Notes

New Methoxylated Fatty Acids from the Caribbean Sponge *Callyspongia fallax*

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The saturated 2-methoxylated fatty acids 2-methoxytetradecanoic acid (**1**), 2-methoxypentadecanoic acid (**2**), and 2-methoxyoctadecanoic acid (**3**) as well as the Δ^6 monoenoic methoxylated fatty acids (6*Z*)-2-methoxy-6-tetradecenoic acid (**4**), (6*Z*)-2-methoxy-6-pentadecenoic acid (**5**), and (6Z)-2-methoxy-13-methyl-6-tetradecenoic acid (**7**) were identified for the first time in nature in the phospholipids from the Caribbean sponge *Callyspongia fallax*. These findings expand the occurrence of 2-methoxylated fatty acids to C_{14}−C_{15} chain lengths and establish new fatty acid biosynthetic possibilities for marine organisms. The novel methoxylated fatty acids could have originated from the phospholipids of a bacterium in symbiosis with the sponge.

Methoxylated fatty acids have been identified from just a few natural sources.[1−6] Some marine examples include the (4*E*)-7-methoxy-4-tetradecenoic acid from the marine cyanobacterium *Lyngbya majuscula*,[1,2] which is antimicrobial against Gram-positive bacteria such as *Staphylococcus aureus*, and the acids 9-methoxypentadecanoic acid and 15-methoxytricosanoic acid identified in the red algae *Schizymenia dubyi*.[3] Other acids have originated from bacteria, such as the acids 11-methoxyheptadecanoic acid and 11-methoxynonadecanoic acid, which occur among others in *Helicobacter pylori*, a bacterium from human gastric mucosae.[4] However, 2-methoxylated fatty acids have been identified only in the phospholipids of sponges.[5,6] These 2-methoxylated fatty acids share the common molecular properties of possessing the *R* configuration at the chiral center and are basically phospholipid bound.[5,6] The first 2-methoxylated fatty acids to be reported were among the phospholipids of the sponge *Higginsia tethyoides*, which contained saturated, monounsaturated, and diunsaturated 2-methoxylated fatty acids with chain lengths between 19 and 28 carbon atoms.[5,6] All of these 2-methoxylated fatty acids were straight-chain fatty acids, and the double bond positions in the monounsaturated fatty acids were mainly encountered at either Δ^{17}, Δ^{18}, Δ^{19}, or Δ^{21}. In addition, the very long chain diunsaturated fatty acids 2-methoxy-5,19-hexacosadienoic acid and 2-methoxy-7,21-octacosadienoic acid were also characterized.[5,6]

While the long-chain 2-methoxylated fatty acids (C_{24}−C_{28}) have been thought to arise from sponge cells, recent examples of short-chain analogues (C_{15}−C_{16}) are postulated to originate from bacteria in symbiosis with sponges.[7−9] In addition, the monoenoic short-chain α-methoxylated fatty acids exhibit moderate antimicrobial activity.[9] Earlier work from our laboratory described the identification and total synthesis of the 2-methoxylated fatty acids (5*Z*)-2-methoxy-5-hexadecenoic acid, (6*Z*)-2-methoxy-6-hexadecenoic acid, and the saturated 2-methoxyhexadecanoic acid from several Caribbean sponges.[8,9] While the saturated C_{16} methoxylated acid did not display any antimicrobial activity against Gram-positive bacteria, the Δ^5 and Δ^6 monounsaturated acids did show moderate activity against either *Staphylococcus aureus* or *Streptococcus faecalis*.[9] However, it is very likely that other short-chain Δ^6 2-methoxylated fatty acids (C_{14} or C_{15}) will also display antimicrobial activity. However, no other short-chain analogues have been reported to date. Therefore, in this paper we report the identification of three saturated and three Δ^6 monounsaturated 2-methoxylated fatty acids from the Caribbean sponge *Callyspongia fallax* Duchassaing and Michelotti (class Demospongiae, order Haplosclerida, family Callyspongidae).

The Caribbean sponge *C. fallax* presented a typical sponge phospholipid profile with phosphatidylethanolamine and phosphatidylserine as the two most abundant phospholipids. Acid methanolysis provided a rather complex phospholipid fatty acid composition of around 76 identifiable fatty acids, as shown in Table 1. Fatty acid chain lengths ranged between C_{14} and C_{27}, mainly consisting of saturated and monoenoic fatty acids. The *iso-anteiso* methyl-branched fatty acids were particularly abundant in this sponge; they made up 26% of the total fatty acid composition. However, a most interesting finding was the identification of a whole series of *iso* monoenoic branched-chain fatty acids with chain lengths between C_{15} and C_{17}. These monoenoic fatty acids had double bonds at either positions Δ^4, Δ^5, Δ^6, Δ^7, or Δ^9, an ample variety of monounsaturation possibilities.[11] However, as expected, the most characteristic fatty acid from *C. fallax* was the (5*Z*,9*Z*)-5,9-hexacosadienoic acid, which was identified in this sponge together with other $\Delta^{5,9}$ diunsaturated very long chain acids such as the (5Z,9Z)-5,9-pentacosadienoic acid and (5*Z*,9*Z*)-5,9-heptacosadienoic acid. All of these fatty acids were characterized as methyl esters by GC−MS, and the double bond positions in the monoenoic esters were elucidated by dimethyl disulfide (DMDS) derivatization as well as by gas chromatographic comparisons with authentic standards.

One of the most interesting series of fatty acids from the phospholipids of *C. fallax* were three saturated 2-methoxylated fatty acids identified as 2-methoxytetradecanoic

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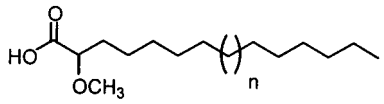
Table 1. Identified Fatty Acids from *C. fallax*

fatty acid	abundance (wt %)
(*Z*)-4-tetradecenoic (14:1)	0.05
(*Z*)-5-tetradecenoic (14:1)	0.05
(*Z*)-6-tetradecenoic (14:1)	0.1
(*Z*)-7-tetradecenoic (14:1)	0.2
tetradecanoic (14:0)	8.0
(4*Z*)-13-methyl-4-tetradecenoic (*i*-15:1)	0.3
(7*Z*)-13-methyl-7-tetradecenoic (*i*-15:1)	0.1
(9*Z*)-13-methyl-9-tetradecenoic (*i*-15:1)	0.1
4,8,12-trimethyltridecanoic (16:0)	0.5
13-methyltetradecanoic (*i*-15:0)	8.7
12-methyltetradecanoic (*ai*-15:0)	2.7
(*Z*)-4-pentadecenoic (15:1)	0.1
(*Z*)-5-pentadecenoic (15:1)	0.1
(*Z*)-6-pentadecenoic (15:1)	0.3
(6*Z*)-2-methoxy-6-tetradecenoic (2-OMe-14:1)[a]	0.3
pentadecanoic (15:0)	6.5
2-methoxytetradecanoic (2-OMe-14:0)[a]	0.2
3-methylpentadecanoic (16:0)	0.2
(4*Z*)-14-methyl-4-pentadecenoic (*i*-16:1)	0.2
(5*Z*)-14-methyl-5-pentadecenoic (*i*-16:1)	0.07
(6*Z*)-14-methyl-6-pentadecenoic (*i*-16:1)	0.03
14-methylpentadecanoic (*i*-16:0)	2.5
(6*Z*)-2-methoxy-13-methyl-6-tetradecenoic (2-OMe-*i*-15:1)[a]	0.3
(6*Z*)-2-methoxy-6-pentadecenoic (2-OMe-15:1)[a]	0.3
(*Z*)-9-hexadecenoic (16:1)	4.0
(*Z*)-11-hexadecenoic (16:1)	0.3
hexadecanoic (16:0)	9.3
2-methoxypentadecanoic (2-OMe-15:0)[a]	0.5
9-methylhexadecanoic (*br*-17:0)	0.8
10-methylhexadecanoic (*br*-17:0)	0.8
(4*Z*)-15-methyl-4-hexadecenoic (*i*-17:1)	0.2
(5*Z*)-15-methyl-5-hexadecenoic (*i*-17:1)	0.2
(6*Z*)-15-methyl-6-hexadecenoic (*i*-17:1)	0.1
(7*Z*)-15-methyl-7-hexadecenoic (*i*-17:1)	0.2
(9*Z*)-15-methyl-9-hexadecenoic (*i*-17:1)	0.1
(6*Z*)-2-methoxy-6-hexadecenoic (2-OMe-16:1)	0.6
15-methylhexadecanoic (*i*-17:0)	2.9
14-methylhexadecanoic (*ai*-17:0)	5.0
(*Z*)-6-heptadecenoic (17:1)	0.5
heptadecanoic (17:0)	2.2
16-methylheptadecanoic (*i*-18:0)	1.3
(*Z*)-9-octadecenoic (18:1)	1.9
(*Z*)-11-octadecenoic (18:1)	4.3
(*Z*)-13-octadecenoic (18:1)	0.3
octadecanoic (18:0)	7.2
(*E*)-11-methyl-12-octadecenoic (19:1)	1.5
17-methyloctadecanoic (*i*-19:0)	0.6
16-methyloctadecanoic (*ai*-19:0)	0.9
nonadecanoic (19:0)	0.6
2-methoxyoctadecanoic (2-OMe-18:0)[a]	0.2
5,8,11,14-eicosatetraenoic (20:4)	1.1
(*Z*)-7-eicosenoic (20:1)	0.2
(*Z*)-11-eicosenoic (20:1)	0.4
(*Z*)-13-eicosenoic (20:1)	0.2
eicosanoic (20:0)	1.1
heneicosanoic (21:0)	0.2
(*Z*)-13-docosenoic (22:1)	0.2
(*Z*)-15-docosenoic (22:1)	0.2
(*Z*)-17-docosenoic (22:1)	0.4
docosanoic (22:0)	1.7
tricosanoic (23:0)	0.4
2-hydroxydocosanoic (*h*-22:0)	0.3
(5*Z*,9*Z*)-5,9-tetracosadienoic (24:2)	0.4
(*Z*)-15-tetracosenoic (24:1)	0.1
(*Z*)-17-tetracosenoic (24:1)	0.4
(*Z*)-19-tetracosenoic (24:1)	0.6
tetracosanoic (24:0)	0.9
(5*Z*,9*Z*)-5,9-pentacosadienoic (25:2)	1.6
2-hydroxytricosanoic (*h*-23:0)	0.1
pentacosenoic (25:1)	0.2
5,9,19-hexacosatrienoic (26:3)	0.5
2-hydroxytetracosanoic (*h*-24:0)	1.2
(5*Z*,9*Z*)-5,9-hexacosadienoic (26:2)	7.4
hexacosenoic (26:1)	0.6
2-hydroxypentacosanoic (*h*-25:0)	0.2
(5*Z*,9*Z*)-5,9-heptacosadienoic (27:2)	1.0

[a] Unprecedented as natural compounds.

acid (**1**), 2-methoxypentadecanoic acid (**2**), and 2-methoxyoctadecanoic acid (**3**). These methoxylated fatty acids were characterized, as methyl esters, on the basis of their GC retention times and MS fragmentations as well as by comparing their MS spectral properties with synthetic samples from the literature.[8,12,13] The GC retention times of the methyl esters from the methoxylated acids **1**−**3** resulted in equivalent chain length (ECL) values of 15.18, 16.12, and 19.21, respectively, implying an unusual substitution, but the fractional values agreed with those reported in the literature for other normal chain 2-methoxylated fatty acid methyl esters.[5,8] The mass spectra of these methyl esters revealed molecular ion peaks at *m*/*z* 272, 286, and 328, respectively, as well as very prominent $M^+ - 59$ (loss of CO_2CH_3) fragmentation peaks at *m*/*z* 213, 227, and 269, respectively. In addition, all of these methyl esters afforded a key fragmentation ion at *m*/*z* 104, corresponding to the McLafferty rearrangement of an α-methoxylated methyl ester.[6] Therefore, the compounds in question were identified as 2-methoxytetradecanoic acid (**1**), 2-methoxypentadecanoic acid (**2**), and 2-methoxyoctadecanoic acid (**3**). It is important to point out that this is the first report of these compounds as naturally occurring fatty acids, but there are several syntheses already reported in the literature for these saturated fatty acids.[12,13]



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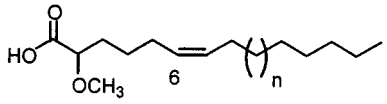
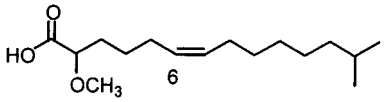
2: n = 2
3: n = 5



4: n = 1
5: n = 2
6: n = 3



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Another interesting series of fatty acids from *C. fallax* were the monounsaturated fatty acids (6*Z*)-2-methoxy-6-tetradecenoic acid (**4**), (6*Z*)-2-methoxy-6-pentadecenoic acid (**5**), (6*Z*)-2-methoxy-6-hexadecenoic acid (**6**), and (6*Z*)-2-methoxy-13-methyl-6-tetradecenoic acid (**7**). With the exception of acid **6**, all of these compounds are unprecedented in nature. The relative GC retention times of their methyl esters, the mass spectra of both the methyl esters and the corresponding DMDS derivatives, and corroboration with synthetic samples provided the basis for their characterization. For example, methyl (6*Z*)-2-methoxy-6-tetradecenoate exhibited unusual chromatographic properties, namely, an ECL value of 14.93, for which the fractional value is well in agreement with those reported in the literature for monounsaturated linear chain α-methoxylated methyl esters.[5] The mass spectrum of methyl (6*Z*)-2-methoxy-6-tetradecenoate contained a molecular ion peak at *m*/*z* 270 and a base peak at *m*/*z* 104, arising from a favorable 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 accounted for by a methoxy group at the 2-position. Important for the structural characterization were also the prominent fragmentation ions at *m/z* 238 ($M^+ - MeOH$), *m/z* 211 ($M^+ - CO_2CH_3$), and *m/z* 179 ($M^+ - CO_2CH_3 - MeOH$), which confirmed the α-methoxylation.[7] The double bond at C-6 was determined by preparing the corresponding DMDS adduct, a convenient method for the location of double bonds by mass spectrometry.[7,14] For example, the mass spectrum of methyl 2-methoxy-6,7-bis(methylthio)tetradecanoate afforded a molecular ion peak at *m/z* 364 and a $M^+ - 59$ peak at *m/z* 305. The double bond was readily determined to be at carbon 6 by the prominent fragmentations at *m/z* 205 $[C_9H_{17}SO_3]^+$ and at *m/z* 159 $[C_9H_{19}S]^+$ since this constitutes cleavage between carbons 6 and 7. There was also an abundant ion at *m/z* 173 $[C_8H_{13}SO_2]^+$ resulting from the loss of methanol from the *m/z* 205 fragment. Upon catalytic hydrogenation (PtO_2), methyl (6*Z*)-2-methoxy-6-tetradecenoate yielded the normal chain methyl 2-methoxytetradecanoate, which was also present in this mixture as a natural component. The experimental data thus support methyl (6*Z*)-2-methoxy-6-tetradecenoate as the most probable structure for this methyl ester. In a similar fashion methyl (6*Z*)-2-methoxy-6-pentadecenoate and methyl (6*Z*)-2-methoxy-6-hexadecenoate were characterized. A GC coelution of methyl (6*Z*)-2-methoxy-6-hexadecenoate from *C. fallax* with a synthetic sample confirmed the *Z* double bond of the natural methyl ester.[9] In addition, a straight line was obtained when the retention times of the α-methoxylated Δ^6-14:1, Δ^6-15:1, and Δ^6-16:1 methyl esters were plotted against chain length, thus supporting the *Z* double bond stereochemistry for the Δ^6-14:1 and Δ^6-15:1 methoxylated methyl esters.

The MS spectral data for methyl (6*Z*)-2-methoxy-13-methyl-6-tetradecenoate were quite similar to that of the straight-chain 2-methoxylated Δ^6 methyl esters, but the methyl branching resulted in a different ECL value. For example, this *iso* methyl ester displayed a smaller ECL value of 15.53, a value that definitely implies methyl branching as compared to an ECL value of 15.86 for methyl (6*Z*)-2-methoxy-6-pentadecenoate.[10] The mass spectrum of this methyl ester revealed a molecular ion at *m/z* 284 and the key ion fragmentations at *m/z* 252 ($M^+ - MeOH$), *m/z* 225 ($M^+ - CO_2CH_3$), *m/z* 193 ($M^+ - CO_2CH_3 - MeOH$), and *m/z* 104 (McLafferty rearrangement). The DMDS adduct (M^+ at *m/z* 378) was also instrumental in locating the double bond position at carbon 6 due to the prominent ions at *m/z* 205 $[C_9H_{17}SO_3]^+$ and *m/z* 173 $[C_{10}H_{21}S]^+$. The *iso* methyl branching was identified by hydrogenating the sample to methyl 2-methoxy-13-methyltetradecanoate, a compound that we recently described from the sponge *Amphimedon complanata*.[10] The hydrogenated compound presented an ECL value of 15.86, well in agreement with what is expected for a saturated *iso* methoxylated C_{15} methyl ester.[10]

The novel fatty acids identified in this paper open a new scheme (2-OCH_3-*n*:0 → Δ6 desaturase → 2-OCH_3-*n*:1, *n* = 14−16) of possible biosynthetic pathways for C_{14}−C_{16} α-methoxylated fatty acids in nature. Despite the fact that the 2-OMe-16:1 Δ^5 fatty acid has been the most ubiquitous methoxylated acid identified so far in marine sponges, in *C. fallax* Δ^6 methoxylated fatty acids predominated. Since most of the corresponding saturated C_{14}−C_{16} 2-methoxylated fatty acids were also found in this sponge, it is logical to assume that they are the biogenetic precursor of the Δ^6 monounsaturated 2-methoxylated fatty acids. We can only speculate at this point as to why only a Δ^6 desaturase operates with the methoxylated fatty acids from this sponge, which is in contrast to other possible alternatives normally encountered in many nonmethoxylated fatty acids, i.e., the Δ^4, Δ^5, or Δ^9 desaturases.

The identification of small amounts (2.4%) of α-methoxylated C_{14}−C_{16} fatty acids in *C. fallax* suggests that these acids are probably constituents of a novel bacterium associated with the sponge. There are several pieces of information that point in this direction. One fact is their low abundance in the sponge, which indicates that they are not major sponge phospholipid constituents. The typical sponge fatty acids are the $\Delta^{5,9}$ fatty acids, and several biosynthetic experiments tend to indicate that short-chain C_{15}−C_{19} fatty acids are actually of bacterial origin.[15] A structural comparison of these α-methoxylated fatty acids with known α-hydroxylated fatty acids also suggests a bacterial origin, as many α-OH C_{15}−C_{17} acids are indeed bacterial in nature.[16] In fact, some myxobacteria contain phosphatidylethanolamines as the major phospholipid with α-hydroxy *iso*-$C_{17:0}$ fatty acids at the 2-position and nonhydroxy fatty acids at the 1-position.[16] Therefore, it is very likely that in *C. fallax* a novel symbiotic marine bacterium could actually contain novel phosphatidylethanolamines with Δ^6 α-methoxylated fatty acids at the 2-position, similar to what is known for the α-hydroxy fatty acids in myxobacteria.[16] However, these 2-methoxylated fatty acids could also occupy the 1-position of the glycerol, as several 1-*O*-alkyl glycerols with a methoxy group at the 2-position of the hydrocarbon chain, such as the 1-*O*-(2-methoxyhexadecyl)glycerol, are known from several marine organisms.[17] These methoxylated alkyl glycerols have antibiotic activity and also inhibit the growth of several tumors.[18]

Experimental Section

General Experimental Procedures. Fatty acid methyl esters were 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 polymethylsiloxane cross-linked with 5% phenyl methylpolysiloxane. The temperature program was as follows: 130 °C for 1 min, then increased at a rate of 3 °C/min to 270 °C, and maintained for 30 min at 270 °C.

Sponge Collection. *Callyspongia fallax* Duchassaing and Michelotti (class Demospongiae, order Haplosclerida, family Callyspongidae) was collected in La Parguera, Puerto Rico, in March, 1991 at 20 m depth by scuba. A voucher specimen (no. 4-32) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras campus.

Extraction and Isolation of Phospholipids. The sponge (48.6 g) was carefully cleaned and cut into small pieces. Extraction with 3 × 250 mL of $CHCl_3$−MeOH (1:1) yielded the total lipids (4.1 g). The neutral lipids (0.11 g), glycolipids (1.03 g), and phospholipids (0.96 g) were separated by column chromatography on Si gel (60−200 mesh) using the procedure of Privett et al.[19]

Preparation and Isolation of Fatty Acid Derivatives. The fatty acyl components of the phospholipids were obtained as their methyl esters (0.09 g) by reaction of the phospholipids (0.91 g) with methanolic HCl followed by column chromatography. The methyl esters were hydrogenated in 10 mL of absolute methanol and catalytic amounts of platinum oxide (PtO_2). The double-bond positions in these compounds were determined by DMDS derivatization following a procedure that was previously described.[14] Mass spectral data for the novel methyl esters, and its derivatives, are presented below.

Methyl 2-methoxytetradecanoate: ECL = 15.18, GC−MS (70 eV) *m/z* 272 $[M]^+$ (1), 227 (5), 214 (15), 213 (100), 199 (1), 194 (1), 185 (5), 171 (1), 166 (1), 157 (1), 153 (5), 143 (2), 139 (2), 135 (3), 125 (12), 111 (32), 104 (7), 98 (23), 97 (61), 85 (19), 84 (16), 83 (71), 81 (16), 71 (57), 69 (74), 67 (18), 58 (17), 57 (51), 55 (73).

Methyl (6*Z*)-2-methoxy-6-tetradecenoate: ECL = 14.93, GC−MS (70 eV) *m*/*z* 270 $[M]^+$ (2), 238 (10), 211 (32), 178 (10), 179 (9), 150 (11), 136 (10), 127 (15), 111 (24), 109 (31), 104 (100), 97 (34), 95 (83), 87 (28), 83 (46), 81 (74), 79 (33), 71 (70), 69 (61), 67 (79), 57 (47), 55 (85).

Methyl 2-methoxy-6,7-bis(methylthio)tetradecanoate: GC−MS (70 eV) *m*/*z* 364 $[M]^+$ (17), 317 (1), 305 (5), 257 (2), 225 (2), 205 (100), 206 (11), 173 (37), 159 (43), 145 (22), 139 (2), 131 (4), 125 (12), 117 (5), 111 (9), 104 (5), 98 (10), 97 (22), 95 (14), 93 (11), 87 (54), 85 (10), 83 (11), 81 (17), 79 (15), 75 (12), 71 (45), 69 (51), 67 (32), 61 (43), 57 (21), 55 (48).

Methyl 2-methoxypentadecanoate: ECL = 16.12, GC−MS (70 eV) *m*/*z* 286 $[M]^+$ (1), 241 (2), 239 (2), 228 (15), 227 (92), 213 (3), 199 (3), 197 (2), 185 (3), 171 (3), 167 (2), 166 (2), 157 (2), 153 (2), 143 (9), 139 (4), 135 (3), 125 (14), 111 (33), 104 (8), 98 (16), 97 (64), 87 (57), 85 (26), 84 (18), 83 (76), 81 (33), 75 (18), 71 (78), 69 (82), 67 (30), 58 (17), 57 (76), 55 (100).

Methyl (6*Z*)-2-methoxy-13-methyl-6-tetradecenoate: ECL = 15.53, GC−MS (70 eV) *m*/*z* 284 $[M]^+$ (1), 252 (5), 225 (15), 193 (6), 192 (7), 150 (5), 136 (5), 127 (11), 111 (22), 109 (22), 104 (100), 97 (40), 95 (51), 87 (38), 83 (46), 81 (51), 79 (22), 71 (54), 69 (67), 67 (57), 57 (58), 55 (85).

Methyl 2-methoxy-13-methyl-6,7-bis(methylthio)tetradecanoate: GC−MS (70 eV) *m*/*z* 378 $[M]^+$ (14), 319 (6), 309 (2), 267 (1), 225 (2), 206 (12), 205 (100), 187 (5), 174 (10), 173 (73), 159 (2), 157 (10), 145 (24), 139 (7), 131 (6), 125 (20), 117 (8), 111 (21), 104 (7), 98 (16), 97 (41), 95 (29), 93 (17), 87 (63), 85 (33), 83 (46), 81 (39), 79 (23), 74 (15), 71 (81), 69 (77), 67 (49), 61 (42), 57 (79), 55 (91).

Methyl (6*Z*)-2-methoxy-6-pentadecenoate: ECL = 15.86, GC−MS (70 eV) *m*/*z* 284 $[M]^+$ (1), 252 (5), 225 (15), 193 (6), 192 (7), 150 (5), 136 (5), 127 (11), 111 (22), 109 (22), 104 (100), 97 (40), 95 (51), 87 (38), 83 (46), 81 (51), 79 (22), 71 (54), 69 (67), 67 (57), 57 (58), 55 (85).

Methyl 2-methoxy-6,7-bis(methylthio)pentadecanoate: GC−MS (70 eV) *m*/*z* 378 $[M]^+$ (16), 319 (5), 267 (1), 225 (1), 206 (11), 205 (100), 187 (7), 174 (9), 173 (73), 159 (2), 157 (10), 145 (24), 139 (2), 131 (3), 125 (12), 117 (4), 111 (8), 104 (5), 98 (11), 97 (37), 95 (26), 93 (15), 87 (68), 85 (14), 83 (27), 81 (28), 79 (19), 74 (18), 71 (40), 69 (54), 67 (48), 61 (81), 57 (22), 55 (93).

Methyl 2-methoxyoctadecanoate: ECL = 19.21, GC−MS (70 eV) *m*/*z* 328 $[M]^+$ (2), 295 (1), 270 (18), 269 (93), 239 (1), 238 (1), 213 (1), 199 (1), 197 (1), 185 (1), 171 (1), 167 (2), 166 (1), 157 (1), 153 (5), 143 (2), 141 (4), 139 (4), 135 (1), 125 (15), 111 (36), 104 (7), 98 (10), 97 (67), 87(14), 85 (45), 84 (16), 83 (77), 81 (24), 75 (7), 71 (100), 69 (80), 67 (24), 58 (18), 57 (90), 55 (81).

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