PERGAMON

Phytochemistry 53 (2000) 129–133

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

5-Lipoxygenase-derived oxylipins from the red alga *Rhodymenia pertusa*

Zhi Dong Jiang, Sharon O. Ketchum, William H. Gerwick*

College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA

Received 25 February 1999; received in revised form 12 July 1999

Abstract

The lipid extract of the temperate red alga *Rhodymenia pertusa* has yielded four eicosanoid metabolites, three of which are new natural products. Using principally NMR and MS techniques, their structures were deduced as 5*R*,6*S*-dihydroxy-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic acid (5*R*,6*S*-diHETE), 5*R**,6*S**-dihydroxy-7(*E*),9(*E*),11(*Z*),14(*Z*),17(*Z*)-eicosapentaenoic acid (5*R**,6*S**-diHEPE), 5-hydroxy-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic acid (5-HETE), 5-hydroxy-6(*E*),8(*Z*),11(*Z*),14(*Z*),17(*Z*)-eicosapentaenoic acid (5-HEPE). The co-occurrence of these metabolites strongly suggests that *R. pertusa* contains a unique 5*R*-lipoxygenase system acting on both arachidonic and eicosapentaenoic acids.

Keywords: *Rhodymenia pertusa*; Rhodymeniaceae; Marine algae; Oxylipin; 5-Lipoxygenase

1. Introduction

Products of the lipoxygenase (LO) pathway in mammalian tissues, especially those deriving from the 5-lipoxygenase branch, are pivotal signaling molecules employed in many physiological and pathophysiological conditions (Drazen, Isreal & O'Byrne, 1999). Therefore, it has been a surprising development in natural products chemistry to discover that evolutionarily ancient marine algae are rich in various classes of eicosanoids (Gerwick & Bernart, 1993; Gerwick, 1994). Lipoxygenase products similar or identical in structure to those found in mammalian systems appear to be pervasive among the different phyla and orders of marine algae (Gerwick, 1999). To date, evidence has been presented in the literature for the occurrence of 5*R*-, 8-, 9*S*-, 12*S*-, and 15*S*-lipoxygenases acting on C20 substrates and ω3-, ω6-, ω9-, and ω10-lipoxygenases acting on C18 substrates (Gerwick, 1994, 1999). Herein we report on the isolation of four new oxylipins from a temperate marine alga, *Rhodymenia pertusa*, which logically emanate from the 5-lipoxygenase manifold. This is only the third report of 5-LO products from a marine alga (Wang & Shimizu, 1990), and the second from the Rhodophyta. Moreover, the nature of the isolated metabolites from this red alga implies the involvement of a 'leukotriene A-type' intermediate in the alga, which if correct, would join a relatively small number of cases wherein a LTA-like substance is produced in a non-mammalian system (Gardner, 1991, 1999).

R. pertusa (Postels and Ruprecht) J. Agardh is a foliaceous red alga belonging to the family Rhodymeniaceae in the order Rhodymeniales. It grows subtidally to a depth of 18 m on the Pacific coast of Oregon and Washington where it reaches a maximum size of 60 cm. Only a few natural products, such as the sterols cholesterol and desmosterol, have been previously reported from this genus (Gibbons, Goad & Goodwin, 1967; Idler, Saito & Wiseman, 1968). Hence, it was of interest to us that subtidal collections of *R. pertusa* from the Puget Sound of Washington gave a lipid extract rich in UV-absorbing and acid-charring

* Corresponding author. Tel.: +1-541-737-5801; fax: +1-541-737-3999.

E-mail address: bill.gerwick@orst.edu (W.H. Gerwick).

0031-9422/00/$ - see front matter
PII: S0031-9422(99)00445-8

metabolites by TLC analysis. Investigation of these has led us to characterize four oxylipins from this algal source, three of which are new and one of which has previously been reported from several different marine life forms (Gerwick, 1999), including a red alga (Guerriero, D'Ambrosio & Pietra, 1990). Oxylipins have not previously been reported from this order of algae.

2. Results and discussion

R. pertusa was extracted by standard methods to give 1.4 gm of a dark green crude lipid extract. Fractionation of this extract over silica gel in the vacuum mode gave two fractions (5 and 6) enriched in UV-absorbing metabolites by TLC. Because these fractions contained substances with poor chromatographic qualities (tailing), they were separately converted to methyl esters by brief treatment with ethereal CH_2N_2 and then further purified by additional silica gel vacuum chromatography and normal phase HPLC (see experimental). The more polar of these fractions (6) yielded two new diols as methyl ester derivatives (**2**, **4**) while the less polar fraction gave two hydroxy-containing methyl esters (**6**, **8**).

Derivative **2** was a colorless oil showing $[\alpha]_D^{23} = -1.4^\circ$ ($c = 0.29$, MeOH) that analyzed for $C_{21}H_{34}O_4$ by a combination of low resolution electron impact mass spectrometry (LR-EIMS) of the bis-TMS ether and ^{13}C NMR analysis. The five degrees of unsaturation inherent in this molecular formula were accounted for by one ester carbonyl (δ 174.2) and four carbon–carbon double bonds (eight olefinic carbon signals between δ 126 and δ 134). The ^1H NMR spin system from C-2 to C-20 was completely revealed by ^1H–^1H COSY analysis. A sharp triplet at δ 2.36 with $J = 7.0$ Hz could be assigned to the C-2 methylene protons by analogy with other oxylipins (Bernart & Gerwick, 1994). These connected in turn to high field methylene protons at C-3 and C-4, the latter of which were coupled to a midfield methine signal at δ 3.70. The carbon shift associated with this latter signal resonated at δ 73.8, identifying it as an oxygen bearing methine. In turn, ^1H–^1H COSY showed this methine was adjacent to a second oxygenated methine center (^1H at δ 4.15; ^{13}C at δ 75.6), thereby defining the 5,6-vicinal diol relationship in derivative **2**. ^1H–^1H COSY allowed deduction of the proximity of three sequential disubstituted double bonds adjacent to this vicinal diol (UV $\lambda_{max} = 260,\ 271,\ 286$). Moreover, the well resolved nature of the six associated protons allowed careful measurement of their coupling constants and thus defined this system to be a *trans*, *trans*, *cis* triene ($J_{7,8} = 15.1$ Hz; $J_{9,10} = 14.8$ Hz; $J_{11,12} = 10.9$ Hz). The partially resolved C-12 proton (δ 5.48) displayed a ^1H–^1H COSY correlation to a bis-allylic methylene at δ 2.94 which in turn showed correlation to two overlapped olefinic protons at C-14 and C-15 (δ 5.37–5.41). The spin system continued first to an allylic methylene at δ 2.05 (C-16) and then to a band of three overlapping methylene resonances at δ 1.32 (C-17, C-18, C-19). The only additional correlation to this latter high field band was a triplet methyl group at δ 0.89 ($J = 6.7$ Hz). The lone remaining unassigned signal was that of a carbomethoxy ester which had been introduced via CH_2N_2 treatment (δ 3.67, *s*). Therefore, from these data, derivative **2** was defined as methyl 5,6-dihydroxy-7*E*,9*E*,11*Z*,14*Z*-eicosatetraenoate.

The absolute stereochemistry of compounds **1** and **2** was established by comparing the ^1H NMR properties and optical rotation of **1**, produced from saponification of **2** in basic MeOH, with these recorded for all four stereoisomers of 5,6-diHETE. In 1989, Kugel et al., synthesized all four stereoisomers by unequivocal methods in order to evaluate their biological properties (Kugel, Lellouche, Beaucourt, Niel, Girard & Rossi, 1989). The ^1H NMR spectrum of algal-derived 5,6-diHETE (**1**) was identical to these published for both 5*S*,6*R*-diHETE and 5*R*,6*S*-diHETE. However, the *R. pertusa* derived 5,6-diHETE (**1**) showed an optical rotation of $[\alpha]_D^{24} = -3^\circ$, comparable in sign only to that of authentic 5*R*,6*S*-diHETE (lit. $[\alpha]_D^{22} = -13^\circ$). The lower rotation of the algal-derived material compared to the synthetic standard may either reflect partial racemization of this sample, or may be due to problems inherent in obtaining good rotational data for compounds of low rotation, low abundance, and poor solubility (Kugel et al., 1989). Hence, the complete structure of metabolite **1** from *R. pertusa* was established as 5*R*,6*S*-dihydroxy-7*E*,9*E*,11*Z*,14*Z*-eicosatetraenoate.

The same HPLC trace yielding derivative **2** possessed a second major natural product, also as a methyl ester derivative (**4**). Derivative **4** was of an obvious close structural resemblance to derivative **2** as shown by ^1H NMR and UV analysis. By ^1H NMR analysis, derivative **4** showed two additional olefinic protons (δ 5.33–5.47), one additional bis-allylic methylene (δ 2.82), and a sharp triplet methyl group at δ 0.98. These features, particularly the latter, are characteristic of ω3 fatty acids. Hence, based on its comparability to derivative **2**, a structure of methyl 5*R*∗,6*S*∗-dihydroxy-7*E*,9*E*,11*Z*,14*Z*,17*Z*-eicosapentaenoate could be assigned to derivative **4**. Stereochemical features of the vicinal diol in metabolite **3** were not examined with derivative **4**, but are proposed as 5*R*,6*S* based on its co-occurrence with metabolite **1**.

Methylation and HPLC of fraction 4 from the original chromatography yielded two additional oxylipin derivatives, **6** and **8**, in small yield (ca. 0.5 mg of

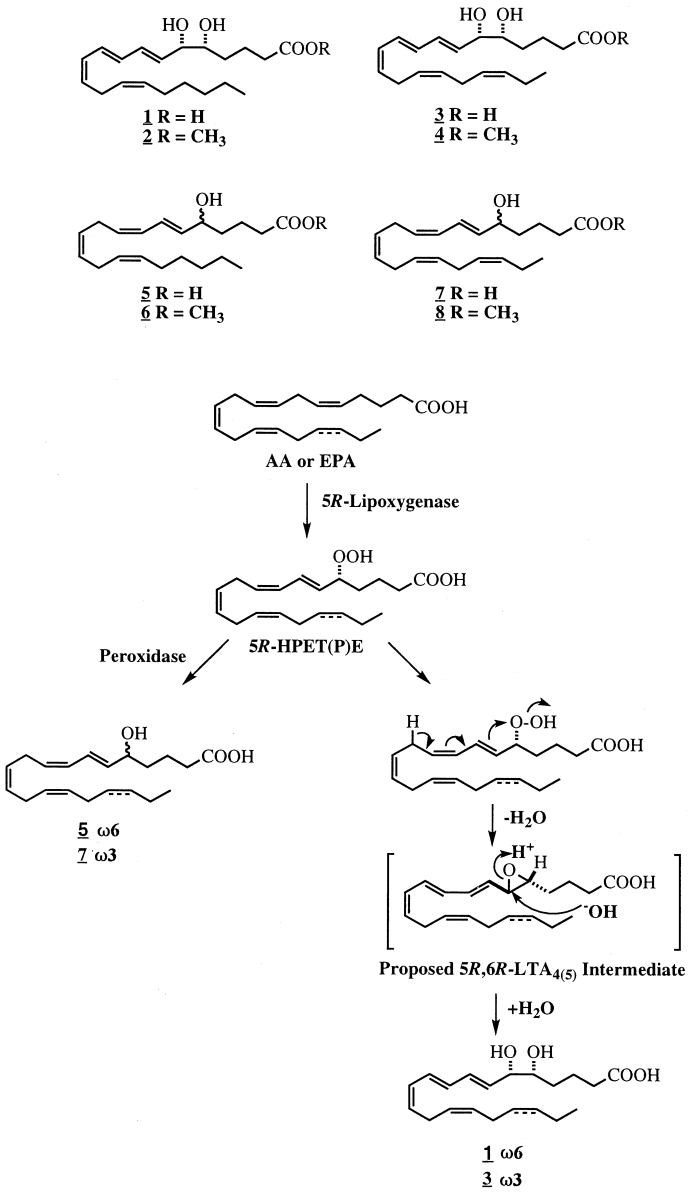

Fig. 1. Biogenetic origin of oxylipins in *R. pertusa* is proposed to involve a 5*R*-lipoxygenase acting on AA or EPA that subsequently produces 5*R*,6*R*-leukotriene A_4 and 5*R*,6*R*-leukotriene A_5 as intermediates.

each). It was apparent from consideration of ^{1}H NMR spectral features that the relationship between **6** and **8** was similar to that between **2** and **4**. Specifically, relative to derivative **6**, derivative **8** showed as points of difference two additional olefinic protons (δ 5.27–5.47), one additional bis-allylic methylene (δ 2.86), and a sharp triplet methyl group at δ 0.98. Hence, it could be concluded that these represented an $\omega6/\omega3$ pair of related substances.

The diene-alcohol nature of derivative **6** was revealed by UV ($\lambda_{max} = 236$), ^{1}H NMR and MS data. GC–MS data of the TMS ether derivative showed substantial fragmentation at C4–C5 (*m*/*z* 305, 15%) and C5–C6 (*m*/*z* 203, 11%), indicating the presence of a C5 alcohol group. A single α-hydroxy proton was present (δ 4.17), in the ^{1}H NMR spectrum, confirming the monohydroxy nature of **6**. Three of the four protons of a conjugated diene system were clearly resolved; analysis of their coupling constant allowed formulation of a 6*E*,8*Z* system. Integration of the unresolved vinyl protons signals at δ 5.27–5.47 indicated that methyl ester derivative **6** was an $\omega6$ derivative; hence, its structure was formulated as 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoate. By similar analysis, derivative **8** was reasoned to be 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoate. While the small amount of isolated sample precluded stereoanalysis of either metabolite **5** or **7**, we propose a 5*R*

stereochemistry based on a biosynthetic origin in common with that of **1** and **3** (Fig. 1).

The four metabolites isolated and characterized from *R. pertusa* (as methyl ester derivatives) strongly support the presence and function of a 5*R*-lipoxygenase in this red alga. This is intriguing because there are relatively few examples of oxylipins in non-mammalian systems that appear to arise from a 5-lipoxygenase-initiated pathway (Hada, Swift & Brash, 1997; Gerwick, 1999). In particular, other red algae have been characterized to mainly produce oxylipins utilizing 8-LO (Graber, Gerwick & Cheney, 1996), 9-LO (Jiang & Gerwick, 1997) and 12-LO (Hamberg & Gerwick, 1993) initiated pathways. In mammals, the 5-LO route is a seminal pathway in which the initial 5*S*-lipoxygenase product, 5*S*-HPETE, is further transformed by 5-LO into LTA_4. Depending upon the tissue, LTA_4 is then converted into either a peptidyl leukotriene (LTC_4), leukotriene B_4, a lipoxin, or 5*S*,6*R*-diHETE (Samuelsson, Dahlen, Lindgren, Rouzer & Serhan, 1987). The one other example of the algal metabolism of a PUFA by a 5*R*-lipoxygenase, the production of bacillariolides I and II by the diatom *Nitzschia pungens* (Wang & Shimizu, 1990), is particularly intriguing because it appears that the initial hydroperoxide is transformed into a reactive epoxy allylic carbocation which then carbocyclizes into a novel C-2–C-6 cyclopentane system (Gerwick, 1996). In the red alga investigated in this study, *R. pertusa*, the fate of the putative LTA_4-like substance appears to follow more closely the mammalian route of metabolism. It is interesting that the marine algal 5-LO's detected to date are apparently both of 5*R* stereospecificity, the opposite stereospecificity of mammalian and some higher plant LO's (e.g. potato) (Shimizu, Radmark & Samuelsson, 1984; Gardner, 1991). It will be particularly insightful to study the structure and evolutionary relationship of these ancient algal lipoxygenases to other classes of plant and animal lipoxygenases so as to understand this alternate stereospecificity.

3. Experimental

3.1. General

Ultraviolet spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer and optical rotation was measured on a Perkin Elmer 141 polarimeter. Nuclear magnetic resonance spectra were obtained on a Bruker AC300 spectrometer with TMS as internal standard. Gas chromatography–electron impact mass spectrometry (GC–EIMS) was performed on a Hewlett-Packard 5810 GC interfaced with a Hewlett-Packard 5971 mass selective detector. High performance liquid chromatography was performed using a Waters M-6000 pump and Waters Lambda-Max 480 UV-detector for detection. Thin layer chromatography used Merck aluminum-backed TLC sheets (silica gel 60 F_{254}). All solvents were distilled prior to use.

3.2. Collection, extraction and isolation

R. pertusa was collected by Scuba diving (−8 to −15 m) at Keystone Ferry, Washington in July 1990 (a voucher specimen is available from WHG as WKF-19/Jul/90-1). A one gallon volume of wet alga (42 g dry weight of extracted alga) was immediately frozen in dry ice at the time of collection and stored. Defrosted alga was homogenized in $CHCl_3$/MeOH (2:1) and warmed to ca. 60° for 30 min. The homogenate was filtered and the $CHCl_3$ layer separated and reduced in vacuo to yield 1.4 g of a dark green oil. The majority of this (1.3 g) was separated into 7 fractions using vacuum silica gel chromatography (VLC, hexanes/EtOAc/MeOH). Fractions 5 (60% EtOAc/hexanes) and 6 (100% EtOAc) were separately methylated with diazomethane and then further purified by VLC (15% EtOAc/hexanes). Following additional HPLC (2 × 30 cm × 4.1 mm Versapack columns, 10% EtOAc/hexanes, detection 254 nm), fraction 5 gave 0.5 mg of methyl 5-HETE (**6**) and 0.5 mg of methyl 5-HEPE (**8**), whereas fraction 6 (VLC eluted with 25–40% EtOAc/hexanes; HPLC Maxsil 50 cm × 10 mm, 35% EtOAc/hexanes) gave 2.9 mg of methyl 5*R*,6*S*-diHETE (**2**) and 2 mg of methyl 5*R**,6*S**-diHEPE (**4**), all as oils.

3.3. Formation of TMS ethers

TMS ethers of hydroxyl-containing oxylipins were formed by reacting 50–200 μg of the compound with three drops dry pyridine, three drops 1,1,1,3,3,3-hexamethyldisilazane, and three drops of chlorotrimethylsilane for 20 min at rt. Reagents and solvents were removed in vacuo and the resulting crusty white solid extracted with hexanes at a concentration of 50 μg oxylipin/ml hexanes. The decanted hexanes were analyzed directly by GC–EIMS.

3.4. Methyl 5R,6S-diHETE (2)

$[\alpha]_D^{23} = -1.4°$ ($c = 0.29$, MeOH); UV (methanol)$\lambda = 260$, 271, 286 nm, $\log \varepsilon = 4.264$, 4.208, 4.143; LR–EIMS of TMS ether *m*/*z* (% rel. int.) 494 (0.4, M^+), 463 (0.5), 404 (1.1), 393 (0.3), 373 (0.5), 261 (6), 203 (100), 171 (48), 147 (20), 129 (50), 113 (39) 99 (53), 73 (80); ^{1}H NMR spectral data (300 MHz, $CDCl_3$) δ 6.55 (1H, *dd*, $J = 11.4$, 14.8 Hz, H-10), 6.37 (1H, *dd*, $J = 10.8$, 15.1 Hz, H-8), 6.21 (1H, *dd*, $J = 10.8$, 14.8 Hz, H-9), 6.03 (1H, *t*, $J = 11.0$ Hz, H-11), 5.75 (1H, *dd*, $J = 7.3$, 15.1 Hz, H-7), 5.48 (1H, *m*,

H-12), 5.41 (1H, *m*, H-15), 5.37 (1H, *m*, H-14), 4.15, (1H, *m*, H-6), 3.70 (1H, *m*, H-5), 3.67 (3H, *s*, –OMe), 2.94 (2H, *t*, $J = 7.3$ Hz, H-13), 2.36 (2H, *t*, $J = 7.0$ Hz, H-2), 2.05 (2H, *m*, H-16), 1.85 (1H, *m*, H-3a), 1.71 (1H, *m*, H-3b), 1.45 (2H, *m*, H-4), 1.23–1.41 (6H, *m*, H-17, H-18, H-19), 0.89 (3H, *t*, $J = 6.7$ Hz, H-20); ^{13}C NMR spectral data (75 MHz, $CDCl_3$) δ 175.2 (C-1), 133.6, 131.6, 131.6, 131.0, 130.4, 129.0, 128.2, 126.9, 75.6, 73.8, 51.6 (–OMe), 33.7 (C-2), 31.5, 31.4, 29.3, 27.2, 26.2, 22.6 (C-19), 21.1, 14.1 (C-20).

3.5. Methyl 5R*,6S*-diHEPE (**4**)

UV (methanol) $\lambda = 266$, 274, 283 nm, $\log \varepsilon = 3.93$, 3.988, 3.922; IR (neat) 2955, 2926, 1739, 1528, 1347, 1270, 1237, 1165, 1114, 1101, 1052, 1013, 997, 952, 941, 720 cm^{-1}; ^{1}H NMR spectral data (300 MHz, $CDCl_3$); δ 6.55 (1H, *dd*, $J = 11.4$, 14.7 Hz, H-10), 6.37 (1H, *dd*, $J = 10.7$, 15.1 Hz, H-8), 6.22 (1H, *dd*, $J = 10.8$, 14.6 Hz, H-9), 6.04 (1H, *t*, $J = 11.0$ Hz), 5.75 (1H, *dd*, $J = 7.3$, 15.1 Hz, H-7), 5.33–5.47 (5H, *m*, H-12, H-14, H-15, H-17, H-18), 4.15 (1H, *m*, H-6), 3.70 (1H, *m*, H-5), 3.67 (3H, *s*, –OMe), 2.97 (2H, *t*, $J = 6.8$ Hz, H-13), 2.82 (2H, *t*, $J = 6.6$ Hz, H-16), 2.36 (2H, *t*, $J = 7.0$ Hz, H-2), 2.08 (2H, *m*, H-19), 1.84 (1H, *m*, H-3a), 1.71 (1H, *m*, H-3b), 1.47 (2H, *m*, H-4), 0.98 (3H, *t*, $J = 7.6$ Hz, H-20).

3.6. Methyl 5-HETE (**6**)

UV (methanol) 236 nm; LR–EIMS of TMS ether *m*/*z* (% rel. int.) 406 (M^+, 5), 352 (5), 305 (15), 281 (4), 255 (26), 203 (11), 190 (10), 129 (19), 91 (31), 73 (100); ^{1}H NMR spectral data (300 MHz, $CDCl_3$); δ 6.52 (1H, *dd*, $J = 11.5$, 15.4 Hz, H-7), 5.98 (1H, *t*, $J = 11.5$ Hz, H-8), 5.71 (1H, *dd*, $J = 7.0$, 15.4 Hz, H-6), 5.27–5.47 (5H, *m*, H-9, H-11, H-12, H-14, H-15), 4.17 (1H, *m*, H-5), 3.67 (3H, *s*, –OMe), 2.96 (2H, *t*, $J = 7.0$ Hz, H-10), 2.84 (2H, *t*, $J = 6.5$ Hz, H-13), 2.35 (2 H, *t*, $J = 7.3$ Hz, H-2), 2.05 (2H, *m*, H-16), 1.69 (2H, *m*, H-4), 1.25–1.47 (8H, *m*, H-3, H-17, H-18, H-19), 0.88 (3H, *t*, $J = 6.7$ Hz, H-20).

3.7. Methyl 5-HEPE (**8**)

^{1}H NMR spectral data (300 MHz, $CDCl_3$); δ 6.53 (1H, *dd*, $J = 11.5$, 15.4 Hz, H-7), 5.98 (1H, *t*, $J = 11.5$ Hz, H-8), 5.71 (1H, *dd*, $J = 7.0$, 15.4 Hz, H-6), 5.27–5.47 (7H, *m*, H-9, H-11, H-12, H-14, H-15, H-17, H-19), 4.17 (1H, *m*, H-5), 3.67 (3H, *s*, –OMe), 2.96 (2H, *t*, $J = 7.0$ Hz, H-10), 2.86 (4H, *m*, $J = 6.5$ Hz, H-13, H-16), 2.35 (2 H, *t*, $J = 7.3$ Hz, H-2), 2.05 (2H, *m*, H-19), 1.69 (2H, *m*, H-3), 1.47 (2H, *m*, H-4), 0.98 (3H, *t*, $J = 7.6$ Hz, H-20).

3.8. Conversion of methyl 5R,6S-diHETE (**2**) into 5R,6S-diHETE (**1**)

The methyl ester **2** (2 mg) was dissolved in 9 ml of MeOH and 1 ml of 10 N NaOH. The reaction was allowed to proceed at rt for 1 h, partially neutralized with 0.8 ml of 10 N HCl, and then adjusted to ca. pH 3 with acetic acid. Precipitated NaCl was filtered, 20 ml of distilled H_2O added, and the resulting solution was extracted with $CHCl_3$ (2×20 ml). The $CHCl_3$ layer was evaporated to give 2 mg of an oil. By TLC this material was a single compound which matched the ^{1}H NMR spectrum of 5*R*,6*S*-diHETE (**1**) and 5*S*,6*R*-diHETE (Kugel et al., 1989), but had a sign of optical rotation consistent only with that of 5*R*,6*S*-diHETE (**1**): $[\alpha]_D^{24} = -3^\circ$ ($c = 0.2$, EtOH), lit. $[\alpha]_D^{22} = -13^\circ$ ($c = 0.1$, EtOH).

Acknowledgements

We thank D. Nagle and P. Proteau for help with algal collections. This work was supported by the Oregon Sea Grant Program under grants R/SH-5 and R/BT-09.

References

Bernart, M. B., & Gerwick, W. H. (1994). *Phytochemistry*, *36*, 1233.
Drazen, J. M., Isreal, E., & O'Byrne, P. M. (1999). *N. Engl. J. Med*, *340*, 197.
Gardner, H. W. (1991). *Biochim. Biophys. Acta*, *1084*, 221.
Gerwick, W. H., & Bernart, M. (1993). In D. H. Attaway, & O. R. Zaborsky, *Advances in marine biotechnology: pharmaceutical bioactive natural products, vol. 1* (pp. 101–152). New York: Plenum Press.
Gerwick, W. H. (1994). *Biochim. Biophys. Acta*, *1211*, 243.
Gerwick, W. H. (1996). *Lipids*, *31*, 1215.
Gerwick, W. H. (1999). In U. Sankawa, D. H. R. Barton, K. Nakanishi, & O. Meth-Cohn, *Comprehensive natural products chemistry*, *vol. 1* (pp. 207–254). New York: Pergamon Press.
Gibbons, G. F., Goad, L. J., & Goodwin, T. W. (1967). *Phytochemistry*, *6*, 677.
Graber, M. A., Gerwick, W. H., & Cheney, D. P. (1996). *Tetrahedron Lett*, *37*, 4635.
Guerriero, A., D'Ambrosio, M., & Pietra, F. (1990). *Helv. Chim. Acta*, *73*, 2183.
Hada, T., Swift, L. L., & Brash, A. R. (1997). *Biochim. Biophys. Acta*, *1346*, 109.
Hamberg, M., & Gerwick, W. H. (1993). *Archiv. Biochem. Biophys*, *305*, 115.
Idler, D. R., Saito, A., & Wiseman, P. (1968). *Sterols*, *11*, 465.
Jiang, Z.-D., & Gerwick, W. H. (1997). *Lipids*, *32*, 231.
Kugel, C., Lellouche, J.-P., Beaucourt, J.-P., Niel, G., Girard, J.-P., & Rossi, J.-C. (1989). *Tetrahedron Lett*, *30*, 4947.
Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A., & Serhan, C. N. (1987). *Science*, *237*, 1171.
Shimizu, T., Radmark, O., & Samuelsson, B. (1984). *Proc. Natl. Acad. Sci. USA*, *81*, 68.
Wang, R., & Y. Shimizu, Y. (1990). *J. Chem. Soc. Chem. Commun.*, 413.