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NOVEL MONOUNSATURATED FATTY ACIDS FROM THE SPONGES *AMPHIMEDON COMPRESSA* AND *MYCALE LAEVIS*

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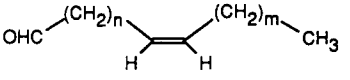
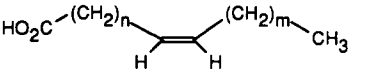
ABSTRACT.—The novel phospholipid aldehydes (*Z*)-17-tricosenal [**1**], (*Z*)-19-pentacosenal [**2**], and (*Z*)-19-hexacosenal [**3**] were characterized in the sponge *Amphimedon compressa*, together with the novel phospholipid fatty acids (*Z*)-16-tricosenoic [**4**], (*Z*)-18-tricosenoic [**5**], (*Z*)-19-pentacosenoic [**8**], (*Z*)-20-heptacosenoic [**9**], and (*Z*)-21-octacosenoic [**10**]. The sponge *Mycale laevis* was shown to have the novel phospholipid fatty acids 16-pentacosenoic [**6**] and 18-pentacosenoic [**7**]. Both Caribbean sponges were also shown to have the acid (5*Z*)-2-methoxy-5-hexadecenoic, an interesting case of α-methoxy substitution. These compounds were found to be present in phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine.

Several monounsaturated phospholipid fatty acids exist in nature, but few cases are known of very-long-chain monounsaturated acids longer than twenty-two carbons (1). However, sponges have provided the most interesting examples of long-chain phospholipid fatty acids since acids with chain-lengths between 24 and 30 carbons have been reported (2). This unusual ability of these marine invertebrates to biosynthesize very-long-chain fatty acids has been responsible for the many interesting structures which have been reported without counterpart in the terrestrial world (3). Two rather common sponge fatty acid biosynthetic pathways are the 18:1n-7 to 26:1n-7 and the 18:1n-9 to 26:1n-9 routes, while the 18:1n-8 to 26:1n-8 pathway has only recently been recognized to exist in these invertebrates (4). The latter biosynthetic pathways can explain the origin of the common sponge phospholipid fatty acids 15-, 16-, and 17-tetracosenoic acids as well as the 17-, 18-, and 19-hexacosenoic acids. Interesting to mention in this respect is that long odd-chain monounsaturated fatty acids are extremely rare in nature, in part due to the fact that these acids are normally found in very small amounts, practically in microgram quantities. Of the few reported examples, we can mention the acid 17-pentacosenoic (25:1n-8), which was recently identified in the phospholipids of the sponge *Geodia gibberosa* (5), thus providing evidence for the biosynthetic route 17:1n-8 to 25:1n-8, and the acid 17-tricosenoic (23:1n-6), which was identified in traces in the sponge *Microciona prolifera* (2). These preliminary results have encouraged us to survey additional sponges for novel odd-chain monounsaturated phospholipid fatty acids longer than twenty-two carbons, since with the advent of new analytical techniques, such as gc-ms, it is now possible to deal with minute amounts of substances and to elucidate these structures unambiguously. In the present study we report the structures of seven new monounsaturated phospholipid fatty acids and three new aldehydes from the Caribbean sponges *Amphimedon compressa* Pallas (family Nephatidae, order Haplosclerida) and *Mycale laevis* Carter (family Mycalidae, order Poecilosclerida). The sponge *A. compressa* was previously shown to contain the important fatty acids 2-hydroxydocosanoic and 2-hydroxytricosanoic (6), while *My. laevis* has



1 n=15, m=4
2 n=17, m=4
3 n=17, m=5



4 n=14, m=5
5 n=16, m=3
6 n=14, m=7
7 n=16, m=5
8 n=17, m=4
9 n=18, m=5
10 n=19, m=5

TABLE 1. The Phospholipid Fatty Acids and Aldehydes from Sponges.

Compounds	Equivalent chain length	Sponge	
		Amphimedon compressa[a] Abundance (%)	*Mycale laevis* Abundance (%)
Aldehydes			
Octadecanal (18:0)	18.56	2.2	—
17-Tricosenal (23:1)[b] **[1]**	23.24	0.3	—
Tricosanal (23:0)	23.32	0.3	—
17-Tetracosenal (24:1)	24.32	5.0	2.3
19-Pentacosenal (25:1)[b] **[2]**	25.24	2.9	—
19-Hexacosenal (26:1)[b] **[3]**	26.20	4.3	—
Fatty Acids			
Tetradecanoic (14:0)	14.00	2.0	1.0
13-Methyltetradecanoic (15:0)	14.59	—	0.3
12-Methyltetradecanoic (15:0)	14.66	Traces	0.1
Pentadecanoic (15:0)	15.00	Traces	0.7
9-Hexadecenoic (16:1)	15.71	0.9	3.0
11-Hexadecenoic (16:1)	15.75	—	0.3
Hexadecanoic (16:0)	16.00	2.6	3.8
2-Methoxy-5-hexadecenoic (16:1)	16.80	0.5	0.6
Heptadecanoic (17:0)	17.00	0.3	1.0
11-Octadecenoic (18:1)	17.71	0.9	3.0
Octadecanoic (18:0)	18.00	2.6	4.1
Nonadecanoic (19:0)	19.00	0.8	0.6
Eicosatetraenoic (20:4)	19.13	0.6	4.7
11-Eicosenoic (20:1)	19.64	0.1	—
13-Eicosenoic (20:1)	19.71	0.1	0.3
Eicosanoic (20:0)	20.00	0.5	1.4
Heneicosanoic (21:0)	21.00	1.0	1.0
13-Docosenoic (22:1)	21.70	—	0.3
15-Docosenoic (22:1)	21.77	0.3	0.1
16-Docosenoic (22:1)	21.85	0.1	—
17-Docosenoic (22:1)	21.91	0.1	—
Docosanoic (22:0)	22.00	—	4.1
16-Tricosenoic (23:1)[b] **[4]**	22.78	0.7	2.9
17-Tricosenoic (23:1)	22.83	0.5	—
18-Tricosenoic (23:1)[b] **[5]**	22.90	0.3	—
Tricosanoic (23:0)	23.00	—	6.2
5,9-Tetracosadienoic (24:2)	23.52	—	0.3
15-Tetracosenoic (24:1)	23.76	—	1.4
17-Tetracosenoic (24:1)	23.86	3.9	3.7
19-Tetracosenoic (24:1)	23.90	0.7	0.1
Tetracosanoic (24:0)	24.00	0.4	8.3
5,9-Pentacosadienoic (25:2)	25.00	—	4.4
16-Pentacosenoic (25:1)[b] **[6]**	24.78	—	0.3
18-Pentacosenoic (25:1)[b] **[7]**	24.87	—	1.1
19-Pentacosenoic (25:1)[b] **[8]**	24.89	1.5	—
Pentacosanoic (25:0)	25.00	—	4.4
24-Methyl-5,9-pentacosadienoic (26:2)	25.25	—	7.1
5,9-Hexacosadienoic (26:2)	25.59	—	17.9
17-Hexacosenoic (26:1)	25.78	—	3.8
19-Hexacosenoic (26:1)	25.87	2.0	3.9
Hexacosanoic (26:0)	26.00	—	1.5
20-Heptacosenoic (27:1)[b] **[9]**	26.80	0.2	—
21-Octacosenoic (28:1)[b] **[10]**	27.80	0.2	—
5,9,23-Nonacosatrienoic (29:3)	28.43	5.0	—
5,9,23-Tricontatrienoic (30:3)	29.43	8.0	—

[a]The other 50% were the previously reported 2-(+)-hydroxydocosanoic acid (45%) and 2-(+)-hydroxytricosanoic acid (6).

[b]These acids and aldehydes are novel in nature.

not been previously analyzed for phospholipid fatty acids. Herein we report the results of our investigation.

RESULTS AND DISCUSSION

The main phospholipids from *A. compressa* and *My. laevis* were found to be phosphatidylethanolamine and phosphatidylserine with lesser amounts of phosphatidylcholine. Transesterification with 1.0 N HCl/MeOH permitted the characterization in microgram quantities of the fatty acids as methyl esters and the aldehydes as dimethyl acetals by gc-ms and chemical degradation (Table 1). *A. compressa* had six main aldehydes, mainly arising from 1-*O*-alk-1-enyl-2-acyl-sn-glycero-3-phosphoethanolamine, commonly known as plasmalogens (7). Of these aldehydes, three were determined to be unprecedented in nature, namely (*Z*)-17-tricosenal [**1**], (*Z*)-19-pentacosenal [**2**], and (*Z*)-19-hexacosenal [**3**]. The aldehyde (*Z*)-19-hexacosenal [**3**] now becomes the longest aldehyde yet isolated from a marine sponge. As dimethyl acetals these aldehydes normally do not afford a molecular ion upon eims, but an ion equivalent to $[M-31]^+$ (loss of a methoxy group) permits the determination of the mol wt. All of these dimethyl acetals had a base peak at *m/z* 75 due to the $[C_3H_7O_2]^+$ fragment. For example, 19-pentacosenal dimethyl acetal presented an $[M-31]^+$ peak at *m/z* 379 and a base peak at *m/z* 75, while 19-hexacosenal dimethyl acetal had an $[M-31]^+$ peak at *m/z* 393 and also a base peak at *m/z* 75. The double bond positions in the dimethyl acetals were initially determined by ms, using the corresponding dimethyl disulfide derivatives (8), and further confirmed by oxidative cleavage with $KMnO_4/NaIO_4$. These results are shown in the Experimental section and in Table 2. For example, 19-hexacosenal dimethyl acetal afforded, upon reaction with dimethyl disulfide and iodine in Et_2O, 19,20-bis(methylthio)hexacosanal dimethyl acetal, which upon eims cleaves efficiently between carbons 19 and 20 affording abundant fragments at *m/z* 373 $[C_{22}H_{45}SO_2]^+$ and at *m/z* 145 $[C_8H_{17}S]^+$, allowing unambiguous determination of the double bond position at C-19. These results were further confirmed by $KMnO_4/NaIO_4$ oxidation of 19-hexacosenal dimethyl acetal, which gave methyl 19,19-

TABLE 2. Dimethyl Esters and Bis(Methoxy) Esters Obtained from the $KMnO_4/NaIO_4$ Cleavage.

Aldehydes and Fatty Acids	Dimethyl Esters and Bis(Methoxy) Esters	Principal Ms Fragmentations *m/z* (rel. int.)
4+6	$MeO_2C-(CH_2)_{14}-CO_2Me$	$[M-31]^+$ 283 (4), 241 (2.6), 209 (2), 168 (1.5), 126 (3), 112 (13), 98 (54), 74 (100)
5+7	$MeO_2C-(CH_2)_{16}-CO_2Me$	$[M-31]^+$ 311 (3), 269 (1), 237 (1.6), 196 (1.3), 154 (2), 112 (14), 98 (54), 74 (100)
8	$MeO_2C-(CH_2)_{17}-CO_2Me$	$[M-31]^+$ 325 (18), 283 (8), 251 (7), 210 (6), 168 (5), 112 (44), 98 (100), 74 (99)
9	$MeO_2C-(CH_2)_{18}-CO_2Me$	$[M-31]^+$ 339 (2), 297 (1), 265 (0.7), 224 (0.6), 182 (0.6), 112 (12), 98 (48), 74 (100)
10	$MeO_2C-(CH_2)_{19}-CO_2Me$	$[M-31]^+$ 353 (1.7), 311 (1), 279 (0.6), 238 (0.5), 196 (0.5), 112 (11), 98 (45), 74 (100)
2+3	$(MeO)_2CH-(CH_2)_{17}-CO_2Me$	$[M-59]^+$ 313 (0.6) $[M-91]^+$ 281 (0.1), 75 (100)

bis(methoxy)nonadecanoate as one of the main fragments (see Table 2). The other two novel aldehydes in *A. compressa* were also characterized in a similar way, i.e., the 17,18-bis(methylthio)tricosanal dimethyl acetal provided major fragments at *m/z* 345 $[C_{20}H_{41}SO_2]^+$ and at *m/z* 131 $[C_7H_{15}S]^+$ in support of a C-17 double bond, while the 19,20-bis(methylthio)pentacosanal dimethyl acetal afforded the key fragments at *m/z* 373 $[C_{22}H_{45}SO_2]^+$ and at *m/z* 131 $[C_7H_{15}S]^+$, thus providing evidence for a C-19 double bond. A careful gc Ft-ir of the mixture presented no absorption in the 960–980 cm^{-1} region, indicating cis rather than trans unsaturation. In fact, with only a few exceptions, sponges are known to biosynthesize exclusively fatty acyl chains with cis double bonds (3).

The fatty acyl components of the phospholipids were characterized as methyl esters by gc-ms, chemical degradation, and capillary gc retention times using the standard equivalent chain length (ECL) values. The total isolated phospholipid fatty acids from the sponges are shown in Table 1. Of particular interest to mention here is that we were able to characterize in both *A. compressa* and *My. laevis* the recently discovered (5*Z*)-2-methoxy-5-hexadecenoic acid (9), whose gross structure was previously elucidated but whose double bond position could not be determined. This methyl ester exhibited unusual chromatographic properties, that is an ECL value of 16.80, implying the presence of polar functionalities. The mass spectrum presented a mol wt 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 $[M-32]^+$, *m/z* 239 $[M-CO_2Me]^+$, and at *m/z* 207. In fact, comparison of this mass spectrum with similar spectra for other 2-methoxy acids (10) confirmed that we have identified a novel 2-methoxyhexadecenoic acid. The double bond position was determined by preparing the corresponding dimethyl disulfide adduct, which afforded a molecular ion peak at *m/z* 392 (4%). The double bond was readily determined to be at C-5 by the fragmentations at *m/z* 201 (31%) $[C_{11}H_{22}SCH_3]^+$ and at *m/z* 191 (100%) $[C_8H_{15}SO_3]^+$, since this constitutes cleavage between C-5 and C-6. There was also a prominent peak at *m/z* 159 (91%), corresponding to the *m/z* 191 fragment with the loss of MeOH. The experimental data thus supported (5*Z*)-2-methoxy-5-hexadecenoic acid as the compound in question.

The most important fatty acids in *A. compressa* were a series of five new monounsaturated fatty acids, while *My. laevis* had two novel fatty acids (Table 1). The total characterization of these acids was possible by means of gc-ms of methyl ester derivatives, ECL values, hydrogenation to the corresponding saturated methyl esters, derivatization to dimethyl disulfides, and oxidative cleavage. The new phospholipid fatty acids were the 16- and 18-tricosenoic acids, 16-, 18-, and 19-pentacosenoic acids, the (*Z*)-20-heptacosenoic acid [**9**], and the (*Z*)-21-octacosenoic acid [**10**]. The (*Z*)-16-tricosenoic acid [**4**] was found to occur in both *A. compressa* and *My. laevis*, while (*Z*)-18-tricosenoic acid [**5**] was only detected in *A. compressa* together with the previously reported (*Z*)-17-tricosenoic acid (2). The 16- and 18-tricosenoic acid methyl esters afforded, after hydrogenation in MeOH with PtO_2 as catalyst, methyl tricosanoate as confirmed by gc co-injection with an authentic sample. This experiment excludes the possibility of any methyl branching. The double bond positions were determined by derivatization to methyl 16,17-bis(methylthio)tricosanoate and methyl 18,19-bis(methylthio)tricosanoate. In the former the double bond was found to be at C-16 from the ms fragments at *m/z* 315 $[C_{18}H_{35}SO_2]^+$ and at *m/z* 145 $[C_8H_{17}S]^+$, while in the second isomer the double bond was determined to be at C-18 from the favorable fragmentations at *m/z* 343 $[C_{20}H_{39}SO_2]^+$ and at *m/z* 117 $[C_6H_{13}S]^+$. These results were nicely confirmed by $KMnO_4/NaIO_4$ oxidation, since methyl 16-tricosenoate cleaved to hexadecanedioic

acid dimethyl ester and methyl heptanoate, while methyl 18-tricosenoate cleaved to octadecanedioic acid dimethyl ester and methyl pentanoate (Table 2).

The 16-, 18-, and 19-pentacosenoic acids were the next group of novel acids identified in the series. The first two were found in *My. laevis*, and the (*Z*)-19-pentacosenoic acid [**8**] was found in *A. compressa*. Each of these compounds, as methyl esters, was converted by catalytic hydrogenation into methyl pentacosanoate, thus confirming that they are all straight-chain methyl esters. The dimethyl disulfide adducts were again key to determining the double bond positions, as methyl 16,17-bis(methylthio)pentacosanoate cleaved nicely between C-16 and C-17 as evidenced by the fragments at *m/z* 315 $[C_{18}H_{35}SO_2]^+$ and at *m/z* 173 $[C_{10}H_{21}S]^+$, thus supporting a double bond at C-16. On the other hand, 18,19-bis(methylthio)pentacosanoate provided the fragmentations at *m/z* 343 $[C_{20}H_{39}SO_2]^+$ and at *m/z* 145 $[C_8H_{17}S]^+$ corresponding to cleavage between carbons C-18 and C-19, while 19,20-bis(methylthio)pentacosanoate cleaved prominently, upon electron impact, between C-19 and C-20 in the key fragments at *m/z* 357 $[C_{21}H_{41}SO_2]^+$ and *m/z* 131 $[C_7H_{15}S]^+$. Oxidative cleavage confirmed our assignments (Table 2).

Two of the most interesting phospholipid fatty acids in the mixture were (*Z*)-20-heptacosenoic acid [**9**] and (*Z*)-21-octacosenoic acid [**10**], since these acids represent some of the longest straight-chain monounsaturated acids yet isolated from a sponge. Upon catalytic hydrogenation (PtO_2/MeOH) these methyl esters were transformed into methyl heptacosanoate and into methyl octacosanoate, thus excluding the possibility of any branching. As dimethyl disulfide adducts, methyl 20,21-bis(methylthio)heptacosanoate cleaved prominently at *m/z* 371 $[C_{22}H_{43}SO_2]^+$ and at *m/z* 145 $[C_8H_{17}S]^+$, while methyl 21,22-bis(methylthio)octacosanoate cleaved to afford the abundant ions at *m/z* 385 $[C_{23}H_{45}SO_2]^+$ and at *m/z* 145 $[C_8H_{17}S]^+$. Oxidative cleavage of methyl 20-heptacosenoate followed by esterification produced eicosanedioic acid dimethyl ester and methyl heptanoate, while oxidative cleavage of methyl 21-octacosenoate provided heneicosanedioic acid dimethyl ester and methyl heptanoate. These results confirmed our structural assignments. A careful gc Ft-ir of the mixture presented no absorption in the 960–980 cm^{-1} region, indicating cis rather than trans unsaturation for all of the new fatty acids reported in this work.

Capillary gc and gc-ms analysis of the fatty acid mixture from *A. compressa* also revealed the presence of two very-long-chain phospholipid fatty acids displaying base peaks at *m/z* 81, a value diagnostic of acids possessing the $\Delta^{5,9}$ unsaturation pattern typical of "demospongic acids" (6). On the basis of capillary gc retention times and ECL values, together with mass spectral comparisons, these acids were characterized as 5,9,23-nonacosatrienoic (29:3) and 5,9,23-tricontatrienoic (30:3). The double bond positions were further corroborated by oxidative cleavage since both of these acids afforded tetradecanedioic acid dimethyl ester ($[M-31]^+$ at *m/z* 255) as one of the fragments.

The results presented in this work are important for several reasons. While the biosynthetic pathway 18:1n-7 to 26:n-7 has been extensively documented in sponges, and all intermediates in this pathway have been identified in one way or the other, we are indirectly presenting evidence in this work that *A. compressa* is capable of extending the latter route to 28:1n-7, i.e., from 18:1n-7 to 28:1n-7. More interesting is that we are also presenting evidence that sponges are capable of biosynthesizing unprecedented odd-chain fatty acids utilizing novel biosynthetic pathways, as indicated by the route 17:1n-7 to 27:1n-7 and the route 17:1n-6 to 25:1n-6. Evidence for the former pathway comes from the isolation of the novel fatty acids (*Z*)-16-tricosenoic [**4**], (*Z*)-18-pentacosenoic [**6**], and (*Z*)-20-heptacosenoic [**8**], which are obviously related by a two-carbon elongation mechanism. The new acid (*Z*)-18-tricosenoic [**5**] is just the first exam-

ple of an n-5 biosynthetic route, and future work might reveal other acids in the same series. The aldehydes arising from the plasmalogens of the sponges, namely (*Z*)-17-tricosenal [**1**], (*Z*)-19-pentacosenal [**2**], and (*Z*)-19-hexacosenal [**3**], all seem to be biosynthetically related to the fatty acids, since the acids (*Z*)-17-tricosenoic, (*Z*)-19-pentacosenoic [**8**], and (*Z*)-19-hexacosenoic were all found in the sponges. These findings add a new dimension to the possible alkenyl chains that sponge plasmalogens can reach, since the longest monounsaturated aldehyde known from sponges was the common (*Z*)-17-tetracosenal (11). Work is in progress to elucidate these complex biosynthetic routes in sponges.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The methyl esters were analyzed by gc-ms using 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. The ^{31}P nmr of the phospholipids was performed at 22° on a GN 300 FT NMR spectrometer at 121.6 MHz. For the acquisition 16K data points were used, and approximately 1000 accumulations were obtained before Fourier transformation of the free induction decay. In a typical run, phospholipids (20–30 mg) were dissolved in 3 ml of $CDCl_3$-CD_3OD (2:1) containing as internal reference triphenylphosphine.

SPONGE MATERIAL.—*A. compressa* and *My. laevis* were collected March 21, 1991, at 3–40 feet near the reef crest and reef base of Caballo Ahogado, La Parguera, Puerto Rico. The sponges were kindly classified by Dr. Vance Vicente from the United States Department of the Interior Fish and Wildlife Service. A voucher specimen of each sponge is on file at the National Museum of Natural History of the Smithsonian Institution.

EXTRACTION AND ISOLATION OF PHOSPHOLIPIDS.—The sponges (ca. 350–400 g of freeze-dried sponge) were carefully cleaned of all nonsponge debris and cut into small pieces. Immediate extraction with 500 ml of $CHCl_3$-MeOH (1:1) yielded the total lipids. The neutral lipids, glycolipids, and phospholipids (100 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 investigated either by preparative tlc using Si gel 60 and CHCl3-MeOH-NH_4OH (63:32:5) as solvent or by ^{31}P nmr.

PREPARATION AND ISOLATION 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). The fatty acid methyl esters and dimethyl acetals thus obtained (5 mg) were not individually isolated, but were characterized in the mixture by separating the components on a fused silica column coated with DB-1. For the location of double bonds, dimethyl disulfide derivatives were prepared by dissolving the esters (2 mg) in dimethyldisulfide (0.2 ml) and adding a solution (0.05 ml) of iodine in Et_2O (60 mg/ml). The solution was heated at 50° for 24 h, and the product was purified as described previously (14). Hydrogenations were carried out in 10 ml of MeOH and catalytic amounts of PtO_2. Mass spectral data for the new dimethyl disulfide adducts are presented below.

17,18-Bis(methylthio)tricosanal dimethyl acetal.—Ms *m/z* (rel. int.) $[M]^+$ 476 (1), $[C_{20}H_{41}SO_2]^+$ 345 (5), $[C_7H_{15}S]^+$ 131 (30), 109 (7), 87 (15), 83 (29), 81 (20), 75 (20), 74 (12), 69 (23), 67 (19), 61 (100), 57 (19), 55 (62).

19,20-Bis(methylthio)pentacosanal dimethyl acetal.—Ms *m/z* (rel. int.) $[M]^+$ 504 (1), $[C_{22}H_{45}SO_2]^+$ 373 (6), 345 (3), 132 (4), $[C_7H_{15}S]^+$ 131 (45), 111 (2), 109 (8), 101 (4), 97 (11), 95 (15), 87 (30), 83 (29), 81 (18), 75 (13), 74 (10), 69 (31), 67 (31), 61 (100), 59 (5), 57 (14), 55 (89).

19,20-Bis(methylthio)hexacosanal dimethyl acetal.—Ms *m/z* (rel. int.) $[M]^+$ 518 (1), $[C_{22}H_{45}SO_2]^+$ 373 (5), 345 (4), 146 (5), $[C_8H_{17}S]^+$ 145 (41), 109 (4), 101 (4), 97 (20), 95 (12), 87 (23), 83 (10), 81 (19), 75 (11), 74 (8), 69 (32), 67 (24), 61 (100), 59 (3), 57 (11), 55 (71).

Methyl 16,17-bis(methylthio)tricosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 460 (1), 316 (3), $[C_{18}H_{35}SO_2]^+$ 315 (13), 284 (2), $[C_{17}H_{31}SO]^+$ 283 (12), 146 (4), $[C_8H_{17}S]^+$ 145 (54), 111 (4), 109 (5), 97 (24), 95 (18), 87 (27), 83 (22), 81 (24), 75 (12), 74 (55), 69 (40), 67 (27), 61 (86), 59 (15), 55 (100).

Methyl 17,18-bis(methylthio)tricosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 460 (1), 330 (3), $[C_{19}H_{37}SO_2]^+$ 329 (21), 298 (4), $[C_{18}H_{33}SO]^+$ 297 (19), 132 (5), 131 (65), 111 (4), 109 (8), 97 (19), 95 (23), 87 (33), 83 (39), 81 (27), 75 (10), 74 (60), 69 (45), 67 (34), 61 (99), 59 (16), 57 (20), 55 (100).

Methyl 18,19-bis(methylthio)tricosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 460 (1), 344 (3), $[C_{20}H_{39}SO_2]^+$

343 (9), $[C_{19}H_{35}SO]^+$ 311 (11), 118 (3), $[C_6H_{13}S]^+$ 117 (66), 111 (5), 109 (9), 97 (15), 95 (19), 87 (35), 83 (22), 81 (21), 75 (12), 74 (72), 69 (83), 67 (31), 61 (97), 59 (24), 57 (22), 55 (100).

Methyl 16,17-bis(methylthio)pentacosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 488 (1), $[C_{18}H_{35}SO_2]^+$ 315 (8), $[C_{17}H_{31}SO]^+$ 283 (6), $[C_{10}H_{21}S]^+$ 173 (10), 111 (5), 109 (11), 97 (20), 95 (34), 87 (35), 83 (33), 81 (33), 75 (9), 74 (48), 69 (54), 67 (37), 61 (64), 59 (21), 57 (48), 55 (100).

Methyl 18,19-bis(methylthio)pentacosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 488 (1), 344 (3), $[C_{20}H_{39}SO_2]^+$ 343 (12), 312 (2), $[C_{19}H_{35}SO]^+$ 311 (10), $[C_8H_{17}S]^+$ 145 (55), 111 (3), 109 (6), 97 (30), 95 (17), 87 (40), 83 (24), 81 (25), 75 (13), 74 (51), 69 (40), 67 (33), 61 (62), 59 (15), 57 (23), 55 (100).

Methyl 19,20-bis(methylthio)pentacosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 488 (1), 358 (6), $[C_{21}H_{41}SO_2]^+$ 357 (30), 326 (5), $[C_{20}H_{37}SO]^+$ 325 (23), 132 (7), $[C_7H_{15}S]^+$ 131 (82), 111 (4), 109 (10), 97 (24), 95 (26), 87 (42), 83 (57), 81 (29), 75 (12), 74 (80), 69 (48), 67 (32), 61 (93), 59 (18), 57 (23), 55 (100).

Methyl 20,21-bis(methylthio)heptacosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 516 (1), $[C_{22}H_{43}SO_2]^+$ 371 (5), $[C_{21}H_{39}SO]^+$ 339 (5), 146 (5), $[C_8H_{17}S]^+$ 145 (42), 111 (7), 109 (10), 97 (27), 95 (16), 87 (45), 83 (20), 81 (28), 75 (10), 74 (61), 69 (39), 67 (30), 61 (74), 59 (17), 57 (23), 55 (100).

Methyl 21,22-bis(methylthio)octacosanoate.—Ms *m/z* (rel. int.) $[M]^+$ 530 (1), $[C_{23}H_{45}SO_2]^+$ 385 (7), $[C_{22}H_{41}SO]^+$ 353 (7), 146 (8), $[C_8H_{17}S]^+$ 145 (51), 111 (6), 109 (9), 101 (5), 97 (28), 95 (20), 87 (41), 83 (22), 81 (23), 75 (11), 74 (84), 69 (51), 67 (26), 61 (79), 59 (16), 57 (27), 55 (100).

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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