Identification of Novel Iso/Anteiso Nonacosadienoic Acids from the Phospholipids of the Sponges Chondrosia remiformis and Myrmekioderma styx

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IDENTIFICATION OF NOVEL ISO/ANTEISO NONACOSADIENOIC ACIDS FROM THE PHOSPHOLIPIDS OF THE SPONGES *CHONDROSIA REMIFORMIS* AND *MYRMEKIODERMA STYX*

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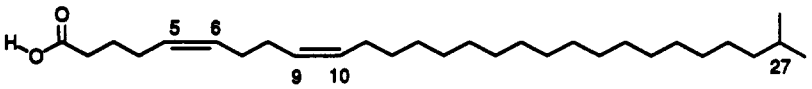
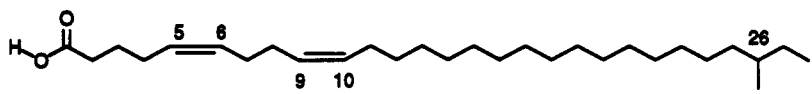
ABSTRACT.—The previously unreported 27-methyl-5,9-octacosadienoic acid [**1**] and 26-methyl-5,9-octacosadienoic acid [**2**] were identified in the phospholipids of the Caribbean sponges *Chondrosia remiformis* and *Myrmekioderma styx*. Both sponges also contain the novel 15-methyl-5,9-hexadecadienoic acid [**3**] in their phospholipids. These results extend the possible chain lengths in $\Delta^{5,9}$ iso/anteiso fatty acids in sponge phospholipids from C_{17} to C_{29}.

Sponges are unusual in that they have very-long-chain fatty acids in their phospholipids (1). Symmetric phospholipids, i.e., those with the same fatty acids at positions sn-1 and sn-2, have been identified from several sponges containing as the principal fatty acid 5,9-hexacosadienoic acid (2). Recent work in sponge biochemistry has established that sponges are also able to incorporate in their phospholipids iso/anteiso methyl branched $\Delta^{5,9}$ fatty acids (3). Examples of very-long-chain $\Delta^{5,9}$ branched acids include the complete series of iso/anteiso 5,9–26:2, 5,9–27:2, and 5,9–28:2 acids, which have been identified in many sponge phospholipids (4). These so-called "demospongic" acids have been shown to originate from the corresponding shorter-chain iso/anteiso bacterial acids (C_{15}–C_{17}) which are also present in sponges (3). The shortest iso/anteiso $\Delta^{5,9}$ fatty acids from sponge phospholipids were reported from *Erylus formosus*, which contained the iso/anteiso 5,9–21:2 acids (5). On the other hand, the longest $\Delta^{5,9}$ fatty acids reported to date have been the iso/anteiso 5,9–28:2 acids known to occur in the phospholipids of several sponges such as *Cribrochalina vasculum* (4). In this work we report the isolation and identification of the unprecedented fatty acids 15-methyl-5,9-hexadecadienoic acid [**3**], which was found in the phospholipids of the sponges *Chondrosia remiformis* Schulze (family Chondrosiidae) and *Myrmekioderma styx* de Laubenfels (family Halichondriidae, order Halichondrida), and the acids 27-methyl-5,9-octacosadienoic [**1**] and 26-methyl-5,9-octacosadienoic [**2**], which were also identified in the phospholipids of *Ch. remiformis* and *M. styx*.

The main phospholipids from *Ch.*



1



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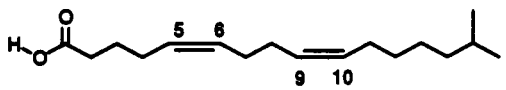

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remiformis and *M. styx* were found to be phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol. These phospholipids were characterized by tlc comparisons with authentic samples (Sigma) and the use of spray reagents such as molybdenum blue and ninhydrin. Acid methanolysis of the phospholipids yielded the fatty acid mixture shown in Table 1. Most of the fatty acids present in the sponges have been previously characterized in the literature. Critical for the characterization were gc retention times of the fatty acid methyl esters, ms data on the methyl esters, their corresponding dimethyl disulfide and pyrrolidide derivatives, and catalytic hydrogenation of the whole fatty acid mixture for easier elucidation of methyl branching.

Our attention was primarily cen-

TABLE 1. The Phospholipid Fatty Acids from *Chondrosia remiformis* and *Myrmekioderma styx*.

Fatty Acid	*Chondrosia remiformis*[a] (wt %)	*Myrmekioderma styx*[b] (wt %)
Tridecanoic (13:0)	0.1	—
11-Methyltridecanoic (14:0)	0.6	—
Tetradecanoic (14:0)	1.4	3.3
4,8,12-Trimethyltridecanoic (16:0)	—	4.6
Methyltetradecanoic (15:0)	0.2	—
13-Methyltetradecanoic (i-15:0)	7.2	6.2
12-Methyltetradecaonic (ai-15:0)	2.1	2.8
Pentadecanoic (15:0)	0.7	2.3
Methylpentadecanoic (16:0)	0.5	—
14-Methylpentadecanoic (i-16:0)	—	2.7
5,9-Hexadecadienoic (16:2)	3.6	—
9-Hexadecenoic (16:1)	6.9	0.9
Hexadecanoic (16:0)	5.2	6.2
15-Methyl-5,9-hexadecadienoic (i-17:2)[c]	0.7	2.3
15-Methyl-9-hexadecenoic (i-17:1)	1.2	3.2
Methylhexadecanoic (17:0)	6.9	5.0
15-Methylhexadecanoic (i-17:0)	1.2	5.0
14-Methylhexadecanoic (ai-17:0)	0.8	1.6
Heptadecanoic (17:0)	0.6	1.2
3,7,11,15-Tetramethylhexadecanoic (20:0)	10.0	—
9-Octadecenoic (18:1)	—	0.6
11-Octadecenoic (18:1)	—	0.9
Octadecanoic (18:0)	4.4	5.6
Methylnonadecanoic (19:0)	3.3	8.2
11-Nonadecenoic (19:1)	3.6	3.0
Eicosatetraenoic (20:4, n-6)	3.0	2.0
18-Methylnonadecanoic (i-20:0)	0.1	0.6
17-Methylnonadecanoic (ai-20:0)	—	0.3
Eicosanoic (20:0)	1.0	1.2
19-Methyleicosanoic (i-21:0)	0.1	0.5
18-Methyleicosanoic (ai-21:0)	0.1	0.1
Heneicosanoic (21:0)	0.1	0.5
2-Hydroxyeicosanoic (h-20:0)	0.2	—
Docosanoic (22:0)	0.3	—
2-Hydroxyheneicosanoic (h-21:0)	0.1	—
2-Hydroxydocosanoic (h-22:0)	0.6	—
17-Tetracosenoic (24:1)	0.1	0.5
Tetracosanoic (24:0)	0.1	—

TABLE 1. Continued.

Fatty Acid	*Chondrosia remiformis*[a] (wt %)	*Myrmekioderma styx*[b] (wt %)
23-Methyltetracosanoic (i-25:0)	0.3	—
22-Methyltetracosanoic (ai-25:0)	0.1	—
Pentacosanoic (25:0)	0.4	6.2
2-Hydroxytetracosanoic (h-24:0)	0.5	—
9-Hexacosenoic (26:1)	—	0.5
24-Methyl-5,9-pentacosadienoic (i-26:2)	—	1.3
5,9-Hexacosadienoic (26:2)	—	1.1
9-Heptacosenoic (27:1)	—	0.2
25-Methyl-5,9-hexacosadienoic (i-27:2)	—	3.9
24-Methyl-5,9-hexacosadienoic (ai-27:2)	—	1.7
5,9-Heptacosadienoic (27:2)	—	1.0
26-Methyl-5,9-heptacosadienoic (i-28:2)	—	1.0
25-Methyl-5,9-heptacosadienoic (ai-28:2)	—	0.3
5,9-Octacosadienoic (28:2)	0.6	0.2
22-Methyl-5,9-octacosadienoic (29:2)	—	2.0
27-Methyl-5,9-octacosadienoic (i-29:2)[c]	0.1	0.3
5,9,23-Nonacosatrienoic (29:3)	0.2	—
26-Methyl-5,9-octacosadienoic (ai-29:2)[c]	0.1	0.2
5,9-Nonacosadienoic (29:2)	—	0.1
5,9,23-Tricontatrienoic (30:3)	29.0	—

[a]Other minor acids identified include: 15:1, br-18:0, and 26:0.

[b]The following aldehydes were also identified arising from the hydrolysis of alkenyl ethers in plasmalogens: 18:0, 21:0, and 25:0.

[c]Not previously found in nature.

tered on three fatty acids, one relatively short and two long-chain acids. The shortest acid, as the methyl ester, displayed a molecular ion peak at *m/z* 280 and a base peak at *m/z* 81. This information revealed a $\Delta^{5,9}$ heptadecadienoic fatty acid methyl ester (FAME). The base peak at *m/z* 81 is characteristic for $\Delta^{5,9}$ fatty acid methyl esters (4). The olefin positions were confirmed by two different methods. The first method of choice was to synthesize the corresponding pyrrolidide (6). The eims of the corresponding pyrrolidide derivative displayed a strong peak at *m/z* 180, due to allylic cleavage between C-7 and C-8, and this confirmed the double bond arrangement. The second independent method was to react this $\Delta^{5,9}$ heptadecadienoic FAME with dimethyl disulfide (7), since it afforded a cyclic thiophene. The ms of the cyclic thiophene confirmed cleavage between C-5 and C-6, yielding the fragments at *m/z* 161 $[C_7H_{13}SO_2]^+$ and *m/z* 245 $[C_{13}H_{25}S]^+$, while fragmentation between C-9 and C-10 afforded fragments at *m/z* 247 $[C_{11}H_{19}S_2O_2]^+$ and *m/z* 159 $[C_9H_{19}S]^+$. Once the double bond positions were unequivocally determined, the stereochemistry was established by capillary gc-Ft-ir (8). The double bonds were determined to be cis since the ir spectra of the methyl ester exhibited absorption around 705 cm^{-1} (out-of-plane bending vibration) and no absorption in the 960–980 cm^{-1} region. Methyl branching remained to be established in this compound since the methyl ester of the $\Delta^{5,9}$ heptadecadienoic fatty acid displayed an equivalent chain-length (ECL) value of 16.11. Catalytic hydrogenation (PtO_2) was the key experiment, since the diunsaturated methyl ester was transformed into the known 15-methyl-

hexadecanoic acid methyl ester, which has an ECL value of 16.64. Therefore, the unknown was characterized as 15-methyl-5,9-hexadecadienoic acid [**3**].

Two unusual very-long-chain FAMEs, identified in both *Ch. remiformis* and *M. styx*, displayed similar mass spectra. One of these esters presented an ECL value of 28.10 and the other ester a value of 28.48. Upon eims both showed a molecular ion at *m/z* 448 as evidence of a nonacosadienoic acid methyl ester and a base peak at *m/z* 81, suggesting a $\Delta^{5,9}$ double bond arrangement. The olefin positions were again confirmed by preparing both the pyrrolidide and dimethyl disulfide derivatives. The pyrrolidide derivatives for these methyl esters presented similar spectra upon eims. The typical base peak at *m/z* 113 (McLafferty rearrangement) was present, and a strong peak at *m/z* 180 (due to allylic cleavage between C-7 and C-8) was also quite conspicuous in favor of $\Delta^{5,9}$ unsaturation. Reaction of both FAMEs with dimethyl disulfide resulted in rearrangement to a cyclic thiophene. The ms of the derivatives confirmed cleavage between C-5 and C-6, affording the fragments at *m/z* 161 $[C_7H_{13}SO_2]^+$ and *m/z* 413 $[C_{25}H_{49}S_2]^+$, while fragmentation between C-9 and C-10 afforded fragments at *m/z* 247 $[C_{11}H_{19}S_2O_2]^+$ and *m/z* 327 $[C_{21}H_{43}S]^+$. While the ms fragmentation pattern of these derivatives correlated well with the $\Delta^{5,9}$ arrangement, methyl branching remained to be established since catalytic hydrogenation (PtO_2) of both original methyl esters did not afford nonacosadienoic acid methyl ester. The methyl branching in these esters was confirmed in several ways. The hydrogenated pyrrolidide derrivatives of the compounds indicated the location of methyl branching. One of the compounds displayed a diminished peak at *m/z* 462 (C_{27}) concurrent with enhanced *m/z* 448 (C_{26}) and *m/z* 476 (C_{28}) fragments, indicative of iso methyl branching (6). The other compound, as the hydrogenated pyrrolidide, displayed a diminished peak at *m/z* 448 (C_{26}) concurrent with enhanced *m/z* 434 (C_{25}) and *m/z* 462 (C_{27}) fragments, indicative of anteiso methyl branching (6). Since in *M. styx* we also isolated previously identified iso/anteiso $\Delta^{5,9}$–27:2 and $\Delta^{5,9}$–28:2 methyl esters, we plotted log retention time (for both the saturated and diunsaturated methyl esters) vs. number of carbon atoms for the two series (iso and anteiso) of the three chain lengths (C_{27}–C_{29}). A straight line was obtained for both the iso and the anteiso series. The double bonds were determined to be cis since all infrared spectra exhibited absorption around 705 cm^{-1} (out-of-plane bending vibration) and no absorption in the 960–980 cm^{-1} region. From all of the experimental data we conclude that we identified 27-methyl-5,9-octacosadienoic acid [**1**] and 26-methyl-5,9-octacosadienoic acid [**2**], which have not been identified before in nature.

A third nonacosadienoic acid, which was isolated from *M. styx* but not *Ch. remiformis*, was characterized as 22-methyl-5,9-octacosadienoic acid and was previously reported from the phospholipids of *Aplysina fistularis* (9).

In summary, this work expands our knowledge of possible iso/anteiso $\Delta^{5,9}$ fatty acids in the phospholipids of sponges from C-17 to C-29. From previous biosynthetic experiments we can postulate the biosynthetic route shown below for the iso/anteiso nonacosadienoic acids (10):

br-17:0↦br-29:0↦br-Δ^5- and Δ^9-29:1↦br-$\Delta^{5,9}$-29:2

Identification of the iso-17:2 $\Delta^{5,9}$ acid is interesting, not only because it has never before been reported in nature, but because it represents a $\Delta^{5,9}$ fatty acid of a chain length typical of bacteria. Are there bacteria capable of using the same biosynthetic routes as sponge cells? Work is in progress trying to answer this question.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—FAMEs were analyzed by gc-ms using a 59970 MS ChemStation (Hewlett-Packard, Palo Alto, CA) equipped with a 30 m×0.32 mm nonpolar fused silica column (Supelco, Bellefonte, PA) with SPB™-1 as the bonded phase. Gc-Ft-ir spectra were recorded on a Nicolet (Madison, WI) 740 FT-IR spectrometer. The sponges were freeze-dried or lyophilized.

COLLECTION OF *CH. REMIFORMIS* AND *M. STYX*.—*M. styx* was collected August 1, 1992, near Mona island, Puerto Rico, at a depth of 25 m. *Ch. remiformis* was collected from the roots of the mangroves in La Parguera, Puerto Rico, at a depth of 1 ft. Voucher specimens of the sponges are available at the Department of Chemistry of the University of Puerto Rico, Rio Piedras, Puerto Rico. Dr. Vance Vicente from the National Marine Fisheries Service (NOAA) classified the sponges.

EXTRACTION AND ISOLATION OF PHOSPHOLIPIDS.—The sponges (40 g) were carefully cleaned of all nonsponge debris and cut into small pieces. Extraction with 250 ml of $CHCl_3$-MeOH (1:1) yielded the total lipids. The neutral lipids, glycolipids, and phospholipids (20 mg) were separated by cc on Si gel (60–200 mesh) using the procedure of Privett *et al.* (11). The phospholipid classes were fractionated by preparative tlc using Si gel 60 and $CHCl_3$-MeOH-NH_4OH (65:35:5) as solvent.

PREPARATION AND ISOLATION OF FATTY ACID DERIVATIVES.—The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction of the phospholipids with methanolic HCl (12) followed by cc purification eluting with *n*-hexane–Et_2O (9:1). For the location of double bonds, pyrrolidides were prepared by direct treatment of the methyl esters with pyrrolidine-HOAc (10:1) in a capped vial (2 h at 100°) followed by ethereal extraction from the acidified solution and purification by preparative tlc (9). The double bond position of the monoenoic acids was elucidated by preparing the corresponding dimethyl disulfide derivatives by dissolving the esters (2 mg) in dimethyl disulfide (0.2 ml), adding a solution (0.05 ml) of I_2 in Et_2O (60 mg/ml), and heating the solution at 50° for 24 h, followed by the standard workup (13). Hydrogenations were carried out in 10 ml of MeOH and catalytic amounts of PtO_2. Spectral data follow for the key fatty acids for this discussion.

Methyl 15-methyl-5,9-hexadecadienoate.—Ms *m/z* (rel. int.) $[M]^+$ 280 (1.8), 270 (1), 266 (0.4), 249 (0.5), 248 (0.5), 241 (0.8), 233 (0.5), 227 (1), 213 (0.9), 206 (1.8), 199 (1.5), 197 (1.7), 193 (0.8), 185 (1), 181 (1.8), 180 (1.3), 173 (1), 171 (1.6), 165 (5), 164 (2.2), 157 (3), 150 (5), 141 (12), 135 (6), 123 (6), 111 (6), 109 (30), 99 (14), 95 (23), 87 (20), 81 (100), 79 (39), 74 (51), 69 (34), 67 (88), 65 (13), 59 (34), 57 (35).

N-*15-Methyl-5,9-hexadecadienoylpyrrolidine.*—Ms *m/z* (rel. int.) $[M]^+$ 319 (1), 306 (0.4), 264 (0.4), 250 (0.7), 234 (0.4), 180 (14), 126 (19), 113 (100), 98 (23), 85 (13), 70 (24), 55 (52).

2-(4-Methoxycarbonyl-1-methylthiobutan-1-yl)-5-(1-methylthio-6-methylhept-1-yl)tetrahydrothiophene.—Ms *m/z* (rel. int.) $[M]^+$ 406 (14), $[M-CH_3SH]^+$ 358 (4), $[C_{11}H_{19}S_2O_2]^+$ 247 (19), $[C_{13}H_{25}S]^+$ 245 (38), $[C_{10}H_{15}SO_2]^+$ 199 (70), $[C_{12}H_{21}S]^+$ 197 (84), $[C_9H_{11}SO]^+$ 167 (55), $[C_7H_{13}SO_2]^+$ 161 (14), $[C_9H_{19}S]^+$ 159 (15), $[C_6H_9SO]^+$ 129 (24), $[C_6H_9O_2]^+$ 113 (15), $[C_5H_5O]^+$ 81 (44).

Methyl 27-methyl-5,9-octacosadienoate.—Ms *m/z* (rel. int.) $[M]^+$ 448 (2), 418 (1), 398 (2), 299 (1), 271 (5), 255 (2.6), 215 (2), 181 (3), 171 (2), 168 (2), 164 (4), 163 (3), 157 (3), 154 (2.7), 150 (8), 141 (15), 136 (9), 133 (5), 131 (7), 129 (4), 123 (5), 121 (8), 109 (30), 105 (9), 99 (12.5), 95 (22), 91 (19), 81 (100), 79 (29), 74 (30), 69 (45), 67 (76), 59 (12), 57 (80).

N-*27-Methyl-5,9-octacosadienoylpyrrolidine.*—Ms *m/z* (rel. int.) $[M]^+$ 487 (2), 234 (0.7), 220 (0.5), 181 (4), 180 (28), 166 (1), 154 (0.5), 140 (0.9), 126 (15), 113 (100), 98 (20), 85 (11), 71 (16), 57 (25), 55 (34).

2-(4-Methoxycarbonyl-1-methylthiobutan-1-yl)-5-(1-methylthio-18-methylnonadecan-1-yl)tetrahydrothiophene.—Ms *m/z* (rel. int.) $[M-CH_3SH]^+$ 526 (3), $[C_{25}H_{49}S_2]^+$ 413 (5.5), $[C_{24}H_{45}S]^+$ 365 (31), $[C_{21}H_{43}S]^+$ 327 (4.5), $[C_{11}H_{19}S_2O_2]^+$ 247 (22), $[C_{10}H_{15}SO_2]^+$ 199 (42), $[C_9H_{11}SO]^+$ 167 (41), $[C_7H_{13}SO_2]^+$ 161 (16), $[C_6H_9SO]^+$ 129 (24), $[C_6H_9O_2]^+$ 113 (14), $[C_5H_5O]^+$ 81 (37).

Methyl 26-methyl-5,9-octacosadienoate.—Ms *m/z* (rel. int.) $[M]^+$ 448 (2.5), 417 (0.4), 416 (1), 404 (1), 306 (0.6), 292 (0.3), 195 (0.5), 181 (3), 167 (1), 163 (1), 155 (0.5), 150 (3), 141 (5), 136 (3.3), 133 (0.8), 123 (3), 121 (2.5), 109 (10), 99 (6), 97 (6), 95 (9), 91 (3), 81 (100), 79 (11), 74 (17), 71 (21), 69 (27), 67 (32).

2-(4-Methoxycarbonyl-1-methylthiobutan-1-yl)-5-(1-methylthio-17-methylnonadecan-1-yl)tetrahydrothiophene.—Ms *m/z* (rel. int.) $[M-CH_3SH]^+$ 526 (3), $[C_{25}H_{49}S_2]^+$ 413 (15.5), $[C_{24}H_{45}S]^+$ 365 (25), $[C_{21}H_{43}S]^+$ 327 (5), $[C_{11}H_{19}S_2O_2]^+$ 247 (39), $[C_{10}H_{15}SO_2]^+$ 199 (69), $[C_9H_{11}SO]^+$ 167 (48), $[C_7H_{13}SO_2]^+$ 161 (18), $[C_6H_9SO]^+$ 129 (28), $[C_6H_9O_2]^+$ 113 (18), $[C_5H_5O]^+$ 81 (42).

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