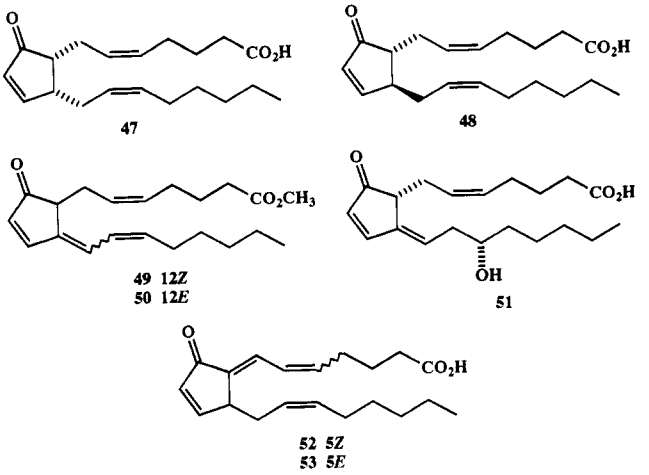
donic acid) into the sponge prostaglandin biosynthetic pathway.[56]

Numerous investigations of the biosynthesis of these coral prostanoids, principally by the Corey group at Harvard and the Brash group at Vanderbilt, have occurred over the past 20 years; however, we still do not have a complete understanding of the prostaglandin biosynthetic pathway in *P. homomalla*.[2] Early investigations illustrated that the coral utilizes a route other than the prostaglandin H synthase (cyclooxygenase) pathway typical of mammalian prostaglandin biosynthesis.[57,58] Subsequently, in work with *P. homomalla*[5] as well as through parallel investigations with several other species of coral (notably, *Pseudoplexaura porosa* and *Clavularia viridis*),[59,60] a pathway has emerged (Figure 7), related to one found in higher plants, which produces the phytohormones phytodienoic acid (**46**) and jasmonic acid (**11**).[19] However, the exact nature of



the substrate (i.e. whether it possesses any preexisting oxidation) which feeds into this coral "allene oxide" pathway is unknown.[5] A number of new prostanoids **47–53** have been isolated from biosynthetic experiments with soft corals.[6] These biosynthetic investigations have been hampered, however, by the inability to develop a coral preparation which is able to perform the entire biosynthetic sequence beginning with arachidonic acid and finishing with chiral PGA_2.

The role of PGA_2 ester production by *Plexaura homomalla* appears to be involved in the chemical defense of these soft-bodied organisms against predation by fish, and perhaps invertebrates as well.[61–64] A series of investigations by Gerhart have convincingly showed that predacious fish are deterred from feeding on these corals because the coral-derived prostaglandins [both (15*R*)-PGA_2 (**33**) and (15*S*)-PGA_2 (**16**)] are

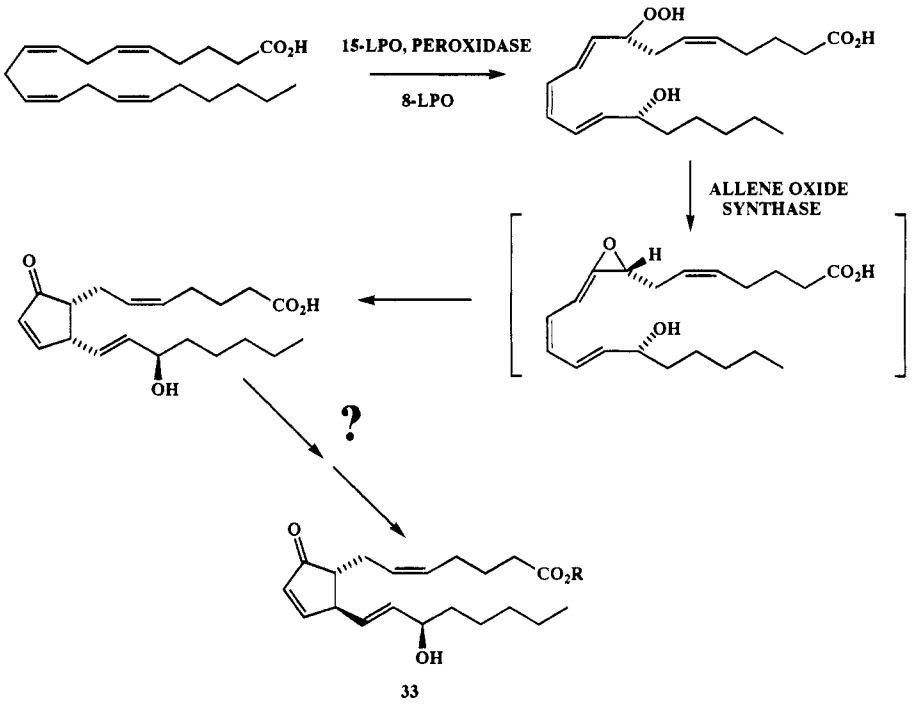


effective emetic agents which subsequently cause a learned aversion in the fish.[61–64] Prostaglandins are well known to possess emetic properties.[27] Unfortunately, there was a flaw in the design of these experiments as only the fully deesterified PGA_2's were evaluated and not any of the esterified forms which exist in the coral or that are produced by partial ester hydrolysis. Concern over this defect was the impetus for a follow-up investigation which evaluated the antifeedant activity of all possible esterified, partial esterified, and free forms of (15*R*)-PGA_2 **33–36** by their incorporation into agar strips.[65] These were attached to ropes anchored in a tropical reef ecosystem. Both of the partially esterified forms (**35** and **36**) as well as free (15*R*)-PGA_2 (**33**) showed significant antifeedant activity while the fully esterified form **34** did not. However, this latter result was less certain due to a poor recovery of test agar strips. While it seems certain that prostaglandins in *P. homomalla* function in nature to protect the coral from predation,[29] these later experiments have served to point out that it remains uncertain what levels of esterification exist in the ecologically relevant PGA_2 derivatives.

While the prostaglandins of *P. homomalla* appear to inhibit predation by most species, one gastropod mollusc, the flamingo tongue snale (*Cymphoma gibbosum*), is a specialist predator adapted to feeding on this coral. Although the gregarious behavior and feeding habits of the *C. gibbosum* appear unrelated to prostaglandin production by the coral,[63,66] it seems that this predator does assimilate prostaglandins from its diet. Specifically, both a small amount of (15*S*)-PGA_2 (**16**) as well as larger quantities of its less toxic decomposition product (15*S*)-PGB_2 (**45**) were detected in the mollusc.[67] It is possible that *C. gibbosum* catalyzes this rearrangement in an effort to detoxify the PGA_2 in its diet.

Another mollusc, the bivalve *Modiolus demissus*, was suggested to contain PGA derivatives as determined by reaction with anti-PGB following reaction with dilute KOH.[33] Prostanoid production in molluscs appears to be involved in osmotic regulation[33,68] and, by virtue of its inhibition with aspirin or indomethacin, produced via prostaglandin H synthase as in mammals. A low-affinity PGA_2 binding site has been described from *M. demissus*.[69]

The red alga *Gracilaria verrucosa* was a source of small amounts of PGA_2 (**16**); however, this may be a dehydration artifact of the more major metabolite in the alga, PGE_2 (**14**, see above).[26]

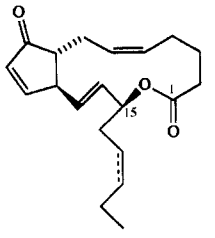


33

Figure 7. Proposed biosynthesis of (15*R*)-prostaglandin A_2 (**33**) by the Caribbean octocoral *Plexaura homomalla* (from ref 5).

Lobophytum carnatum from the coast of Vietnam was recently investigated for both its fatty acid and oxylipin content and found to contain a very high level of polyunsaturated fatty acids as well as methyl and ethyl esters of PGA_2 (**39** and **54**) and its degradation product PGB_2 **55** and **56**.[70] The ethyl esters were likely artifacts of storage in ethanol. However, since these identifications were made solely on the basis of HPLC and GC retention times versus standards, as well as by mass spectrometry, absolute stereochemical features were not rigorously established.

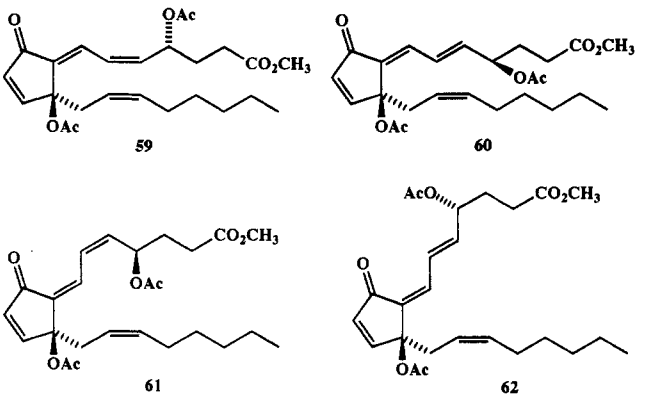
The nudibranch *Tethys fimbria* is a source of PGA_2 1,15-lactone (**57**) and PGA_3 1,15-lactone (**58**), prostanoids related in structure and function to those of the PGE and PGF series described above from this source.[39,40]



57) ω7
58) ω3

5. Clavulones and Related Metabolites from the Soft Coral Clavularia viridis

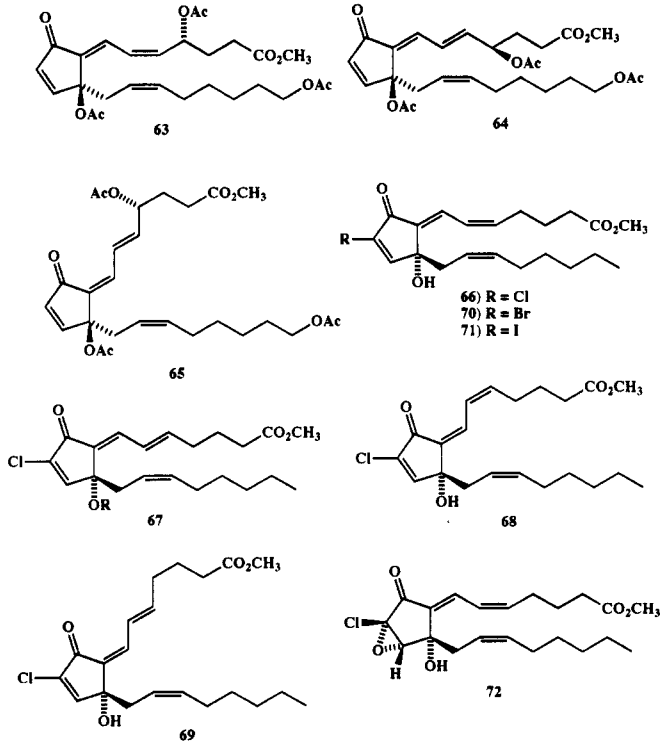
The Okinawan soft coral *Clavularia viridis* has been a rich source of structurally novel and biologically active oxylipins. The first of these, reported simultaneously by two groups in 1982,[71,72] were a mixture of four geometrical isomers, clavulone I (**59**), clavulone II (**60**), clavulone III (**61**), and claviridenone a (**62**). In both reports, a combination of spectroscopic methods and limited chemical degradations were used to assign the planar structures. Clavulones I–III (**59–61**) were reported to possess antiinflammatory activity in a fertilized chicken egg assay (30 μg/mL).[71] The absolute stereochemistry of these prostanoids was deduced using degradative and chiroptical techniques by both groups the following year.[73,74] Both laboratories deduced the correct absolute stereochemistry, however, applied the wrong stereodescriptor to the C12 center (12*R* was applied; 12*S* was pictured and ultimately correct).[75] Several stereospecific syntheses of the clavulones have confirmed their structures and stereochemistry.[76–79]



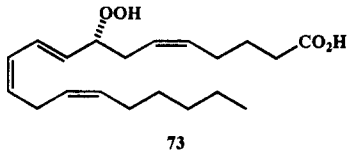
59 60 61 62

A number of derivatives of these clavulone natural products have subsequently been isolated from *C. viridis* and defined using similar techniques, including the 20-acetoxy derivatives of clavulones I–III (**63–65**),[80] C10 chlorine-containing metabolites chlorovulones I–IV (**66–69**),[75,81] the bromo and iodo analogs (**70** and **71**) of chlorovulone I,[82] and the C10,11-epoxide analog of chlorovulone I (**72**).[83]

The biosynthesis of these *C. viridis* prostanoids has been the source of intense interest and experimentation. While early thoughts on how these unique oxylipins were produced envisioned a variant of the endoperoxide pathway,[84] it was subsequently shown that lipoxygenase pathways of arachidonic acid metabolism were more



prevalent in the coral. Specifically, various cell-free preparations of *C. viridis* (as well as other corals, e.g. *Pseudoplexaura porosa*)[59] were shown to transform exogenous arachidonic acid to 8(*R*)-hydroperoxyeicosatetraenoic acid (8(*R*)-HPETE, **73**).[60] This presumed 8-lipoxygenase product was then shown to be converted by coral enzymes to a cyclopentanoid product, termed "preclavulone-A" (**47**).[59,60] The planar structure and relative stereochemistry of cyclopentanoid **47** was established based on extensive chemical degradations and synthesis.[60] The close structural similarity of preclavulone-A to 12-oxophytodienoic acid (**46**) and isojasmonic acid (**12**), lipoxygenase derived products from higher plants,[19] suggested it might be formed via analogous transformations.[85] Hence, it was envisioned (Figure 8) that lipoxygenase-derived 8(*R*)-HPETE (**73**) is first transformed to an allene oxide (**2**),



then opened to an oxidopentadienyl cation intermediate, which finally cyclizes to preclavulone-A (**47**). While preclavulone-A possesses an obviously close structural relationship to the naturally occurring clavulones, it has subsequently been found that allene oxide **2** undergoes spontaneous nonenzymatic rearrangement yielding racemic preclavulone-A.[86,87] Thus, it remains uncertain what relationship the *in vitro* synthesis of racemic preclavulone-A has to the *in vivo* biosynthesis of chiral clavulones.

The clavulones and their halogenated derivatives, along with the structurally related punaglandins (see below), have shown remarkable cytotoxicity and antiproliferative activity against several transformed cell lines. Clavulone I (**59**) shows excellent antiproliferative

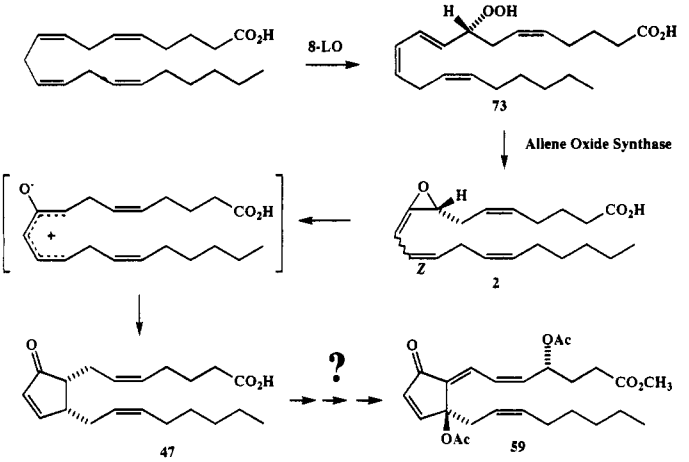


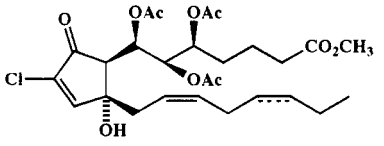
Figure 8. Proposed biosynthesis of the clavulones in the Okinawan soft coral *Clavularia viridis* (from ref 60).

activity to HL60 cells (ED_{50} = 0.4 μM) with cytotoxicity observed at levels greater than 1 μM,[88] while clavulone II (**60**) in L1210 cells showed good cytotoxicity (IC_{50} = 0.6 μM).[89] Clavulone I (**59**) was shown to inhibit DNA synthesis leading to an accumulation of cells in G_1 phase.[88] Evaluation of analogs of the clavulones and PGA_2 in these assays has led to the following structure–activity (antiproliferative and cytotoxic) relationships in this structure class: (1) the C10–11 olefin or epoxide is essential for activity, (2) a halogen atom at C10 increases the activity of the clavulones (Cl = F > Br = I > H), (3) the C12 hydroxyl is also required for full activity, (4) the C5–8 diene potentiates these activities, and (5) the stereochemistry of the C12 and C15 hydroxyl groups is not important to the activity.[90–92] *In vivo* testing of a few of these *Clavularia* products has been promising (clavulone II (**60**) at 10 mg kg^{-1} day^{-1} for 5 days gave a T/C = 151[131] or at 20 mg kg^{-1} day^{-1} gave a T/C of 160).[89] The clavulones have also been described to have potent (0.45 μM) positive chronotropic effects on the spontaneous beating rate of cultured myocardial cells from fetal mouse hearts.[93]

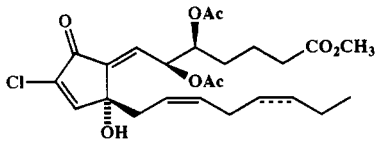
6. Punaglandins from the Octocoral *Telesto reisii*

The Hawaiian octocoral *Telesto reisii* produces biologically active oxylipins related in structure to those from *C. viridis* (see above).[94] At the time of their discovery, these four punaglandins (1–4; **74–77**) were the only ones to naturally contain halogen atoms. Their planar structures were determined by spectroscopic methods, principally, high-field ^1H NMR. Some elements of the relative stereochemistry were proposed based on the products formed from elimination of acetic acid, coupling constants, and nOe experiments. However, stereospecific syntheses in a number of laboratories soon led to a revision in the relative stereochemistry for punaglandins 3 (**76**) and 4 (**77**), and also defined the full absolute stereochemistry for these metabolites as 5*S*,6*S*,12*R*.[94–99] Punaglandin 3 (**76**) was extremely potent in inhibiting L1210 leukemia cell proliferation (IC_{50} = 0.04 μM)[95] and caused a significant life extension in Ehrlich ascites tumor bearing mice (T/C = 180 at 5 mg kg^{-1} day^{-1} for 5 days).[89] This *in vivo* activity was subsequently confirmed in other studies (punaglandin

3 (76) at 5 mg kg^{-1} day^{-1} gave T/C = 151; punaglandin 4 (77) at 5 mg kg^{-1} day^{-1} gave T/C 179).[91]



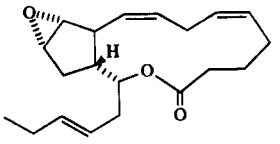
74) ω6
75) ω3



76) ω6
77) ω3

7. Macrolactone from the Red Alga Laurencia hybrida

A monocarbocyclic oxylipin, 78, closely related to hybridalactone (79), was also isolated from the red alga *Laurencia hybrida* and its structure tentatively reported in the same publication as hybridalactone.[100] The structural assignment was based principally on ^{1}H NMR data in comparison with the better described hybridalactone (79, see below).



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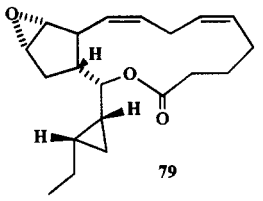

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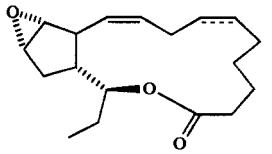
8. Ecklonialactones from the Brown Alga Ecklonia stolonifera

Two novel oxylipins with weak abalone antifeedant activity have been isolated from the Japanese kelp *Ecklonia stolonifera* and their structures determined as ecklonialactones A (80, 0.65%) and B (81, 0.33%) by spectrochemical techniques and X-ray crystallography.[101] Ecklonialactone A (80) was subjected to a

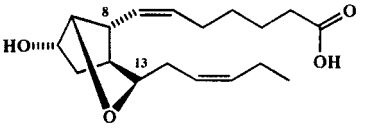


80) Δ 6
81) Δ 9

detailed spectroscopic analysis to determine the nature of functional groups and two-dimensional NMR methods were employed to piece the molecule together; this structure and relative stereochemistry were confirmed by X-ray crystallography. Ecklonialactone B (81) was hypothesized as the 6,7-dihydro derivative of ecklonialactone A (80) from its highly comparable data set. This was confirmed by direct comparison of the perhydro derivatives produced from both compounds. It is interesting to note that the relative positions of functional groups as well as the relative stereochemistry in ecklonialactones A (80) and B (81) is the same as found in hybridalactone (79, see above and Table II). The differences in positions of the rings, olefins, and sites of oxidation in the ecklonialactones and hybridalactone structure classes derives from the fact that they originate from 18 and 20 carbon precursors, respectively. Hence, a biogenesis analogous to that proposed and partially substantiated for hybridalactone[102] has been proposed for ecklonialactones A (80) and B (81) beginning with stearidonic and linolenic acids, respectively.[3]

9. Cymathere Ethers from the Brown Alga Cymathere triplicata

The large brown kelp *Cymathere triplicata* is an edible alga from the state of Washington and British Columbia which gives off a detectable odor of cucumbers when exposed at low tide. These odoriferous components of the cucumber are known to arise from lipoxygenase metabolism of polyunsaturated fatty acids followed by hydroperoxide lyase chain scission.[103] The extract of a frozen collection of the alga was revealed to contain a wealth of oxylipin natural products, two of which [cymathere ether A (82) and cymathere ether B (83)] were isolated and defined by spectroscopic analysis, principally of the methyl ester derivatives.[9]



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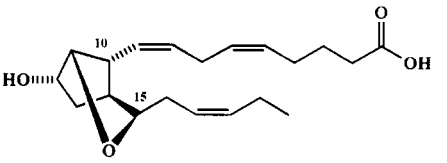
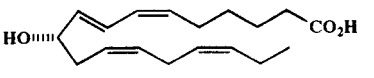


83

By these techniques, these metabolites were shown to be homologous cyclopentyl ether-containing metabolites which logically arise from stearidonic and eicosapentaenoic acid precursors, respectively. The relative stereochemistries were determined by ^{1}H NMR coupling constants and nOe studies, and absolute stereochemistry inferred by isolation of the cometabolites 10(*S*)-hydroxyoctadeca-6(*Z*),8(*E*),12(*Z*),15(*Z*)-tetraenoic acid (84) and 12(*S*)-hydroxyeicosa-5(*Z*),8(*Z*),10-(*E*),14(*Z*)-tetraenoic acid (85). These hydroxy-con-



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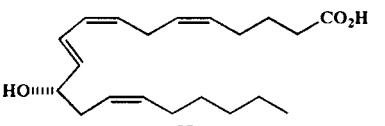

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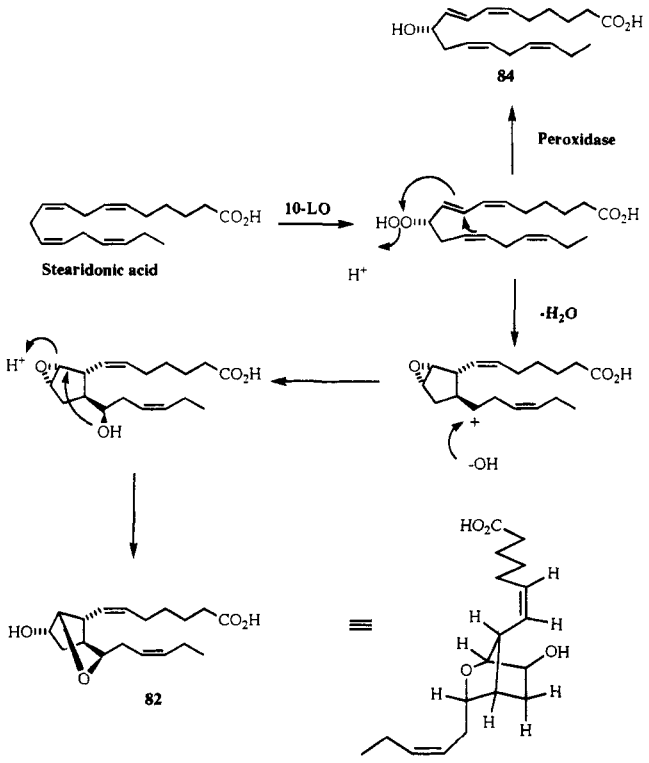


Figure 9. Proposed biogenesis and three-dimensional representation of cymathere ether A (82) and 10(*S*)-hydroxyoctadeca-6(*Z*),8(*E*),12(*Z*),15(*Z*)-tetraenoic acid (84) in the brown alga *Cymathere triplicata* (from ref 9).

taining oxylipins are proposed as products of alternate metabolisms of the key intermediate hydroperoxides (Figure 9). The three-dimensional representations of these ether-containing oxylipins illustrate that they are in fact substituted oxynorbornanes, and as such, they represent unprecedented locked conformation prostaglandin analogs. In the proposed biogenetic pathway to cymathere ether A (82), the hydroperoxide is converted to an epoxide with concomitant formation of the cyclopentyl ring and water addition to C13. Internal epoxide opening by the C13 alcohol yields the cymathere ethers (Figure 9).

10. Bacillariolides from the Diatom Nitzschia pungens

In an effort to identify constituents other than domoic acid in the marine diatom *Nitzschia pungens* which would explain the severity of human intoxications from shellfish grown in the presence of a natural bloom of this alga, the methanol extract of cultured diatom cells was investigated for unique constituents.[104] Two new compounds were isolated, bacillariolide I (86, 0.25% wet weight of cells) and bacillariolide II (87, 0.0036% wet weight of cells), and their structures defined by spectroscopic techniques, principally NMR. While prostaglandins are known to possess emetic properties[27] and PGE_2 deriving from a red alga is implicated as the causative agent in food poisoning in Japan,[26] it remains unknown whether the bacillariolides participate in the shellfish poisonings seen in Canada and elsewhere. It was speculated that the bacillariolides (86 and 87) may derive from anionic opening of a 5,6-epoxide intermediate, itself deriving from rearrangement of a 5-lipoxygenase introduced hydroperoxide (Figure 10). While it was suggested that this hydroperoxide rearrangement

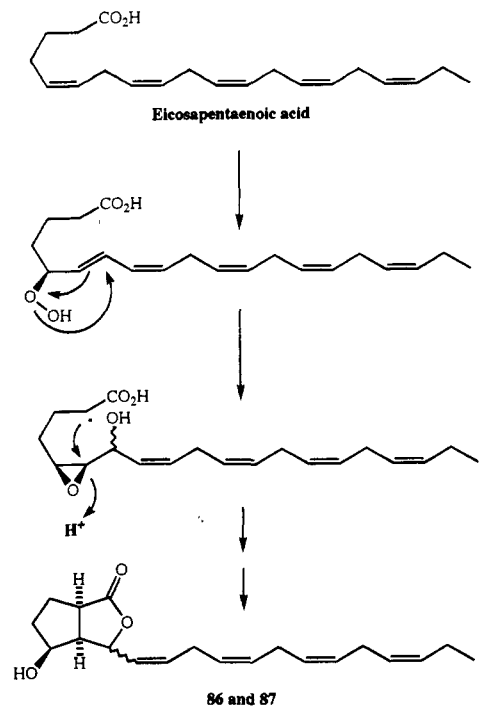
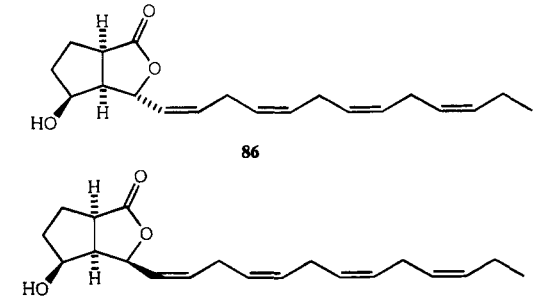


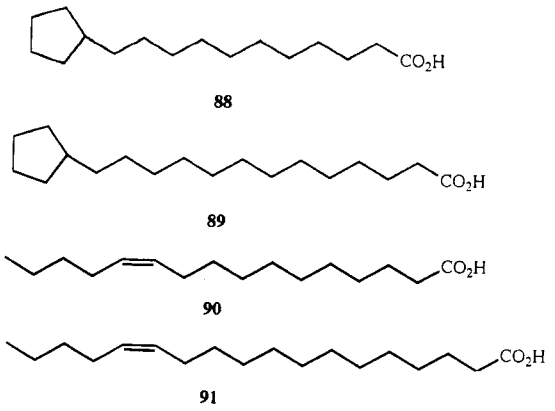
Figure 10. Proposed biogenesis of bacillariolides I (86) and II (87), modified from Shimizu (from ref 104).



occurs with nonstereospecific addition of OH^- to C7, other epoxy alcohols of this sort have been shown to arise from intramolecular rearrangement of the hydroperoxide intermediate.[105]

11. Fatty Acids with Terminal Cyclopentyl Rings from Red Algae of the Solieriaceae

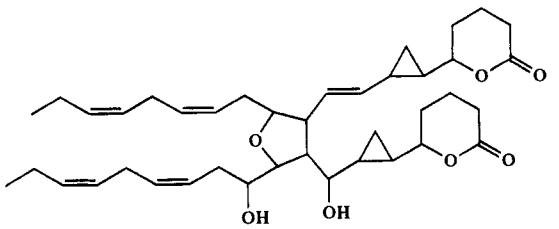
Study of the fatty acid compositions of five algae from the Solieriaceae (Gigartinales), *Agardhiella tenera*, *Anatheca montagnei*, *Euchema cottonii*, *E. spinosum*, and *Meristotheca senegalensis*, revealed the presence of several unique compounds of potential chemotaxonomic significance.[106] GC–MS methodology of the methyl ester derivatives using authentic standards defined one known but highly unusual cyclopentyl-containing fatty acid (dihydrohyndocarpic acid, 88) and a second new but homologous cyclopentyl-containing fatty acid (dihydrochaulmoogric acid, 89). Two new ω5-containing fatty acids [18:1ω5 (90) and 16:1ω5 (91)] were also described and were proposed as precursors to the cyclized species. Alternatively, a short-chain acid containing a preexisting cyclopentyl ring could function as a starter unit to fatty acid biosynthesis (H. Floss, personal communication).



III. Bicarbocyclic Oxylipins

A. Aplydilactone from the Sea Hare *Aplysia kurodai*

As part of a search for biologically active molecules from marine animals, the Yamada group isolated a highly unusual dimeric oxylipin from the sea hare *Aplysia kurodai*, aplydilactone (**92**), which possessed very weak phospholipase A_2 activating activity (2-fold at 50 mM).[107,108] Aplydilactone was assembled from



partial structures deduced from spectroscopic analysis of the natural product and several key derivatives. Connection of these partial structures required more extensive degradative, including a partial hydrogenation followed by ozonolysis. Aplydilactone is unique among oxylipin natural products in its dimeric nature, with the two units likely both deriving from eicosapentaenoic acid. The monomeric units are highly similar in structure to those of the constanolactones **4** and **5**, cyclopropane lactones from a red alga (see above). As sea hares in general, and *Aplysia* spp. in particular, are well known to assimilate and sequester the unique secondary metabolites of their algal diets, presumably as a way of enhancing their own defense against predation, it is likely that aplydilactone or its monomeric precursors also originate in an algal source. Dimerization could be occurring by enzymatic processes either in the alga or the sea hare, or conceivably, could occur via nonenzymatic processes in the sea hare digestive gland. Mechanistically, this unsymmetrical dimerization might result from an acid catalyzed dehydration (Figure 11),[2] or alternatively, involve coupling of lipoxygenase derived radicals generated under anaerobic conditions.[103]

B. Hybridalactone from the Red Alga *Laurencia hybrida*

While the red algal genus *Laurencia* is well recognized for producing a wide variety of terpenes and polyketides

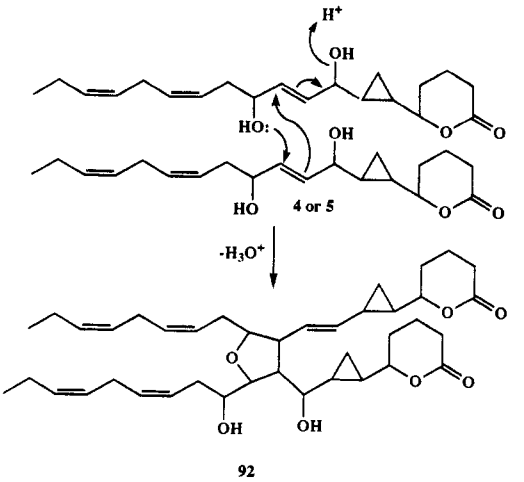


Figure 11. Proposed biogenesis of aplydilactone (**93**) involving the acid-catalyzed unsymmetrical dimerization of constanolactone (**4** and **5**) monomeric units (from ref 2).

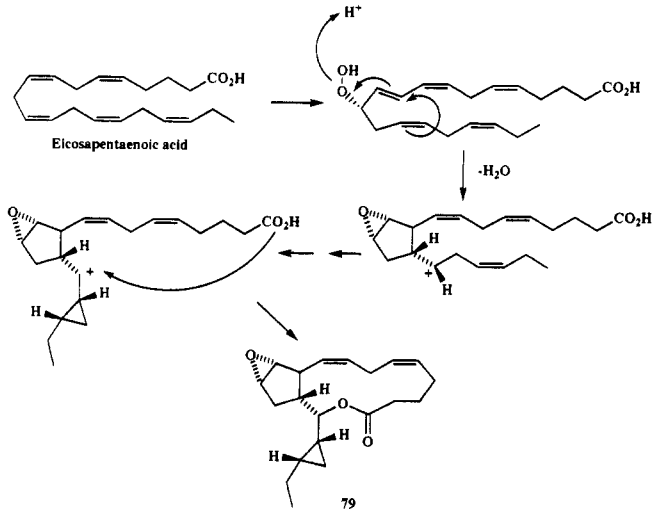


Figure 12. Proposed biogenesis of hybridalactone (**79**) by the red alga *Laurencia hybrida* (from ref 7).

which contain halogen atoms, a species from Great Britain, *Laurencia hybrida*, has been a source of two novel oxylipins containing cyclopentyl and lactone rings.[100] The major compound, hybridalactone (**79**), was isolated in about 0.5% yield from the lipid extract and its tetracyclic structure (cyclopropyl, cyclopentyl, epoxide, lactone rings) was defined on the basis of a combination of spectroscopic data. Relative stereochemistry in **79** was suggested from a combination of coupling constant data for the natural product and a derivative formed *via* methanolysis (C12) of the epoxide. The minor substance **78**, which is monocarbocyclic, is discussed above.

This fascinating structure has several features of obvious structural similarity to the prostaglandins, however, interestingly, the positions of rings in the chain and nature of the oxidized atoms suggests that it has a different biosynthetic origin than the mammalian substances. These features prompted Corey and coworkers to propose a unique biosynthetic origin for hybridalactone (**79**) based on initiation by a 12-lipoxygenase.[102] It was envisioned that cationic oxirane formation is followed by carbocyclization to a cyclopentyl allylic cation (Figure 12). This allylic carbocation enters a complex cyclopropyl–cyclobutyl–cyclopropyl

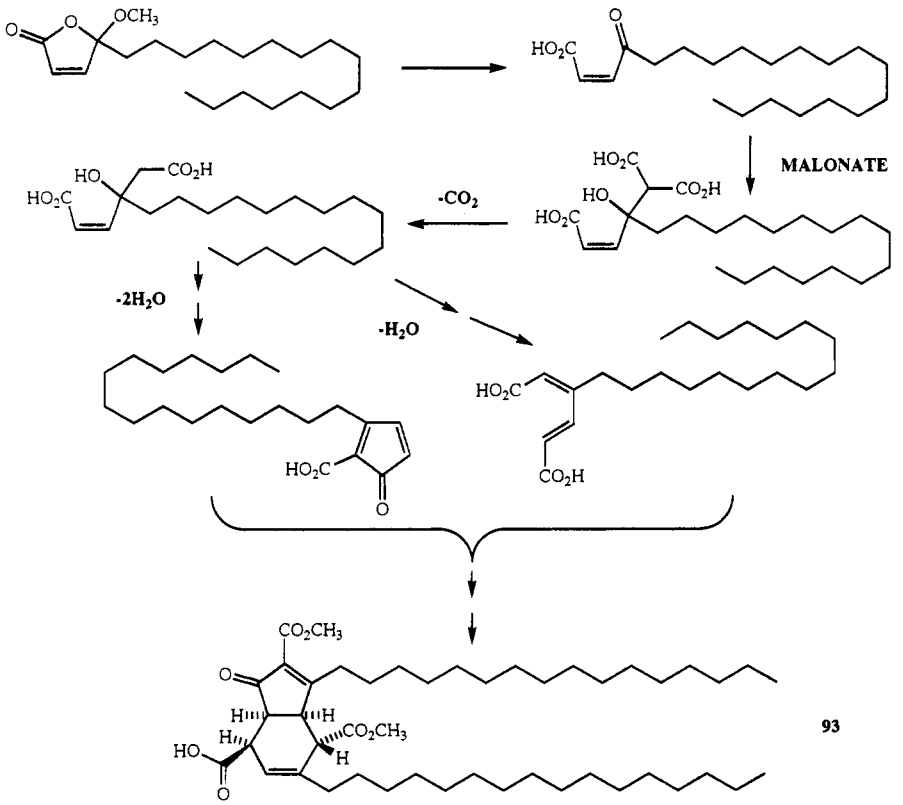
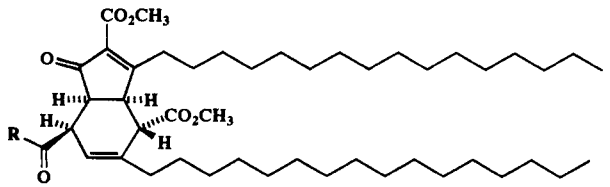


Figure 13. Proposed biogenesis of the manzamenone skeleton (**93–98**) by the sponge *Plakortis* sp. (from ref 110).

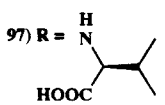
manifold to give the C15 cation which is quenched by lactonization. In combination with conformational analysis, this biogenetic proposal predicted, beginning with a 12(*S*)-hydroperoxide, a 10*S*,11*R*,12*S*,14*R*,15*S*,-16*R*,17*S* absolute stereochemistry for hybridalactone (**79**). This proposal was confirmed by stereospecific total synthesis.[109] Synthetic and natural hybridalactone samples showed identical spectroscopic and chiroptical features. Separate corroboration of these predictions of absolute stereochemistry was obtained by X-ray crystallography of a heavy-atom derivative of hybridalactone (**79**).[102]

C. Manzamenones from the Sponge *Plakortis* sp.

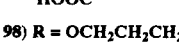
Recently, six novel fatty acid-derived bicarbocyclic compounds, manzamenones A–F (**93–98**), were isolated and identified from Okinawan collections of the sponge *Plakortis* sp.[110] The new compounds were isolated as



93) R = OH
94) R = OCH_3
95) R = OCH_2CH_3
96) R = NH_2



97) R =



98) R = $OCH_2CH_2CH_2CH_3$

fairly major metabolites and their structures deduced by a combination of mass spectral and 2-D NMR methods. The identity of the C_{16} alkyl chains at C3 and C9 in these metabolites was suggested from electron impact mass spectral fragment ions which apparently arise from the separate and independent loss of these chains. As *Plakortis* sponges have been a rich source of cyclic peroxides and butenolides,[111] the biogenesis of these bicarbocyclic metabolites has been envisioned to involve an opened butenolide intermediate which condenses with malonate and then undergoes decarboxylation and dehydration to form two monomeric units. An enantioselective intermolecular *endo*-type [4 + 2] cycloaddition of these units is then envisioned to produce the manzamenone skeleton (Figure 13). Manzamenone A (**93**) is reported to show weak inhibitory activity to protein kinase C.[110]

IV. Concluding Remarks

Marine organisms produce a wide range of natural product structural classes, including terpenes, polyketides, peptides, alkaloids, phlorotannins, and others.[112] However, recognition that these life forms utilize simple polyunsaturated fatty acids to form complex oxylipins resembling those produced by mammalian systems has developed only in the last few years. Given the potent biological activity of this structure class in mammalian systems, it is likely that these interesting marine analogs will possess impressive pharmacological activities and stimulate their development as new pharmaceutical agents and pharmacological tools (e.g. **59–77**). Study of how these unique marine oxylipins are formed will certainly lead to the discovery of new enzymes with novel reactivity and substrate specificity, some of which may be useful in a synthetic sense. For the most part, we have little appreciation of why marine organisms have developed this metabolic capacity, that

is, what role these oxylipins play in the physiology and ecology of these life forms. Thus, while an adequate number of discoveries of oxylipins have occurred to permit us to appreciate the breadth of their occurrence, we have practically no understanding of the mechanisms of their biosynthesis, their significance to the organisms that produce them, or their potentially useful pharmacological properties. It is both the bane as well as the source of much enthusiasm in marine natural products chemistry that we always seem to generate more questions than we answer.

V. Acknowledgments

Philip Proteau and Dale Nagle helped in the compilation of some of the literature reviewed here and in the construction of some of the graphics. I thank Philip Proteau for critically reading the manuscript. Research on marine oxylipin chemistry in the authors laboratory has been supported by the Oregon Sea Grant Program (R/SH-5).

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