201. Novel Hydroxyicosatetraenoic and Hydroxyicosapentaenoic Acids and a 13-Oxo Analog. Isolation from a Mixture of the Calcareous Red Algae *Lithothamnion corallioides* and *Lithothamnion calcareum* of Brittany Waters

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A mixture of the red calcareous algae *Lithothamnion corallioides* CROUAN and *Lithothamnion calcareum* (PALLAS) ARESCHOUGH, from Brittany waters, gives, on ethanolic extraction, the novel icosanoids ethyl 13-hydroxyarachidonate (= (+)-(all-*Z*)-ethyl 13-hydroxyicosa-5,8,11,14-tetraenoate; (+)-**1**)), ethyl 13-hydroxytimnodonate (= (+)-(all-*Z*)-ethyl 13-hydroxyicosa-5,8,11,14,17-pentaenoate ((+)-**2**), and (5*Z*,9*E*,11*E*,14*E*)-ethyl 8-hydroxy-13-oxoicosa-5,9,11,14-tetraenoate (**3**). Moreover, the ethyl esters of four known icosatetraenoic acids are contained in the above algae. Structural elucidations of these metabolites are based mainly on NMR data.

1. Introduction. – It is well known that arachidonic acid is involved in the biosynthesis of mammalian prostaglandins (*via* the endoperoxide route [1]), leukotrienes (*via* (5*S*,6*E*,8*Z*,11*Z*,14*Z*)-5-(hydroperoxy)icosatetraenoic acid [2]), thromboxanes [3], and marine prostanoids (*via* (8*R*,5*Z*,9*E*,11*Z*,14*Z*)-8-(hydroperoxy)icosa-5,9,11,14-tetraenoic acid [4]). Owing to the physiological importance of these compounds, any new oxygenated derivative of arachidonic and related acids deserves attention.

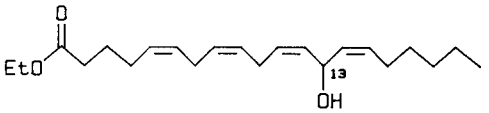
The distribution of such compounds is mainly in the sea, typical examples being the prostanoids produced by gorgonians, telestaceans [4], and algae [5]. The sea has also yielded a configurationally unspecified 12-hydroxyicosapentaenoic acid (from algae and invertebrates [6]), (+)-(12*S*,5*Z*,8*Z*,10*E*,14*Z*)-12-hydroxyicosa-5,8,10,14-tetraenoic and (+)-(5*Z*,8*Z*,10*E*,14*Z*,17*Z*)-12-hydroxyicosa-5,8,10,14,17-pentaenoic acid[1]) (from the sponge *Echinochalina mollis* of the Coral Sea [7a]), and (+)-(8*R*,5*Z*,9*E*,11*Z*,14*Z*)-8-hydroxyicosa-5,9,11,14-tetraenoic and (+)-(8*R*,5*Z*,9*E*,11*Z*,14*Z*,17*Z*)-8-hydroxyicosa-5,9,11,14,17-pentaenoic acid (from the black coral *Leiopathes* sp. of South Indian Ocean [8]).

We report here on the ethyl esters of three new oxygenated icosatetraenoic and icosapentaenoic acids, and four known hydroxyicosatetraenoic acids isolated from red calcareous algae of the genus *Lithothamnion* (subclass Florideiphyceae, order Cryptonemiales, family Corallinaceae). These compounds (which are notable for their relationship to physiologically important metabolites in mammals) represent a conspicuous addition to the extremely limited natural-product chemistry of the Cryptonemiales. In fact, although algae of this order bear much importance as components or barrier reefs, and their remains (Maërl) are used in the pharmaceutical industry and for sewage process,

[1]) For this compound, previously isolated from the red seaweed *Murrayella periclados* [7b], the absolute configuration 12*S* was assigned from optical-rotation data in comparison with (12*S*)-methyl ester [7b].

while some of them are in use in folk medicine, their unusual natural-product chemistry is limited to some amino acids, such as carnosadine [8a] and lividine [8b], extracted from *Grateolupia* species.

2. Results and Discussion. – 2.1. *(+)-(all-Z)-Ethyl 13-Hydroxyicosa-5,8,11,14-tetraenoate* ((+)-**1**). This substance has a ^{13}C-NMR spectrum (*Table*) with 22 resonances for 2 Me, 9 CH_2 groups at C and a CH_2 group at a heteroatom, 8 olefinic CH, a methine group bearing an *O*-substituent, and an estereal C=O. These resonances and ^{1}H-NMR



(+)-**1**

resonances for an EtO and a Me group (*t*; see *Exper. Part*) suggest a linear C_{20} fatty acid esterified by EtOH. The presence of a secondary diallylic alcohol function is suggested both by the δ(H) 1.65 (br. *s*), which undergoes a shift with the temperature and exchange with D_2O, and the δ(H) 5.29 (*m*), which is largely superimposed to the resonances of the olefinic CH protons and could only be assigned by HETCOR. Moreover, the presence of two diallylic CH_2 groups is suggested by the δ(H) 2.82 (br. *t*) and 2.91 (*m*). The C(1)–C(5)

Table. *^{13}C-NMR Data of Compounds (+)-***1**, *(+)-***2**, *and* **3** *in $CDCl_3$* (observed shifts $\Delta\delta$ for (+)-**1** and (+)-**2** in the presence of [Eu(fod)$_3$] at the ratio [[Eu(fod)$_3$]]/[sub] $\approx$ 0.2 are reported within square brackets)

C-Atom	(+)-**1**		(+)-**2**		**3**
C(1)	173.70 (*s*)	[a)]	173.70 (*s*)	[a)]	173.71 (*s*)
C(2)	33.72 (*t*)	[1.61]	33.71 (*t*)	[1.83]	33.63 (*t*)
C(3)	24.79 (*t*)	[0.96]	24.79 (*t*)	[1.08]	24.71 (*t*)
C(4)	26.59 (*t*)	[0.43]	26.59 (*t*)	[0.48]	26.69 (*t*)
C(5)	129.15 (*d*)	[0.21]	129.17 (*d*)	[0.22]	132.78 (*d*)
C(6)	128.80 (*d*)	[0.21]	128.87 (*d*)	[0.22]	125.05 (*d*)
C(7)	25.64 (*t*)	[0.22]	25.63 (*t*)	[0.24]	35.10 (*t*)
C(8)	127.54 (*d*)	[0.18]	127.46 (*d*)	[0.21]	71.28 (*d*)
C(9)	128.58 (*d*)	[0.23]	128.56 (*d*)	[0.25]	144.84 (*d*)
C(10)	26.10 (*t*)	[0.45]	26.10 (*t*)	[0.45]	128.24 (*d*)
C(11)	129.69 (*d*)	[0.61]	129.96 (*d*)	[0.61]	142.05 (*d*)
C(12)	131.63 (*d*)	[0.74]	131.45 (*d*)	[0.82]	128.46 (*d*)
C(13)	63.79 (*d*)	[2.88]	63.79 (*d*)	[2.90]	189.55 (*s*)
C(14)	130.95 (*d*)	[0.72]	131.22 (*d*)	[0.73]	129.15 (*d*)
C(15)	132.30 (*d*)	[0.52]	130.38 (*d*)	[0.53]	148.30 (*d*)
C(16)	27.82 (*t*)	[0.37]	26.02 (*t*)	[0.37]	32.67 (*t*)
C(17)	29.32 (*t*)	[0.14]	126.37 (*d*)	[0.14]	27.86 (*t*)
C(18)	31.53 (*t*)	[0.12]	132.63 (*d*)	[0.14]	31.39 (*t*)
C(19)	22.56 (*t*)	[0.07]	20.60 (*t*)	[0.09]	22.46 (*t*)
C(20)	14.05 (*q*)	[0.05]	14.22 (*q*)	[0.02]	13.99 (*q*)
CH_3CH_2O	14.26 (*q*)	[0.44]	14.26 (*q*)	[0.49]	14.27 (*q*)
CH_3CH_2O	60.30 (*t*)	[1.40]	60.29 (*t*)	[1.55]	60.37 (*t*)

a) Not detected.

and C(15)–C(20) moieties are established by COSY experiments. These data, coupled with the evidence that the olefinic C=C functions lack conjugation (λ_{max} below 210 nm) and are 1,2-disubstituted suggest the structure of an arachidonic acid esterified by EtOH and hydroxylated at either C(7), C(10), or C(13). That the latter is the position of choice is indicated – once all C–H correlations are assigned by HETCOR – by ^{1}H-NMR spectra in the presence of increasing amounts of [Eu(fod)$_3$] which reveal that H–C(10) and H–C(16) undergo the same shift (*Table*).

The (*Z*)-configuration at C(11)=C(12) and C(14)=C(15) rests on $J \approx 11$ Hz between the respective protons in the presence of *ca.* 0.2 equiv. of [Eu(fod)$_3$]. That also C(5)=C(6) and C(8)=C(9) have the (*Z*)-configuration is indicated by δ(C) 26.59, 25.64, and 26.10 for C(4), C(7), and C(10), respectively, as expected for steric crowding [9][2]).

2.2. *(+)-(all-Z)-Ethyl 13-Hydroxyicosa-5,8,11,14,17-pentaenoate* ((+)-**2**). This substance has a ^{13}C-NMR spectrum similar to that of (+)-**1**, though two CH$_2$ *t* are replaced by two olefinic CH *d*, and 3 H–C(20) appears as a neat *t*[3]). From the observation that two out the three diallylic CH$_2$ groups undergo the same shift on addition of [Eu(fod)$_3$]

(+)-**2**

(*Table*), positions C(7) and C(16) are ruled out for the OH group. Moreover, the OH group must be at C(13), as the ^{13}C-NMR spectra for the C(1)–C(13) part of (+)-**1** and (+)-**2** are superimposable. The assignment of the (*Z*)-configuration at the C=C groups is based on similar arguments as for (+)-**1**.

The diallylic alcohol function of both (+)-**1** and (+)-**2** is a rare feature of icosanoids; at least within icosatetraenoic and icosapentaenoic acids, OH-substituted at C(13), no such structural feature has ever been reported. The most closely related example concerns the methyl ester of a linear diunsaturated C$_{18}$ fatty acid deriving from the Ag$^+$-assisted ring opening in the presence of H_2O_2 of a vinylcyclopropyl-bromide precursor, where a diallylic (*Z,E*)-hydroperoxide function occurs at C(11) [11].

2.3. *(5Z,8E,11E,14E)-Ethyl 8-Hydroxy-13-oxoicosa-5,9,11,14-tetraenoate* (**3**). This substance reveals ^{13}C-NMR signals for 22 C-atoms: 2 Me, 8 CH$_2$ at C and 1 CH$_2$ at a heteroatom, 8 olefinic CH, 1 CH at a heteroatom, 1 estereal C=O, and 1 enonic C=O. In combination with the ^{1}H-NMR observation of an EtO and a Me-group *t* and the NMR

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[2]) The δ(C) assignments are based both on HETCOR experiments without and with added [Eu(fod)$_3$] and by comparisons with the data for similar compounds [7a] [9]. Attempts to determine the absolute configuration of (+)-**1** by *Mosher*'s method [10a] failed, although we used the mild conditions of *Trost*'s variant [10b]. Thus, compound (+)-**1**, when treated with α-(trifluoromethyl)-*O*-methylmandelic acid and oxalyl chloride/DMF (method 3 in [10b]), decomposed completely into material that could not be isolated; when compound (+)-**1** was treated with α-(trifluoromethyl)-*O*-methylmandelic acid and DCC/DMAP (method 1 in [10b]), a mixture of polyolefinic compounds was formed that did not contain the expected ester; from three *m*'s at δ(H) ($CDCl_3$) 5.7, 6.0, and 6.5, we suspect that a *trans/cis* conjugated diene system has formed. Because of this, no attempt was made to apply *Mosher*'s method [10] to the very minor compound (+)-**2**.

[3]) In the case of (+)-**1**, the corresponding *t* is distorted as expected for a typical fatty acid.

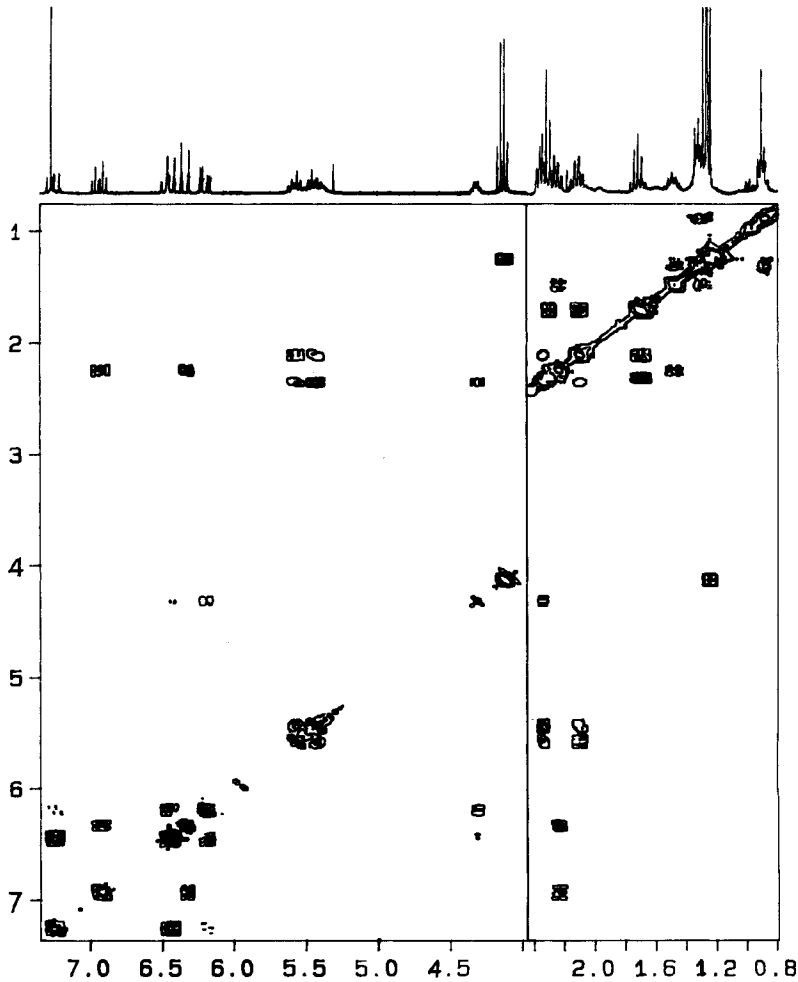


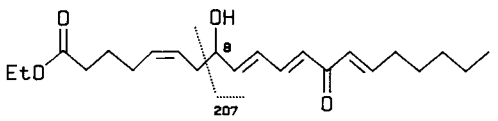
Figure. *Low-field* (left) *and high-field* (right) *$^1H,^1H$-COSY-45 of* **3**. The corresponding 1D-^{1}H-NMR spectra are shown along the highest sides. 512 FID's were acquired with 2048 digital points each. The data matrix was zero-filled to 2048 × 2048 and a pseudo *Gauss*ian echo filter was used in both time dimensions. After *Fourier* transformation, the data were folded and shown as a contour plot.

data for (+)-**1** and (+)-**2**, this suggests the ethyl ester of a linear fatty acid which bears an OH and a C=O function. COSY experiments (*Fig.*), coupled with delayed COSY experiments that reveal long-range correlations, in particular between H–C(12) and H–C(14), give support to the structure for the whole chain C(1)–C(20). In further support, the *β* positions to the C=O group are revealed from the low-field shift of the ^{1}H and ^{13}C resonances for both H–C(11) (7.25 and 142.05 ppm) and H–C(14) (6.92 and 148.30 ppm), while the C(8) position for the OH group is indicated by the mass fragment with *m/z* 207. The (*Z*)- and (*E*)-configurations at the C=C groups rest on *J* values of *ca.* 11 and 15 Hz, respectively (*Exper. Part*).

During attempts to determine the absolute configuration by *Horeau*'s method, as with leiopathic acid [9], compound **3** decomposed.

Another case of hydroxy-oxo-icosanoid is the hydroxyicosapentaenoic acid ptilodene, isolated from the red marine seaweed *Ptilota filicina* (Ceramiales) [12].

2.4. *Esters (+)-***4**, *(+)-***5**, *(+)-***6**, *and (+)-(12*S*,5*Z*,8*Z*,10*E*,14*Z*)-Ethyl 12-Hydroxyicosa-5,8,10,14-tetraenoate.* Compounds (+)-**4** [13a], (+)-**5** [13b], and (+)-**6** [13c] have been obtained by total synthesis which confirmed the structures deduced from UV, MS, and comparison with reference compounds for metabolites either endogenous or ob-



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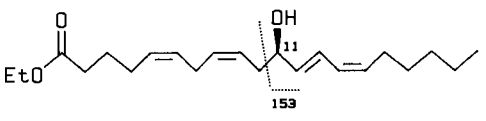
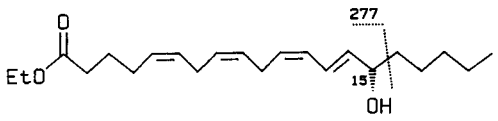
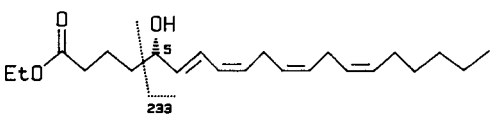


(+)-**4**



(+)-**5**



(+)-**6**

tained from enzymatic oxidation of arachidonic acid [14]. No ^{13}C-NMR data were reported and the ^{1}H-NMR data [13] are not detailed, so that our NMR data in the *Exper. Part* may turn useful in the elucidation of new metabolites of this important class.

(+)-(12*S*,5*Z*,8*Z*,10*E*,14*Z*)-Ethyl 12-hydroxyicosa-5,8,10,14-tetraenoate was already found in our laboratory as methyl ester in the extracts of the marine sponge *Echinochalina mollis* of the Coral Sea [7].

We thank Dr. *J. Cabioch* for the identification of the algae, the *Station Zoologique de Roscoff* for collection and laboratory facilities, in particular Mr. *A. Maron* for the dredging, and the C.N.R. and the M.P.I. (Progetti di Interesse Nazionale), Roma, for financial support.

Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. HPLC: either *Merck-LiChrosorb Si-60* (7 μm). Reverse-phase HPLC: *Merck-LiChrosorb RP18* (7 μm) column. All HPLC columns were 25 × 1 cm. Polarimetric data: *Jasco-DP-181* polarimeter. UV(λ_{max} in nm, ε in mol^{-1} lcm^{-1}): *Perkin-Elmer Lambda 3* spectrophotometer. NMR: *Varian-XL-300* (^{1}H-NMR at 299.94 MHz, ^{13}C-NMR at 75.43 MHz); within square brackets are reported low-field shifts, $\Delta\delta$, induced by [Eu(fod)$_3$] at [[Eu(fod)$_3$]]/[substrate] ≈ 0.2; the superimposed resonances of CH protons, without reporting the multiplicities, were extracted from the slices parallel to the F_2 axis in ^{1}H, ^{13}C-shift correlation experiments; all spectra were carried out in $CDCl_3$; δ's (ppm) relative to internal Me_4Si (= 0 ppm) and J's in Hz; the notation 'small' indicates $J < 0.5$ Hz). MS: home-built quadrupole mass spectrometer based on the *ELFS-4-162-8 Extranuclear* quadrupole [15].

2. *Collection and Isolation.* The algae, *Lithothamnion corrallioides* CROUAN and *Lithothamnion calcareum* (PALLAS) ARESCHOUGH, were collected in mixture from Maërl dredging in the Bay of Morlaix, Brittany, in March 1986. The algae were immediately immersed and kept for some months in 95% EtOH. The liquid was then

decanted and the procedure repeated. The aq. residue from evaporation of this mixture at the water pump was extracted with petroleum ether (giving 4.19 g of residue after evaporation). Gradient ($H_2O \rightarrow$MeOH) reverse-phase flash chromatography (*RP-18*) of this residue, collecting fractions of 200 ml of each, gave products (+)-**1**–(+)-7 in *Fractions 8* and *9*. The residue (0.22 g) from evaporation of *Fraction 8* was subjected to *Amberlyst-A21* chromatography with petroleum ether/Et_2O 1:1 in order to remove fatty acids; the residue was subjected to reverse-phase HPLC eluting **3** (4.5 mg) at t_R 9.5 min (MeCN/H_2O 7:3). The residue from evaporation of *Fraction 9* was subjected to *Amberlist-A21* as above, and the residue was subjected to HPLC with hexane/AcOEt 17:3 to give *Fractions 1* and *2* (5 and 18 mg, resp., after evaporation). *Fraction 1* was subjected to HPLC with hexane/AcOEt 93.5: 6.5 to give (+)-**7**, (+)-**5**, and (+)-**4** (1.5, 1.4, and 0.9 mg at t_R 15.3, 16.0, and 16.8 min, resp.). *Fraction 2* was subjected to reverse-phase HPLC with MeCN/H_2O 4:1 (UV monitoring at λ = 205 nm) to give (+)-**2**, (+)-**1**, and (+)-**6** (4.0, 9.5, and 2.0 mg at t_R 10, 13, and 17 min, resp.)[4]).

3. *Ethyl 13-Hydroxyrachidonate* (= *(+)-(all-Z)-Ethyl 13-Hydroxyicosa-5,8,11,14-tetraenoate*; (+)-**1**). $[\alpha]^{20}$ (λ [nm]) = +1.5 (589), +4.9 (577), +5.5 (546), +12.2 (435), +21.3 (365; *c* = 0.6, EtOH). UV (EtOH): below 210. ^{1}H-NMR: 1.25 [0.34] (*t*, *J* = 7.0, CH_3CH_2O); 4.12 [1.01] (*q*, *J* = 7.0, CH_3CH_2O); 2.30 [0.97] (*t*, *J* = 7.5, 2 H–C(2)); 1.70 [0.60] (*tt*, *J* = 7.5, 7.5, 2 H–C(3)); 2.08 [0.37] (*m*, 2 H–C(4)); 5.38 [0.15] (H–C(5), H–C(6), H–C(9)); 2.82 [0.20] (br. *dd*, *J* = 6.4, 6.4, 2 H–C(7)); 5.35 [0.15] (H–C(8)); 2.91 [0.36] (55 Hz wide *m*, 2 H–C(10)); 5.42 [0.34] (H–C(11)); 5.48 [0.80, 0.26] (H–C(12), H–C(15)); 5.29 [1.29] (H–C(13)); 5.45 [0.75] (H–C(14)); 2.12 [0.30] (*m*, 2 H–C(16)); 1.37 [0.15] (*m*, 2 H–C(17)); 1.30 [0.07] (*m*, 2 H–C(18), 2 H–C(19)); 0.88 [0.05] (distorted *t*, *J* = 7.0, 3 H–C(20)); 1.65 [3.72] (br. *s*, OH). MS: 330 (10, $[M - H_2O]^{+\cdot}$), 91 (100).

4. *(+)-(all-Z)-Ethyl 13-Hydroxyicosa-5,8,11,14,17-pentaenoate* ((+)-**2**). $[\alpha]^{20}$ (λ [nm]) = +2.1 (589), +5.1 (577), +5.4 (546), +9.6 (435), +14.7 (365; *c* = 0.33, EtOH). UV (EtOH): below 210. ^{1}H-NMR: 1.24 [0.32] (*t*, *J* = 7.0, CH_3CH_2O); 4.11 [1.19] (*q*, *J* = 7.0, CH_3CH_2O); 2.29 [1.14] (*t*, *J* = 7.4, 2 H–C(2)); 1.69 [0.73] (*tt*, *J* = 7.4, 7.4, 2 H–C(3)); 2.10 [0.42] (br. *dt*, *J* = 7.4, 7.0, 2 H–C(4)); 5.36 [0.21] (H–C(5)); 5.38 [0.19] (H–C(6), H–C(9)); 2.80 [0.26] (br. *dd*, *J* ≈ 6.3, 6.3, 2 H–C(7)); 5.35 [0.22] (H–C(8)); 2.9 [0.43 and 0.31] (70 Hz wide *m*, 2 H–C(10), 2 H–C(16)); 5.46 [0.34 and 0.29] (H–C(11), H–C(15)); 5.48 [0.82 and 0.85] (H–C(12), H–C(14)); 5.30 [1.38 and 0.19] (H–C(13), H–C(17)); 5.43 [0.06] (H–C(18)); 2.06 [0.12] (br. *dq*, *J* = 7.0, 7.0, 2 H–C(19)); 0.96 [0.02] (*t*, *J* = 7.0, 3 H–C(20)); 1.57 [4.13] (br. *s*, OH). MS: 328 (6, $[M - H_2O]^{+\cdot}$), 91 (100).

5. *(5Z,9E,11E,14E)-Ethyl 8-Hydroxy-13-oxoicosa-5,9,11,14-tetraenoate* (**3**). $[\alpha]^{20}$ (λ [nm]) = *ca.* 0 (589), +2.3 (577), +0.5 (546), +4.9 (435), −1780 (365; *c* = 0.22, EtOH). UV (EtOH): 288 (19000). ^{1}H-NMR: 1.25 (*t*, *J* = 7.0, CH_3CH_2O); 4.13 (*q*, *J* = 7.0, CH_3CH_2O); 2.31 (*t*, *J* = 7.2, 2 H–C(2)); 1.71 (*tt*, *J* = 7.2, 7.2, 2 H–C(3)); 2.09 (br. *dt*, *J* = 7.2, 7.2, 2 H–C(4)); 5.56 (*dtt*, *J* = 10.8, 7.2, 1.4, H–C(5)); 5.45 (*dtt*, *J* = 10.8, 7.3, 1.4, H–C(6)); 2.35 (br. *dd*, *J* = 7.3, 7.0, 2 H–C(7)); 4.31 (br. *dt*, *J* = 7.0, 5.4, H–C(8)); 6.20 (*dddd*, *J* = 15.3, 5.4, 1.3, 1.3, H–C(9)); 6.45 (*dddd*, *J* = 15.3, 11.1, 1.6, 0.8, H–C(10)); 7.25 (br. *dd*, *J* = 15.3, 11.1, H–C(11)); 6.42 (*ddd*, *J* = 15.3, 1.6, 1.3, H–C(12)); 6.33 (*dt*, *J* = 15.8, 1.5, H–C(14)); 6.92 (*dt*, *J* = 15.8, 6.9, H–C(15)); 2.54 (*ddt*, *J* = 6.9, 6.9, 1.5, 2 H–C(16)); 1.49 (*m*, 2 H–C(17)); 1.31 (*m*, 2 H–C(18), 2 H–C(19)); 0.89 (highly distorted *t*, *J* ≈ 6.4, 3 H–C(20)); 2.8 (br. *s*, OH). MS: 362 (4, $M^{+\cdot}$), 344 (6, $[M - H_2O]^{+\cdot}$), 317 (5, $[M - C_2H_5O]^+$), 299 (3, $[317 - H_2O]^+$), 221 (8), 207 (81), 91 (60), 55 (100).

6. *(+)-(11R,5Z,8Z,12Z,12E,14Z)-Ethyl 11-Hydroxyicosa-5,8,12,14-tetraenoate* ((+)-**4**). $[\alpha]^{20}$ (λ [nm]) = +5.5 (589), +11.8 (546), +25.8 (435), 36.9 (365; *c* = 0.05, $CHCl_3$). ^{1}H-NMR: 1.26 (*t*, *J* = 7.1, CH_3CH_2O); 4.12 (*q*, *J* = 7.1, CH_3CH_2O); 2.31 (*t*, *J* = 7.5, 2 H–C(2)); 1.70 (*tt*, *J* = 7.5, 6.8, 2 H–C(3)); 2.11 (br. *dt*, *J* ≈ 7, 7, 2 H–C(4)); 5.37 (H–C(5)); 5.38 (H–C(6)); 2.81 (br. *dd*, *J* ≈ 7, 7, 2 H–C(7)); 5.57 (*dtt*, *J* = 10.8, 7.0, 1.4, H–C(8)); 5.43 (H–C(9)); 2.36 (*m*, 2 H–C(10)); 4.22 (*m*, H–C(11)); 5.70 (*dd*, *J* = 15.3, 4.9, H–C(12)); 6.53 (*dddd*, *J* = 15.3, 11.1, 1.2, 1.2, H–C(13)); 5.97 (br. *dd*, *J* = 11.1, 11.1, H–C(14)); 5.44 (H–C(15)); 2.17 (br. *dt*, *J* ≈ 7, 7, 2 H–C(16)); 1.38 (*m*, 2 H–C(17)); 1.30 (*m*, 2 H–C(18), 2 H–C(19)); 0.89 (distorted *t*, *J* ≈ 6.5); 1.57 (br. *s*, OH). ^{13}C-NMR: 14.27 (*q*, CH_3CH_2O); 60.29 (*t*, CH_3CH_2O); C(1) not detected; 33.73 (*t*, C(2)); 24.79 (*t*, C(3)); 26.61 (*t*, C(4)); 128.55 (*d*, C(5)); 129.15 (*d*, C(6)); 25.83 (*t*, C(7)); 131.27 (*d*, C(8)); 124.31 (*d*, C(9) or C(13)); 35.44 (*t*, C(10)); 72.13 (*d*, C(11)); 134.81 (*d*, C(12)); 124.93 (*d*, C(13) or C(9)); 127.61 (*d*, C(14)); 133.21 (*d*, C(15)); 27.44 (*t*, C(16)); 29.31 (*t*, C(17)); 31.48 (*t*, C(18)); 22.56 (*t*, C(19)); 14.07 (*q*, C(20)). MS: 330 (13, $[M - H_2O]^{+\cdot}$), 167 (42), 153 (80), 91 (61), 55 (100).

7. *(+)-(15S,5Z,8Z,11Z,13E)-Ethyl 15-Hydroxyicosa-5,8,11,13-tetraenoate* ((+)-**5**). $[\alpha]^{20}$ (λ [nm]) = +2.2 (589), +7.6 (577), +9.9 (546), +16.4 (435; *c* = 0.09, EtOH). ^{1}H-NMR: 1.26 (*t*, *J* = 7.2, CH_3CH_2O); 4.13 (*q*, *J* = 7.2,

[4]) Due to the difficulty of separating the two species, no effort has been spent to investigate which metabolite belongs to which species of *Lithothamnion*.

CH_3CH_2O); 2.31 (*t*, *J* = 7.5, 2 H–C(2)); 1.70 (*tt*, *J* = 7.5, 6.7, 2 H–C(3)); 2.11 (br. *dt*, *J* ≈ 7, 7, 2 H–C(4)); 5.39 (H–C(5), H–C(8), H–C(9), H–C(11)); 5.42 (H–C(6)); 2.81 (br. *dd*, *J* ≈ 6.5, 6.5, 2 H–C(7)); 2.96 (br. *dd*, *J* ≈ 6.5, 6.5, 2 H–C(10)); 6.01 (br. *dd*, *J* = 11.1, 11.0, H–C(12)); 6.53 (*dddd*, *J* = 15.3, 11.1, 1.1, 1.1, H–C(13)); 5.71 (*dd*, *J* = 15.3, 4.7, H–C(14)); 4.15 (*m*, H–C(15)); 1.3 (*m*, 2 H–C(16), 2 H–C(17), 2 H–C(18), 2 H–C(19)); 0.89 (distorted *t*, *J* ≈ 6.5). ^{13}C-NMR: 14.27 (*q*, CH_3CH_2O); 60.33 (*t*, CH_3CH_2O); 173.77 (*s*, C(1)); 33.71 (*t*, C(2)); 24.78 (*t*, C(3)); 26.57 (*t*, C(4)); 128.71 (*d*, C(5)); 129.08 (*d*, C(6)); 25.66 (*t*, C(7)); 128.63 (*d*, C(8)); 128.03 (*d*, C(9)); 26.11 (*t*, C(10)); 130.15 (*d*, C(11)); 127.56 (*d*, C(12)); 125.26 (*d*, C(13)); 136.69 (*d*, C(14)); 72.78 (*d*, C(15)); 37.32 (*t*, C(16)); 25.15 (*t*, C(17)); 31.80 (*t*, C(18)); 22.63 (*t*, C(19)); 14.09 (*q*, C(20)). MS: 330 (18, $[M - H_2O]^{+\cdot}$), 303 (2, $[M - C_2H_5O]^{+}$), 285 (2, $[303 - H_2O]^{+}$), 277 (3), 91 (100), 55 (78).

8. *(+)-(5S,6E,8Z,11Z,14Z)-Ethyl 5-Hydroxyicosa-6,8,11,14-tetraenoate* ((+)-**6**). $[\alpha]^{20}$ (λ [nm]) = +13.2 (589), +10.5 (546), +24,0 (435; *c* = 0.07, C_6H_6). UV (EtOH): 237 (26500). ^{1}H-NMR: 1.21 (*t*, *J* = 7.0, CH_3CH_2O); 4.13 (*q*, *J* = 7.0, CH_3CH_2O); 2.35 (*t*, *J* = 7.4, 2 H–C(2)); 1.71 (*tt*, *J* = 7.4, 7.2, 2 H–C(3)); 1.60 (*m*, 2 H–C(4)); 4.18 (*m*, H–C(5)); 5.71 (br. *dd*, *J* = 15.3, 4.9, H–C(6)); 6.55 (br. *dd*, *J* = 15.3, 11.0, H–C(7)); 6.01 (br. *dd*, *J* = 11.0, 11.0, H–C(8)); 5.28–5.52 (superimposed H–C(9), H–C(11), H–C(12), H–C(14), H–C(15)); 2.97 (br. *dd*, *J* ≈ 7, 7, 2 H–C(10)); 2.82 (br. *dd*, *J* ≈ 7, 7, 2 H–C(13)); 2.04 (br. *dt*, *J* ≈ 7, 7, 2 H–C(16)); 1.37 (*m*, 2 H–C(17)); 1.30 (*m*, 2 H–C(18), 2 H–C(19)); 0.89 (distorted *t*, *J* ≈ 6.5). ^{13}C-NMR: 14.07 (*q*, CH_3CH_2O); 60.32 (*t*, CH_3CH_2O); 173.58 (*s*, C(1)); 36.61 (*t*, C(2)); 20.83 (*t*, C(3)); 34.07 (*t*, C(4)); 72.34 (*d*, C(5)); 135.95 (*d*, C(6)); 125.73 (*d*, C(7)); 129.00 (*d*, C(8)); 130.65 (*d*, C(9) or C(15)); 26.10 (*t*, C(10)); 127.32 (*d*, C(11)); 127.43 (*d*, C(12)); 25.67 (*t*, C(13)); 127.89 (*d*, C(14)); 130.61 (*d*, C(15) or C(9)); 27.24 (*t*, C(16)); 29.70 (*t*, C(17)); 31.52 (*t*, C(18)); 22.58 (*t*, C(19)); 14.25 (*q*, C(20)). MS: 348 (2, $M^{+\cdot}$), 330 (7, $[M - H_2O]^{+\cdot}$), 303 (2, $[M - C_2H_5O]^{+}$), 285 (6, $[303 - H_2O]^{+}$), 247 (8), 233 (5), 91 (100), 55 (78).

9. *(+)-(12S,5Z,8Z,10E,14Z)-Ethyl 12-Hydroxyicosa-5,8,10,14-tetraenoate*. $[\alpha]^{20}$ (λ [nm]) = +2.9 (589), +11.3 (546), +16.9 (435; *c* = 0.07, $(CD_3)_2CO$). Except for the esterifying alcohol, NMR data coincide with those reported for the methyl ester [7a].

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