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NOVEL NATURALLY OCCURRING α-METHOXY ACIDS FROM THE PHOSPHOLIPIDS OF CARIBBEAN SPONGES[1]

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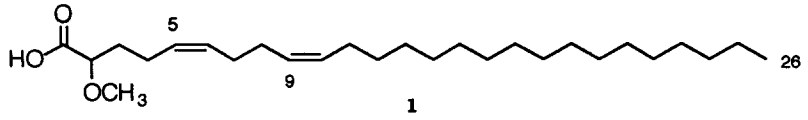
Abstract: The novel very long chain (5Z,9Z)-2-methoxy-5,9-hexacosadienoic acid (**1**) was identified in the phospholipids of the Caribbean sponge *Tropsentia roquensis*. The new acid (5Z)-2-methoxy-5-hexadecenoic acid (**2**) was also identified in the phospholipids of the sponge *Mycale laxissima* while the acid (6Z)-2-methoxy-6-hexadecenoic acid (**3**) was found to be present in the phospholipids of the Caribbean sponge *Spheciospongia cuspidifera*.. The double bond positions in the monounsatutared acids were determined by GC/MS on the corresponding dimethyldisulfide adducts and the double bond stereochemistry was elucidated by GC/FTIR. These fatty acids were found in phosphatidylethanolamine.

Phospholipids with α-substituted fatty acids are quite rare in nature but sponges have provided the most striking examples. Two particularly interesting cases have been the α-methoxy and α-hydroxy phospholipid bound fatty acids. For example, recent work by *Ayanoglu et al.*[2] on the African sponge *Higginsia tethyoides* has revealed the intriguing α-methoxy acids 2-methoxy-17-tetracosenoic acid, 2-methoxy-19-hexacosenoic acid, and 2-methoxy-21-octacosenoic acid. We also recently encountered a series of phospholipids possessing α-hydroxy acids, namely the acids 2-hydroxydocosanoic and 2-hydroxytricosanoic which were isolated from the sponge *Amphimedon compressa*[3]. *Ayanoglu et al.*[4] have also recently uncovered another class of demospongic acids, namely totally saturated 2-acetoxy C_{22}-C_{30} acids from the sponge *Polymastia gleneni*. These probably are the only known examples of 2-oxo-substituted fatty acids in the phospholipids of sponges, mostly occurring in phosphatidylethanolamine. Interesting to mention in this respect is that these acids are normally found in very small amounts, practically in milligram quantities, but with the advent of new analytical techniques, such as GC/MS and GC/FTIR, it is now possible to deal with minute amounts of substances and to unambiguously elucidate these structures. We now wish to report the isolation and structure elucidation of three new phospholipid acids, namely the long chain acid (5Z,9Z)-2-methoxy-5,9-hexacosadienoic acid (**1**) from *Tropsentia roquensis*,



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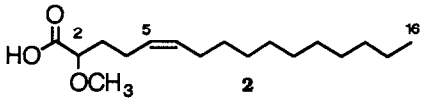
(5Z)-2-methoxy-5-hexadecenoic acid (**2**) from the phospholipids of the sponge *Mycale laxissima*, and (6Z)-2-methoxy-6-hexadecenoic acid (**3**) from the phospholipids of the Caribbean sponge *Spheciospongia cuspidifera* .



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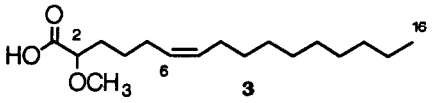

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Capillary GC and GC/MS analysis of the mixture gave the principal acids described in Table 1 for *M. laxissima* , *S. cuspidifera* , and *T. roquensis*. As can be seen from the Table, *S. cuspidifera* has a series of α-hydroxy fatty acids, the most abundant being those between C_{22}-C_{25} carbons. These acids were characterized as mixtures by GC/MS as well as by comparing their equivalent chain length (ECL) values with known standards.

Table 1. Principal Fatty Acids from *M. laxissima* , *S. cuspidifera*, and *T. roquensis* [a].

Fatty Acid	Abundance (%)		
	M.laxissima	*S. cuspidifera*	*T. roquensis*
Tetradecanoic (14:0)	3	7	2
4,8,12-Trimethyltridecanoic (16:0)	-	11	4
Pentadecanoic (15:0)[b]	2	-	7
9-Hexadecenoic (16:1)	3	2	3
Hexadecanoic (16:0)	14	10	16
Heptadecanoic (17:0)	2	3	10
Octadecenoic (18:1)[c]	4	5	-
2-Methoxy-5-hexadecenoic (16:1)	1	-	-
2-Methoxy-6-hexadecenoic (16:1)	-	4	-
Octadecanoic (18:0)	11	5	3
Nonadecanoic (19:0)[b]	6	6	11
Eicosanoic (20:0)	2	5	1
Heneicosanoic (21:0)	1	2	1
2-Hydroxydocosanoic (22:0)	-	2	-
2-Hydroxytricosanoic (23:0)	-	1	-
2-Hydroxytetracosanoic (24:0)	-	5	-
2-Hydroxypentacosanoic (25:0)	-	2	-
5,9-Tetracosadienoic (24:2)	-	-	4
5,9-Pentacosadienoic (25:2)	6	4	4
5,9-Hexacosadienoic (26:2)	15	18	27
2-Methoxy-5,9-hexacosadienoic (26:2)	-	-	3

a) Some minor acids are not included in this table.

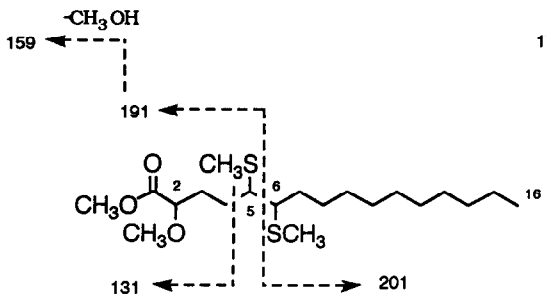
b) These normally were a mixture of the branched iso-anteiso acids.

c) Includes the Δ9 and Δ11 isomers.

The most interesting fatty acid from the sponge *T. roquensis* turned out to be a new methoxy acid (3% abundance) which was characterized as (5Z,9Z)-2-methoxy-5,9-hexacosadienoic acid (**1**) from its spectral data. The corresponding fatty acid methyl ester **1a** exhibited a long retention time in GC (ECL= 26.2), possibly due to polar functionalities, and the mass spectrum displayed a molecular ion at *m/z* 436. Important fragmentations confirming the presence of a 2-methoxy substituent were observed at *m/z* 104 ($C_4H_8O_3^+$, 100%) arising from a McLafferty rearrangement, *m/z* 404 (M^+-CH_3OH, 2%), *m/z* 377 (M^+-$COOCH_3$, 3%) and *m/z* 345 (M^+-$COOCH_3$ + CH_3OH, 3%). The double bond positions were determined by permanganate-periodate oxidation. The new methoxy fatty acid methyl ester in question could be easily separated from the other fatty acid methyl esters in the mixture by preparative TLC using silica gel and hexane/ether (9:1, v/v) as solvent. Oxidation of the purified methyl ester followed by esterification in 1.2N HCl/MeOH afforded methyl heptadecanoate as one of the fragments, thus establishing between carbons 9 and 10 the last double bond in the chain. The second double bond was found to be between carbons 5 and 6 from the spectrum of the corresponding pyrrolidide derivative **1b** since a prominent peak at *m/z* 210 ($C_{12}H_{20}NO_2^+$, 4%), resulting from allylic cleavage between C_7 and C_8, was observed[5]. The double bond stereochemistry was found to be *cis* since there was no absorption at 980-968 cm^{-1}.

The most interesting fatty acid methyl ester from *M.laxissima* (1% abundance) presented quite a challenge for its characterization due to the small amounts of this acid in a complex mixture of more than 25 fatty acid methyl esters. Capillary GC on a DB-1 30m column and GC/MS became the principal tools for the characterization. The unknown ester exhibited unusual chromatographic properties, that is an equivalent chain length (ECL) value of 17.20. The mass spectrum presented a molecular weight at *m/z* 298 (1%) and a base peak at *m/z* 104, arising from a McLafferty rearrangement. The additional 30 amu, when compared with the typical base peak of saturated fatty acid methyl esters at *m/z* 74, could only be provided by a methoxy group at the 2-position. Important for the characterization were also fragmentations at *m/z* 266 (M^+-MeOH, 2%) , *m/z* 239 (M^+- $COOCH_3$,5%) , and *m/z* 207 (M^+-$COOCH_3$ + CH_3OH, 2%). In fact, comparison of this mass spectrum with similar spectra for other 2-methoxy acids[2] confirmed that we have identified a novel 2-methoxyhexadecenoic acid. The double bond position was determined by preparing the corresponding dimethyldisulphide adduct, a convenient method for the location of double bonds by mass spectrometry[6,7]. The mass spectrum of the corresponding dimethyldisulphide adduct afforded a molecular ion peak at *m/z* 392 (M^+, 4%). The double bond was readily determined to be at carbon 5 by the fragmentations at *m/z* 201 ($C_{11}H_{22}SCH_3^+$, 31%) and *m/z* 191 ($C_8H_{15}SO_3^+$, 100%) since this constitutes cleavage between carbons 5 and 6. There was also a prominent peak at *m/z* 159 (91%) corresponding to the *m/z* 191 fragment with the loss of methanol; these fragmentations are shown in **4**. The experimental data thus supported the methyl ester of (5Z)-2-methoxy-5-hexadecenoic acid (**2**) as the new compound. The double

bond stereochemistry was found to be *cis* since there was no prominent absorption in the 980-968 cm^{-1} region .



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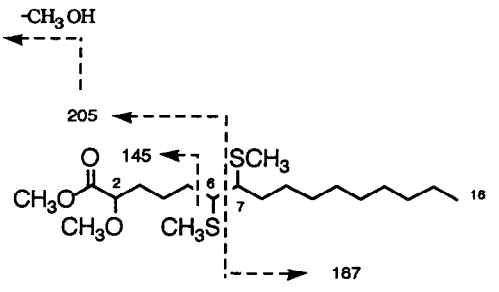

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The new methoxy fatty acid methyl ester (4% abundance) from *S. cuspidifera* also exhibited a long retention time in GC (equivalent chain length value of 17.22), unusual for the molecular weight of this ester. The mass spectrum presented a molecular weight at *m/z* 298 (1%) and the same base peak at *m/z* 104 (McLafferty rearrangement). Other key fragmentations were also found at *m/z* 266 (M^+-MeOH, 6%), *m/z* 239 (M^+- $COOCH_3$, 5%) and *m/z* 207 (M^+-$COOCH_3$ + CH_3OH, 6%). Therefore, this suggested that the compound in question was the same or an isomer of **2**. The dimethyldisulphide adduct was again prepared as described in the Experimental and this unequivocally determined the double bond position to be between carbons 6 and 7. In this case, the molecular ion peak was also observed at *m/z* 392 (M^+, 3%) and the double bond was deduced from the prominent fragments at *m/z* 187 ($C_{10}H_{20}SCH_3^+$, 37%) and *m/z* 205 ($C_9H_{17}SO_3^+$, 100%) since this constitutes cleavage between carbons 6 and 7 as exemplified in **5**. There was also a prominent peak at *m/z* 173 (37%) corresponding to the 205 fragment with the concomitant loss of methanol. The double bond stereochemistry was also found to be *cis* since there was no absorption at 980-968 cm^{-1}. The unknown turned out to be the fatty acid methyl ester of (6Z)-2-methoxy-6-hexadecenoic acid (**3**) which is also unprecedented in nature.

The acids reported here were mainly found in phosphatidylethanolamine. Naturally occurring 2-hydroxy fatty acids are known to possess the R configuration and the determination by *Ayanoglu et al.*[8] that methoxy fatty acids from sponges have the R configuration at the α-carbon, tends to indicate that the compounds presented here also possess the R configuration. The biosynthetic origin of these novel acids is presently a matter of speculation and much biosynthetic work is needed in order to put in perspective the origin of these novel α-methoxy acids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES. The sponges were collected in July, 1989 near the shelf edge of La Parguera, Puerto Rico at a depth of 15 m. The sponges (500 g) were

washed in sea water, carefully cleaned of all nonsponge debris and cut into small pieces. Immediate extraction with 600 mL of chloroform/ methanol (1:1, v/v) yielded the total lipids. The neutral lipids, glycolipids and phospholipids (100 mg) were separated by column chromatography on silica gel (60-200 mesh) using a procedure similar to that of Privett *et al.*[9], where silica gel is used instead of silicic acid. The phospholipid classes were fractionated by preparative thin-layer chromatography (TLC) using silica gel G and chloroform/methanol/ water (25:10:1, v/v/v) as solvent. The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction of the phospholipids with methanolic hydrogen chloride[10] followed by column chromatographic purification and elution with hexane/ diethyl ether (9:1, v/v). The resulting methyl esters were analyzed by gas chromatography-mass spectrometry using either a Hewlett Packard 5995 A gas chromatograph-mass spectrometer or a Hewlett Packard 59970 MS ChemStation equipped with a 30 m X 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 mass spectra of the key fatty acid methyl esters follows.

Methyl 2-methoxy-5,9-hexacosadienoate (**1a**) MS (70eV) m/z (relative intensity), 436 (M^+, 0.1), 404 (2), 377(3), 345(3), 193(1), 180(2), 171(3), 166(4), 165(5), 159(2), 151(4), 135(7), 139(26), 138(10), 121(8), 111(23), 107(14), 104(100), 97(13), 95(15), 93(10), 91(6), 84(7), 81(38), 79(39), 71(17), 67(30), 57(23), 55(31).

Methyl (5Z)-2-methoxy-5-hexadecenoate (**2a**) MS (70eV) *m/z* (relative intensity), 298 (M^+,1), 266 (M^+-CH_3OH,12), 239(M^+-CO_2CH_3,5), 207(1.7), 206(2.6), 180(1), 150(3), 139(3), 135(3), 127(2), 123(4), 121(4), 117(2), 111(12), 109(8), 104(100), 97(16), 95(13), 93(16), 89(9), 87(6), 83(12), 81(14), 79(11), 75(5), 71(7), 69(14), 67(16), 57(10), 55(21).

Methyl (6Z)-2-methoxy-6-hexadecenoate (**3a**) MS (70 eV) m/z (relative intensity), 298(M^+,1), 266(M^+-CH_3OH,4), 239(M^+-CO_2CH_3,20), 207(6), 206(7), 180(6), 150(11), 139(5), 138(8), 136(10), 127(8), 123(13), 121(12), 117(6), 111(18), 109(19), 104(100), 97(21), 95(39), 93(17), 88(12), 87(16), 83(18), 81(35), 79(19), 74(16), 71(30), 69(25), 67(37), 57(22), 55(48).

Dimethyldisulphide adducts. Dimethyldisulphide derivatives were prepared by dissolving the esters (2 mg) in dimethyldisulphide (0.2mL) and adding a solution (0.05mL) of iodine in diethyl ether (60mg/mL). The solution was heated at 50°C for 24h followed by the standard purification method[7]. Spectral data for the key acids follows.

Methyl 2-methoxy-5,6-bis(methylthio)hexadecanoate (**4**) MS(70 eV) *m/z* (relative intensity), 392 (M^+, 4), 201(31), 191 (100), 160(10), 159(91), 131(52), 111(17), 97(23), 95(11), 87(18), 85(13), 83(19), 81(13), 79(13), 75(15), 74(17), 71(27), 69(26), 67(19), 61(33), 59(17), 57(20), 55(39).

Methyl 2-methoxy-6,7-bis(methylthio)hexadecanoate (**5**) MS(70 eV) *m/z* (relative intensity), 392 (M^+, 3), 206(9), 205(100), 187(37), 173(37), 145(23), 125(12), 97(32), 95(16), 93(11), 87(64), 85(12), 83(28), 81(21), 79(12), 75(11), 71(45), 69(27), 67(29), 61(44), 57(19), 55(46).

N-acylpyrrolidide derivatives. Were prepared by direct treatment of the methyl esters with pyrrolidine/acetic acid (10:1, v/v) in a capped vial (2 hr at 100 °C) followed by ethereal

extraction from the acidified solution and purification by preparative TLC. Spectral data for the long-chain acid follows.

N-2-methoxyhexacosa-5,9-dienoylpyrrolidine (**1b**) MS (70eV) m/z (relative intensity), 475(M^+,1), 210(4), 196(2), 179(3), 178(2), 156(2), 144(5), 143(100), 135(3), 128(27), 126(11), 121(5), 114(3), 113(4), 107(3), 98(23), 81(12), 79(12), 69(12), 67(16), 58(12), 57(16), 55(34).

Permanganate-periodate oxidation. A stock oxidant solution of sodium metaperiodate (2.09g) and potassium permanganate (0.04g) in water (100ml) was prepared. This solution (1 ml) together with potassium carbonate solution (1 ml; 2.5g/l) was added to the methyl ester (1 mg) in *tert* -butanol (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 sulphuric acid, and excess oxidant was destroyed with sodium bisulfite. The solution was extracted thoroughly with diethyl ether (3 X 4 ml). The organic layer was dried over sodium sulphate and removed in a stream of nitrogen at room temperature. The products were methylated with 1.2N HCl/MeOH for GC analysis.

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