Three New and Bioactive Icosanoids from the Temperate Red Marine Alga *Farlowia mollis*

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Three new dihydroxyicosanoids, 12(R),13(R)-dihydroxy-icosa-5(Z),8(Z),10(E),14(Z)-tetraenoic acid, 12(R),13(R)-dihydroxyicosa-5(Z),8(Z),10(E),14(Z),17(Z)-pentaenoic acid and 10(R*),11(R*)-dihydroxyoctadeca-6(Z),8(E),12(Z)-trienoic acid, have been isolated from a previously un-studied temperate red marine alga, *Farlowia mollis* (Cryptonemiales, Rhodophyta). The structures of these new metabolites have been deduced from detailed nuclear magnetic resonance and mass spectrometry analyses on stabilized diacetate-methyl esters and stereochemistry deduced by ¹H NMR couplings and CD analysis of a dibenzoate derivative. Collectively, these new natural products modulate fMLP-induced superoxide anion gen-eration in human neutrophils, inhibit the conversion of arachidonic acid to lipoxygenase products by human neutrophils, and inhibit the functioning of the dog kidney Na⁺/K⁺ ATPase.
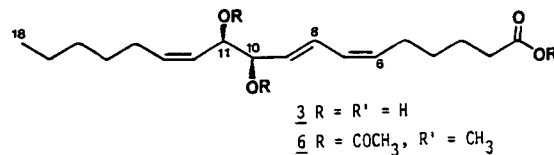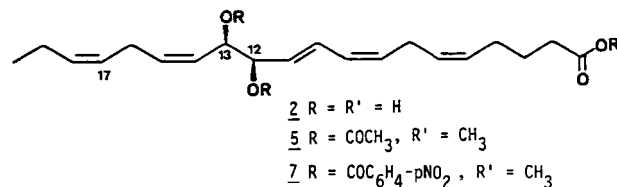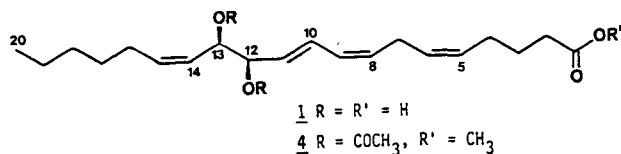*Lipids 24,* 256–260 (1989).

Recently, we have reported on the isolation of several new icosanoids from the red seaweeds *Ptilota filicina, Mur-rayella periclados* and *Platysiphonia miniata* (1–4). Our continued survey of Oregon seaweeds for new natural products with potential biomedicinal application has now identified another red alga, *Farlowia mollis* (Harv. & Bail.) Farl. & Setch., as a rich source of structurally novel and physiologically active icosanoids. The complete structures of these unstable diols (1,2,3) were efficiently solved by application of 2D-nuclear magnetic resonance (NMR) and CD methodologies on stabilized derivatives. Further-more, a metabolite recently isolated from *P. filicina,* 5(Z),7(E),9(E),14(Z),17(Z)-icosapentaenoic acid, as well as all Z 5,8,11,14,17-icosapentaenoic acid (EPA), were also present in the organic extract of this temperate seaweed.

EXPERIMENTAL METHODS

General. Ultraviolet spectra were recorded on an Amin-co DW-2a UV-Vis spectrophotometer and infrared spec-tra (IR) were recorded on a Nicolet 5 DXB FT 15 spec-trophotometer. Circular dichroism was measured on a Jasco 41A spectropolarimeter. NMR spectra were re-corded on a Bruker AM 400 NMR spectrometer and all shifts are reported relative to an internal TMS standard. Low resolution mass spectra (LRMS) were obtained on a Varian MAT CH7 spectrometer, whereas high resolu-tion mass spectra (HRMS) were obtained on a Kratos MS 50 TC. High performance liquid chromatography (HPLC) was done using a Waters M-6000 pump, U6K injector and

R 401 differential refractometer, whereas thin layer chro-matograms (TLC) were made using Merck aluminum-backed TLC sheets (silica gel 60 F_{254}). All solvents were distilled from glass prior to use.

Collection, extraction and isolation. Farlowia mollis was collected from exposed intertidal pools (-0.5 to $+0.5$ m) at Cape Perpetua on the Oregon coast in August 1986. The seaweed was preserved by freezing until workup, at which time the defrosted alga (103 g dry wt) was homogenized in warm $CHCL_3/MeOH$ (2:1, v/v). The mix-ture was filtered and the solvents were removed *in vacuo* to yield 600 mg of a dark green tar which displayed an-tibiotic activity to *S. aureus* and *E. coli.* The extract was fractionated by silica gel chromatography in the vacuum mode (10 cm × 9 cm, Merck TLC-grade Kieselgel), and metabolites were progressively eluted with increasingly polar mixtures of isooctane and ethyl acetate. The known compounds, 5(Z),7(E),9(E),14(Z),17(Z)-icosapentaenoic acid and all Z 5,8,11,14,17-icosapentaenoic acid (EPA), eluted in 20–40% EtOAc/isooctane (ca. 2–3% of the lipid extract each) and were identified by comparison of their 400 MHz ¹H NMR features with authentic standards. Those eluting with 50% EtOAc/isooctane were a mixture of fatty acids and contained diols 1, 2 and 3. Treatment of a portion of these fractions with CH_2N_2 afforded a mix-ture of methyl esters that was subsequently chromato-graphed on normal phase HPLC (μ-Porasil Z-module, 65% EtOAc/isooctane) which removed residual pigments from the fatty acid mixture. After observations using NMR, the fatty acid mixture was acetylated using excess acetic anhydride/pyridine (1/1) and the resulting mixture was separated by normal phase HPLC (2 × 3.9 mm × 25 mm μ-Porasil, 10% EtOAc/isooctane) to yield 10 mg of 4, 15 mg of 5 and 4 mg of 6.

1 R = R' = H
4 R = COCH₃, R' = CH₃

2 R = R' = H
5 R = COCH₃, R' = CH₃
7 R = COC₆H₄-pNO₂, R' = CH₃

3 R = R' = H
6 R = COCH₃, R' = CH₃

Methyl 12(R),13(R)-diacetoxy 5(Z),8(Z),10(E),14(Z)-icosatetraenoic acid (4). Compound 4 was a colorless oil showing the following: UV (MeOH) λ_{max} 236 nm (log ε = 4.532); IR (CHCL$_3$) 3022, 3019, 2931, 1734, 1372, 1246, 1222, 1217, 1210, 1026 cm^{-1}; $[\alpha]_D{}^{25}$ = +2.24° (c = 0.63, CCL$_4$); low resolution electron impact mass spectrometry (LR EIMS) m/z (rel. intensity) 374 (M$^+$ − AcOH, 9.1), 314 (M$^+$ − 2 AcOH, 4.4), 265 (26.1), 225 (22.0), 223 (82.7), 205 (36.7), 191 (20.5), 173 (12.8), 169 (22.6), 163 (17.3), 131 (14.5), 127 (62.9), 95 (11.2), 91 (14.4), 83 (15.3), 81 (23.3), 79 (16.6), 67 (20.2), 57 (20.2), 55 (20.6), 43 (100); HR EIMS m/z obs. 374.24768 (M$^+$ − AcOH, C$_{23}$H$_{34}$O$_4$ requires 374.24757; for ^{1}H NMR and ^{13}C NMR data see Table 1.

Methyl 12(R),13(R)-diacetoxy-5(Z),8(Z),10(E),14(Z),17(Z)-icosapentaenoic acid (5). Compound 5 was also isolated as a colorless oil and showed UV (MeOH) λ_{max} 236 nm (log ε = 4.432); IR (CHCL$_3$) 3020, 2965, 2955, 1734, 1434, 1372, 1223, 1220, 1208, 1025 cm^{-1}; $[\alpha]_D{}^{25}$ = +3.72° (c = 1.13, CCL$_4$); LR EIMS m/z (rel. intensity) 372 (M$^+$ − AcOH, 6.6), 312 (M$^+$ − 2AcOH, 4.7), 265 (29.6), 237 (17.0), 224 (15.5), 223 (100), 205 (48.8), 191 (28.0), 173 (21.0), 163 (28.6), 147 (13.7), 145 (20.2), 141 (16.6), 135 (10.1), 131 (28.9), 129 (11.9), 125 (32.5), 121 (13.2), 109 (14.7), 107 (93.0), 105 (19.0), 97 (10.1), 95 (24.0), 93 (20.3), 91 (36.3), 83 (22.8), 81 (35.0), 80 (12.5), 79 (53.2), 77 (13.2), 71 (16.3), 69 (15.6), 59 (13.1), 57 (20.3), 55 (37.1); for ^{1}H NMR and ^{13}C NMR data see Table 1.

Methyl 10(R),11(R*)-diacetoxy 6(Z),8(E),12(Z)-octadecatrienoic acid (6)*. Compound 6 was also isolated as a colorless oil showing the following: UV (MeOH) λ_{max} 234 nm (log ε = 4.224); IR (neat) 3014, 2954, 2930, 2858, 1742, 1457, 1437, 1371, 1241, 1226, 1168, 1114, 1026 cm^{-1}; LR EIMS m/z (rel. intensity) 348 (M$^+$ − AcOH, 13.5), 239 (25.4), 237 (10.7), 223 (15.9), 198 (14.6), 197 (73.5), 180 (12.5), 179 (100), 169 (24.4), 165 (62.4), 147 (25.0), 137 (10.8), 127 (63.1), 119 (19.4), 109 (21.1), 107 (13.7), 105 (11.7), 95 (10.8), 93 (10.6), 91 (14.6), 83 (10.8), 81 (22.4), 79 (14.3), 67 (18.9), 57 (19.4), 55 (16.9), 43 (78.9); for ^{1}H NMR and ^{13}C NMR data see Table 1.

Formation of methyl 12(R),13(R)-bis(p-nitrobenzoyl)-5(Z),8(Z),10(E),14(Z),17(Z)-icosapentaenoic acid (7) from diol 2. A portion of crude extract (1.2 g) was treated with CH$_2$N$_2$ and chromatographed over silica gel in the vacuum mode (EtOAc/isooctane). Fraction 3 (30% EtOAc/isooctane) contained mainly methyl ester derivatives of diols 1–3 and was refluxed overnight with p-nitrobenzoyl chloride in CH$_2$Cl$_2$ and pyridine (1:1) with catalytic amounts of dimethylaminopyridine (DMAP). The reaction was terminated with the addition of ice and then H$_2$O and the products extracted with Et$_2$O. The Et$_2$O was sequentially washed with portions of saturated NaHCO$_3$ (2 × 25 ml), 5% HCl (2 × 25 ml), and H$_2$O (1 × 25 ml). A rapid vacuum chromatography of this material gave a fraction enriched in 7, which was further purified over HPLC (Alltech RSIL 10 μ 50 cm × 10 mm, 15% EtOAc/isooctane, 1.3 mg). Pure derivative 7 showed the following: UV (MeOH) λ_{max} = 240 nm (log ε = 4.874); $[\alpha]_D{}^{25}$ = +21.0° (MeOH); CD (MeOH) $\Delta\varepsilon$ = −19.8, +34.6 (λ_{max} = 235, 256 nm); ^{1}H NMR (bz-d-6, 400 MHz) δ 0.97 (t, 3H, J = 7.7 Hz, CH$_3$CH$_2$-), 1.70 (p, 2H, J = 7.4 Hz), 2.09 (m, 4H), 2.31 (t, 2H, J = 7.4 Hz), 2.93 (t, 2H, J = 6.9 Hz), 3.03 (bt, 2H, J = 6 Hz), 3.66 (s, 3H), 5.30–5.49 (m, 5H), 5.52 (m, 3H), 5.88 (dd, 1H, J =

3.8, 8.2 Hz), 6.03 (t, 1H, J = 11.0 Hz), 6.17 (dd, 1H, J = 3.8, 9.5 Hz), 6.80 (dd, 1H, J = 14.7, 11.0 Hz), 8.16 (d, 2H, J = 8.7 Hz, Ar-H), 8.18 (d, 2H, J = 8.0, Hz, Ar-H), 8.28 (d, 2H, J = 8.7 Hz, Ar-H), 8.30 (d, 2H, J = 8.0 Hz, Ar-H).

Evaluation of superoxide anion production by human neutrophils stimulated with the diols 1–3. Ca. 150 to 200 ml of whole blood (ACD anticoagulant) was drawn from various donors by certified members of the Red Cross or Good Samaritan Hospital (Corvallis, Oregon). The blood was immediately subjected to dextran precipitation (6% Dextran, M.W. = 70,000, Pharmacia). Ca. 60 ml of whole blood were mixed with 40 ml 6% Dextran, and the cells were allowed to stand for 1 hr, undisturbed, in a 100-ml plastic graduated cylinder. After this time, most of the RBCs had settled to the bottom leaving a straw-colored leukocyte-rich plasma on top. This plasma was drawn off and centrifuged at 120 × g for 20 min at 4°C. The supernatant was discarded, and the cells were shocked with ice-cold distilled H$_2$O (ca. 10% the volume of the original plasma suspension), and after 30 sec their isotonicity restored by the addition of 0.6 M NaCl (33% the volume of added H$_2$O. The cells were washed with PBS (Sigma, w/o Ca^{+2}), and then subjected to a discontinuous gradient centrifugation using Ficoll-Paque (Pharmacia). Ca. 3 × 10^7 cells in 8 ml PBS were layered onto 4 ml Ficoll-Paque in a 15-ml glass centrifuge tube and then centrifuged at 250 × g for 20 min at 4°C. The pellet of cells at the bottom of the tube contained ca. 99% PMNLs by microscopic examination. These cells were washed with PBS and then resuspended using Hank's buffered saline solution (Sigma, with Ca^{+2}, w/o phenol red (HBSS)) at a concentration of 6.0 × 10^6 cells/ml (5). The cells were counted using a hemocytometer and their viability (>95% for all assays) determined using a trypan blue (0.5% in PBS) exclusion assay in which only damaged cells take up the dye.

The following protocol was used to measure the production of superoxide anion by isolated human neutrophils (6,7). Isolated neutrophils (1–2 hr old, 3–4 × 10^6/0.5 ml Hank's buffered saline solution (HBSS)) were incubated for 10 min at 37°C. Cytochalasin B (5 μg/ml) was added to the cells and the mixture was incubated for 3 min, or if no cytochalasin B was used, the incubation time of 3 min was maintained. Test lipids (0.10–10 μM) were then added to the cells, and the mixture was incubated at 37°C for 2 min. Cytochrome C was then added to the mixture (final conc. 10 mM in HBSS), and followed immediately by fMLP or LTB$_4$, if they were to be tested. The final assay volume of 2.0 ml was then incubated at 37°C for 15 min and then stopped by the addition of 10 μl of superoxide dismutase (1 mg/0.3 ml HBSS). The cells were pelleted by centrifugation (200 × g for 10 min) and the supernatant was measured for absorbances between 500–570 nm (reduced cytochrome C λ_{max} = 550 nm). Blanks were used to obtain baseline values and controls produced by the addition of 10 μl of superoxide dismutase (SOD) before cytochalasin B, fMLP or LTB$_4$ were added to the cells.

RESULTS AND DISCUSSION

The temperate red alga *Farlowia mollis* was originally collected in our survey efforts from the Oregon coast in 1986 and the lipid extract showed antimicrobial activity to

several human pathogens and the occurrence of potentially novel compounds by TLC (blue char upon acidification at 0.13 R_f). Furthermore, the crude extract of *F. mollis* displayed good inhibitory activity to dog kidney Na^+/K^+ ATPase (IC_{50} = 38 $\mu g/ml$) and hog gastric mucosa H^+/K^+ ATPase (IC_{50} = 9.4 γ/ml). Thus, larger collections were made in August 1986, May 1987 and June 1987, all of which contained the same unusual appearing blue-charring compounds. Bioautography against *E. coli* indicated that polar compounds in the extract were responsible for the antimicrobial activity. Hence, the material collected in August of 1986 was vacuum chromatographed over silica gel to rapidly yield mixtures enriched in these polar blue-charring diols (1-3).

In order to stabilize and more easily separate compounds 1-3, they were first treated with CH_2N_2 and later with acetic anhydride in pyridine, to produce diacetate-methyl ester derivatives. This mixture of derivatives was then easily separated by HPLC to yield (in order of their polarity) a purple-charring compound, (4), a blue-charring compound, (5), and a grey-blue-charring compound, (6).

The diacetate-methyl ester derivative of 4 was a colorless and optically active oil which gave a measurable M^+ − acetate peak by HR EIMS affording a molecular formula of $C_{25}H_{38}O_6$ (7° unsaturation). The IR spectrum for 4 showed an intense carbonyl stretch for multiple

esters ($\gamma_{C=O}$ = 1734 cm^{-1}), the protonic consequences of which were readily observed in the ^1H NMR (Table 1) and defined two acetates and one methyl ester. By ^{13}C NMR, the remaining four degrees of unsaturation presented as olefinic bonds (Table 1), and two of these formed a conjugated system (λ_{max} = 236 nm).

The overall structure of derivative 4 was readily approachable by ^1H-^1H COSY experiments and lead to the generation of two partial structures which accounted for all of the atoms in the molecule. The first partial structure began with a sharp 2H triplet at δ 2.09, which was readily identified from comparisons with model compounds as belonging to the C-2 protons of a fatty acid, in this case in the form of a methyl ester. Sequential correlations between these C-2 protons and those at C-3, C-4 and C-5 defined a normal Δ 5 unsaturated fatty acid (Table 1). The other proton of the Δ 5 olefin was located at δ 5.29 and was additionally correlated to a bisallylic methylene at δ 2.78 (H_2-7). The C-7 methylene was a triplet and was therefore coupled to one other proton, located at δ 5.38 from the COSY experiment. The C-8 proton was coupled by 11.0 Hz to its olefin partner (δ 5.98) and, thus, defined a *cis* geometry for this double bond. This latter proton was coupled to another olefin proton at δ 6.83 (H-10), which in turn was correlated to its olefin partner (δ 5.78, H-11) by 15.0 Hz and, therefore, was of

TABLE 1

NMR Data for the Methyl Ester Diacetate Derivatives of Three Icosanoid Natural Products From *Farlowia mollis*[a]

C#	Compound 4				Compound 5				Compound 6			
	^1H			^{13}C[b]	^1H			^{13}C[c]	^1H			^{13}C[d]
	δ	J	(Hz)	δ	δ	J	(Hz)	δ	δ	J	(Hz)	δ
1	—	—	—	173.18	—	—	—	173.18	—	—	—	—[e]
2	2.09	t	7.3	33.32	2.09	t	7.3	33.31	2.01	t	7.4	33.85
3	1.57	tt	7.3,7.3	25.03	1.57	tt	7.3,7.3	25.02	1.43	tt	7.4,7.6	24.65
4	1.91	dt	7.3,7.3	26.75	1.91	dt	7.3,7.3	26.75	1.15	tt	7.6,7.2	29.13
5	5.25	m	—	129.86	5.25	m	—	129.86	1.92	dt	7.2,7.2	27.60
6	5.29	m	—	128.16	5.29	m	—	—[e]	5.31	td	7.2,11.0	134.44
7	2.78	bdd	7.3,7.3	26.43	2.77	bdd	7.2,7.2	26.42	5.97	bdd	11.0,11.0	127.93
8	5.38	td	7.3,11.0	132.44	5.40	m	—	132.48	6.75	dd	11.0,15.0	130.91
9	5.98	bdd	11.0,11.0	127.93	5.97	bdd	11.2,11.2	127.90	5.78	dd	15.0,8.1	127.04
10	6.83	dd	11.0,15.0	130.75	6.82	dd	11.2,15.2	130.84	5.87	dd	8.1,3.7	75.61
11	5.78	dd	15.0,8.2	127.38	5.76	dd	15.2,8.2	127.23	6.21	dd	3.7,8.2	70.65
12	5.87	dd	8.2,3.7	75.58	5.85	dd	8.2,3.8	75.47	5.60	m	—	124.02
13	6.20	dd	3.7,8.0	70.61	6.19	dd	3.8,8.5	70.54	5.60	m	—	136.91
14	5.59	m	—	124.02	5.55	m	—	124.13	2.21	m	—	28.40
15	5.59	m	—	136.93	5.59	m	—	135.02	1.30	m	—	29.46
16	2.22	m	—	28.40	3.03	m	—	26.75	1.25	m	—	31.71
17	1.29	tt	6.9,6.9	29.47	5.40	m	—	126.31	1.22	m	—	22.87
18	1.23	m	—	31.72	5.40	m	—	133.04	0.89	t	6.8	14.22
19	1.23	m	—	22.89	2.03	dq	7.3,7.3	20.88				
20	0.86	t	6.8	14.22	0.91	t	7.3	14.38				
OMe	3.65	s	—	50.98	3.65	s	—	50.99	3.65	s	—	50.98
OAc	1.70	s	—	20.64	1.70	s	—	20.69	1.70	s	—	20.65
OAc	1.75	s	—	20.70	1.75	s	—	20.69	1.70	s	—	20.65
C=0	—	—	—	169.51(2C)	—	—	—	169.46(2C)	—	—	—	169.46(2C)

[a] Chemical shift values in ppm relative to TMS as an internal standard operating at 9.398 T. All spectra obtained in deuterated benzene.
[b] Assignments made from a ^1H-^{13}C heteronuclear 2D shift correlation spectroscopic experiment and by comparison with model compounds[1,2].
[c] Assignments made by comparing with (4) and with model compounds[1,2].
[d] Assignments made by comparing with (4), (5) and with model compounds[1,2].
[e] Peak not observed in spectrum.

NEW ICOSANOIDS FROM THE MARINE ALGA *FARLOWIA*

trans geometry. The H-11 proton was coupled to a proton α to an acetate located at δ 5.87 (H-12, 75.58 ppm from HETCOR) and this was adjacent to another such α-acetoxy proton at δ 6.20 (H-13, 70.61 ppm). This partial structure terminated with a correlation between the H-13 proton and a two proton olefin multiplet at δ 5.59 (H-14,15).

The second partial structure in derivative 4 began with a poorly defined methyl triplet at δ 0.86 which showed correlations to a 4H multiplet at δ 1.23 (H_2-19, H_2-18). The multiplet was further coupled to a 2H multiplet at δ 1.29 (H_2-17), itself correlated to an allylic doublet of triplets at δ 2.22 (H_2-16). Finally, this latter band was also correlated to the 2H olefin multiplet at δ 5.59 (H-15,14).

Combination of these spin system-derived partial structures accounted for all of the atoms in the molecule and could be put together in only a single manner, thus giving the constitutive structure for diol 4. The stereochemistry of the C5-6 olefin was given by the ^{13}C NMR chemical shift of the adjacent *bis*-allylic methylene. A value of δ 26.43 for this carbon atom (assigned from the HETCOR experiment) defines both adjacent olefins as possessing Z stereochemistries (8,9). Similarly, the shift of δ 28.40 for C-16 defines a Z stereochemistry for the C14-15 olefin. The relative configurations at C-12 and C-13 were defined as threo from the diagnostic couplings (J = 3.7 Hz) between these protons (see discussion which follows for 5) (10). The absolute stereochemistry was deduced as 12R, 13R by virtue of this threo relationship and the comparable optical rotations of derivatives 4 and 5. Determination of the absolute stereochemistry of diol 2 and, thus, of derivative 5, was by CD as will be discussed. Hence, the structure of diol 1 was deduced as 12(R),13(R)-dihydroxyicosa-5(Z),8(Z),10(E),14(Z)-tetraenoic acid.

The spectroscopic features (IR, UV, optical rotation and NMR) of the diacetate-methyl ester derivative 5 were similar to those obtained for the same derivative of diol 4 (Table 1). Further, the LR EIMS of derivative 5 showed a highly analogous pattern of cleavage relative to compound 4. However, several significant peaks (i.e., M+ − HOAc) in the mass spectrum of 5 were 2 amu units less than in 4 and indicated that it contained an additional olefin. All of the proton and carbon resonances ascribable to protons and carbons at C-1 through C-15 were nearly identical in compounds 4 and 5. However, two additional olefin resonances and one additional *bis*-allylic methylene resonance were observed in the ^{1}H NMR spectrum of derivative 5 relative to derivative 4. Further, the terminal methyl group was slightly further down field and was coupled to an allylic methylene at C-19, positioning the new olefin between C-17 and C-18. This olefin was of the Z stereochemistry as revealed by characteristic carbon shifts of δ 26.75 for C-16 and δ 20.88 for C-19 (1,8,9).

In a simple diol such as 5 (i.e., without the confounding effects of 1-3 interactions from additional substituents at C-10, C-11, C-14 or C-15), the C-12-C-13 rotamer with antioriented alkyl groups will predominate as the lowest energy conformation (10). Hence, a threo arrangement between protons at C-12 and C-13 was indicated by measurement of a diagnostically small coupling constant (J = 3.8 Hz) (10). The *bis*-(p-nitrobenzoate) derivative 7 showed a bisignate CD curve with a positive maximum at 256 nm and a negative maximum at 235 nm and, thus,

could be assigned to heterochromophoric exciton coupling between the C-8 to C-11 diene and p-nitrobenzoate at C-12 (11,12). This positive split Cotton effect indicates a right-hand screwness between these groups and defines the stereochemistry at 12 as R (12-15). The 12R, 13R stereoisomer is the only one consistent with (a) the threo arrangement of protons at C-12 and C-13, (b) the anti arrangement of alkyl chains and (c) a righthand screwness between benzoate and diene chromophores (Fig. 1). Hence, diol 2 was the ω-3 analog of compound 1, or 12(R),13(R)-dihydroxyicosa-5(Z),8(Z),10(E),14(Z),17(Z)-pentaenoic acid. The R stereochemistry at C-12 correlates to the same relative arrangement of atoms as found in 12-(S)-HETE and 12-(S)-HEPE, hydroxyicosanoids we have recently isolated from other red marine algae (3,4).

Derivative 6 of diol 3 was isolated from related chromatography fractions and again showed very similar spectroscopic (IR, UV, LR EIMS and NMR) features to derivatives 4 and 5. The mass spectrum of 6 gave analogous peaks as for 4, however, they occurred at 26 amu lower, indicating that it was derived from an 18-carbon fatty acid. Derivative 6 showed nearly identical ^{1}H and ^{13}C NMR bands for atoms at C-6 to C-18, as assigned to C-8 to C-20 in derivative 4 (Table 1). From ^{1}H-^{1}H COSY data, sequential correlations were observed between methylene groups at C-2 to C-5, as well as a correlation from H_2-5 to an olefin proton at δ 5.31 (C-6). Although the threo relative stereochemical relationship between protons at C-12 and C-13 was intact in 6 (J = 3.7), correlation to the absolute stereochemistry in
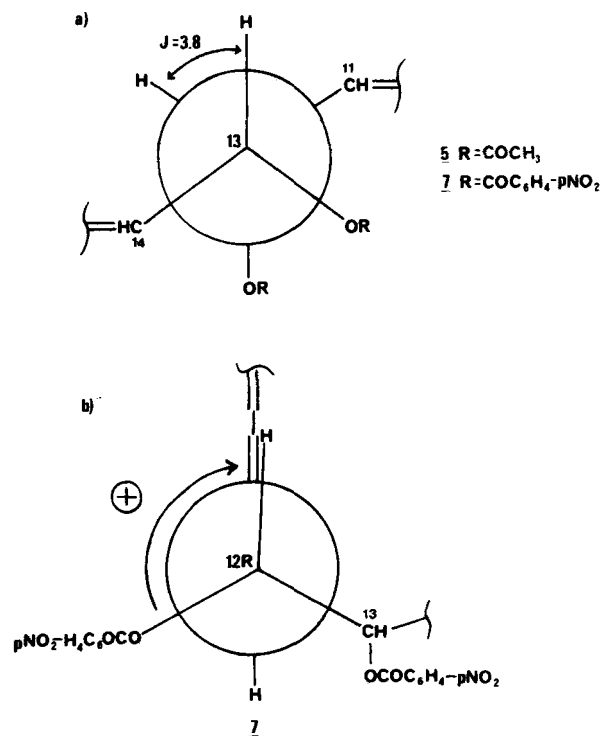

a) J=3.8

5 R=COCH$_3$

7 R=COC$_6$H$_4$-pNO$_2$

b)

7

FIG. 1. Newman projection of (a) C-13-C-12 in derivatives 5 and 7 showing threo relationship of protons based on their coupling constant, and (b) C-12-C-11 in derivative 7 showing the clockwise (positive split Cotton effect) relationship of the C-12 p-nitrobenzoate chromophore and the C-8 to C-11 diene chromophore.

TABLE 2

Superoxide Anion Production in Human Neutrophils
Stimulated by a 1:1.5 Mixture of Diols 1 and 2[a]

	Donor 1[b]	Donor 2[b]	Donor 3[c]
Control (SOD,fMLP) w/o CytoB	ND	ND	ND
Control (SOD,fMLP) w/CytoB	ND	ND	ND
Control (SOD,LTB$_4$	ND	ND	ND
Control (cells,no SOD)	—	—	ND
fMLP (20^{-7}M)	20.8	20.4[c]	14.6
1+2 (10^{-5}M)	19.4	2.1	5.5
1+2 (10^{-6}M)	22.2	ND	2.7
1+2 (10^{-7}M)	23.7	1.4	2.6
1+2 (10^{-5} fMLP (10^{-7}M)	17.5	17.1	9.5
1+2 (10^{-6}M) fMLP (10^{-7}M)	24.7	—	—
1+2 (10^{-7}M) fMLP (10^{-7}M)	21.8	21.8	16.7

[a]3.0×10^6 cells per assay, O_2^- production expressed as total nmoles/3.0×10^6 cells.

[b]Single measurement.

[c]Average of two replicates.

ND, none detected; —, experiment not performed.

derivative 5 was precluded as the compound decomposed before rotational data could be recorded. Hence, 3 was an 18-carbon analog (10(R*),11(R*)-dihydroxyoctadeca-6(Z),8(E),12(Z)-trienoic acid) of diol 1, in which the olefin and hydroxy functionalities were in the same positions in the two compounds relative to their methyl termini.

A mixture of diols 1 and 2 (1:1.5) were evaluated for several pharmacological properties expected in analogs of leukotriene, lipoxin and diHETE-type natural products. In Table 2, values are reported for the production of superoxide anion by human neutrophils stimulated under various experimental conditions. The mixture appears to be a weak primary stimulator of superoxide anion production. However, at 10^{-5} M, it inhibited fMLP-stimulated superoxide anion production in ranges between 15% and 34% of control values for three donors. This inhibitory activity is comparable with the activities of the prostaglandins PGE$_1$ and PGI$_2$ at equivalent concentrations (7).

The 1:1.5 mixture of diols 1 and 2 showed weak activity in preliminary testing for inhibition of 5-lipoxygenase

activity in A23187-stimulated human polymorphonuclear leukocytes (38% inhibition at 10^{-4} M). Other dihydroxyicosanoids are known inhibitors of lipoxygenase activity (16). Further, the mixture was only moderately inhibitory to the dog kidney Na$^+$/K$^+$ ATPase preparation (54% inhibition at 10^{-4} M, 35% at 10^{-5} M).

REFERENCES

1. Lopez, A., and Gerwick, W.H. (1987) Lipids 22, 190–194.
2. Lopez, A., and Gerwick, W.H. (1988) Tetrahedron Lett. 29, 1505–1506.
3. Bernart, M.W., and Gerwick, W.H. (1988) Tetrahedron Lett. 29, 2015–2018.
4. Moghaddam, M.F., Gerwick, W.H., and Ballantine, D.L. (1989) Prostaglandins, in press.
5. Babior, B.M., and Cohen, H.F. (1981) in Leukocyte Function; Methods in Hematology (Cline, M.J., ed.) Vol. 3, pp. 1–2, Churchill Livingstone, London, England.
6. Beckman, J.K., Gay, J.C., Brash, A.R., Lukens, J.N., and Oates, J.A. (1985) Lipids 20, 318–322.
7. Fantone, J.C., and Kinnes, D.A. (1983) Biochem. Biophys. Res. Commun. 113, 506–512.
8. Tulloch, A.P., and Bergter, L. (1980) Lipids 14, 996–1002.
9. Rakoff, H.J., and Emken, E.A. (1983) J. Am. Oil Chem. Soc. 60, 546–552.
10. Moore, R.E. (1985) in Progress in the Chemistry of Organic Natural Products (Herz, W., Grisebach, H., Kirby, G.W., and Tamm, Ch., eds.) Vol. 48, pp. 81–201, Springer-Verlag, Wein, Germany.
11. Salvadori, P., and Ciardelli, F. (1973) in Fundamental Aspects and Recent Developments in Optical Rotary Dispersion and Circular Dichroism (Ciardelli, F. and Salvadori, P., eds.) pp. 3–24, Heyden & Son, Ltd., London, England.
12. Harada, N., and Nakanishi, K. (1983) Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry, pp. 85–95 and 254–259, University Science Books, Mill Valley, California.
13. Nakanishi, K., Park, M.H., Vazquez, J.T., and Wiesler, W.T. (1987) in Stereochemistry of Organic and Bioorganic Transformations (Bartmann, W., and Sharpless, K.B., eds.) Vol. 17, pp. 303–319, VCH Publishers, Weinheim, Germany.
14. Gonnella, N.C., and Nakanishi, K. (1982) J. Am. Chem. Soc. 104, 3775–3776.
15. D'Auria, M.V., Minale, L., Riccio, R., and Uriarte, E. (1988) Experientia 44, 719–720.
16. Parker, C.W. (1987) Ann. Rev. Immunol. 5, 65–84.