Identification of 2-Methoxyhexadecanoic Acid in *Amphimedon compressa*

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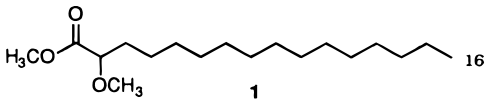
The phospholipid fatty acid composition of *Amphimedon compressa* was reinvestigated and the 2-methoxyhexadecanoic acid was identified for the first time in nature. Structure characterization was accomplished by means of gas chromatography−mass spectrometry and total synthesis from commercially available 2-hydroxyhexadecanoic acid.

Naturally occurring α-methoxy fatty acids have been identified only from the phospholipids of sponges.[1−3] Some examples include normal-chain saturated 2-methoxy fatty acids between 19 and 24 carbons and very long-chain monounsaturated fatty acids such as (2*R*,-21*Z*)-2-methoxy-21-octacosenoic acid, which was the first-reported naturally occurring α-methoxy fatty acid from a phospholipid.[2] Just recently, we identified 2-methoxy-5-hexadecenoic acid and 2-methoxy-6-hexadecenoic acid from the phospholipids of several Caribbean sponges.[3] We also established that the 2-methoxy-5-hexadecenoic acid can be found more often in the phospholipids of sponges, but the Δ6 analogue is quite rare.[4] Despite all of these efforts, the parent and perhaps more classic 2-methoxyhexadecanoic acid has neither been isolated nor identified from any natural source. The closest naturally occurring structure known is a glycerol ether, namely 1-*O*-(2-methoxyhexadecyl) glycerol, which was isolated from Greenland shark liver oil.[5] In the present communication we report the first naturally occurring 2-methoxyhexadecanoic acid, which we found in a reinvestigation of the sponge *Amphimedon compressa* Pallas (order Haplosclerida, family Nephatidae).[4]

The main phospholipids from the present *A. compressa* were also identified as phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine.[4] A reexamination of the fatty acid composition of this *A. compressa* resulted in a similar fatty acid composition to that which we previously reported, which the key fatty acids are 5,9,23-nonacosatrienoic acid and 5,9,23-tricontatrienoic acid, together with a series of unusual monounsaturated fatty acids ranging in length between C_{22} and C_{26}.[4] The 2-methoxy-5-hexadecenoic acid was also identified in this fatty acid mixture. Essential for the characterization were GC retention times of the corresponding fatty acid methyl esters, MS data on the methyl esters and their corresponding dimethyl disulfide derivatives, and catalytic hydrogenation of the whole fatty acid methyl ester mixture for easier elucidation of methyl branching.

Our main interest was centered on the new compound, 2-methoxyhexadecanoic acid, which was characterized as the methyl ester in 0.5% relative abundance as compared to the other 30 fatty acids in the mixture. The GC retention time of methyl ester **1** corresponded to an equivalent chain-length (ECL) value of 17.22, implying unusual branching. The MS of **1** displayed a molecular ion peak at *m*/*z* 300 and a strong $M^+ - 59$ peak at *m*/*z* 241, together with a small peak at *m*/*z* 104 (McLafferty rearrangement).[4] This information strongly suggested methyl 2-methoxyhexadecanoate (**1**) as the most probable structure. Final structure characterization was achieved by total synthesis. Methyl 2-methoxyhexadecanoate (**1**) is a known synthetic compound, but its synthesis was achieved through a synthetic scheme ending with the reaction of methyl 2-bromohexadecanoate with sodium methoxide.[6,7] We employed a simpler synthesis through the double methylation of commercially available 2-hydroxyhexadecanoic acid (Matreya, Inc.). Several methods were initially tried for this transformation, for example, direct double methylation with CH_2N_2, reaction of the α-hydroxy acid with NaOH and methyl sulfate, and reaction with MeI in refluxing K_2CO_3. None of these procedures was effective in this double methylation, presumably in part due to hydrogen bonding of the α-hydroxy to the carbonyl. Our best results, however, were obtained by reacting 2-hydroxyhexadecanoic acid with NaH and MeI in DMSO, which afforded the desired methyl 2-methoxyhexadecanoate (**1**) in 70−80% yields. Synthetic methyl ester **1** was co-injected in GC with the isolated sample from *A. compressa*, and they proved to be identical. Complete NMR data for **1** was also obtained and is presented in the Experimental Section for comparison.[8] α-Methoxy fatty acids from sponges are known to have the *R* configuration at the α-carbon, and **1** may well follow this trend.[1]



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The identification of 2-methoxyhexadecanoic acid in *A. compressa* has biosynthetic relevance. For example, the commonly occurring 2-methoxy-5-hexadecenoic acid in sponges may well originate from Δ5 desaturation on the 2-methoxyhexadecanoic acid, which is reported here for the first time as a naturally occurring fatty acid. Work is in progress elucidating the origin of unusual fatty acids in marine invertebrates.

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Experimental Section

General Experimental Procedures. Fatty acid methyl esters were analyzed by GC in a Hewlett−Packard 5890A Series II gas chromatograph equipped with a fused silica capillary column (30 m × 0.32 mm i.d.) containing either SE−54 or SPBTM^{-1} (carrier gas He). Analyses were performed as previously described. Samples were also analyzed by GC−MS at 70 eV using a Hewlett−Packard 5972A MS ChemStation equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethyl siloxane cross-linked with 5% phenyl methylpolysiloxane. NMR data were collected in a Bruker Avance DPX 300 spectrometer. *J* values are given in Hz.

Sample Collection. *A. compressa* was collected November 1, 1997, at Cayo Enrique, Lajas, Puerto Rico, at a depth between 1 and 2 m. The sponge was washed in seawater, carefully cleaned of all nonsponge debris, and lyophilized. A voucher specimen is available at the Department of Chemistry of the University of Puerto Rico, Río Piedras campus.

Extraction and Isolation of Phospholipids. The sponge (250 g) was carefully cleaned and cut into small pieces. Extraction with 2 × 250 mL of $CHCl_3$−MeOH (1:1) yielded the total lipids (6.7 g). The neutral lipids, glycolipids, and phospholipids (480 mg) were separated by column chromatography on Si gel (60−200 mesh) using the procedure of Privett et al.[9]

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 followed by column chromatography.[4] The double-bond positions in the polyunsaturated fatty acids were determined by preparing the corresponding dimethyl disulfide derivatives.[4] Hydrogenations were carried out in 10 mL of MeOH and catalytic amounts of PtO_2.

Methyl 2-Methoxyhexadecanoate (**1**). Into a two-necked round-bottom flask provided with a magnetic stirrer and under a nitrogen atmosphere, were placed 0.10 g (0.37 mmol) of 2-hydroxyhexadecanoic acid in 3 mL of DMSO. Separately, two equivalents of NaH were dissolved in 1 mL of DMSO (also under a nitrogen atmosphere) and added dropwise, after which the reaction mixture was stirred at room temperature for 10 min. An excess of MeI was then added, and the reaction mixture was further stirred for 20 min. After this time the reaction mixture was diluted with hexane−ether (1:1) and washed twice with H_2O to remove the DMSO. The organic phase was dried over $MgSO_4$, filtered, and evaporated in vacuo, affording 0.08 g (0.26 mmol, 73%) of **1**. Spectral data for **1** is presented below.

Methyl 2-methoxyhexadecanoate (**1**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.18 (1H, dd, J = 7.2, 2.9 Hz, H-2), 3.78 (3H, s, $-OCH_3$), 3.37 (3H, s, CO_2CH_3), 1.6−1.8 (2H, m, H-3), 1.2−1.4 (24H, m, $-CH_2-$), 0.87 (3H, t, J = 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.8 (s, C-1), 80.7 (q, $-OCH_3$), 58.0 (d, C-2), 52.40 (q, CO_2CH_3), 32.8 (t), 31.9 (t), 29.7 (t), 29.64 (t), 29.61 (t), 29.5 (t), 29.4 (t), 29.33 (t), 29.29 (t), 24.7 (t), 22.7 (t), 14.1 (q, C-16); GC−MS (70 eV) *m/z* 300 $[M]^+$ (2), 242 (17), 241 (100), 208 (1), 207 (2), 153 (1), 138 (1), 125 (6), 123 (2), 111 (19), 109 (3), 104 (4), 99 (2), 97 (39), 95 (8), 85 (11), 83 (42), 81 (11), 71 (43), 69 (37), 67 (13), 58 (10), 57 (34), 55 (39).

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