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IDENTIFICATION AND CHARACTERIZATION OF TWO NEW METHYLICOSADIENOIC ACIDS FROM *ERYLUS FORMOSUS*

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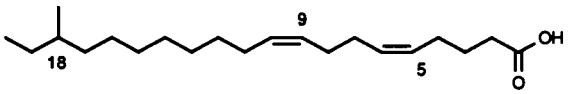
ABSTRACT.—The novel fatty acids 18-methyl-5,9-icosadienoic acid [**1**] and 19-methyl-5,9-icosadienoic acid [**2**] were identified in the Caribbean sponge *Erylus formosus*. Other interesting fatty acids identified were the branched acids 3-methylpentadecanoic acid and 3-methylhexadecanoic acid, which are known to possess larvicidal activity. The most stable conformation of the new acid 19-methyl-5,9-icosadienoic acid is also presented as predicted by Molecular Mechanics (MM2) calculations. The sterol composition of *E. formosus* is also reported.

Sponges have been a constant source of structurally unusual and interesting fatty acids. Common phospholipid fatty acids from marine sponges include 5,9-hexacosadienoic (26:2), which occurs in most known sponges (1), 5,9-heptacosadienoic (27:2), and 5,9-octacosadienoic (28:2). Branched fatty acids of longer than usual chain-length have also recently been encountered in several sponges. For example, work by Ayanoglu and coworkers (2,3) with the sponges *Petrosia ficiformis* and *Jaspis stellifera* has revealed the presence of the unusual phospholipid fatty acids 25-methyl-5,9-hexacosadienoic and 24-methyl-5,9-hexacosadienoic, interesting cases of iso and anteiso terminal methyl branching in these so-called "demospongic" acids. Recently we reported that the sponge *Ectyoplasia ferox* also contains the $\Delta^{5,9}$-28:2 iso and anteiso isomers, i.e., the very long chain phospholipid fatty acids 26-methyl-5,9-heptacosadienoic and 25-methyl-5,9-heptacosadienoic, these being the longest pair of $\Delta^{5,9}$ iso and anteiso phospholipid fatty acids from any marine sponge (4). The $\Delta^{5,9}$-26:2 iso and anteiso pair, i.e., the acids 24-methyl-5,9-pentacosadienoic and 23-methyl-5,9-pentacosadienoic, were also recently isolated from the sponge *Cribrochalina vasculum* (5). Therefore, the only reported pairs of branched iso-anteiso $\Delta^{5,9}$ fatty acids from sponges are those acids ranging in length from 26 to 28 carbons. In our search for novel acids of unusual biosynthetic origin around Puerto Rico we have now found that the sponge *Erylus formosus* Sollas (subclass Tetractinomorpha, order Choristida) contains the iso-anteiso $\Delta^{5,9}$-21:2 pair, i.e., the hitherto unreported 18-methyl-5,9-icosadienoic acid [**1**] and 19-methyl-5,9-icosadienoic acid [**2**]. The only previous report of natural products from sponges of the genus *Erylus* is the isolation of eryloside A, a 4-methylated steroidal glycoside that was recently isolated from the sponge *Erylus lendenfeldi* (6).

The complete phospholipid fatty acid composition of *E. formosus* is presented in Table 1. The fatty acids accounted for 1% of the total sponge material. The



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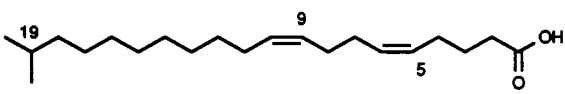

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TABLE 1. The Phospholipid Fatty Acids from *Erylus formosus*.

Fatty Acid	Abundance (%)
Tetradecanoic (14:0)	1.8
13-Methyltetradecanoic (iso-15:0)	17.0
12-Methyltetradecanoic (anteiso-15:0)	1.6
3-Methylpentadecanoic (16:0)	0.6
Hexadecenoic (16:1)	0.5
Methylpentadecanoic (16:0)	1.5
Hexadecanoic (16:0)	10.0
3-Methylhexadecanoic (17:0)	1.0
15-Methylhexadecanoic (iso-17:0)	6.3
14-Methylhexadecanoic (anteiso-17:0)	6.0
5,9-Octadecadienoic (18:2)	0.5
Octadecenoic (18:1)	1.8
Octadecanoic (18:0)	5.0
Methyloctadecanoic (19:0)	9.9
5,9-Icosadienoic (20:2)	8.0
19-Methyl-5,9-icosadienoic (21:2)[a]	1.3
18-Methyl-5,9-icosadienoic (21:2)[a]	0.5
Heneicosanoic (21:0)	0.7
Tricosanoic (23:0)	1.7
Tetracosanoic (24:0)	0.3
Pentacosanoic (25:0)	2.3
24-Methyl-5,9-pentacosadienoic (iso-26:2)	2.6
5,9-Hexacosadienoic (26:2)	1.5
25-Methyl-5,9-hexacosadienoic (iso-27:2)	4.7
24-Methyl-5,9-hexacosadienoic (anteiso-27:2)	9.5
5,9-Octacosadienoic (28:2)	2.6
5,9-Nonacosadienoic (29:2)	0.8

[a]These acids are unprecedented in nature.

most striking feature of the composition is the presence of a series of branched very long chain fatty acids possessing the unusual $\Delta^{5,9}$ desaturation pattern. Of prime importance to this report was the presence of two novel acids characterized as 18-methyl-5,9-icosadienoic acid [**1**] and 19-methyl-5,9-icosadienoic acid [**2**]. The complete characterization of these two acids was possible by means of gc-ms fragmentation patterns of their corresponding methyl ester derivatives as well as chemical degradation. The methyl esters of acids **1** and **2** presented molecular ion peaks at $[M]^+$ 336 and base peaks at *m/z* 81, confirming the proposed structures. The base peak at *m/z* 81 is unique for the $\Delta^{5,9}$ system, practically an ms fingerprint for these methyl esters (5). Upon catalytic hydrogenation (PtO_2) these fatty acid methyl esters were transformed into 18-methylicosanoic acid methyl ester ($[M]^+$ 340), which presented an equivalent chain length (ECL) value of 20.72, and into 19-methylicosanoic acid methyl ester ($[M]^+$ 340), which presented an ECL value of 20.60. ECL values are very informative about methyl substitution in fatty acyl chains, and the typical values obtained in this case match exactly the observed values for anteiso and iso methyl esters, respectively (5). For example, iso methyl esters tend to elute first with typical fractional chain lengths (FCL) of 0.60–0.65 followed by the anteiso methyl esters with values of 0.70–0.75 (7). The anteiso branching in 18-methylicosanoic acid methyl ester was further corroborated by its ms spectrum, because a peak at *m/z* 311 $[M-29]^+$, 2.5% abundance) was more conspicuous than a peak at *m/z* 309 $[M-31]^+$ (1.5% abundance). In order to confirm the pro-

posed structures, we cleaved the methyl esters of acids **1** and **2** with $KMNO_4$/$NaIO_4$ followed by esterification with HCl/MeOH. The short chain fatty acid methyl esters 10-methylundecanoic and 9-methylundecanoic ($[M]^+$ 214) were thus obtained. The identity of these structures was corroborated by gc co-injection with authentic standards and comparing their mass spectrum with the literature (8). The latter experiment established the last double bond in both chains at C-9 and also supports the iso-anteiso branching. For example, an iso methyl fatty ester can be distinguished from the corresponding straight-chain compound by the presence of a small ion at $[M-65]^+$ and a doublet at $[M-57]^+$ and $[M-56]^+$. The $[M-65]^+$ peak results from the $[CH_3OCO(CH_2)_8CH\text{-}CH_3]^+$ fragment plus the loss of MeOH and H_2O, while the $[M-57]^+$ and $[M-56]^+$ peaks result from fragmentation at the terminal methyl branching (8). In the case of 10-methylundecanoic acid methyl ester, the fragments were observed at *m/z* 157 $[M-57]^+$, *m/z* 158 $[M-56]^+$, and at *m/z* 149 $[M-65]^+$. A gc-Ft-ir spectrum was obtained for the methyl esters of 18-methyl-5,9-icosadienoic acid [**1**] and 19-methyl-5,9-icosadienoic acid [**2**], and no absorption was observed in the 960–980 cm^{-1} region, thus confirming the cis configuration for the double bonds. All of our experimental data then supports the proposed structures **1** and **2** which represent a new set of $\Delta^{5,9}$-iso-anteiso acids of intermediate chain length.

Due to the novelty of these demospongic $\Delta^{5,9}$ fatty acids, we ran a Molecular Mechanics (MM2) calucation on the new acid 19-methyl-5,9-icosadienoic [**2**]. We used the MM2 program in the ChemDraft II computational package provided by C-Graph Software, Inc. After several different conformations, a minimum energy (9.08 kcal) was obtained for the conformation depicted in Figure 1. The most stable conformation for the acyl chain is obviously the one which presents the adjacent methylene groups in the anti form. The fatty acid in the phospholipid can attain a maximum length of ca. 24.4 Å but is bent at the first cis double bond by an angle of ca. 116°–120° and bent again in the opposite direction at the second cis double bond by a similar deformation of 116°–120°. The net result is a total bend of ca. 150°, which is not so dramatic considering the presence of two cis double bonds in the molecule. The total calculated dipole moment was 2.45 D.

Two rather interesting branched saturated fatty acids were also isolated from *E. formosus*, namely 3-methylpentadecanoic acid and 3-methylhexadecanoic acid. In the case of 3-methylpentadecanoic acid methyl ester, mass spectral analysis revealed a mol wt of 270, suggesting 16 carbons in the molecule, and the base peak at *m/z* 74 confirmed it to be a fatty acid methyl ester. This compound was inert upon catalytic hydrogenation, excluding the possibility of any unsaturation. The mass spectrum of the fatty acid methyl ester was very revealing,

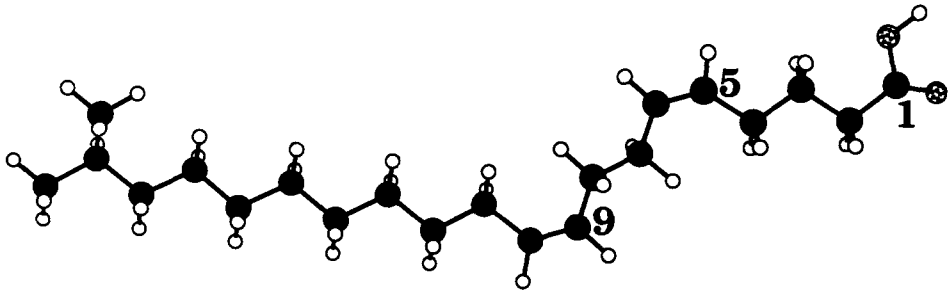

FIGURE 1. Most stable conformation for 19-methyl-5,9-icosadienoic acid [**2**] as predicted by Molecular Mechanics (MM2) calculations.

especially a peak at *m/z* 101 $[C_5H_9O_2]^+$ with an impressive abundance of 86%, which clearly indicates methyl branching at C-3 due to a favorable cleavage between C-3 and C-4. The compound in question is 3-methylpentadecanoic acid methyl ester. Characterization of 3-methylhexadecanoic acid methyl ester followed an analogous course, namely a molecular ion at *m/z* 284 and a base peak at *m/z* 74, as well as cleavage between C-3 and C-4, giving rise to the diagnostic peak at *m/z* 101 (95% abundance). 3-Methylalkanoic acids have been reported to occur in Surakhany crude oil (9) and in rabbit fat (10). The importance of the latter compounds lies in the fact that they have been found to possess larvicidal activity against the southern house mosquito *Culex pipiens quinquefasciatus* (11).

The phospholipid composition of *E. formosus* was analyzed with the help of tlc and ^{31}P nmr. The principal phospholipids in this sponge were phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidylcholine.

Because sterols are known to coexist with phospholipids in cell membranes, we decided to take a look at the sterols from *E. formosus* in detail to see if unusual methyl branching was to be found in the sterol mixture. With the help of hplc, ^1H nmr (300 MHz), ms, and comparison with authentic samples, the following sterols were isolated and identified: cholesterol (10% abundance of the total sterol mixture), sitosterol (57%), stigmasterol (2%), 24-methylcholesterol (9%), cholestanol (7%), 24-methylcholesta-5,22-dienol (3%), and fucosterol (12%). Therefore, *E. formosus* presented a common sponge sterol composition.

E. formosus is an interesting sponge because it contains a pair of iso-anteiso $\Delta^{5,9}$-21:2 fatty acids, adding a new dimension to the possible $\Delta^{5,9}$ branched very long chain "demospongic" acids. On the basis of previous biosynthetic experiments with the sponges *Microciona prolifera* and *J. stellifera* (3), we can postulate that the new iso or anteiso acids reported in this work could arise by chain elongation of the iso-15:0 or anteiso-15:0 primers, respectively, to their corresponding branched long-chain methylicosanoic acids. These resulting 21:0 acids could then be desaturated to introduction of either the $\Delta 5$ or $\Delta 9$ double bond first, followed by $\Delta 9$ or $\Delta 5$ desaturation, respectively.

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.

SPONGE MATERIAL.—*E. formosus* was collected 7 July 1989, near the shelf edge of La Parguera, Puerto Rico at a depth of 80 ft. The sponge was 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 (70 mg) were separated by cc on Si gel (60–200 mesh) using a procedure similar to that of Privett *et al.* (12). The phospholipid classes were either investigated 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 (13) followed by cc purification eluting with *n*-hexane–Et_2O (9:1). Hydrogenations were carried out in 10 ml of absolute MeOH and catalytic amounts of PtO_2.

18-Methyl-5,9-icosadienoic acid methyl ester [**1**].—Ms *m/z* (rel. int.) $[M]^+$ 336 (3), 321 (0.2), 306 (0.3), 305 (0.7), 304 (1.3), 287 (1.5), 267 (1.5), 262 (1.6), 257 (0.6), 248 (0.6), 235 (0.9), 233 (0.5), 222 (1.3), 221 (1.9), 213 (0.3), 209 (0.7), 208 (1), 207 (0.6), 205 (0.5), 199 (0.7), 195 (1.8), 194 (3), 182 (2), 181 (3.7), 179 (1), 177 (1.5), 169 (1.4), 168 (2), 167 (3), 164 (4.5),

163 (4), 154 (4), 152 (2.6), 150 (14), 149 (10), 145 (1.5), 141 (27), 136 (13), 135 (11), 123 (9), 119 (3), 115 (2), 111 (11), 110 (20), 109 (48), 108 (11), 107 (8), 105 (3), 101 (2.5), 99 (16), 97 (23), 95 (31), 93 (12), 91 (8), 87 (10), 85 (12), 83 (35), 82 (36), 81 (100), 80 (23), 79 (27), 74 (25), 69 (48), 68 (25), 67 (77), 57 (46); ir ν max ($CHCl_3$) 2969, 2939, 2885, 1745, 1462, 1378, 1310, 1244, 1169, 1057, 722 cm^{-1}.

19-Methyl-5,9-icosadienoic acid methyl ester [**2**].—Ms *m/z* (rel. int.) $[M]^+$ 336 (2.4), 323 (0.6), 304 (0.8), 287 (0.9), 262 (1.6), 252 (0.6), 249 (0.6), 230 (0.8), 229 (1.9), 222 (0.9), 221 (1.3), 208 (1), 199 (1), 195 (1), 194 (1.4), 193 (1), 191 (1.2), 181 (2), 180 (1), 179 (1.2), 175 (1), 169 (0.9), 168 (1), 165 (2.5), 164 (3.6), 163 (3), 157 (1.9), 154 (2), 151 (2.6), 150 (8.5), 149 (7.6), 147 (1.9), 143 (2.7), 141 (17.7), 140 (8), 137 (5.5), 136 (10.5), 135 (8.5), 133 (2.5), 131 (3.5), 129 (3.6), 125 (4.8), 123 (7.7), 121 (8), 119 (4.6), 115 (2.5), 112 (3), 110 (13.7), 109 (36), 105 (5), 101 (2.4), 99 (14), 97 (20.8), 95 (24), 93 (12), 91 (10.7), 87 (10), 85 (8), 83 (26), 82 (30), 81 (100), 80 (20), 79 (29), 74 (25), 71 (22), 69 (44), 68 (18), 67 (72), 59 (17), 57 (45); ir ν max ($CHCl_3$) 2969, 2939, 2885, 1745, 1462, 1378, 1310, 1244, 1169, 1057, 722 cm^{-1}.

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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