Novel 10-Hydroxydocosapolyenoic Acids from Deep-Water Scleractinian Corals

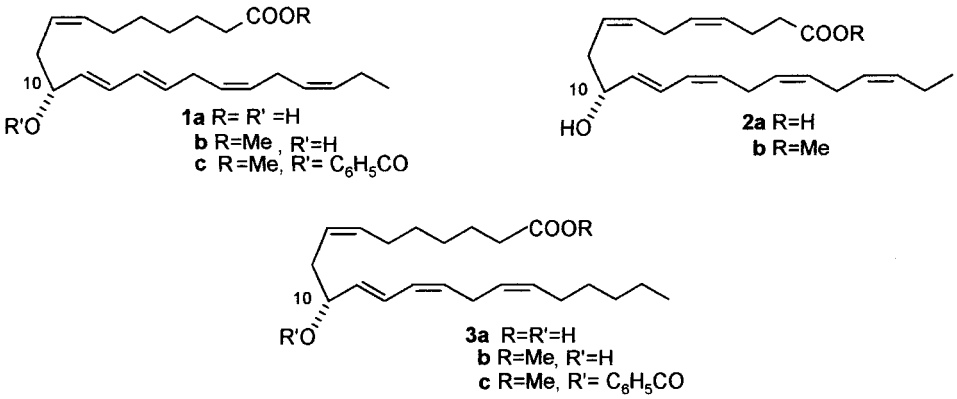
by **Ines Mancini**[a]), **Antonio Guerriero**[a]), **Graziano Guella**[a]), **Torkild Bakken**[b]), **Helmut Zibrowius**[c]), and **Francesco Pietra**[a])[d])*

[a]) Laboratorio di Chimica Bioorganica, Università di Trento, I-38050 Povo-Trento
[b]) Department of Natural History, Norwegian University of Science and Technology, N-7034 Trondheim
[c]) Centre d'Océanologie de Marseille, Station Marine d'Endoume, F-13007, Marseille
[d]) Centro Linceo Interdisciplinare Beniamino Segre, Accademia Nazionale dei Lincei, I-00165 Roma

The new 10-hydroxydocosapolyenoic acids (10*R*,7*Z*,11*E*,13*E*,16*Z*,19*Z*)-10-hydroxydocosa-7,11,13,16,19-pentaenoic acid (**1a**) and (10*R**,4*Z*,7*Z*,11*E*,13*Z*,16*Z*,19*Z*)-10-hydroxydocosa-4,7,11,13,16,19-hexaenoic acid (**2a**) were isolated as methyl esters **1b** and **2b**, respectively, following CH_2N_2 treatment of the EtOH extract of the scleractinian coral *Madrepora oculata* from deep-water of the southern Indian Ocean. From the same species from the Norwegian Sea, **1b** and the methyl ester **3b** of the new (10*R*,7*Z*,11*E*,13*Z*,16*Z*)-10-hydroxydocosa-7,11,13,16-tetraenoic acid (**3a**) were analogously isolated, while from the untreated extract, the free acid **3a** itself could be isolated. The absolute configuration of **1a** and **3a** was established by the chiral exciton coupling of the C(10) benzoate esters **1c** and **3c**. Other known 10-hydroxydocosapolyenoic acids and 8-hydroxyeicosapolyenoic acid were also isolated from *M. oculata* from both locations. These results imply the intervention of a rare lipooxygenase with high ω13 specificity. In an examination of several other deep-water scleractinians, hydroxypolyenoic acids were found only in *Lophelia pertusa* from the northeastern Atlantic Ocean.

1. Introduction. – Among Anthozoa, the Octocorallia have yielded a plethora of unusual secondary metabolites, whereas the Hexacorallia, in particular the species-rich order Scleractinia, have given a limited variety of compounds. The list is confined to non-reef-building species of the suborders Dendrophylliina (unusual indole alkaloids [1] and polyoxazole macrolides [2]) and to reef-building species in the orders Astrocoeniina (polyacetylenes [3][4]) and Faviina (triterpenoids that otherwise are typical of terrestrial plants [5] and HIV-inhibiting polysaccharides [6]). As concerns deep-water scleractinians, only unusual sterols of *Deltocyathus magnificus* (Caryophylliina) have been reported [7]. This is not surprising in view of the difficulty in collecting these deep water cnidarians. We describe here hydroxypolyenoic acids of a rare type from these organisms.

2. Results and Discussion. – 2.1. *New 10-Hydroxydocosapolyenoic Acids* **1a**–**3a**. The new 10-hydroxydocosapolyenoic acids **1a** and **2a** were initially isolated as methyl esters **1b** and **2b** following CH_2N_2 treatment of the EtOH extract of the scleractinian coral *Madrepora oculata*, collected near St. Paul Island in the southern Indian Ocean. Treated likewise, the same species from the Trondheimsfjord, Norway, gave the new 10-hydroxydocosapolyenoic acid methyl ester **3b**. Free acids **3a** and **1a** were isolated from the untreated EtOH extract of the coral.



The composition $C_{22}H_{34}O_3$ for **1a** was established by both NMR (*Table*) and MS data (*Exper. Part*) of derivative **1b**, indicating the presence of C=C and C=O unsaturations and the absence of rings. The ^{13}C-NMR data (*d* at δ_C 72.08) suggests the presence of a secondary alcohol C-atom, whose position is established by MS fragments at *m/z* 191 (C(9)–C(10) bond breaking) and 173 (loss of H_2O from the *m/z* 191 fragment). (*E*,*E*)Conjugation is suggested by a stronger UV absorption at 231 nm than expected for a (*Z*,*E*)-conjugated compound, while the position and configuration of the other C=C bonds are based on ^{1}H,^{1}H coupling constants, ^{1}H,^{1}H COSY correlation maps, and comparison with data for related hydroxypolyenoic fatty acids [8–10].

The benzoyl derivative **1c** shows a negative first and positive second split *Cotton* effect (*Exper. Part*), in agreement with a negative exciton chirality between the conjugated polyene and benzoate chromophores. From the negative sign of the first *Cotton* effect, the absolute configuration *R* can be assigned to C(10) according to the allylic chirality method [11].

Structure **3a** is also fully supported by the NMR data in the *Table* and the MS data in the *Exper. Part* for the methyl ester **3b**. The composition $C_{23}H_{38}O_3$ from HR-MS data suggests one C=C bond less than in **1b**. This is confirmed by the fragment ions at *m/z* 193 ($C_{13}H_{21}O^+$) and 175 (loss of H_2O from *m/z* 193). The NMR spectra (*Table*) confirm that the structure of the C(1) to C(10) moiety is identical to the same portion in both **1b** and **4b** [10], which is reported below. Moreover, the resonance of Me(22) (δ_H 0.87, *i.e.*, by 0.1 ppm at higher field than for both **1b** and **2b**) is consistent with a longer saturated terminal chain in **3b**. The configurations at the C=C bonds are supported by the *J* coupling-constant values. The absolute configuration *R* is assigned to C(10) by the allylic chirality method [11] applied to the benzoyl derivative **3c**, as described above.

Structure **2a** also rests on NMR (*Table*) and MS data (*Exper. Part*) for derivative **2b**, in comparison with **3b** and **4b**. Thus, the NMR signals of the olefinic portion C(4)=C(5) of **2b** replace the resonances of two CH_2 groups of **4b** [10], while the NMR signals of both the C(4)=C(5) and C(19)=C(20) portions replace those of four CH_2 groups of **3b**. There is a parallel correspondence in the MS data (*Exper. Part*). (*Z*)-Configuration for the double bond C(13)=C(14) rests on the typical ^{1}H,^{1}H coupling (*J*(13,14) = 11 Hz) and upfield δ(C) value for C(15) (26.08(*t*)) with respect to the corresponding position in **1b** (30.36 (*t*)). The absolute configuration was not assessed in this case, and

Table. *^1H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **1b, 2b,** *and* **3b**. δ in ppm rel. to $SiMe_4$, J in Hz.

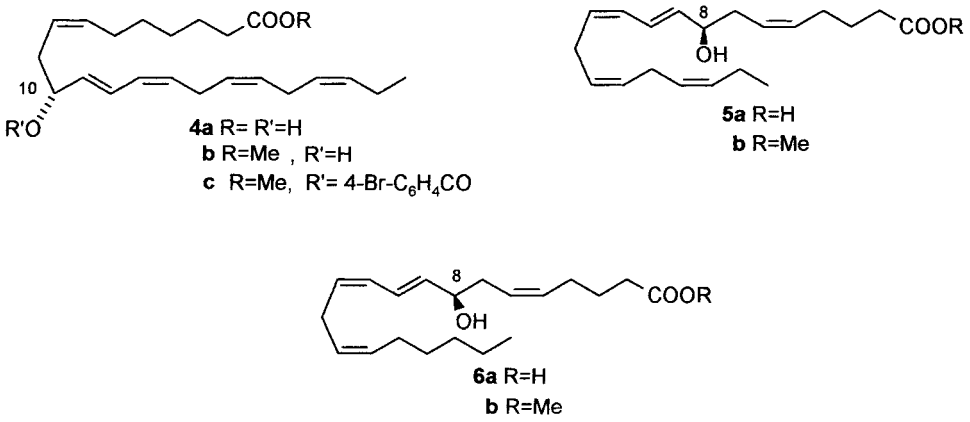
Atom	**1b**		**2b**		**3b**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
C(1)	–	174.26 (*s*)	–	173.62 (*s*)	–	not det.
C(2)	2.30 (*t*, *J* = 7.5)	34.04 (*t*)	[a])	33.95 (*t*)	2.30 (*t*, *J* = 7.5)	34.02 (*t*)
C(3)	1.65 (*m*)	24.81 (*t*)	[a])	22.81 (*t*)	1.66 (*m*)	24.81 (*t*)
C(4)	[b])	28.76 (*t*)	[c])	129.06 (*d*)	[b])	28.75 (*t*)
C(5)	[b])	29.19 (*t*)	[c])	128.02 (*d*)	[b])	29.19 (*t*)
C(6)	2.05 (br. *q*, *J* = 7.5) [d])	27.22 (*t*)	2.84 (br. *t*, *J* = 7.1) [d])	25.79 (*t*)	2.05 (*m*)	27.23 (*t*) [e])
C(7)	5.54 (*dtt*, *J* = 11.0, 7.1, 1.4)	133.37 (*d*)	5.53 (*dtt*, *J* = 10.8, 7.1, 1.4)	131.14 (*d*)	[c])	133.29 (*d*)
C(8)	[e])	124.68 (*d*)	[c])	124.99 (*d*)	[c])	124.56 (*d*)
C(9)	2.33 (*m*) [d])	35.37 (*t*)	[a])	35.40 (*t*)	2.35 (br. *q*, *J* = 6.5)	35.39 (*t*)
C(10)	4.27 (*m*)	72.08 (*d*)	4.25 (*m*)	72.01 (*d*)	4.23 (*m*)	72.10 (*d*)
C(11)	5.62 (br. *dd*, *J* = 15.0, 6.5)	133.16 (*d*)	5.73 (br. *dd*, *J* = 15.2, 6.5)	135.53 (*d*)	5.72 (br. *dd*, *J* = 15.3, 6.5)	135.45 (*d*)
C(12)	6.05 (*ddd*, *J* = 15.0, 10.3, 1.5)	129.73 (*d*) [f])	6.57 (*ddt*, *J* = 15.2, 11.0, 1.3)	125.57 (*d*)	6.56 (*ddt*, *J* = 15.3, 11.0, 1.3)	125.62 (*d*)
C(13)	6.22 (*ddt*, *J* = 15.0, 10.3, 0.9)	133.00 (*d*) [f])	6.00 (br. *t*, *J* = 11.0)	127.91 (*d*)	5.98 (br. *t*, *J* = 11.0)	127.73 (*d*) [f])
C(14)	5.69 (*dt*, *J* = 15.0, 6.5)	130.71 (*d*) [f])	[c])	130.47 (*d*)	[c])	130.84 (*d*) [g])
C(15)	2.86 (br. *t*, *J* = 6.5) [d])	30.36 (*t*)	2.97 (br. *t*, *J* = 6.7) [d])	26.08 (*t*)	2.93 (br. *t*, *J* = 7.2)	27.13 (*t*) [e])
C(16)	[e])	126.70 (*d*)	[c])	127.36 (*d*)	[c])	127.02 (*d*) [f])
C(17)	[e])	129.41 (*d*) [f])	[c])	128.94 (*d*)	[c])	130.88 (*d*) [g])
C(18)	2.79 (br. *t*, *J* = 7.0) [d])	25.48 (*t*)	2.82 (br. *t*, *J* = 6.9) [d])	25.55 (*t*)	2.05 (*m*)	26.11 (*t*)
C(19)	5.32 (*ddt*, *J* = 10.5, 7.9, 1.4)	126.91 (*d*)	5.32 (*ddt*, *J* = 10.5, 6.9, 1.4)	126.90 (*d*)	[b])	29.70 (*t*)
C(20)	[e])	132.09 (*d*)	[c])	132.11 (*d*)	[b])	31.50 (*t*)
C(21)	2.07 (br. *dq*, *J* = 7.5, 7.0) [d])	20.56 (*t*)	2.08 (br. *dq*, *J* = 7.5, 7.0) [d])	20.57 (*t*)	[b])	22.57 (*t*)
C(22)	0.97 (*t*, *J* = 7.5)	14.27 (*q*)	0.97 (*t*, *J* = 7.5)	14.26 (*q*)	0.87 (*t*, *J* = 7.5)	14.08 (*q*)
MeO	3.67 (*s*)	51.49 (*q*)	3.67 (*s*)	51.59 (*q*)	3.65 (*s*)	51.49 (*q*)
OH	1.60 (br. *s*)	–	1.75 (br. *d*, *J* = 5.0)	–	submerged	–

[a]) Superimposed in the range 2.28 – 2.44. [b]) Superimposed in the range 1.25 – 1.40. [c]) Superimposed in the range 5.34 – 5.49. [d]) COSY spectra show homoallylic couplings. [e])[f])[g]) These data can be interchanged within each group.

the assumption of configuration *R* in **2a** is merely for analogy with co-occurring (and thus of implied similar biogenesis) **1a**, **3a**, and (see below) **4a**.

From a literature search, no match was found for structure **1a**, while a flat structure corresponding to **2a** was suggested by comparison with the UV data (only UV shoulder) and MS fragmentation of a silyl derivative of a very minor fraction obtained by an *in vitro* rat liver microsome oxidation of docosahexaenoic acid [12], or by comparison with the sole MS fragmentation of a very minor fraction obtained by an α-tocopherol-aided autoxidation of docosahexaenoic acid [13]. Similarly, a flat structure corresponding to **3a** was suggested by comparison with the sole MS fragmentation of silyl derivatives of very minor fractions (two in this case, without offering any explanation as to the difference) obtained by an *in vitro* oxidation of 7,10,13,16-docosatetraenoic acid by human platelets [14]. These scanty data [13][14] cannot be accepted as evidence for structures **2a** or **3a**, even when not considering the configurations.

2.2. *Known 10-Hydroxydocosapolyenoic Acid* **4a** *and 8-Hydroxyeicosapolyenoic Acids* **5a** *and* **6a**. Methyl esters **4b** and **5b** were isolated as the most abundant hydroxypolyenoic derivatives, besides minor **6b**, from *Madrepora oculata* from St. Paul Island, following a treatment of the raw EtOH extract with diazomethane. The same methyl esters were also isolated from the diazomethane-treated EtOH extract of the same species from the Trondheimsfjorden, Norway. These esters were separated by extensive HPLC. From a small portion of the untreated extract of this coral, free acid **6a** and mixtures **4a**/**6a**, **6a**/**1a**, and **6a**/**1a**/**3a** were isolated.



4a R= R'=H
b R=Me , R'=H
c R=Me, R'= 4-Br-C_6H_4CO

5a R=H
b R=Me

6a R=H
b R=Me

Structure **4a** corresponds to leiophatic acid, originally isolated from the antipatharian *Leiopathes* sp. [10a], now reassigned by *Grasshoff* to *Leiopathes glaberrima* [10b]. The original assignment (10*R*) by correlation with (+)-(*R*)-hydroxysuccinic acid [10a] has now been confirmed from chiral exciton coupling [11] of the 4-bromobenzoate derivative **4c** (*Exper. Part*). It must be pointed out that **4a** cannot represent a contamination of *M. oculata* of southern Indian Ocean by *L. glaberrima*, although the two anthozoans were collected in the same trawl near St. Paul Island. In fact, *L. glaberrima*, preserved and worked up like *M. oculata*, gave both **4a** and its ethyl ester [10a], whereas *M. oculata* gave **4a** only. Moreover, the overall composition of the

mixture of hydroxypolyenoic acids was markedly different for the two species. Finally, **4a** was present also in the Norwegian sample of *M. oculata.*

(5*Z*,9*E*,11*Z*,14*Z*,17*Z*)-8-Hydroxyeicosa-5,9,11,14,17-pentaenoic acid (8-HEPE; **5a**) was previously isolated from various marine organisms: as a minor component of the antipatharian *L. glaberrima* [10], as a component of the starfish *Patiria miniata* [15], and as the hatching factor of the barnacles *Balanus balanoides* [16][17] and *Elminius modestus* [18]. It was also shown that **5a** is produced from eicosatetra- and eicosapentaenoic acids by blood cells of the crab *Carcinus maenas* under stimulation by a calcium ionophore [19].

The hydroxyeicosatetraenoic acid **6a** (8-HETE) was previously found, *inter alia*, in both *L. glaberrima* [10] and *P. miniata* [15], and was also obtained, like **5a** above, from *C. maenas* blood cells [19].

3. Perspective. – Hydroxyeicosa- and hydroxydocosatetraenoic acids [20] and pentaenoic analogues are intermediates of the arachidonic acid cascade in mammals. Recently, compounds of this class have been found accumulated in several unrelated marine organisms, like red algae [8][21], sponges [9], an antipatharian [10], and a starfish [15], and the biosynthesis of these compounds from coralline algae [8] has been recently carried out with cell-free preparations [22]. Hydroxypolyenoic acids are also contained in certain seed oils from terrestrial plants [23]. Additional interest lies in the vital role that these acids play in marine invertebrates, like (*R*)-8-HETE **6a** in starfish oocyte maturation [24]. Further references to these and other fatty acids can be found in a recent extensive review [25].

From an examination of other deep-water scleractinians, 10-hydroxypolyenoic acids appear to be confined to certain species only and with largely variable abundance, even on an intraspecific basis. Thus, while a sample of *Lophelia pertusa* from the northeastern Atlantic Ocean contained the same series of hydroxypolyenoic acids as *M. oculata* from the southern Indian Ocean, albeit at much lower concentrations, northeastern Atlantic *M. oculata* only contained **5a**, and none of these acids were found in *Letepsammia formosissima*, *Deltocyathus magnificus, Stephanocyatus spiniger*, and *Javania lamproticum* from the Loyalty Islands.

The findings reported here for *M. oculata* and *L. pertusa* add deep-water scleractinians (to date as the only examples in the order Scleractinia) to the list of producers of hydroxypolyenoic acids. These belong to the same class as those of the antipatharian *L. glaberrima* [10], implying the intervention of a lipooxygenase with high ω13 specificity. In mammalian enzymes, this activity has rarely been observed, and if so then as a very minor pathway [12][14].

The differences in the distribution of hydroxypolyenoic acids observed here for the Atlantic sample of *M. oculata* can hardly be attributed to differences in associated microbial populations in view of the very similar hydroxypolyenoic acid profile for the southern Indian Ocean and the Norwegian samples of *M. oculata*. The taxonomy of this species, like that of other deep water scleractinians, is currently based on morphological characters of the calcareous skeleton [26]. It would be desirable to also take into account characters of the soft parts (polyps and, in the case of colonial species, connecting tissues), such as anatomical details, nematocysts, and intraspecific aggression behavior. Molecular-genetic characters, such as from allozyme or genome

studies, should also be considered since the hydroxypolyenoic acids described here must reflect the operation of functional genes. To our knowledge, no genome or allozyme analysis has been reported for deep-water scleractinians, which represent a largely different world from reef scleractinians for which such data are available in relation to spatial distances [27]. Thus, the way is long before the differences observed here for *M. oculata* from the different areas may possibly be considered as resulting from intraspecific geographic differentiation.

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Experimental Part

General. Reversed-phase flash chromatography (FC): *Merck LiChroprep RP-18* (40–63 μm). HPLC: *Merck LiChrospher* (10 μm), 25 × 10 cm column, flux 5 ml/min. TLC: *Merck silica gel 60 PF_{254}*. NMR: *Varian XL-300*; ^{1}H 299.94 MHz and ^{13}C 75.43 MHz; in $CDCl_3$; δ in ppm rel. to internal Me_4Si (= 0 ppm) and *J* in Hz; assignments from DEPT and ^{1}H, ^{13}C COSY. UV: *Perkin-Elmer-Lambda-3* spectrophotometer; λ_{max} in nm, ε in $mol^{-1} \cdot l \cdot cm^{-1}$. Polarimetric data: *JASCO-DIP-181* polarimeter; $[\alpha]_D$ in 10^{-1} deg · ml · g^{-1}; in EtOH for **4b** and **5b**, observing inversion of sign in $CHCl_3$. CD: *Jasco-J-710* spectropolarimeter; in EtOH; $\Delta\varepsilon$ (λ) (λ_{max} in nm, ε in $mol^{-1} \cdot l \cdot cm^{-1}$). EI-MS: *Kratos MS80* with home-built data system; *m/z* (rel. %).

Biological Material. For all species (Cnidaria, Anthozoa, Scleractinia), the conventional suborder and family attribution is indicated. Coral samples were obtained by trawl (CP), dredge (DW), or by submersible dive (cruise *CALSUB*). On board, the corals were put into glass jars (if possible closely packed), and these were filled with EtOH. The material was then stored in the cold and dark, both on board and in the laboratory, but transfer of variable length was at ambient temperature. After a few months, the solvent was decanted and stored in the dark at −20° for several years (7 in the case of the Indian Ocean *M. oculata*), depending on the year of collection. *Deltocyathus magnificus* MOSELEY, 1876 (Caryophylliina, Caryophylliidae): sample 526M, 0.1 l of EtOH extract, Loyalty Islands, SW Pacific (15.2.1989, Alis, cruise *Musorstom-6*, stat. CP413, 20°40.10′S, 167°03.50′E, depth 463 m). *Javania lamprotichum* MOSELEY, 1880 (Caryophylliina, Flabellidae): sample 544M, 0.1 l of EtOH extract, Loyalty Islands, SW Pacific, Cyana, cruise *CALSUB* (27.2.1989, dive 1024/30/9, 20°53′S, 167°03′E, depth 561 m); sample 545M, 1 l of EtOH extract, same area, same cruise (5.3.1989, dive 1029/35/14, 20°38′S, 166°56.6′E, depth 566 m). *Letepsammia formosissima* MOSELEY, 1876 (Fungiina, Micrabaciidae): sample 523M, 1 l of EtOH extract, Loyalty Islands, SW Pacific, Alis, cruise *Musorstom-6* (21.2.1989, stat. CP 464, 21°02.30′S, 167°31.60′E, depth 430 m); sample 525M, 1 l of EtOH extract, same area, same cruise (15.2.1989, stat. CP 415, 20°40.20′S, 167°03.95′E, depth 461 m). *Lophelia pertusa* LINNAEUS, 1758 (Caryophylliina, Caryophylliidae): sample 490M, 2 l of EtOH extract, Galicia Seamount, NE Atlantic, Le Noroit, cruise *Seamount-1* (18.10.1987, stat. DW106, 42°41.6′N, 11°48.5′W, depth 765 m). *Madrepora oculata* LINNAEUS, 1758 (Faviina, Oculinidae): sample 449M, 9 l of EtOH extract, Saint-Paul Island, S. Indian Ocean, Marion-Dufresne cruise *MD-50* (20.7.1986, stat. 27-CP129, 38°43.53′S, 77°26.50E, depth 290 m); sample 489M, 2 l of EtOH extract, Galicia Seamount, NE Atlantic, Le Noroit, cruise *Seamount-1* (18.10.1987, stat. DW106, 42°41.6′N, 11°48.5′W, depth 765 m); sample 863M, 2 l of EtOH extract, Agdenes, in the Trondheimsfjord, Norway (16.06.1998, DW, 63°38.85′ N, 09°46.00′ E, depth 350–380 m). *Stephanocyathus spiniger* MARENZELLER, 1888 (Caryophylliina, Caryophylliidae): sample 529M, 1 l of EtOH extract, Loyalty Islands, SW Pacific, Alis, cruise *Musorstom-6* (21.2.1989, stat. CP465, 21°03.55′S, 167°32.25′E, depth 480 m).

Isolation of the Metabolites. The EtOH extract of *Madrepora oculata* from the Indian Ocean (449M) was evaporated, and the aq. residue was extracted with petroleum ether to give an oily residue (10 g) which was subjected to reversed-phase gradient-elution FC (H_2O to MeOH). The MeOH fraction was evaporated to give 0.78 g of residue which was dissolved in Et_2O and treated with CH_2N_2; the mixture was evaporated and the residue subjected to HPLC (hexane/AcOEt 9:1) to give, in the order, **4b** (7.1 mg, t_R 13 min), **6b**/**1b**/**2b** (t_R 14–16 min), and **5b** (4.4 mg, t_R 17 min). The mixture **6b**/**1b**/**2b** was subjected to HPLC (hexane/*i*-PrOH 49:1) which yielded pure **2b** (1.3 mg, t_R 7.5 min), **1b** (0.7 mg, t_R 8.3 min), and **6b** (1.5 mg, t_R 8.8 min). On similar extractions and CH_2N_2 treatment, the Galician *Madrepora oculata* (489M) and *Lophelia pertusa* (490M) gave residues of

1.5 g and 0.7 g, respectively. Both extracts showed UV-absorbing TLC spots and olefinic ^{1}H-NMR signals for hydroxypolyenoic acid methyl esters that were evaluated by HPLC co-elution with authentic compounds. The Galician *M. oculata* contained **5a** only, and in extremely low abundance, whereas *L. pertusa* contained the same hydroxypolyenoic acids as *M. oculata* from St Paul Island, albeit in much lower abundance.

The EtOH extract from Norwegian *M. oculata* (863M) was filtered and evaporated at r.t. The residue was first extracted with hexane and then with AcOEt and the residues from evaporation of the two extracts, being similar on TLC, were combined to give an oily material (1.3 g) that was subjected to reversed-phase gradient-elution FC (H_2O to MeOH) collecting 28 fractions of 50 ml each. A small amount of the combined *Fr. 6–8* was subjected to reversed-phase HPLC (*LiChrosorb RP18*, MeCN/H_2O 7:3), under UV monitoring at 240 nm, to give **5a** (t_R 6.3 min) as the major compound and **4a**/**6a** 1:2 (t_R 8.4 min). A small portion of *Fr. 9* was subjected to HPLC under the same conditions as above to give **3a** (t_R 6.8 min) as the major compound and **1a**/**6a** 1:1 (t_R 7.1 min). A better separation of the components of *Fr.* **6–8** was obtained by reversed-phase HPLC (*LiChrospher RP18*, MeCN/H_2O 7:3 containing 1% of CF_3COOH) under UV monitoring at 220 nm, showing peaks at t_R 7.6, 10.8 and 11.7 min in a 2:2:1 integration ratio. These, however, corresponded (NMR) to dehydration products of **4a–6a**. Although dehydration could be prevented by the addition of Et_3N to the CF_3COOH-containing mixture before evaporation, the problem of freeing the mixture from the ammonium salt induced us to treat the residual *Fr. 6–8* and the acids **4a–6a** already separated with ethereal CH_2N_2 under standard conditions. This was followed by evaporation of the solvents and HPLC (hexane/i-PrOH 97.5:2.5) of the residue under UV monitoring at 240 nm to give **4b** (t_R 7.8 min), **6b** (t_R 9.1 min) and **5b** (t_R 9.8 min) in the ratios of abundance 2:1:0.5. Analogous treatment of *Fr. 9–12*, to which were added the free acids **6a**, **1a**, and **3a** previously obtained by HPLC of *Fr. 9*, led to **3b** (t_R 7.5 min), **1b** (t_R 8.1 min), **6b**, and **5b** in the ratios of abundance 6:4:0.2:0.1. Global amounts of the isolated methyl ester were thus 0.9 mg (**4b**), 1.9 mg (**5b**), 2.0 mg (**6b**), 0.4 mg (**1b**) and 0.6 mg (**3b**). The rather small quantities of extracts from *Stephanocyatus spiniger, Deltocyathus magnificus, Letepsammia formosissima,* and *Javania lamprotichum*, worked up as above up to the CH_2N_2-addition stage, did not reveal NMR signals for hydroxypolyenoic acids.

*(10*R,*7Z,11*E,*13*E,*16Z,19Z)-Methyl 10-Hydroxydocosa-7,11,13,16,19-pentaenoate* (**1b**): $[\alpha]_D^{20} = -7.4$ ($c = 0.05$, EtOH). UV (EtOH): 231 (40700). MS: 360 (0.7, $M^{+\cdot}$), 342 (1.2, $[M-H_2O]^{+\cdot}$), 329 (0.6), 251 (3.3), 191 (19, $C_{13}H_{19}O^+$), 173 (15, $[191-H_2O]^{+\cdot}$), 163 (7), 147 (9.2), 133 (8.2), 131 (28), 121 (37), 55 (100). HR-MS: 360.2657 ± 0.003 ($C_{23}H_{36}O_3^{+\cdot}$; calc. 360.2646), 342.255 ± 0.003 ($C_{23}H_{34}O_2^{+\cdot}$; calc. 342.256).

*(10*R**,*4Z,7Z,11*E,*13Z,16Z,19Z)-Methyl 10-Hydroxydocosa-4,7,11,13,16,19-hexaenoate* (**2b**): $[\alpha]_D^{20} = -8.0$ ($c = 0.10$, EtOH). UV(EtOH): 234 (24700). MS: 358 (0.9, $M^{+\cdot}$), 340 (1.4, $[M-H_2O]^{+\cdot}$), 327 (0.7), 191 (23, $C_{13}H_{19}O^+$), 173 (20, $[191-H_2O]^{+\cdot}$), 55 (88), 41 (100).

*(10*R,*7Z,11*E,*13Z,16Z)-Methyl 10-Hydroxydocosa-7,11,13,16-tetraenoate* (**3b**): $[\alpha]_D^{20}$: too small to be taken with confidence. UV(EtOH): 232 (18000). MS: 362 (0.7, $M^{+\cdot}$), 344 (3, $[M-H_2O]^{+\cdot}$), 331 (2), 193 (100), 175 (16), 57 (40). HR-MS: 362.280 ± 0.003 ($C_{23}H_{38}O_3^{+\cdot}$; calc. 362.282).

Benzoyl Derivatives. To **1b** (0.4 mg, 0.0011 mmol) in dry pyridine (0.3 ml), benzoyl chloride (0.010 ml) and a catalytic amount of 4-(dimethylamino)pyridine were added. The mixture was stirred at r.t. overnight and then evaporated, and the residue was filtered through *LiChrolut RP18 Merck* and washed with MeCN. The filtrate was evaporated and the residue subjected to reversed-phase HPLC (MeCN, 1 ml/min) to give pure **1c** (t_R 7.1 min, 0.4 mg, 78%).

Treated likewise, **3b** (0.5 mg, 0.0014 mmol) gave benzoate **3c** (t_R 7.5 min, 0.7 mg, 78%).

To **4b** (1.7 mg, 0.0047 mmol) in dry pyridine (0.5 ml) was added 4-bromobenzoyl chloride (0.0091 mmol), and the mixture was stirred at r.t. overnight. The pure 4-bromobenzoate **4c** (2 mg, 78%) was isolated by prep. TLC (hexane/Et_2O 55:45 (R_f 0.8).

Data of **1c**: UV (EtOH) 233 (32000). CD: +21.0 (223), −33.0 (240). ^{1}H-NMR: 2.30 (*t, J* = 7.5, 2 H–C(2)); 1.65 (*m*, 2 H–C(3)); 1.25–1.40 (*m*, 2 H–C(4), 2 H–C(5)); 2.05 (*m*, 2 H–C(6), 2 H–C(21)); 5.28–5.54 (*m*, H–C(7), H–C(8), H–C(14), H–C(16), H–C(17), H–C(19), H–C(20)); 2.52 (*m*, 2 H–C(9)); 5.56 (*q, J* = 6.9, H–C(10)); 5.72 (*dd, J* = 15.0, 6.9, H–C(11)); 6.63 (br. *dd, J* = 15.0, 10.8, H–C(12)); 5.98 (br. *t, J* = 10.8, H–C(13)); 2.94 (br. *t, J* = 7.0, 2 H–C(15)); 2.81 (br. *t, J* = 6.5, 2 H–C(18)); 0.96 (*t, J* = 7.5, Me(22)); 3.65 (*s*, MeO); 8.02 (br. *d, J* = 8.1, Ph); 7.41 (*m*, Ph). MS 464 (0.8, $M^{+\cdot}$), 433 (0.5), 359 (0.7), 105 (100).

Data of **3c**: UV (EtOH) 230 (29000). CD: +17.0 (224), −41.0 (241). ^{1}H-NMR: 2.27 (*t, J* = 7.5, 2 H–C(2)); 1.60 (*m*, 2 H–C(3)); 1.25–1.40 (*m*, 2 H–C(4), 2 H–C(5), 2 H–C(19), 2 H–C(20), 2 H–C(21)); 2.05 (*m*, 2 H–C(6), 2 H–C(18)); 5.28–5.50 (*m*, H–C(7), H–C(8), H–C(14), H–C(16), H–C(17)); 2.53 (*m*, 2 H–C(9)); 5.56 (*q, J* = 6.9, H–C(10)); 5.72 (*dd, J* = 15.2, 6.9, H–C(11)); 6.63 (br. *dd, J* = 15.2, 10.8, H–C(12)); 5.98 (br. *t, J* = 10.8, H–C(13)); 2.93 (br. *t, J* = 7.0, 2 H–C(15)); 0.87 (*t, J* = 7.2, Me(22)); 3.66 (*s*, MeO); 8.02 (br. *d, J* = 8.1, Ph); 7.41 (*m*, Ph). MS: 466 (0.5, $M^{+\cdot}$), 435 (0.4), 361 (0.7), 345 (2), 105 (100), 77 (18).

Data of **4c**: UV(EtOH): 242(39000). CD: +26.2(232), −50.0(250). ^{1}H-NMR: 2.28 (*t*, *J* = 7.5, 2 H−C(2)); 1.60 (*quint*, *J* = 7.5, 2 H−C(3)); 1.32 (*m*, 2 H−C(4), 2 H−C(5)); 2.05 (*m*, 2 H−C(6), 2 H−C(21)); 5.27–5.53 (*m*, H−C(7), H−C(8), H−C(14), H−C(16), H−C(17), H−C(19), H−C(20)); 2.53 (*m*, 2 H−C(9)); 5.56 (*q*, *J* = 7.0, H−C(10)); 5.72 (*dd*, *J* = 15.0, 6.9, H−C(11)); 6.63 (br. *dd*, *J* = 15.0, 10.8, H−C(12)); 5.98 (br. *t*, *J* = 10.8, H−C13)); 2.95 (br. *t*, *J* = 7.0, 2 H−C15)); 2.79 (br. *t*, *J* = 6.5, 2 H−C(18)); 0.96 (*t*, *J* = 7.5, Me(22)); 3.67 (*s*, MeO); 7.89, 7.47 (*AB*, *J* = 8.7, Ph).

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