HYDROXYICOSATETRAENOIC, HYDROXYICOSAPENTAENOIC, HYDROXYDOCOSAPENTAENOIC, AND HYDROXYDOCOSA-HEXAENOIC ACIDS FROM THE SPONGE *ECHINOCHALINA MOLLIS* OF THE CORAL SEA

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ABSTRACT.—It is reported that the demosponge *Echinochalina mollis* contains in relatively large amounts (+)-(12R*,5Z,8Z,10E,14Z,17Z)-12-hydroxy-5,8,10,14,17-icosapentaenoic acid, (+)-(12S,5Z,8Z,10E,14Z)-12-hydroxy-5,8,10,14-icosatetraenoic acid, (+)-(12R*,4Z, 7Z,10Z,12E,16Z,19Z)-14-hydroxy-4,7,10,12,16,19-docosahexaenoic acid, and (+)-(12R*, 4Z,7Z,10Z,12E,16Z)-14-hydroxy-4,7,10,12,16-docosapentaenoic acid. The latter three acids are detected for the first time in a marine invertebrate. The acids are isolated as the methyl esters (+)-**1**, (+)-**2**, (+)-**3**, and (+)-**4**.

12-Hydroxyicosapentaenoic (**1,2**) and 12-hydroxyicosatetraenoic acids (**1, 3–10**) and 14-hydroxydocosapentaenoic (**11**) and 14-hydroxydocosahexaenoic acids (**12,13**) have been identified in terrestrial mammals (11,12) and as products of either enzymatic (1,4,6,11,12) or oxidative nonenzymatic (2,13) transformation of unsaturated fatty acids. 12-Hydroxyicosapentaenoic acid has also been detected in subtrace amounts in marine invertebrates and algae (3,6). Finally, prostaglandin E$_2$, contained in the edible red seaweeds *Gracilaria chorda* and *Gracilaria verrucosa*, was the cause of deadly intoxica-

(+)-**1**

(+)-**2**

(+)-**3**

(+)-**4**
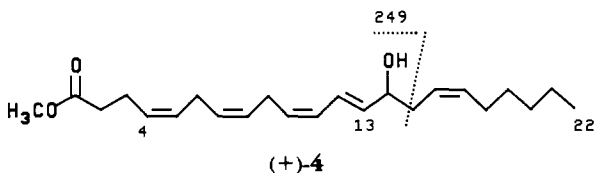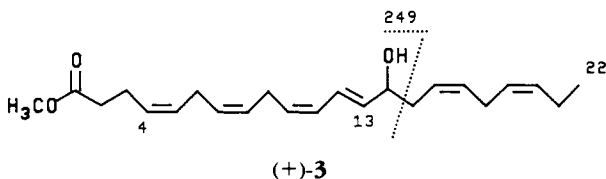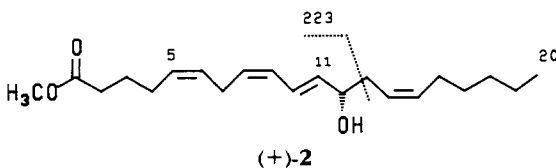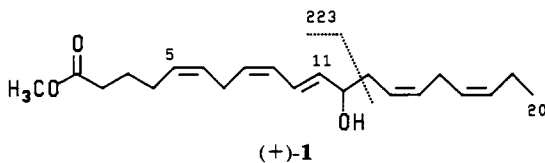
tions in Japan in 1980 and 1982 (14), and the same icosanoid was found in *Gracilaria lichenoides* from West Australian waters (15).

We report here on 12-hydroxyicosapentaenoic acids and on 14-hydroxydocosapentaenoic and 14-hydroxydocosahexaenoic acids identified for the first time in a marine invertebrate along with 12-hydroxyicosatetraenoic acid. Notably, these acids are present in the marine organisms in relatively large amounts.

Cc of the CH_2Cl_2 extract of the marine sponge *Echinochalina mollis* Levi, first on Polyamid and then on an ion-exchange support, followed by reversed-phase flash chromatography, led to a mixture of unsaturated fatty acids. To improve the separation, the mixture was esterified and separated by Si gel hplc into four esters that were eluted in the order (+)-**4**, (+)-**2**, (+)-**3**, and (+)-**1**.

The ^{13}C-nmr data for (+)-**1** in Table 1 reveal 10 olefinic CH groups, 7 CH_2 groups, one O-bearing CH group, an aliphatic Me, and a COOMe group, which amounts to the composition $C_{21}H_{32}O_3$. The mass spectrum shows an ion corresponding to $[M - H_2O]^+$ as the highest mass ion. Because there is only one methyl group and all olefinic bonds are 1,2-disubstituted, the ester must be linear. The fragments C-2–C-5, C-6–C-14 and C-15–C-20 were established by double-quantum filtered COSY experiments (16). The C-14–C-15 connection is based on delayed COSY experiments (17) while the C-5–C-6 connection is demanded by disubstitution at all double bonds. The mass spectrum shows the fragment *m/z* 223 which indicates cleavage at C-12–C-13 and thus the C-12 position for the OH group.

TABLE 1. ^{13}C-nmr Data (in CDCl$_3$).

Carbon	Compound			
	(+)-**1**	(+)-**2**	(+)-**3**	(+)-**4**
C-1	174.07 s	174.07 s	173.61 s	173.60 s
C-2	33.43 t	33.43 t	33.97 t	33.98 t
C-3	24.75 t	24.75 t	22.84 t	22.84 t
C-4	26.59 t	26.59 t	128.48 d	128.46 d
C-5	128.33 d	128.36 d	129.26 d	129.27 d
C-6	129.25 d	129.24 d	25.64 t	25.64 t
C-7	26.13 t	26.13 t	127.68 d	127.71 d
C-8	130.24 d	130.16 d	127.89 d	128.01 d
C-9	127.88 d	127.93 d	26.15 t	26.15 t
C-10	125.38 d	125.30 d	130.18 d	130.10 d
C-11	135.74 d	135.85 d	127.97 d	127.89 d
C-12	71.94 d	72.01 d	125.40 d	125.31 d
C-13	35.35 t	35.38 t	135.78 d	135.88 d
C-14	124.71 d	124.33 d	71.95 d	72.02 d
C-15	131.59 d	133.64 d	35.37 t	35.40 t
C-16	25.75 t	27.44 t	124.72 d	124.33 d
C-17	126.77 d	29.31 t	131.60 d	133.64 d
C-18	132.19 d	31.53 t	25.75 t	27.44 t
C-19	20.60 t	22.58 t	126.78 d	29.31 t
C-20	14.28 q	14.10 q	132.19 d	31.53 t
C-21	—	—	20.60 t	22.59 t
C-22	—	—	14.28 q	14.24 q
MeO	51.54 q	51.54 q	51.61 q	51.60 q

Carbon assignments in Table 1 are based on ^1H-^{13}C chemical-shift correlation experiments (18) and fully support the assigned structure (+)-**1**.

The stereochemistry of the C-8–C-12 fragment rests on the characteristic uv absorption at λ max 236 nm (19). This and the *Z* configuration at both C-14 and C-17

find confirmation in the pattern of ^{1}H-^{1}H coupling constants of Table 2. The Z configuration at C-5 is supported both by the typically shielded ^{13}C resonances for C-4 and C-7 (19) (Table 1) and by chemical-shift calculations (20).

The (12R)-hydroxyacid analogue of (+)-**1** has been reported, without either chiroptical or nmr data, as deriving from the action of the lipoxygenase of the sea urchin *Strongylocentrotus purpuratus* on (5Z,8Z,11Z,14Z,17Z)-icosapentaenoic acid (1). Racemic **1** has been described as a product of autoxidation of methyl icosapentaenoate followed by NaBH$_4$ reduction, though no direct structural proof has been given (2).

TABLE 2. ^{1}H nmr Data (in CDCl$_3$).

Proton	Compound			
	(+)-**1**	(+)-**2**	(+)-**3**	(+)-**4**
H-2	2.32 (t, J = 7.4)	2.32[a] (t, J = 7.2)	2.38[b] (m)	2.37[b] (m)
H-3	1.70 (tt, J = 7.4, 7.4)	1.70 (tt, J = 7.2, 7.2)	2.38[b] (m)	2.37[b] (m)
H-4	2.12[a] (m)	2.10 (br dt, J = 7.2, 6.8)	5.40[b]	5.39[b]
H-5	5.39[b]	5.39[b]	5.40[b]	5.39[b]
H-6	5.39[b]	5.39[b]	2.85[a] (br dd, J = 6.9, 6.9)	2.85 (br dd, J = 7.0, 7.0)
H-7	2.93 (br dd, J = 7.0, 7.0)	2.92 (br dd, J = 6.8, 6.8)	5.40[b]	5.39[b]
H-8	5.39[b]	5.39[b]	5.40[b]	5.39[b]
H-9	5.98 (br dd, J = 11.0, 11.0)	5.99 (br dd, J = 10.9, 10.9)	2.96 (br dd, J = 7.0, 7.0)	2.97 (br dd, J = 6.9, 6.9)
H-10	6.56 (dddd, J = 15.1, 11.0, 1.2, 1.2)	6.56 (dddd, J = 15.0, 10.9, 1.3, 1.3)	5.40[b]	5.39[b]
H-11	5.73 (br dd, J = 15.1, 6.2)	5.72 (br dd, J = 15.0, 6.5)	6.00 (br dd, J = 11.0, 11.0)	6.00 (br dd, J = 10.9, 10.9)
H-12	4.24 (br dt, J = 6.2, 6.5)	4.22 (br dt, J = 6.5, 6.5)	6.57 (dddd, J = 15.0, 11.0, 1.2, 1.2)	6.57 (dddd, J = 15.1, 10.9, 1.3, 1.3)
H-13	2.37[a] (m)	2.34[a] (m)	5.73 (br dd, J = 15.0, 6.3)	5.72 (br dd, J = 15.1, 6.4)
H-14	5.42[a]	5.39[b]	4.23 (br dt, J = 6.3, 6.3)	4.22 (br dt, J = 6.4, 6.4)
H-15	5.54 (dtt, J = 10.7, 7.1, 1.4)	5.56 (dtt, J = 10.8, 7.2, 1.4)	2.35[a] (m)	2.33[a] (m)
H-16	2.81 (br dd, J = 7.1, 7.1)	2.05 (br dt, J = 7.2, 7.2)	5.40[b]	5.39[b]
H-17	5.31 (dtt, J = 10.7, 7.0, 1.4)	1.29[b]	5.55 (dtt, J = 10.8, 7.0, 1.3)	5.57 (dtt, J = 10.9, 7.1, 1.3)
H-18	5.39[b]	1.29[b]	2.81[a] (br dd, J = 7.1, 7.1)	2.05 (dt, J = 7.1, 7.1)
H-19	2.08[a] (m)	1.29[b]	5.30 (dtt, J = 10.8, 7.1, 1.4)	1.30[b] (m)
H-20	0.91 (t, J = 7.6)	0.89 (t, J = 6.9)	5.40[b]	1.30[b] (m)
H-21	—	—	2.07 (dq, J = 7.5, 7.5)	1.30[b] (m)
H-22	—	—	0.97 (t, J = 7.5)	0.99 (t, J = 7.3)
MeO	3.67 (s)	3.67 (s)	3.67 (s)	3.68 (s)
OH	1.90 (br s)	1.92 (br s)	1.88 (br s)	1.86 (br s)

[a]Partially superimposed.
[b]Superimposed.

Structure (+)-**2** is assigned on the basis of similarity of nmr data (Tables 1 and 2) with those for ester (+)-**1**. The free acid of (+)-**2** has been reported by radioimmunoassay, obviously without either spectra or chiroptical data, to be present in subtrace amounts in *Euglena gracilis* (Euglenophyta) (6), in the sponge *Terpios zeteki* (3), in the mollusks *Aplysia californica* (3) and *Helix aspersa* (3), and in the sea urchin *Arbacia* sp. (3). In contrast with these marine organisms (3,6), the hydroxycarboxylic acid precursor of (+)-**2** is accumulated in our sponge. The (12*R*)-hydroxyacid analogue of (+)-**2** has been reported from the above sea-urchin lipoxygenase (1) or from rat-liver microsomal cytochrome P-450 (4) on arachidonic acid. Finally, ^{1}H-nmr data have been reported for synthetic (±)-**2** (10), (12*R*)-**2** (7), and (12*S*)-**2** (8,9).

The C-9–C-20 portion of the ^{13}C-nmr spectrum of (+)-**3** is superimposable to the C-7–C-20 portion of the ^{13}C-nmr spectrum of (+)-**1** (Table 1). The remaining spectral portions show that (+)-**3** has two olefinic CH signals more than (+)-**1** to form a third (Z) CH_2-CH=CH-CH_2 group. The position of the extra double bond with (+)-**3** can be unambiguously assigned on the basis that the 22.84 t is typical of a CH_2 in the β position of a COO group. Double quantum COSY experiments (16) confirm the assignments. The Z configuration at both C-4 and C-7 rests on high-field shift of C-3, C-6, and C-9 (19,20).

The carboxylic acid analogue of (+)-**3** has been reported, without chiroptical data, from human-platelet lipoxygenase (12) on (4Z,7Z,10Z,13Z,16Z,19Z)-docosahexaenoic acid. Compound (±)-**3** has been described from the autoxidation of (4Z,7Z,10Z,12E, 16Z,19Z)-docosahexaenoic acid (13) without giving any direct structural proof, however.

The C-1–C-15 portion of the ^{13}C-nmr spectrum of (+)-**4** is superimposable to the C-1–C-15 portion of (+)-**3**. Moreover, the C-9–C-22 portion of the ^{13}C-nmr spectrum of (+)-**4** is superimposable to the C-7–C-20 portion of (+)-**2**. This points to structure (+)-**4**, which is confirmed by both COSY and delayed COSY experiments (17).

The parent acid of (+)-**4** has already been reported as a product of in vitro transformation of (4Z,7Z,10Z,13Z,16Z)-docosapentaenoic acid by human blood platelets (11). Neither chiroptical data nor any direct structural proof have been offered, however.

EXPERIMENTAL

COLLECTION AND WORKUP.—The sponge was collected in June 1986, around Noumea and was identified by Professor C. Levi, from the Muséum National d'Histoire Naturelle, Paris. The genus *Echinochalina* comprises a few rare species from Australian waters. The sponge can probably be classified in the family Clathriidae, order Poecilosclerida, in spite of the aberrant structure of its skeleton.[1] The fresh sponge (approximately 3 liters in volume) was lyophilized and then extracted with 80% EtOH. The extract was partly evaporated and then partitioned between H_2O and CH_2Cl_2. The organic layer was evaporated to dryness to leave a dark sticky residue (3.0 g) that was subjected to gradient-elution chromatography on 100 g Polyamid 6S [MeOH-H_2O (4:1) to pure MeOH] collecting 10 fractions of 100 ml each. Fractions 1–4, obtained with MeOH/H_2O mixtures of composition ranging from 4:1 to 9:1 were evaporated to give a sticky residue (0.3 g) which was chromatographed on the weakly basic ion-exchange resin Amberlyst A-

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[1]Personal communication from Professor C. Levi.

21, eluting first with Et$_2$O and then with MeOH to remove nonacidic substances. Further elution with MeOH-HOAc (95:5) (devised to recover acidic substances) led to an eluate which was partly evaporated and then subjected to RP-18 flash chromatography, eluting first with H$_2$O to remove HOAc and then with MeOH/H$_2$O mixtures, first of composition 85:15 and then 9:1. The eluates from the 85:15 and 9:1 mixtures were evaporated, and the residue was dissolved in ethereal CH$_2$N$_2$. The mixture was evaporated, and the residue was subjected to hplc with hexane-EtOAc (92:8) to give (+)-**4** (1.5 mg, Rt = 23 min), (+)-**2** (3.5 mg, Rt = 36 min), (+)-**3** (3.5 mg, Rt = 38.5 min), and (+)-**1** (2.1 mg, Rt = 41 min).

(+)-(12R*,5Z,8Z10E,14Z,17Z)-METHYL-12-HYDROXY-5,8,10,14,17-ICOSAPENTAENOATE [(+)-**1**].—Colorless oil, [α]^{20} +7.4° (589), +13.1° (577), +14.9° (546), +27.4° (435), +38.3° (365) (c = 0.18, Me$_2$CO); uv (MeOH) 236 nm (ε 25200); ms *m/z* (rel. int.) [M − H$_2$O]$^+$ 314 (9), 223 (43), 205 (77), 191 (37), 173 (55), 163 (68), 145 (60), 131 (73), 91 (90), 79 (100).

(+)-(12S,5Z,8Z,10E,14Z)-METHYL-12-HYDROXY-5,8,10,14-ICOSATETRAENOATE [(+)-**2**].—Colorless oil, [α]^{20} +12.7° [lit. (9) +13°] (589), +13.7° (577), +17.1° (546), +30.9° (435), +55.9° (365) (c = 0.29, Me$_2$CO); uv (MeOH) 236 nm (ε 31200); ms *m/z* (rel. int.) [M − H$_2$O] 316 (10), 223 (18), 205 (61), 191 (31), 173 (61), 163 (92), 145 (61), 131 (73), 91 (100), 79 (85).

(+)-(12R*,4Z,7Z,10Z,12E,16Z,19Z)-METHYL-14-HYDROXY-4,7,10,12,16,19-DOCOSAHEXA-ENOATE [(+)-**3**].—Colorless oil, [α]^{20} +7.2° (589), +8.9° (577), +11.0° (546), +19.5° (435), +32.9° (365) (c = 0.29, Me$_2$CO); uv (MeOH) 236 nm (ε 25100); ms *m/z* (rel. int.) [M − H$_2$O]$^+$ 340 (2), 249 (8), 231 (8), 217 (6), 205 (9), 199 (32), 91 (100), 79 (64).

(+)-(12R*,4Z,7Z,10Z,12E,16Z)-METHYL-14-HYDROXY-4,7,10,12,16-DOCOSAPENTAENOATE [(+)-**4**].—Colorless oil, [α]^{20} +8.0° (589), +11.2° (577), +13.6° (546), +26.4° (435), +38.4° (365) (c = 0.12, Me$_2$CO); uv (MeOH) 236 nm (ε 24500); ms *m/z* (rel. int.) [M − H$_2$O]$^+$ 342 (5), 249 (11), 231 (11), 217 (14), 205 (16), 199 (31), 91 (100), 79 (68).

ACKNOWLEDGMENTS

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