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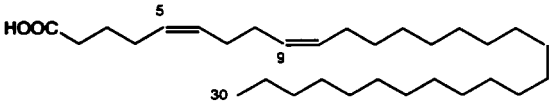
NOVEL VERY LONG CHAIN FATTY ACIDS FROM THE SPONGE *PETROSIA PELLASARCA*

NÉSTOR M. CARBALLEIRA* and ELBA D. REYES

Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

ABSTRACT.—A new series of novel very long chain phospholipid fatty acids were isolated from the Caribbean sponge *Petrosia pellasarca*. These new acids include the complete iso-anteiso 29:0 series, i.e., the novel 27-methyloctacosanoic acid and 26-methyloctacosanoic acid as well as the new 5,9-triacontadienoic acid [**1**]. The biosynthetically intriguing 19,22,25,28,31-tetratriacontapentaenoic acid [**2**] was also identified in *P. pellasarca*. These findings open up new possibilities for fatty acid biosynthesis hitherto unprecedented in terrestrial organisms and suggest a possible β-oxidation pathway deficiency in this organism, analogous to Zellweger syndrome. These phospholipid fatty acids were mainly encountered in phosphatidylethanolamine.

Marine sponges of the Class Demospongiae contain high levels of characteristic C_{24}–C_{30} fatty acids and are unique in that they seem to be able to biosynthesize these very long chain fatty acids with amazing ease (1). Recent studies have shown that many of these "demospongic acids" possess unusual unsaturation and/or methyl branching not found in the fatty acids of other more common organisms. For example, Litchfield and co-workers have identified the 26:2 $\Delta^{5,9}$ and 26:3 $\Delta^{5,9,19}$ fatty acids in the sponge *Microciona prolifera* (1) as well as 28:2 $\Delta^{5,9}$ and 28:3 $\Delta^{5,9,19}$ in *Xestospongia halichondroides* (2) and 30:3 $\Delta^{5,9,23}$ in *Chondrilla nucula* (3). Interesting to note in this context is that the acid 30:2 $\Delta^{5,9}$ has not yet been isolated in nature despite the fact that the above-mentioned triunsaturated 30:3 $\Delta^{5,9,23}$ fatty acid has been encountered in several sponges. Other interesting fatty acids isolated from sponges include the very long chain acids 15,18,21,24-triacontatetraenoic (30:4 ω6) and 15,18,21,24,27-triacontapentaenoic (30:5 ω3), which were also isolated by Litchfield *et al.* (4) from the sponge *Cliona celata*. Recently, we reported on the isolation of a 34:4 acid from the sponge *Amphimedon compressa* (5), which seems to be a four-carbon extension of the 30:4 acid previously reported by Litchfield *et al.* (4). These very long chain ω6 and ω3 fatty acids are of importance inasmuch as they have been reported to accumulate in the brain of patients with Zellweger syndrome, a severe neurodegenerative disorder due to a deficiency in the peroxisomal β-oxidation pathway (6). Omega-3 fatty acids in general have been of importance in recent years because they have been related to the lowering of blood fats,



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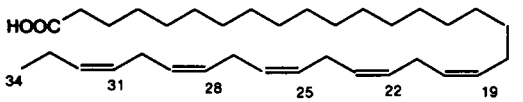

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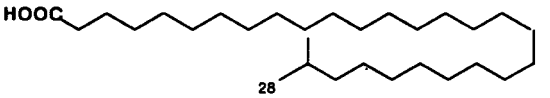

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making hormone-like prostaglandins, and decreasing the tendency toward blood clotting. In our search for novel fatty acids of unique biochemical origin around Puerto Rico we have found that the sponge *Petrosia pellasarca* (de Laubenfels) (order Haplosclerida, family Petrosiidae) contains an amazing array of unprecedented very long-chain fatty acids, which includes the hitherto unreported 5,9-triacontadienoic acid [**1**] as well as the very long-chain fatty acid 19,22,25,28,31-tetratriacontapentaenoic acid [**2**]. The sponge *P. pellasarca* was also shown to contain a novel series of C_{29} iso-anteiso acids, i.e., the acids 27-methyloctacosanoic [**3**] and 26-methyloctacosanoic, the longest set of iso-anteiso acids yet isolated from a sponge.

RESULTS AND DISCUSSION

The phospholipid composition of *P. pellasarca* was analyzed with the help of tlc and ^{31}P nmr. The principal phospholipids in this sponge were phosphatidylethanolamine (almost 60%), phosphatidylserine, and phosphatidylinositol. Much to our surprise, we detected only traces of phosphatidylcholine. The phospholipid fatty acid composition of *P. pellasarca* is shown in Table 1. This fatty acid composition was of interest because it presented 9% of a new very long-chain fatty acid that was characterized as 5,9-triacontadienoic [**1**]. The characterization of this acid was possible by mass spectral data of the methyl ester and pyrrolidide derivative, as well as comparison with other similar

TABLE 1. The Phospholipid Fatty Acids from *Petrosia pellasarca*.

Fatty Acid	Abundance (%)
Tetradecanoic (14:0)	3.8
4,8,12-Trimethyltridecanoic (16:0)	3.9
13-Methyltetradecanoic (i-15:0)	4.9
12-Methyltetradecanoic (a-15:0)	3.7
14-Methylpentadecanoic (i-16:0)	0.2
9-Hexadecenoic (16:1)	7.4
Hexadecanoic (16:0)	13.2
15-Methylhexadecanoic (i-17:0)	2.6
14-Methylhexadecanoic (a-17:0)	5.4
Heptadecanoic (17:0)	1.1
9-Octadecenoic (18:1)	0.7
11-Octadecenoic (18:1)	0.9
Octadecanoic (18:0)	2.6
Methyloctadecanoic (19:0)	6.4
5,8,11,14-Icosatetraenoic (20:4)	1.0
Icosanoic (20:0)	0.7
7,10,13,16-Docosatetraenoic (22:4)	1.1
25-Methylhexacosanoic (i-27:0)	1.2
24-Methylhexacosanoic (a-27:0)	0.7
9-Octacosenoic (28:1)	2.4
Octacosanoic (28:0)	4.7
27-Methyloctacosanoic (i-29:0)[a]	1.6
26-Methyloctacosanoic (a-29:0)	1.8
Nonacosanoic (29:0)	2.3
5,9-Triacontadienoic (30:2)[a]	9.4
Triacontanoic (30:0)	2.8
Hentriacontanoic (31:0)	0.6
Dotriacontanoic (32:0)	0.5
19,22,25,28,31-Tetratriacontapentaenoic (34:5)	12.4

[a]These compounds are unprecedented in nature.

systems isolated before by us from several sponges. The $\Delta^{5,9}$ unsaturation was confirmed by a base peak at *m/z* 81 for the corresponding fatty acid methyl ester (only found for this double bond combination) and transformation upon catalytic hydrogenation to triacontanoic acid methyl ester (30:0), which co-eluted from the gc with an authentic sample. The pyrrolidide derivative of the fatty acids with the typical $\Delta^{5,9}$ unsaturation displays a very intense peak at *m/z* 180 (C_7) due to doubly activated allylic cleavage between C-7 and C-8 (7). The last double bond in the chain of compound **1** was confirmed to be at C-9 by oxidative cleavage. Upon either reductive ozonolysis in 7% BF_3/MeOH or permanganate-periodate oxidation followed by esterification in 1.2 N HCl/MeOH, methyl heneicosanoate was obtained as one of the fragments. This C-21 methyl ester was characterized by gc-ms because it presented a molecular ion peak $[M]^+$ at *m/z* 340 and a base peak at *m/z* 74. Both double bonds in compound **1** were determined to have the cis stereochemistry on the basis of no prominent absorption at 980–968 cm^{-1} in the ir spectrum. Acid **1** has never been encountered before in nature (only the 30:3 $\Delta^{5,9,23}$ acid has been reported) and becomes the longest fatty acid with the $\Delta^{5,9}$ unsaturation to be isolated from a sponge or any other living organism.

A very interesting fatty acid (12.4% abundance) was also obtained in the mixture and was characterized as 19,22,25,28,31-tetratriacontapentaenoic acid methyl ester [**2**], an extremely long fatty acid that has never before been encountered in any sponge. It was possible to characterize this acid with the help of a recently published paper that reports the mass spectra of very long chain polyenoic fatty acids of the ω-3 and ω-6 series (8). Key abundant fragmentations in the mass spectrum of our isolated fatty acid methyl ester were observed at *m/z* 215, 201, 187, 173, 159, 145, 131, and 108, together with the low abundance of a critical peak at *m/z* 150 which is typical for the ω-6 series (8). Compound **2** also had the characteristic base peak at *m/z* 79, no ir absorption at 980–968 cm^{-1}, indicating cis rather than trans unsaturation, and no absorption bands in the 220–300 nm region, proving the absence of conjugated double bonds. Upon catalytic hydrogenation (PtO_2) compound **2** was transformed into tetratriacontanoic acid (34:0), which excludes the possibility of any branching. More importantly, the last double bond in fatty acid **2** was clearly determined to be at C-19 because upon either reductive ozonolysis in 7% BF_3/MeOH or permanganate-periodate oxidation followed by esterification in 1.2 N HCl/MeOH, nonadecanedioic acid dimethyl ester was obtained as one of the fragments. The dimethyl ester presented an $[M-31]^+$ peak at *m/z* 325 and a base peak at *m/z* 98. Despite the fact that fatty acid **2** has been isolated before in patients with Zellweger syndrome (6), this is the first time that it has been detected in sponge phospholipids, namely in phosphatidylethanolamine. Therefore, this fatty acid represents the longest acid isolated to date from any marine sponge and indicates that this particular organism is capable of extending the common 22:5 ω3 fatty acids, normally found in terrestrial animals, to C_{34} chain lengths.

The fatty acid composition of *P. pellasarca* was also of interest because it presented several series (21% of the total fatty acid composition) of iso-anteiso fatty acids, namely the C_{15}, C_{17}, C_{27}, and C_{29} series. Although the C_{15}–C_{17} series are common, the C_{27}–C_{29} series are rare. These acids were readily characterized by their typical equivalent chain length (ECL) values; for example, the iso compound elutes first with typical fractional chain lengths (FCL) of 0.60–0.65 followed by the anteiso compound with values of 0.70–0.75. Ms results confirmed our assignments. The new series of iso-anteiso acids, i.e., the novel 27-methyloctacosanoic acid [**3**] and the not so common 26-methyloctacosanoic acid (9), were the most important series isolated from *P. pellasarca*. Characterization was achieved by means of gc-ms and co-injections with authentic samples. The novel 27-methyloctacosanoic acid [**3**] displayed, as the methyl ester, a molecular ion at *m/z* 452, consistent with the formula $C_{30}H_{60}O_2$. The spectrum also in-

cluded a number of features characteristic of fatty acid methyl esters, including a base peak at *m/z* 74 and prominent fragments at *m/z* 87 and *m/z* 143. Critical for identifying the iso-anteiso branching in this series was the observation that the fatty acid methyl esters displayed ECL values of 28.62 and 28.75, all typical values of iso and anteiso fatty acid methyl esters (10). In fact, a plot of retention time vs. number of carbon atoms for the complete series of iso-anteiso fatty acids yielded a straight line, thus confirming the methyl substitution in these compounds.

In summary, *P. pellasarca* is an interesting sponge because it is able to biosynthesize very long chain fatty acids. Although the biosynthesis of shorter-chain polyenoic fatty acids has been investigated in great detail, relatively little is known about the biosynthesis of the polyenoic very long chain fatty acids. Our data show that *P. pellasarca* accumulates very long chain polyenoic fatty acids, an unusual finding that could probably be explained in terms of a β-oxidation pathway deficiency. The fact that these very long chain fatty acids were found in phosphatidylethanolamine implies that they may participate in the membrane lipid bilayers of the sponge. If so, the extra bulk of their longer chains would probably make that bilayer thicker and more rigid than normal.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The methyl esters were analyzed by gc-ms using either a Hewlett Packard 5995 A gas chromatograph-mass spectrometer or a Hewlett Packard 59970 MS ChemStation equipped with a 30 m × 0.25 mm nonpolar fused silica column coated with DB-1. Gc/Ft-ir spectra were recorded on a Nicolet 740 FT IR spectrometer. The ^{31}P nmr of the phospholipids was performed at 22° on a GN 300 FT NMR spectrometer at 121.6 MHz. For the acquisition 16K data points were used, and approximately 1000 accumulations were obtained before Fourier transformation of the free induction decay. In a typical run, phospholipids (20–30 mg) were dissolved in 3 ml of $CDCl_3$-CD_3OD (2:1) containing as internal reference triphenylphosphine.

SPONGE MATERIAL.—*P. pellasarca* was collected July 7, 1989, near the shelf edge of La Parguera, Puerto Rico, at a depth of 80 ft. The sponge was kindly classified by Dr. Vance Vicente. A voucher specimen is on file at the museum of the Department of Biology of the University of Puerto Rico, Rio Piedras campus.

EXTRACTION AND ISOLATION OF PHOSPHOLIPIDS.—The sponge (500 g) was washed in sea water, carefully cleaned of all nonsponge debris, and cut into small pieces. Immediate extraction with 700 ml of $CHCl_3$-MeOH (1:1) yielded the total lipids. The neutral lipids, glycolipids, and phospholipids (100 mg) were separated by cc on Si gel (60–200 mesh) using a procedure similar to that of Privett *et al.* (11). The phospholipid classes were investigated either by preparative tlc using Si gel G and $CHCl_3$-MeOH-H_2O (25:10:1) as solvent or by ^{31}P nmr.

PREPARATION OF FATTY ACID DERIVATIVES.—The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction of the phospholipid fraction with methanolic HCl (12) followed by cc purification eluting with *n*-hexane–Et_2O (9:1). For the location of double bonds, *N*-acylpyrrolidide derivatives were prepared by direct treatment of the methyl esters with pyrrolidine-HOAc (10:1) in a capped vial (3 h at 100°) followed by ethereal extraction from the acidified solution and purification by preparative tlc (7). Hydrogenations were carried out in 10 ml of absolute MeOH and catalytic amounts of PtO_2. Mass spectral data of the key fatty acid methyl esters for this discussion follows.

5,9-Triacontadienoic acid methyl ester [**1**].—Ms *m/z* (rel. int.) $[M]^+$ 462 (5), 433 (0.6), 431 (2), 419 (0.1), 388 (1.4), 377 (0.1), 363 (0.2), 362 (0.7), 349 (0.5), 348 (1.5), 335 (0.4), 321 (1), 320 (2.6), 307 (0.2), 306 (0.5), 293 (0.3), 279 (0.4), 278 (1.2), 265 (0.4), 264 (1), 251 (1), 250 (1), 237 (0.7), 236 (1.5), 223 (1), 222 (1.6), 209 (2), 208 (2), 195 (5), 191 (1), 187 (1), 182 (12), 181 (11), 168 (11), 164 (14), 157 (1), 154 (8), 151 (10), 150 (35), 149 (16), 143 (3), 141 (32), 137 (16), 136 (29), 135 (18), 124 (14), 123 (19), 122 (13), 110 (31), 109 (58), 99 (16), 96 (48), 94 (23), 85 (16), 82 (50), 81 (100), 80 (26), 74 (21), 71 (23), 67 (63), 57 (65).

5,9-Triacontadienoic acid pyrrolidide.—M/s *m/z* (rel. int.) $[M]^+$ 501 (4), 181 (3), 180 (13), 126 (16), 113 (100), 98 (9), 85 (8), 72 (6), 70 (8), 57 (6), 55 (13).

19,22,25,28,31-Tetratriacontapentaenoic acid methyl ester [**2**].—M/s *m/z* (rel. int.) $[M]^+$ 512 (2.4), $[M-29]^+$ 483 (0.8), $[M-31]^+$ 481 (1), 469 (0.5), 458 (0.4), 444 (1.5), 443 (3), 441 (0.3), 430 (1),

429 (0.6), 423 (0.3), 416 (1), 415 (0.5), 411 (0.9), 403 (1.3), 397 (0.5), 390 (0.5), 389 (0.6), 383 (0.5), 376 (2), 371 (0.4), 369 (0.5), 341 (0.6), 255 (0.7), 229 (3), 216 (4), 215 (10), 203 (2), 201 (11), 200 (1), 199 (3), 188 (4), 187 (8), 175 (14.6), 173 (12), 161 (14), 160 (5.7), 159 (16), 149 (14), 148 (15), 147 (20), 145 (25), 134 (23), 133 (33), 131 (36), 129 (9), 122 (21), 121 (33), 119 (45), 117 (30), 111 (9), 108 (56), 107 (32), 105 (41), 95 (55), 93 (58), 91 (69), 87 (22), 83 (27), 81 (56), 80 (57), 79 (100), 77 (21), 74 (36), 67 (82).

27-Methyloctacosanoic acid methyl ester [**3**].—M/s *m/z* (rel. int.) $[M]^+$ 452 (32), 409 (7), 395 (1), 367 (3), 353 (5), 339 (2), 311 (3), 297 (5), 255 (7), 241 (6), 199 (22), 185 (18), 171 (6), 157 (13), 143 (64), 129 (18), 111 (21), 101 (20), 97 (23), 87 (79), 83 (24), 75 (50), 74 (100), 71 (28), 69 (31), 57 (52).

OZONOLYSIS OF FATTY ACID DERIVATIVES.—Into a 4-ml screw-cap vial was placed the fatty acid (2–3 mg) and 7% BF_3/MeOH (2 ml). Ozone was bubbled in briskly (2 min at −78°) followed by capping and heating of the solution in an oil bath at 100° for 1 h. After cooling, H_2O (2 ml) was added, the aqueous phase was extracted with hexane (2 × 5 ml) and dried over Na_2SO_4, and the solvent was finally evaporated.

PERMANGANATE/PERIODATE OXIDATION.—A stock oxidant solution of sodium metaperiodate (2.09 g) and $KMnO_4$ (0.04 g) in H_2O (100 ml) was prepared. This solution (1 ml) together with K_2CO_3 solution (1 ml; 2.5 g/liter) was added to the methyl ester (1 mg) in *t*-BuOH (1 ml) in a test tube, and the mixture was shaken thoroughly at room temperature (1 h). At the end of this time, the solution was acidified with one drop of concentrated H_2SO_4, and excess oxidant was destroyed with $NaHSO_3$. The solution was extracted thoroughly with Et_2O (3 × 4 ml). The organic layer was dried over Na_2SO_4 and removed in a stream of N_2 at room temperature. The products were methylated with 1.2 N HCl/MeOH for gc analysis.

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