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Biologically active oxylipins from seaweeds

William H. Gerwick[1,2], Philip J. Proteau[1], Dale G. Nagle[1], Mitchell L. Wise[2], Zhi D. Jiang[1], Matthew W. Bernart[1] & Mats Hamberg[3]
[1] *College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, USA;* [2] *Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331, USA;* [3] *Department of Physiological Chemistry, Karolinska Institute, S-104 01 Stockholm, Sweden*

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Abstract

Our previous research has shown that many red algae metabolize polyunsaturated fatty acids to oxidized products resembling the eicosanoid hormones from mammals. We have extended these studies to members of the Phaeophyceae and Chlorophyta and find they also possess similar biosynthetic pathways. From several we have identified novel prostaglandin-like substances. Studies of the molecular mechanisms by which some of these marine oxylipins are formed have revealed that novel oxidative reactions are utilized. Understanding of these biosynthetic pathways in detail has allowed their utilization to produce research biochemicals of high value, such as 12*S*-hydroperoxyeicosatetraenoic acid (12*S*-HPETE). Because of their biological properties, seaweed-derived oxylipins have potential utility as pharmaceuticals and research biochemicals.

Introduction

Marine algae are a well recognized source of polyunsaturated fatty acids (PUFAs), primarily as components of complex lipids (Pohl & Zurheide, 1979) (Figs 1–3). In humans, these PUFAs are metabolized by a variety of oxidative enzymes to the prostaglandins, thromboxanes, hydroxyeicosatetraenoic acids and leukotrienes. Collectively known as eicosanoids, these substances are of seminal importance to the maintenance of normal mammalian physiology.

Thus, while the occurrence of PUFAs in the algae has been known for some time, only recently have we come to recognize that these life forms also metabolize these fatty acids in oxidative pathways analogous to those found in animals (Gerwick *et al.*, 1990). Over the past 6 years, efforts in our laboratories have focussed on the discovery, structural description, mechanism of biosynthesis, and biological properties of such compounds from marine algae representing all of the major groups (Table 1). Some of these substances have been simple hydroxy acids identical to eicosanoids found in humans. Despite their simple structure, many are astoundingly potent in eliciting physiological responses in mammalian tissues. For example, 12*S*-hydroxyeicosatetraenoic acid (12*S*-HETE, 1) can induce migration of smooth muscle cells at concentrations as low as 10^{-15} M (Gurr & Harwood, 1991). In animals, and now realized also in the marine algae, these hydroxy-acids derive from the corresponding hydroperoxides which are themselves produced by various lipoxygenase enzymes. These hydroperoxides are crucial and highly re-

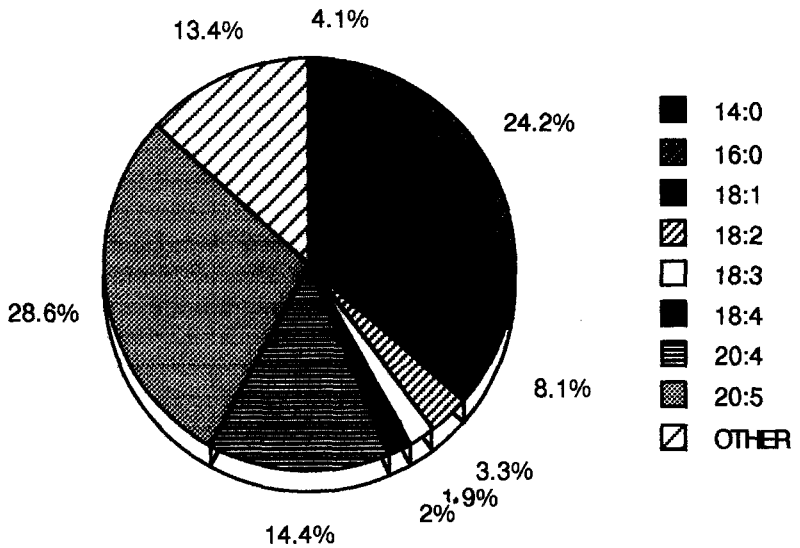


Fig. 1. Average composition of fatty acids in complex lipids from 19 members of the Rhodophyta (21 measurements) (data from Pohl & Zurheide, 1979).

active intermediates which are metabolized in both systems to a diversity of products, such as alcohols, diols, epoxy-alcohols, aldehydes, and enol ethers. A remarkable finding in this work with marine oxylipins (Gerwick *et al.*, 1991) is that these hydroperoxide intermediates are metabolized by additional biosynthetic routes that are without parallel in animals and lead to the production of a variety of carbocyclic and carbon-chain rearranged products.

This paper will consider some of our published and unpublished discoveries of oxylipins from the

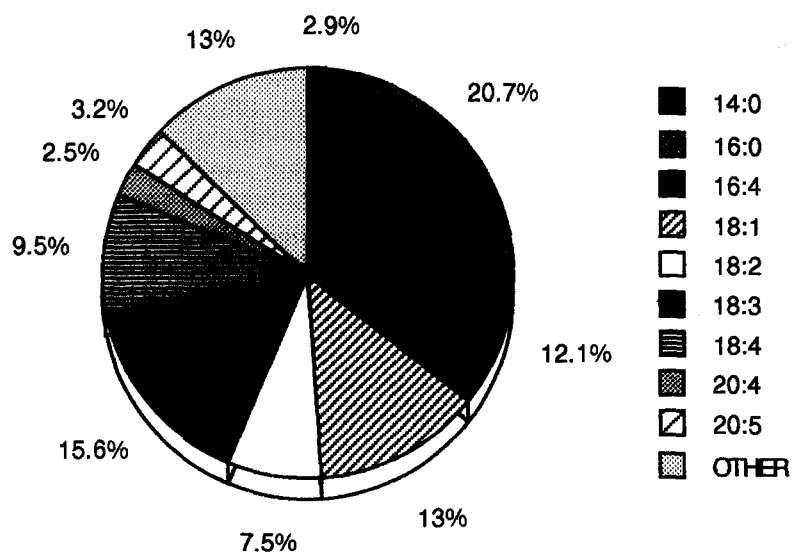


Fig. 2. Average composition of fatty acids in complex lipids from 11 members of the Chlorophyta (12 measurements) (data from Pohl & Zurheide, 1979).

Table 1. Macroalgal species found to contain oxylipins.

- Rhodophyta
  - Rhodophyceae
    - Gelidiales
      - Gelidiaceae
        - *Gelidium latifolium* Bornet & Thuret
    - Corallinales
      - Corallinaceae
        - *Bossiella orbigniana* Silva
        - *Lithothamnion corallioides* Crouan
        - *Lithothamnion calcareum* (Pallas) Areschough
    - Gigartinales
      - Dumontiaceae
        - *Constantinea simplex* Setchell
        - *Farlowia mollis* (Harvey & Bailey) Farlow & Setchell
    - Gracilariales
      - Gracilariaceae
        - *Gracilaria edulis* (Gmelin) Silva (as *G. lichenoides*)
        - *Gracilaria verrucosa* = *G. pacifica* (Abbott)
        - *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto & Foldvik
    - Rhodymeniales
      - Rhodymeniaceae
        - *Rhodymenia pertusa* (Postels & Ruprecht) J. G. Agardh
    - Ceramiales
      - Ceramiaceae
        - *Neoptilota asplenioides* (Esper) Kylin *ex* Scagel, Garbary, Golden & Hawkes
        - *Ptilota filicina* J. G. Agardh
      - Rhodomelaceae
        - *Laurencia hybrida* (DeCandole) Lenormand
        - *Laurencia spectabilis* Postels and Ruprecht
        - *Murrayella periclados* (C. Agardh) Schmitz
      - Delesseriaceae
        - *Cottoniella filamentosa* Borgesen
        - *Platysiphonia miniata* (C. Agardh) Borgesen
        - *Polyneura latissima* (Harvey) Kylin
- Chrysophyta
  - Phaeophyceae
    - Chordariales
      - Notheiaceae
        - *Notheia anomala* (Bailey & Harvey)
    - Laminariales
      - Laminariaceae
        - *Ecklonia stolonifera* Okamura
        - *Laminaria setchellii* Silva
        - *Laminaria saccharina* (Linnaeus) Lamouroux
        - *Laminaria sinclairii* (harvey *ex* Hooker f. & Harvey) Farlow, Anderson & Eaton
        - *Cymathere triplicata* (Postels & Ruprecht) J. G. Agardh
- Chlorophyta
  - Bryopsidophyceae
    - Cladophorales
      - Cladophoraceae
        - *Cladophora columbiana* Collins
    - Codiolophyceae
    - Acrosiphoniales
      - Acrosiphoniaceae
        - *Acrosiphonia coalita* (Ruprecht) Scagel, Garbary, Golden & Hawkes

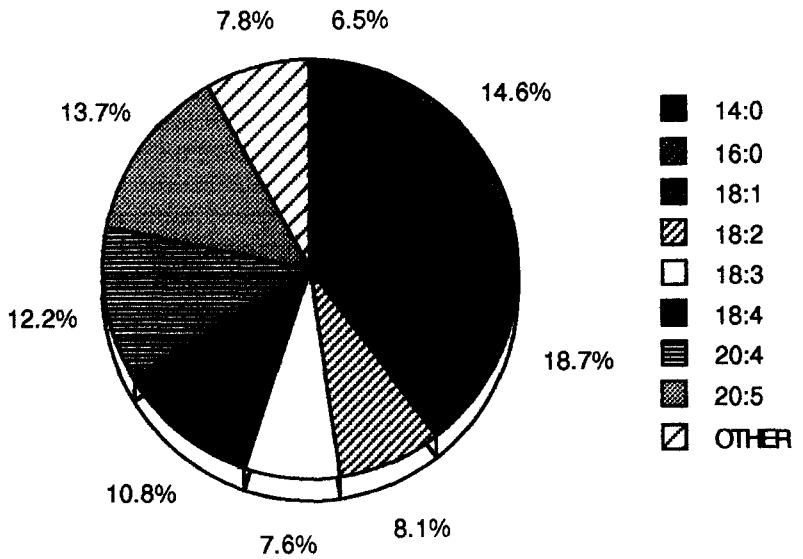
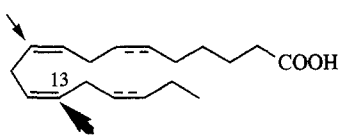
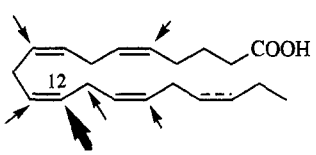
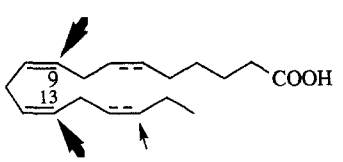
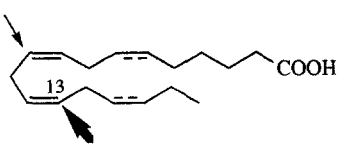
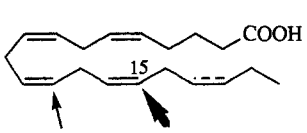
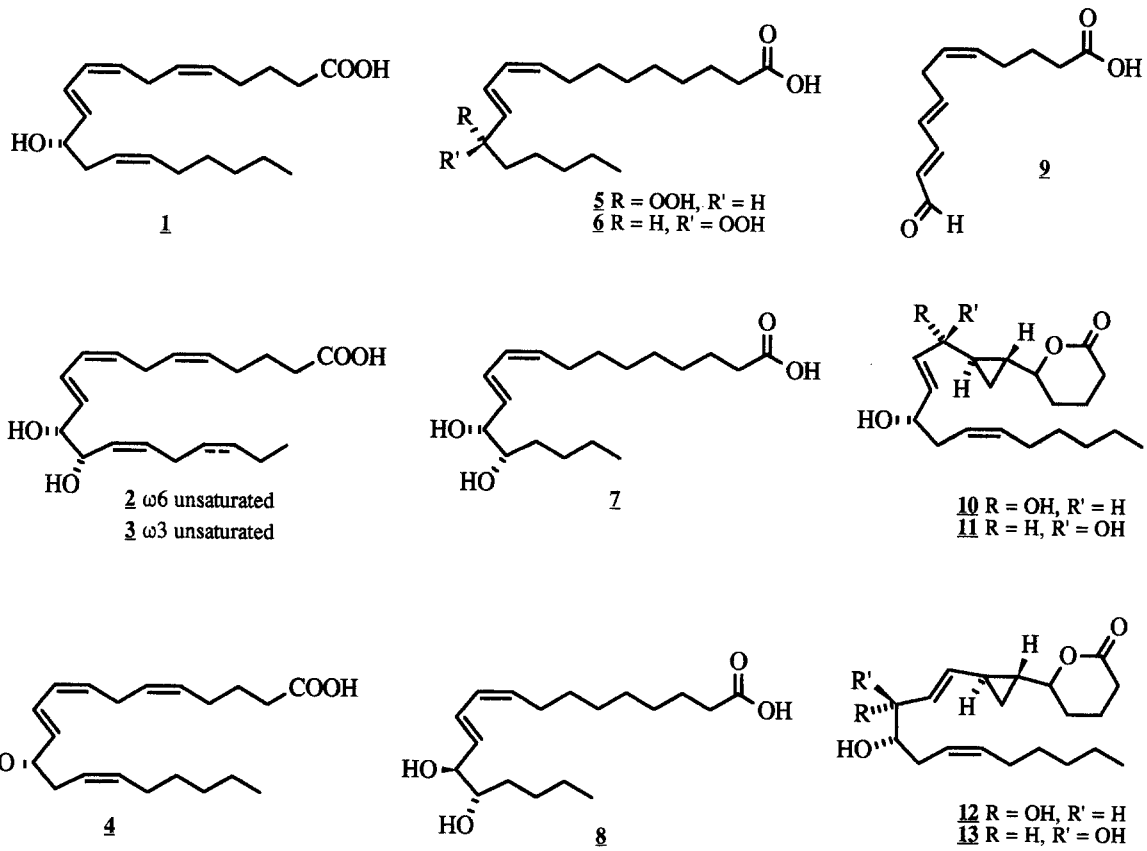


Fig. 3. Average composition of fatty acids in complex lipids from 19 members of the Phaeophyceae (22 measurements) (data from Pohl & Zurheide, 1979).

marine algae in light of their proven or likely biogenetic origin. Although still limited in scope, patterns of oxylipin metabolism are emerging from the different algal groups in terms of the nature of the fatty acids utilized and the sites oxidized in primary biosynthetic events (Table 2).

Table 2. Patterns of Oxylipin metabolism in three groups of marine algae.

Group	C_{18} Acids	C_{20} Acids
Rhodophyta		
Chlorophyta		unknown
Phaeophyceae		

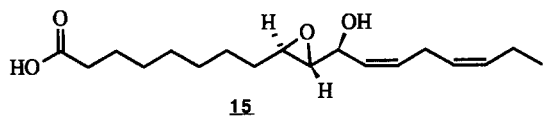


Materials and methods

Algae were collected intertidally at low tide or subtidally by SCUBA using hand tools. All work reported here is with algae frozen with dry ice at the time of collection and maintained frozen until workup. Voucher specimens have been deposited in the Herbarium at Oregon State University. Specimens were partially defrosted and then repetitively extracted in $CHCl_3$/MeOH (2:1) with gentle heating. Upon concentration of these extracts, salts and polar materials were removed by partitioning between $CHCl_3$ and water. The $CHCl_3$ layers were dried *in vacuo* to dark green oily syrups. Extracts were chromatographed over silica gel in the vacuum mode (Coll & Bowden, 1986) using increasing concentrations of EtOAc in hexane. Fractions containing oxylipins by thin layer chromatography (TLC) were further purified using HPLC employing a RSIL 10μ semi-preparative column (50 cm × 10 mm, Alltech, Deerfield) or Versapack 10 μ analytical columns (2 × 30 cm × 4.1 mm, Alltech, Deerfield). All of the oxylipins described herein were colorless or light yellow oils. Structure elucidations employed instrumentation and techniques previously described (Gerwick *et al.*, 1992). Merck aluminum-backed TLC sheets were used for TLC, and all solvents were distilled prior to use.

Collection, extraction and isolation of methyl ester derivative 20

Cladophora columbiana Collins was collected from the high intertidal zone at Boiler Bay, Oregon on 28 May 1990 and kept frozen until workup. The crude $CHCl_3$/MeOH (2:1) extract yielded, following *in vacuo* concentration, 13.2 g of dark green oil (832 g dry weight of algae). A portion



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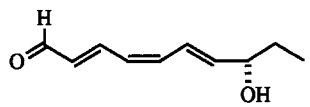

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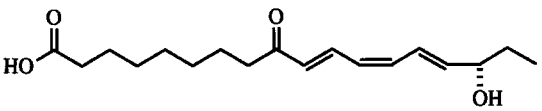

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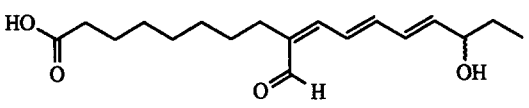

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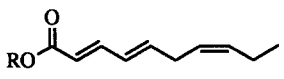
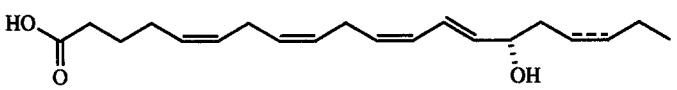
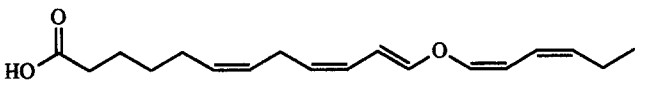


19 R = H
20 R = CH_3



21 Δ5,8,11,13
22 Δ5,8,11,13,17



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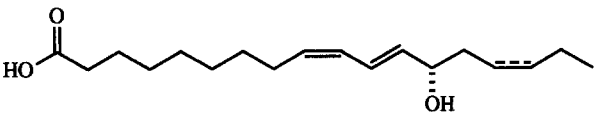
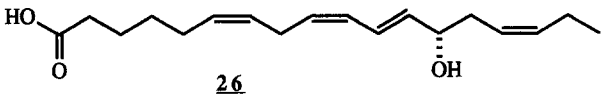


24 Δ9,11
25 Δ9,11,15



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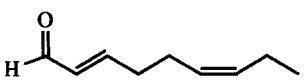

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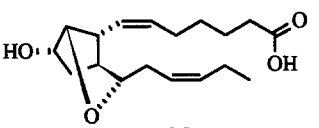

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(11.5 g) of this extract was chromatographed over silica gel in the vacuum mode with increasing percentages of EtOAc in hexane. The fraction eluting with 20% EtOAc/hexane (802 mg) contained a UV-absorbing spot by TLC with char characteristics suggestive of an oxylipin. Hence, 300 mg of this fraction was subjected to a second vacuum chromatography using the same solvents. Materials eluting with 10% EtOAc/hexane were combined and repetitively chromatographed using preparative TLC (3% MeOH/CH_2Cl_2 followed by 50% EtOAc/hexane). This crude material was methylated with CH_2N_2/Et_2O and then chromatographed once again over silica gel in the vacuum mode. Impure derivative 20 eluted in 2% EtOAc/hexane (10.1 mg) and was then subjected to semi-preparative HPLC (RSIL silica 10 μm, 50 cm × 10 mm, 0.75% EtOAc in hexane, 7.0 ml min^{-1}) to give pure derivative 20 as a colorless oil (*ca* 5 mg). IR (CCl_4) 3013, 2963, 2933, 2875, 1720, 1643, 1435, 1338, 1266, 1137, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.27 (1H, *dd*, J = 16.4, 9.9 Hz, H-3), 6.20 (1H, *bdd*, J = 15.0, 10.2 Hz, H-4), 5.09 (1H, *dt*, J = 15.0, 7.0 Hz, H-5), 5.79 (1H, *d*, J = 15.4 Hz, H-2), 5.51 (1H, *bdt*, J = 10.6, 7.1 Hz, H-8), 5.40 (1H, *bdt*, J = 10.6, 7.1 Hz, H-7), 3.67 (3H, *s*), 2.92 (2H, *bt*, J = 7.1 Hz, H_2-6), 2.03 (2H, *bp*, J = 7.1 Hz, H_2-9), 0.96 (3H, *t*, J = 7.5 Hz, H_3-10); low resolution gas chromatography-mass spectrometry (5.84 min retention time, 25 m Ultrasphere-1; 70 eV, relative intensity) obs. M^+ at *m/z* 180 (9), 149 (15), 137 (16), 121 (62), 111 (52), 91 (78), 79 (100), 55 (23).

Results

Oxylipins from the Rhodophyta

Algae of the Rhodophyta have been the most prolific source of both known and new oxylipins to date. One of the earlier discoveries of a novel oxylipin from this group was from the Oregon intertidal alga *Farlowia mollis* (Harv. & Bail.) Farl. & Setch. Originally investigated for a pronounced gram-positive antimicrobial activity associated with the extract, three novel and homologous oxylipins were isolated and structurally defined by spectrochemical methods (Solem *et al.*, 1989). Two of these, 12*R*, 13*S*-dihydroxyeicosatetraenoic acid (12*R*, 13*S*-diHETE, 2) and 12*R*, 13*S*-dihydroxyeicosapentaenoic acid (12*R*, 13*S*-diHEPE, 3) have been the continuing focus of both structural (Jiang & Gerwick, 1990; Jiang & Gerwick, 1991a, 1991b), biosynthetic (Moghaddam & Gerwick, 1990; Moghaddam & Gerwick, 1991; Gerwick *et al.*, 1991), and pharmacological investigations (K. E. Orwig, S. Leers-Sucheta, M. F. Moghaddam, Z. D. Jiang, W. H. Gerwick & F. Stormshak, 1992). Shortly after our original report of the isolation of these substances from *F. mollis*, we discovered the same metabolites, as well as a wide variety of other oxylipins, from an abundant Oregon alga, *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto & Foldvik (Jiang & Gerwick, 1991a).

Our biosynthetic investigations have utilized this latter alga due to its greater local availability. Initially, we found crude acetone powder preparations to be active at metabolizing exogenous arachidonic acid to a variety of products, including 12-HETE, which is also a natural product of *Gracilariopsis lemaneiformis* (Moghaddam & Gerwick, 1990). Subsequent experiments showed the lipoxygenase and hydroperoxide isomerase (see below) enzymes were readily extractable into aqueous buffers and remained in the supernatant fraction resulting from high speed centrifugation (105 000 ***g***). Development of this cell free system obviated the need for using living algal tissue, a difficult system to work with and nearly impenetrable to exogenously applied substrates (Barrow, 1983). Later, we found that the major product of these arachidonic acid incubations was in fact 12*R*, 13*S*-diHETE (2) (Moghaddam & Gerwick, 1991). Furthermore, in this latter work we investigated the source of oxygen atoms at the 12 and 13 position by incubations under an atmosphere of $^{18}O_2$ and showed that both of these hydroxyl groups derive from molecular oxygen. These studies were followed up with a more detailed and rigorous set of experiments which demonstrated that both oxygen atoms derived from the same oxygen molecule (Gerwick *et al.*, 1991). Through both trapping and incubation experiments, the conversion of arachidonic acid to 12*R*,13*S*-diHETE was shown to be a two step process. The first step involves a lipoxygenase (MW *ca* 85 000-88 000 Da, Hamberg & Gerwick, unpublished observations) showing specificity for C-12 in arachidonic acid and which formed the intermediate hydroperoxide, 12*S*-hydroperoxyeicosatetraenoic acid (12*S*-HPETE, 4). A chromatographically separable catalytic activity (hydroperoxide isomerase, MW *ca* 50 000 Da, Hamberg & Gerwick, unpublished observations) is responsible for transforming free 12*S*-HPETE (4) into 12*R*,13*S*-diHETE (2) by loss of a hydrogen at C-13 and moving the distal oxygen of the hydroperoxide functionality to this site. By feeding a diversity of semi-synthetic hydroperoxides we were able to show that the enzyme is capable of accepting a broad range of substrates, requires no activation of the site to be oxidized, and introduces this latter oxygen with complete stereospecificity. Furthermore, this stereospecificity is conferred without regard to the hydroperoxide stereochemistry as incubation of 13*S*-HPOD (5) and 13*R*-HPOD (6) lead to erythro (7) and threo (8) diastereomers, respectively.

Knowledge of the mechanism of biosynthesis of this unique oxylipin has enabled us to harness this system to produce a number of useful and potentially useful biochemicals. Depending on the circumstances of the incubation, we are able to produce high yields of either 12*S*-HETE (1), 12*S*-HPETE (4), or 12*R*,13*S*-diHETE (2) from exogenous arachidonic acid. Biosynthetic production of these compounds provides the opportunity to

make these compounds from radioactive precursors, a point of considerable utility which we have subsequently used in the exploration of their biological properties. Additionally, the enzymes can be provided with structural analogs of the natural substrates so as to produce unique analogs (*i.e.* compounds 7 and 8). In effect, once defined, these enzymes become functional tools in the laboratory synthesis of useful biochemicals.

The two vicinal diol products, 12*R*,13*S*-diHETE (2) and 12*R*,13*S*-diHEPE (3), have a number of interesting biological properties. As originally reported (Solem *et al.*, 1989), a mixture of 2 and 3 was weakly inhibitory to human 5-lipoxygenase obtained from polymorphonuclear leukocytes and showed some inhibition of the dog kidney derived Na^+/K^+ ATPase. More recently, we have discovered that the methyl ester of pure metabolite 3 is effective at causing a pronounced reduction in serum progesterone in female sheep in their tenth day of estrous (injection into ovarian artery), similar to the action of prostaglandin $PGF_{2\alpha}$. This is accompanied by a greater number of treated animals entering estrus 3 days after injection than control treated sheep (methyl eicosapentaenoate). *In vitro* experiments using slices of corpus luteum incubated with 12*R*,13*S*-diHEPE (3) produced less progesterone upon treatment with luteinizing hormone then control (arachidic acid, docosatetraenoic acid) treated slices. In contrast, equivalent amounts of progesterone were produced in tissue slices treated with either arachidic acid, docosatetraenoic acid, or 12*R*, 13*S*-diHEPE (3) and subsequently challenged with 8-bromo-cyclic adenosine monophosphate. Luteal cells incubated with [1-^{14}C]-12*R*, 13*S*-diHEPE (produced biosynthetically) revealed that the majority of the compound associated with the plasma membrane fraction. Hence, based on these data it appears that 12*R*,13*S*-diHEPE (3) is capable of interfering with luteal function in the ewe and that this may happen by altering luteal response to luteinizing hormone.

In structural terms, some of the most fascinating oxylipins isolated to date from a red seaweed come from the small mushroom shaped alga *Constantinea simplex* Setch. (Nagle & Gerwick, 1990). Interestingly, it was only the young blade material which displayed a wealth of natural products by TLC analysis. Subsequently, we isolated two previously encountered hydroxy-acids, 12*S*-HETE (2) and 12*S*-HEPE (3), and an aldehyde (9) which suggested the occurrence of 12-lipoxygenase metabolism in this alga. Continued isolation efforts then yielded the novel metabolites constanolactone A (10) and B (11). A 12-lipoxygenase initiated biogenesis for the constanolactones was envisioned (Fig. 4). Three as yet unpublished findings support this biogenetic proposal: 1) the stereochemistry at C-12 was determined by GC analysis of a degradative fragment to be *S*, as predicted in Fig. 4, and 2) cell free biosynthesis of the constanolactones (10, 11) using acetone powders of fresh frozen *C. simplex* in an atmosphere enriched in $^{18}O_2$ followed by mass spectral analysis showed that only the oxygen at C-12 derives from molecular oxygen, again as predicted; and 3) we have recently isolated two new constanolactones F (12) and G (13) which logically derive from 1,2-opening of proposed epoxide intermediate 14 (Fig. 4). While still the subject of ongoing investigation, it appears likely from these observations that the constanolactones derive from 12-lipoxygenase metabolism of arachidonic acid.

Oxylipins from the Chlorophyta

Only quite recently (Bernart, Whatley & Gerwick, work in progress) have we discovered that marine macrophytic green algae also metabolize polyunsaturated fatty acids to oxylipins. The principle focus of our efforts in this group has been with the Oregon Chlorophyte *Acrosiphonia coalita* (Rupr.) Scagel, Garbary, Golden & Hawkes. This alga produces a wide assortment of oxylipin natural products that emanate from apparent 9-lipoxygenase metabolism of 18 carbon precursors (Fig. 5). Intramolecular rearrangement of a 9-hydroperoxide formed by lipoxygenase metabolism logically explains the occurrence of epoxy alcohols (Pace-Asciak, 1984), such as 15, in *A. coalita*. Additional oxidation by an apparent 16-lipoxygenase

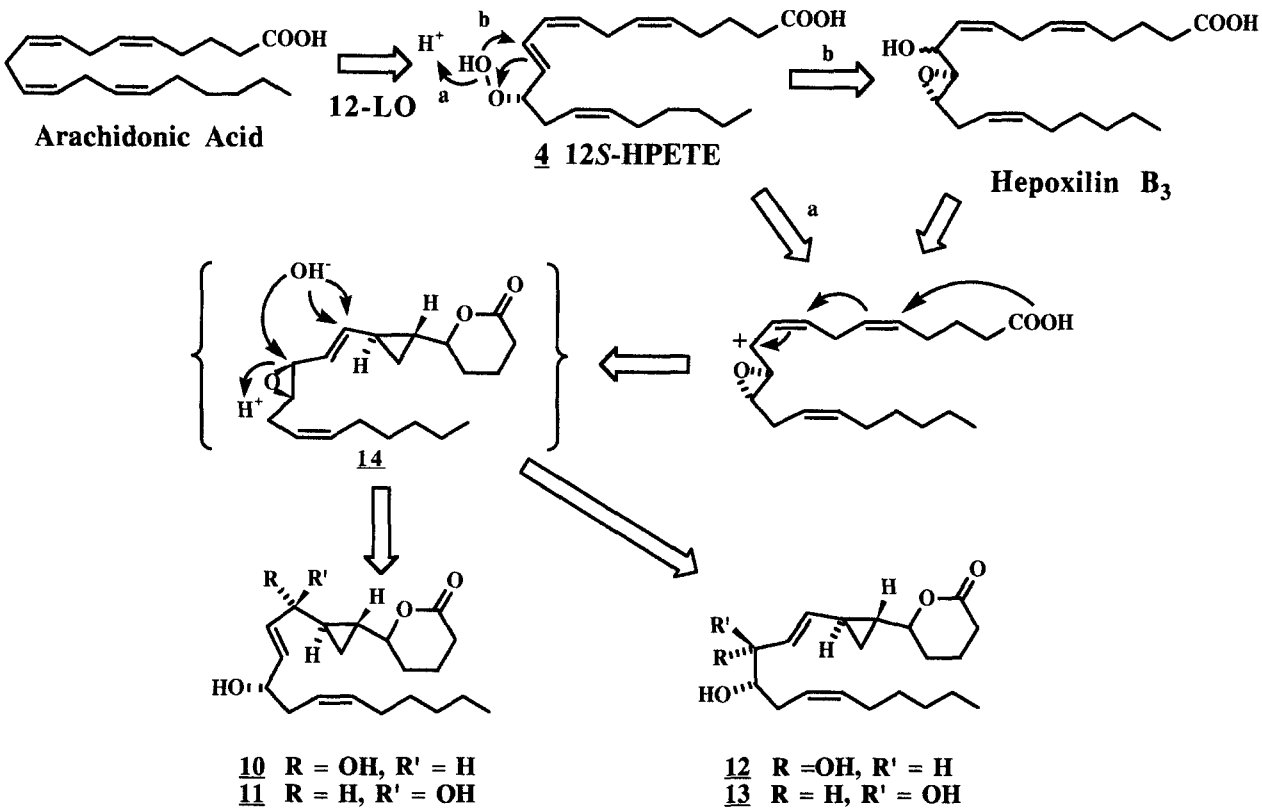

Fig. 4. Proposed biogenesis of metabolites from *Constantinea simplex*.

followed by further metabolism may give rise to chain cleaved aldehydes such as 16 or trienones such as 17. Most interestingly, we have also found that *A. coalita* is capable of transforming C-18 polyunsaturated fatty acids into branched oxylipins such as trienal 18. The branching carbon, which likely represents excision of C-9 from the precursor chain, is without precedent in mammalian or terrestrial plant oxylipins. Metabolite 16 was shown to possess moderate inhibitory activity to *Candida albicans* (10 mm zone of inhibition at 100 μg/disc) using the sensitivity disc assay.

We have also found another green alga from the Oregon coast, *Cladophora columbiana*, to be a source of oxylipin chemistry (19). The frozen algal material was extracted for its lipids and a new compound, with TLC characteristics typical of an oxylipin, isolated following conversion to its methyl ester derivative 20. The methyl ester derivative of this new oxylipin (20) was a colorless oil and analyzed for $C_{11}H_{16}O_2$ by consideration of its mass spectral and other spectroscopic features. Further, its ^{1}H NMR spectrum was highly informative of its relatively simple structure (see experimental) and specific irradiation experiments were used to decipher the entire sequence of methylenes and olefins along the fatty acid-derived chain. By these data, derivative 20 was shown to be methyl 2(*E*),4(*E*),7(*Z*)-decatrienoate, and hence, the natural product 19 is the corresponding free acid. Metabolite 19 could be envisioned to arise from hydroperoxide lyase cleavage (Sekiya *et al.*, 1983; Vick, 1991) of a 9-hydroperoxyoctadeca-10(*E*), 12(*Z*), 15(*Z*)-tr: enoic acid precursor, itself formed by the action of a 9-lipoxygenase on α-linolenic acid (Fig. 6).

Oxylipins from the Phaeophyceae

Our first work resulting in the isolation of an oxylipin from a member of the Phaeophyceae was

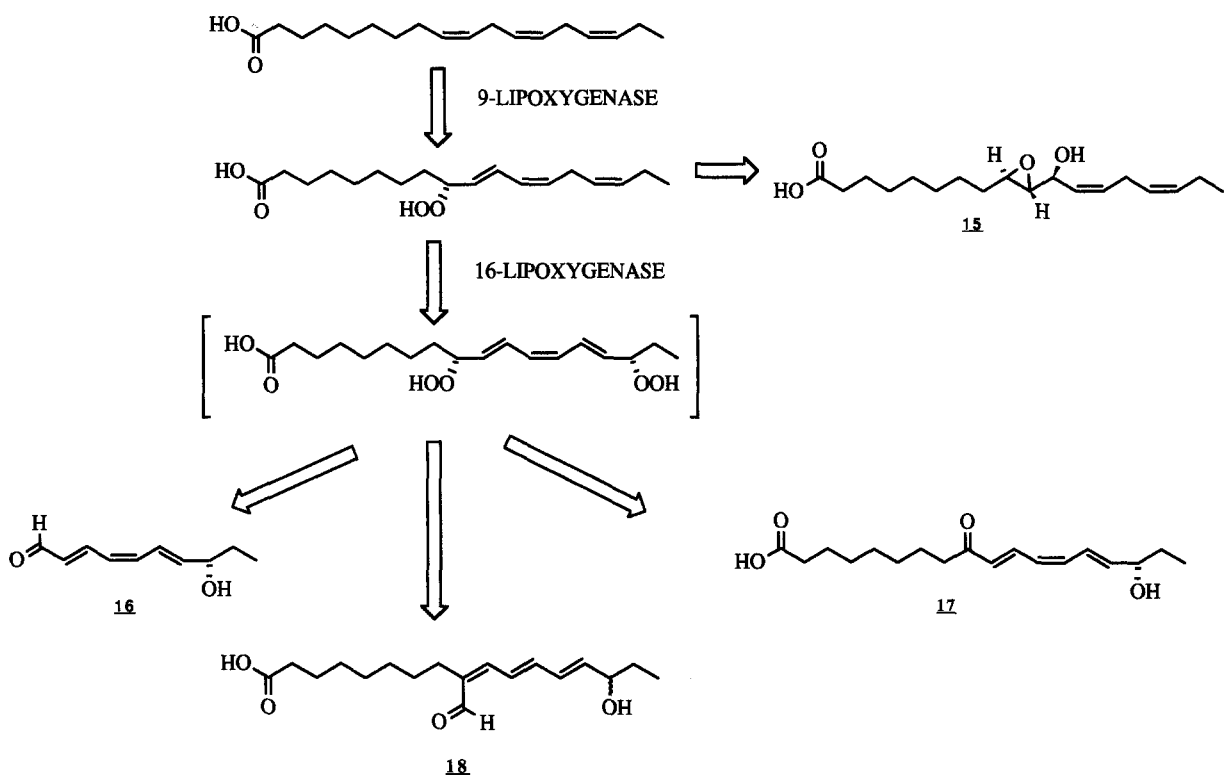

Fig. 5. Proposed oxylipin metabolism in *Acrosiphonia coalita*.

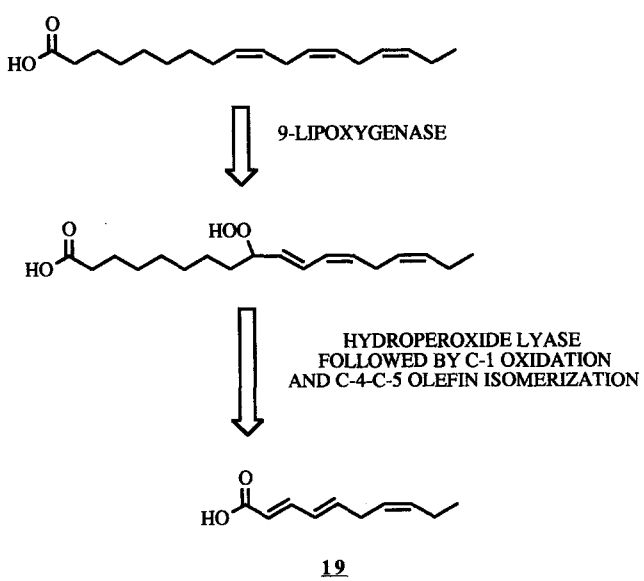

Fig. 6. Proposed biogenesis of truncated fatty acid 19 in *Cladophora columbiana*.

with an Oregon species of *Laminaria, L. sinclairii* (Harvey *ex* Hooker f. & Harvey) Farlow, Anderson & Eaton. From this alga we were able to isolate in reasonable yield two simple hydroxy acids (21, 22) which ultimately derive from arachidonic acid and eicosapentaenoic acid, respectively. More interestingly, we found in less polar fractions a C-18 polyunsaturated fatty acid derived compound which possessed a 'broken' carbon chain spanned by an ether oxygen (23). Furthermore, the ether linking carbons of both fragments in 23 are olefmic so as to produce a divinyl ether linkage. Subsequently, we have isolated several of these same substances, as well as a number of products from metabolism of various 18-carbon fatty acids (24-26), from two other species of *Laminaria, L. setchellii* Silva and *L. saccharina* (Linnaeus) Lamouroux (Table 3, Proteau & Gerwick, work in progress).

Cymathere triplicata (Postels & Ruprecht) J. G. Agardh, a large brown kelp common to the pro-

Table 3. Occurrence of various oxylipins in three species of *Laminaria*.[a]

Compound	*L. sinclairii*	*L. setchellii*	*L. saccharina*
15*S*-HETE (21)	0.3	0.03	0.4
15*S*-HEPE (22)	0.4	0.1	0.3
Bis-enol ether (23)	0.4	n.d.[b]	n.d.
13*S*-HODE (24)	0.15	0.015	<0.1
13*S*-HOTE (25)	0.3	0.04	<0.1
13*S*-HODTA (26)	0.4	0.4	0.1

[a] Yields based on percentage of lipid extract as determined by isolation and gravimetric measurement.
[b] n.d. = not detected.

tected bays of Northern Washington, gives off a pronounced odor of cucumbers when exposed at low tide. In the cucumber, this odor is known to arise from lipoxygenase metabolism of polyunsaturated fatty acids to give unsaturated aldehyde fragments such as 27 (Gardner, 1991). Investigation of the lipid extractables from *C. triplicata* yielded appreciable quantities of 27 as well as of several new compounds (e.g. cymathere ether A 28). The new compounds were isolated in pure form as the methyl esters and their structures determined by various spectrochemical methods (Proteau & Gerwick, 1992). These new compounds possess a cyclopentane ring in the approximate middle of a fatty acid chain, in close similarity to the prostanoids. However, in this *Cymathere* metabolite the presence of an additional ether ring 'locks' the conformation of the ring and side chains into an unusual arrangement relative to that of the common prostanoids. Unfortunately, these compounds have been incompletely evaluated for biological properties to date. Again, they likely arise from lipoxygenase-initiated metabolism, but in this case proceeding through a pathway (Fig. 7) analogous to that proposed for hybridalactone (Corey *et al.*, 1984).

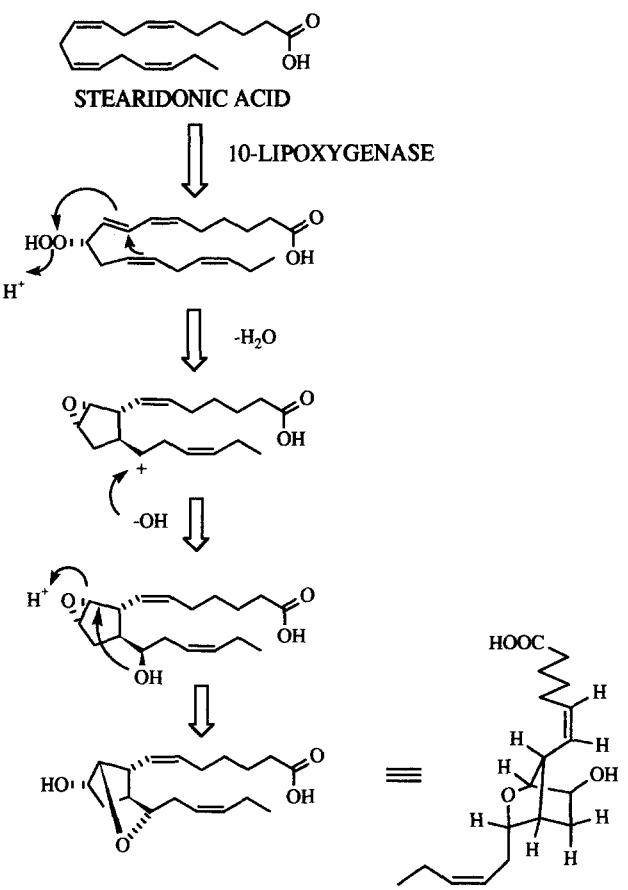

Fig. 7. Proposed biogenesis and 3-D structural representation of cymathere ether A (28) from *Cymathere triplicata*.

Discussion

The above has provided a few brief examples of various oxylipins that we have isolated from marine algae. While most of these arise via metabolic pathways analogous to those found in mammalian sources, it is important to note that many of the products formed in the algae are novel substances. Their analogous nature to the human derived substances makes them good candidates for pharmacologically-active analogs which possess some but not all of the activities of the endogenous mammalian substances. In this regard, they could prove useful as either pharmaceutical agents or tools in pharmacological research. Further, while the above examples have drawn on only a few projects from our laboratory, our continued work in this area shows this to be a metabolic capability not restricted to only a few species. Rather, as revealed in Table 1, this is a

general metabolism that has widespread occurrence in the algae.

While we are continuing to discover new algal species which contain oxylipins, some general trends have emerged from our work as well as that of others (Table 2). In the Rhodophyta, we have observed principally the metabolism of 20-carbon fatty acids, although some metabolism of 18-carbon acids is seen as well. This trend is consistent with the overall profile of polyunsaturated fatty acids in the Rhodophyta (Fig. 1). The majority of products coming from 18-carbon acids logically derive from a lipoxygenase acting at C-13 (ω6) while those deriving from 20-carbon acids appear to mainly derive from a lipoxygenase acting at C-12 (ω9). In the Chlorophyta, we have only observed metabolism of 18-carbon acids, again consistent with the overall profile of available polyunsaturated fatty acids in these algae (Fig. 2). These 18-carbon acids appear to become initially oxidized by action of a lipoxygenase at C-9 or C-13. These positional specificities are the same as observed in some higher plant lipoxygenases and may reflect the dual specificities of a single enzyme (Kuhn *et al.*, 1990). In the Phaeophyceae we have observed products deriving from metabolism of both 18-carbon and 20-carbon fatty acids with the majority becoming initially oxidized at C-13 (ω6) in the 18-carbon class and C-15 (ω6) in the 20-carbon class. As shown in Fig. 3, the Phaeophyceae possess an abundance of precursor polyunsaturated fatty acids of both the C-18 and C-20 classes. Further, in these algae there appears to be a common theme of ω6-lipoxygenation in both of these carbon-chain classes.

Description of these new oxylipins in the algae is crucial to the development of a fuller appreciation of their biochemistry, and hence, their physiology and ecology. Since some of these algal oxylipins are structurally identical to those obtained from mammalian tissues, the algae may serve as a resource of these valuable biochemicals (Gerwick *et al.*, 1990). Additionally, as we develop a greater understanding of the pathways and enzymes utilized by the algae to produce these oxylipins, it is possible that these algal enzymes may become useful models for analogous biochemical reactions in humans. Finally, detailed knowledge of the reaction mechanisms by which the algal enzymes manipulate their organic substrates will enhance the utilization of these enzymes as tools in synthetic chemistry.

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