Unusual Lipids in the Caribbean Sponges Amphimedon viridis and Desmapsamma anchorata

Néstor M. Carballeira, and Fathi Shalabi

More About This Article

The permalink http://dx.doi.org/10.1021/np50110a004 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

UNUSUAL LIPIDS IN THE CARIBBEAN SPONGES
AMPHIMEDON VIRIDIS AND *DESMAPSAMMA ANCHORATA*

NÉSTOR M. CARBALLEIRA* and FATHI SHALABI

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, San Juan, Puerto Rico 00931-3346

ABSTRACT.—The phospholipid fatty acid composition of the Caribbean sponge *Amphimedon viridis* was studied, revealing the presence of the previously unreported substances 5,13-nonadecadienoic acid, 4-nonadecenoic acid, and 15-heneicosenoic acid. In *A. viridis* the very long-chain 5,9,23-nonacosatrienoic acid and 5,9,23-tricosatrienoic acid predominated in the mixture. The fatty acid composition of the sponge *Desmapsamma anchorata* was revised and 62 phospholipid fatty acids were characterized. The rare 8,13-octadecadienoic acid and 18-tetracosenoic acid were also characterized in the mixture. The two possible stereochemical isomers of 7-methyl-6-hexadecenoic acid, i.e., 7-methyl-6(Z)-hexadecenoic acid and the 7-methyl-6(E)-hexadecenoic acid were identified in both sponges, but *D. anchorata* had larger amounts. This is the first time that both stereoisomers have been identified in a sponge. The previously reported 7-methyl-8-hexadecenoic acid was not found in *D. anchorata*. Unprecedented aldehydes, comprising 15- and 17-docosenal, were also identified in the sponges.

The large variety of possible phospholipid fatty acids that sponges are capable of biosynthesizing is now a well-established fact (1,2). An interesting group of sponges has been *Amphimedon* spp., whose phospholipids present fatty acids with well-defined routes for either monounsaturated very long-chain fatty acids and/or polyunsaturated fatty acids (3,4). For example, the sponge *Amphimedon compressa* has been shown to contain the very long-chain fatty acids 5,9,23-nonacosatrienoic and 5,9,23-tricontatrienoic as the principal fatty acids in phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine isolated from the sponge. A large number of monounsaturated fatty acids of the *n*-7 family were also identified, most likely the metabolic precursors to the $\Delta^{5,9,23}$ fatty acids isolated from *A. compressa* (3). A second study of the Demosponge *Amphimedon complanata* also demonstrated that the key fatty acid in the mixture was the 5,9,23-tricontatrienoic acid. However, in *A. complanata* we also identified a novel 11,15-icosadienoic acid, as part of the phospholipid-bound fatty acids, together with 7-methyl-6-hexadecenoic acid (4). The 7-methyl-6-hexadecenoic acid has been isolated before in whale oils by both Pascal and Ackman (5) and Sano (6), but no double-bond stereochemistry was identified. For example, are the (Z), (E), or both stereoisomers present in nature? Are these acids of bacterial origin? In the present work we studied the phospholipid fatty acid composition of the Caribbean sponge *Amphimedon viridis* Duchassaing & Michelotti (family Haliclonidae, order Haplosclerida). We wanted to compare the fatty acid profile of *A. viridis* with that of other previously analyzed *Amphimedon* spp. At the same time, we wanted to identify new fatty acids which could give us novel insights into fatty acid biosynthetic pathways in marine organisms. We have also included in the present paper the revised fatty acid composition of the sponge *Desmapsamma anchorata* (family Esperiopsidae, order Poecilosclerida) (7).

RESULTS AND DISCUSSION

The main phospholipids from *A. viridis* and *D. anchorata* were found to be phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI). Transesterification of the total phospholipids with 1.0 N HCl/MeOH allowed the characterization of the individual fatty acids as methyl esters by gc-ms. The principal fatty acids of the sponges are shown in Table 1. Most of the identified fatty acids have been characterized before in one way or another, but several

TABLE 1.   The Total Phospholipid Fatty Acids and Aldehydes from *A. viridis* and *D. anchorata*.

Compound	Amphimedon viridis[a] (wt %)	Desmapsamma anchorata[a] (wt %)
Fatty Acids		
9-Tetradecenoic (14:1)	0.1	—
Tetradecanoic (14:0)	1.3	2.1
4-Pentadecenoic (15:1)	0.1	—
9-Pentadecenoic (15:1)	0.1	—
13-Methyltetradecanoic (*i*-15:0)	0.3	0.9
12-Methyltetradecanoic (*ai*-15:0)	—	0.2
Pentadecanoic (15:0)	0.3	1.0
14-Methylpentadecanoic (*i*-16:0)	0.2	1.2
13-Methylpentadecanoic (*ai*-16:0)	—	0.4
9,12-Hexadecadienoic (16:2)	0.2	—
5-Hexadecenoic (16:1)	0.1	—
6-Hexadecenoic (16:1)	—	0.2
9-Hexadecenoic (16:1)	2.4	1.5
11-Hexadecenoic (16:1)	0.3	0.4
2-Methoxy-5-hexadecenoic (16:1)	0.4	0.8
Hexadecanoic (16:0)	3.9	9.9
7-Methyl-6(*E*)-hexadecenoic [2b]	0.1	2.9
7-Methyl-6(*Z*)-hexadecenoic [2a]	0.2	5.2
15-Methylhexadecanoic (*i*-17:0)	0.3	0.8
14-Methylhexadecanoic (*ai*-17:0)	0.4	3.1
5-Heptadecenoic (17:1)	—	0.1
9-Heptadecenoic (17:1)	0.2	—
11-Heptadecenoic (17:1)	0.2	—
Heptadecanoic (17:0)	0.8	0.4
15-Methylheptadecanoic (*ai*-18:0)	—	0.5
3,7,11,15-Tetramethylhexadecanoic (20:0)	—	0.7
5,9-Octadecadienoic (18:2)	2.9	—
5,11-Octadecadienoic (18:2)	0.8	—
8,13-Octadecadienoic (18:2)	—	0.2
5-Octadecenoic (18:1)	0.1	—
9-Octadecenoic (18:1)	1.2	0.9
11-Octadecenoic (18:1)	3.3	3.2
13-Octadecenoic (18:1)	0.1	—
Octadecanoic (18:0)	3.8	4.2
5,13-Nonadecadienoic [1] (19:2)[b]	0.2	—
4-Nonadecenoic (19:1)[b]	0.3	0.2
5-Nonadecenoic (19:1)	0.1	—
6-Nonadecenoic (19:1)	0.1	0.3
11-Nonadecenoic (19:1)	0.1	—
13-Nonadecenoic (19:1)	0.3	—
17-Methyloctadecanoic (*i*-19:0)	0.5	0.3
16-Methyloctadecanoic (*ai*-19:0)	0.3	0.1
Nonadecanoic (19:0)	1.4	1.2
5,8,11,14-Eicosatetraenoic (20:4 *n*-6)	3.5	0.2
5,8,11,14,17-Eicosapentaenoic (20:5 *n*-3)	1.4	0.1
5,9-Eicosadienoic (20:2)	1.0	—
5,11-Eicosadienoic (20:2)	1.2	—
5,13-Eicosadienoic (20:2)	1.4	—
7,13-Eicosadienoic (20:2)	0.6	—
11,14-Eicosadienoic (20:2)	0.2	—
5-Eicosenoic (20:1)	0.1	—
7-Eicosenoic (20:1)	0.9	—
11-Eicosenoic (20:1)	0.2	0.2

TABLE 1. Continued.

Compound	Amphimedon viridis[a] (wt %)	Desmapsamma anchorata[a] (wt %)
13-Eicosenoic (20:1)	0.7	0.2
15-Eicosenoic (20:1)	0.1	—
16-Methylnonadecanoic (20:0)	—	0.4
18-Methylnonadecanoic (i-20:0)	—	0.4
Eicosanoic (20:0)	0.7	3.6
7-Heneicosenoic (21:1)	0.1	—
11-Heneicosenoic (21:1)	0.1	—
13-Heneicosenoic (21:1)	0.1	—
15-Heneicosenoic (21:1)[b]	0.1	—
19-Methyleicosanoic (i-21:0)	—	0.2
18-Methyleicosanoic (ai-21:0)	—	0.2
Heneicosanoic (21:0)	1.0	2.2
4,7,10,13,16-Docosapentaenoic (22:5 n-6)	0.4	—
4,7,10,13,16,19-Docosahexaenoic (22:6 n-3)	0.7	—
7,10,13,16-Docosatetraenoic (22:4 n-6)	1.0	—
10,13,16,19-Docosatetraenoic (22:4 n-3)	0.5	—
7,13-Docosadienoic (22:2)	0.2	—
7,15-Docosadienoic (22:2)	0.9	—
19-Methylheneicosanoic (ai-22:0)	—	0.5
5-Docosenoic (22:1)	—	0.1
13-Docosenoic (22:1)	0.2	0.2
15-Docosenoic (22:1)	0.2	0.4
17-Docosenoic (22:1)	0.1	—
Docosanoic (22:0)	0.8	19.2
21-Methyldocosanoic (i-23:0)	—	1.5
16-Tricosenoic (21:1)	0.2	0.1
17-Tricosenoic (23:1)	0.2	—
18-Tricosenoic (23:1)	0.1	—
Tricosanoic (23:0)	0.2	1.0
5,9-Tetracosadienoic (24:2)	—	0.2
15-Tetracosenoic (24:1)	—	0.1
17-Tetracosenoic (24:1)	1.7	0.4
18-Tetracosenoic (24:1)	0.1	0.1
19-Tetracosenoic (24:1)	0.2	—
Tetracosanoic (24:0)	0.1	9.0
5,9-Pentacosadienoic (25:2)	1.2	0.9
9-Pentacosenoic (25:1)	—	0.1
17-Pentacosenoic (25:1)	0.2	—
18-Pentacosenoic (25:1)	0.1	0.1
19-Pentacosenoic (25:1)	1.2	—
Pentacosanoic (25:0)	—	1.8
5,9,19-Hexacosatrienoic (26:3)	—	0.7
5,9-Hexacosadienoic (26:2)	0.2	10.0
17-Hexacosenoic (26:1)	0.1	0.7
19-Hexacosenoic (26:1)	1.2	0.6
Hexacosanoic (26:0)	—	0.5
20-Heptacosenoic (27:1)	0.1	—
21-Heptacosenoic (27:1)	0.1	—
5,9,19-Octacosatrienoic (28:3)	2.2	0.2
5,9,21-Octacosatrienoic (28:3)	7.5	—
19-Octacosenoic (28:1)	0.1	0.1
21-Octacosenoic (28:1)	0.2	—
5,9,23-Nonacosatrienoic (29:3)	15.6	—
5,9,23-Tricosatrienoic (30:3)	17.6	—

TABLE 1.   Continued.

Compound	Amphimedon viridis[a] (wt %)	Desmapsamma anchorata[a] (wt %)
Aldehydes		
15-Docosenal (22:1)[b]	0.2	0.04
17-Docosenal (22:1)[b]	—	0.03
16-Tricosenal (23:1)	0.3	0.2
17-Tricosenal (23:1)	0.3	0.02
15-Tetracosenal (24:1)....................................	—	0.03
17-Tetracosenal (24:1)....................................	2.0	0.2
17-Pentacosenal (25:1)	0.2	0.2
19-Pentacosenal (25:1)	1.0	—
17-Hexacosenal (26:1)....................................	—	0.05
19-Hexacosenal (26:1)....................................	0.5	0.06

[a]Some minor acids were not identified. Abundance in wt %.
[b]Not recognized to exist in nature before.

fatty acids deserve special mention. One important observation is that *A. viridis* displayed very long-chain polyunsaturated fatty acids, in particular the 5,9,23-nonacosatrienoic acid (29:3) and the 5,9,23-tricosatrienoic acid (30:3). These acids have been isolated before in other *Amphimedon* spp. and seem to be characteristic for the Haliclonidae (4).

All monounsaturated fatty acids in the biosynthetic routes *n*-6, *n*-7, and *n*-9 from 15–16 carbons to 27–28 carbons were identified in the sponges. This is the first report of such a complete list of fatty acid intermediates in a marine organism. For example, all monounsaturated fatty acids between $15:1\Delta^9$ and $27:1\Delta^{21}$ were identified in *A. viridis*. In particular, 15-heneicosenoic acid is worthy of consideration since it has not been identified before in nature. Its characterization was performed by utilizing gc-ms on both the dimethyldisulfide and pyrrolidide derivatives for better confirmation of the structure. The corresponding methyl 15,16-bis(methylthio)heneicosanoate displayed a $[M]^+$ at m/z 432 and key fragmentations at m/z 301 $[C_{17}H_{33}SO_2]^+$ and at m/z 131 $[C_7H_{15}S]^+$, which defined the double bond at C-15. This was confirmed by the mass spectrum of the corresponding pyrrolidide derivative (8). The N-heneicos-15-enoylpyrrolidine displayed a $[M]^+$ at m/z 377 and a difference of 12 mass units between m/z 280 (C_{14}) and m/z 292 (C_{15}) indicating the double bond to be at C-15. Hydrogenation (PtO_2) of the methyl ester resulted in methyl heneicosanoate and excluded the possibility of any branching. The ir spectra of all of the methyl esters exhibited an absorption around 718 cm^{-1} (out-of-plane bending vibration) and not even traces of an absorption in the 960-980 cm^{-1} region, thus indicating cis rather than trans stereochemistry (9).

Although of probable cyanobacterial origin, the shortest monounsaturated fatty acids arising from the direct action of a desaturase were also characterized in these sponges, particularly in *A. viridis*. This was of interest because some novel structures were characterized. For example, the cis Δ^5 fatty acids, 5-hexadecenoic (16:1), 5-heptadecenoic (17:1), 5-octadecenoic (18:1), 5-nonadecenoic (19:1), 5-eicosenoic (20:1), and 5-docosenoic (22:1) acids, were all identified in the sponges indicating the presence of an active Δ^5 desaturase in these organisms. The corresponding Δ^9 desaturase activity was also evident in the sponges as the monounsaturated fatty acids 9-tetradecenoic (14:1), 9-pentadecenoic (15:1), 9-hexadecenoic (16:1), 9-heptadecenoic (17:1), 9-octadecenoic (18:1), and 9-pentacosenoic (25:1) acids were also identified (Table 1). However, the less common Δ^6 desaturase activity was also encountered in the sponges. For example, the acids 6-

hexadecenoic (16:1) and 6-nonadecenoic (19:1) were characterized. The rarer 4-pentadecenoic (15:1) and 4-nonadecenoic (19:1) acids were also identified. These Δ^4 acids could have arisen via Δ^6 desaturation followed by chain-shortening as Sprecher *et al*. have recently shown that there are no known Δ^4 desaturases (10). The 4-nonadecenoic acid is novel, and it was characterized by synthesizing the corresponding pyrrolidide and dimethyl disulfide derivative of the methyl ester. The *N*-nonadec-4-enoylpyrrolidine displayed a difference of 12 mass units between fragments at *m/z* 126 (C_3) and *m/z* 138 (C_4) indicating a double bond at C-4. The abundant fragment observed at *m/z* 166 (41%) resulted from allylic cleavage between C-6 and C-7, a typical fragment observed in the mass spectrum of the pyrrolidide derivatives of Δ^4 fatty acids (8). The double-bond position was unequivocally confirmed by the mass spectrum of the corresponding methyl 4,5-bis(methylthio)-nonadecanoate. The key fragments were observed at *m/z* 147 $[C_6H_{11}SO_2]^+$ and *m/z* 257$[C_{16}H_{33}S]^+$, thus indicating the double bond to be at C-4. Again, catalytic hydrogenation converted the methyl 4-nonadecenoate into methyl nonadecanoate, thus excluding the possibility of any methyl branching. The ir spectra indicated cis rather than trans stereochemistry.

Of the methyl branched monounsaturated fatty acids in the sponges, two were of considerable interest. Both fatty acid methyl esters with a $[M]^+$ at *m/z* 282 presented the same fragmentation pattern in ms. However, their retention time in capillary gc [special performance capillary column (HP-5MS) crosslinked with 5% Ph Me silicone] was different inasmuch as one methyl ester had an ECl of 16.25 and the other an ECl of 16.56, and both methyl esters eluted before the *iso*-17:0 methyl ester (Figure 1). The pyrrolidine derivative of both acids was identical, displaying a prominent peak at *m/z* 208 and a diminished intensity peak at *m/z* 180. This implies methyl substitution at C-7, which was corroborated by hydrogenating both methyl esters to the known 7-methylhexadecanoic acid methyl ester. The double-bond positions in both acids were determined to be at C-6 by a difference of 12 mass units between fragments at *m/z* 154 (C_5) and *m/z* 166 (C_6) in the mass spectrum of the corresponding pyrrolidide derivatives. Therefore, we have to conclude from the experimental data that we have identified both stereoisomers of the 7-methyl-6-hexadecenoic acid. We further corroborated our structural assignments by gc co-injection with an authentic sample provided to us by Dr. V.R. Mamdapur, who recently synthesized the 7-methyl-6-hexadecenoic acid (11). This acid was mistakenly
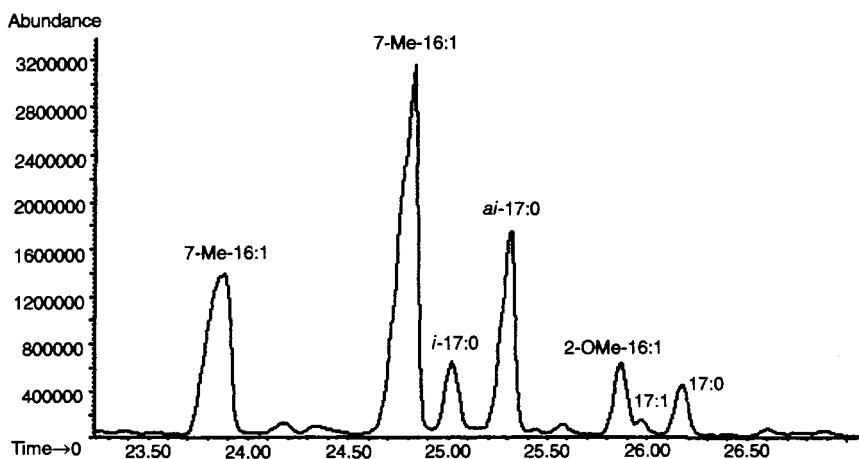

FIGURE 1.   Capillary gc separation of isomeric methyl 7-methyl-6-hexadecenoates. (See Experimental for details.)

reported before by us from *D. anchorata* as 7-methyl-8-hexadecenoic acid (7). One of the stereoisomers of the 7-methyl-6-hexadecenoic acid was identified before by us from *A. complanata* (4), but the compound was first reported from whale oils by both Pascal and Ackman (5) and Sano (6). However, this is the first report of both stereoisomers in a sponge. We believe that the compounds are of bacterial origin because we have also recently identified the same isomers in other invertebrates, such as in the fireworm *Hermodice carunculata* and the anemone *Stoichactis helianthus* (N. Carballeira, unpublished results).

Both sponges contained reasonable amounts of methyl branched fatty acids. The iso-anteiso methyl branching was predominant, but other isomers were identified. Of special recognition was the identification of the unusual 16-methylnonadecanoic acid in *D. anchorata*. The pyrrolidide derivative was key to the location of methyl branching. The corresponding pyrrolidide displayed a molecular ion peak at m/z 365 and more abundant than usual flanking peaks at m/z 294 (C_{15}) and m/z 322 (C_{17}), in addition to a diminished peak at m/z 308 (C_{16}). This indicates methyl substitution at C-16 (8). The 16-methylnonadecanoic acid was identified before in the sponge *Dysidea fragilis* (12).

Several diunsaturated fatty acids were characterized in the sponges, in particular the typical sponge phospholipid very long-chain fatty acids 5,9-pentacosadienoic acid and 5,9-hexacosadienoic acid. We also characterized a complete series of eicosadienoic acids of intermediate chain-length with unsaturations at positions 5,9-, 5,11-, 5,13-, 7,13-, and 11,14. The most interesting dienoic acid in *A. viridis* was 5,13-nonadecadienoic acid [1]. Although the 5,13 diunsaturation has been observed before in eicosadienoic acids, it has never been detected in odd-chain acids such as a nonadecadienoic acid. Character-ization of the dienoic acid was possible by means of double dimethyl disulfide addition to afford the methyl 5,6,13,14-tetrakis(methylthio)nonadecanoate. This compound had a $[M]^+$ at m/z 496. Cleavage between C-5 and C-6 afforded the fragments at m/z 161 $[C_7H_{13}SO_2]^+$ and m/z 335, but the latter fragment readily loses a mole of CH_3SH to give a fragment at m/z 287 $[C_{16}H_{31}S_2]^+$. Alternatively, cleavage between C-13 and C-14 afforded the fragments at m/z 131 $[C_7H_{15}S]^+$ and m/z 365, but the latter ion also readily loses a mole of CH_3SH resulting in a detectable fragment at m/z 317 $[C_{16}H_{29}S_2O_2]^+$. These fragmentations, therefore, confirm the double-bond positions between C-5 and C-6 and between C-13 and C-14. To further confirm the results, the corresponding *N*-nonadeca-5,13-dienoylpyrrolidine was also prepared. The derivative had a $[M]^+$ at m/z 347 and 12 mass units spacing between m/z 140 (C_4) and m/z 152 (C_5) as well as between m/z 250 (C_{12}) and m/z 262 (C_{13}) were observed, corroborating the double-bond positions

1

2a

2b
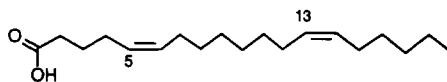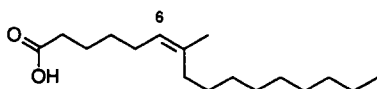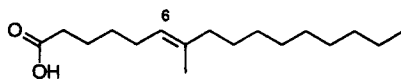
at Δ^5 and Δ^{13}. Catalytic hydrogenation converted the methyl 5,13-nonadecadienoate into methyl nonadecanoate, thus excluding the possibility of any methyl branching. The ir spectrum also indicated cis rather than trans stereochemistry.

Eleven different monounsaturated aldehydes, essentially arising from 1-O-alkenyl phospholipids or plasmalogens, were identified in trace amounts in the sponges (Table 1). The aldehydes were initially characterized by gc-ms as dimethyl acetals (from reaction of the alkenyls with HCl/MeOH). While several aldehydes have been identified before, others are new and/or have never been identified in marine organisms. For example, the 15- and 17-docosenal are unprecedented. The double-bond positions were determined by dimethyl disulfide derivatization. However, when the initially isolated dimethyl acetals were submitted to the dimethyl disulfide reaction, the acetal function-ality reversed to the ketone. For example, reaction of the dimethyl acetal of 15-docosenal afforded 15,16-bis(methylthio)docosanal which has a $[M]^+$ at m/z 416 and prominent fragmentations at m/z 271 $[C_{16}H_{31}SO]^+$ and m/z 145 $[C_8H_{17}S]^+$. On the other hand, the dimethyl acetal of 17-docosenal afforded the 17,18-bis(methylthio)docosanal, which presented a $[M]^+$ at m/z 416 and the more conspicuous fragmentations at m/z 117 $[C_6H_{13}S]^+$ and m/z 299 $[C_{18}H_{35}SO]^+$, indicating a double bond at Δ^{17}. The other aldehydes in the mixture were characterized in a similar fashion.

In this work we have expanded our present knowledge of possible fatty acids in sponges. Studies are in progress towards the understanding of novel fatty acid pathways in the marine environment.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Fatty acid methyl esters were analyzed by gc-ms using a 5972A MS ChemStation (Hewlett-Packard, Palo Alto, CA) equipped with a 30 m× 0.25 mm special performance capillary column (HP-5MS) crosslinked with 5% phenyl methyl silicone. The temperature program was as follows: 130° for 2 min, then increased at 3°/min to 270° and maintained for 40 min. The carrier gas was He at a pressure of 10 psi. Gc-Ftir spectra were recorded on a Nicolet (Madison, WI) 740 Ft-ir spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a GE 300 MHz spectrometer. The sponges were freeze-dried or lyophilized before analysis.

ANIMAL MATERIAL.—*Desmapsamma anchorata* was collected 21 January, 1987, near Cayo Enrique, La Parguera, Puerto Rico, at a depth of 50 ft. *Amphimedon viridis* was collected December 1993, near Cayo Enrique, La Parguera, Puerto Rico, at a depth of 1 ft. Voucher specimens of the sponges are available at the Department of Chemistry, University of Puerto Rico, Río Piedras, Puerto Rico.

EXTRACTION AND ISOLATION OF PHOSPHOLIPIDS.—The sponges (65–90 g, dry wt) were carefully cleaned of all non-sponge debris and cut into small pieces. Extraction with 2×250 ml of CHCl$_3$-MeOH (1:1) yielded the total lipids (ca. 6 g). The neutral lipids, glycolipids, and 40 mg of phospholipids were separated by cc on Si gel (60–200 mesh) using the procedure of Privett *et al.* (13). The phospholipid classes were fractionated by prep. tlc using Si gel 60 and CHCl$_3$-MeOH-NH$_4$OH (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 (14) followed by cc purification eluting with *n*-hexane-Et$_2$O (9:1). The double-bond positions of the monoenoic and dienoic fatty acids were elucidated by preparing the corresponding dimethyl disulfide derivatives by dissolving the esters (2 mg) in dimethyl disulfide (0.2 ml) and adding a solution (0.05 ml) of I$_2$ in Et$_2$O (60 mg/ml), heating the solution at 50° for 24 h, followed by the standard workup (15). *N*-Acylpyrrolidide derivatives were prepared by direct treatment of the methyl esters with pyrrolidine-HOAc (10:1) in a capped vial (93 h at 100°) followed by Et$_2$O extraction from the acidified solution and purification by prep. tlc. Hydrogenations were carried out in 10 ml of MeOH and catalytic amounts of PtO$_2$. Spectral data for the key fatty acids for this discussion follow.

Spectral data for all fatty acid methyl esters from A. viridis.—Ir ν max 3018, 2955, 2832, 1740, 1465, 1425, 1155, 1050, 718 cm^{-1}; ^{1}H nmr (300 MHz, CDCl$_3$) δ 0.88 (t, CH_3CH_2-), 1.25 (m, -CH_2-), 1.61 (m, -CH=CH-CH$_2$-CH_2-), 2.06 (m, -CH_2-CH=CH-), 2.30 (t, -CH_2-CO$_2$CH$_3$), 2.82 (m, -CH=CH-CH_2-CH=CH-), 3.66 (s, CH$_3$O), 5.35 (m, CH=CH).

Methyl 4,5-bis(methylthio)nonadecanoate.—Ms *m/z* [M]$^+$ 404 (3), [M−CH$_3$SH]$^+$ 356 (2), 327 (2), [C$_{16}$H$_{33}$S]$^+$ 257 (100), [C$_6$H$_{11}$SO$_2$]$^+$ 147 (86), 123 (8), [C$_5$H$_7$SO]$^+$ 115 (59), 97 (34), 95 (27), 87 (60), 79 (14), 74 (22), 69 (47), 67 (35), 61 (42), 57 (40), 55 (65).

N-*Nonadec-4-enoylpyrrolidine.*—Ms *m/z* [M]$^+$ 349 (5), 334 (0.5), 320 (1), 306 (1), 292 (2), 278 (2), 264 (1), 250 (2), 236 (4), 222 (8), 208 (6), 194 (6), 180 (7), 166 (41), 152 (12), 138 (4), 126 (26), 113 (100), 98 (42), 95 (10), 70 (25), 57 (14), 55 (40).

Methyl 5,6,13,14-tetrakis(methylthio)nonadecanoate.—Ms *m/z* [M]$^+$ 496 (5), [M−C$_2$H$_6$S$_2$]$^+$ 402 (1), [M−C$_3$H$_9$S$_3$]$^+$ 355 (3), 353 (2), [C$_{16}$H$_{29}$S$_2$O$_2$]$^+$ 317 (18), 288 (3), [C$_{16}$H$_{31}$S$_2$]$^+$ 287 (19), [C$_{15}$H$_{25}$SO$_2$]$^+$ 269 (16), 241 (13), [C$_{14}$H$_{21}$O$_2$]$^+$ 221 (11), 192 (2), [C$_{14}$H$_{23}$]$^+$ 191 (6), [C$_{13}$H$_{17}$O]$^+$ 189 (10), [C$_7$H$_{13}$SO$_2$]$^+$ 161 (58), [C$_7$H$_{15}$S]$^+$ 131 (71), 130 (10), [C$_6$H$_9$SO]$^+$ 129 (63), 97 (16), 95 (32), 91 (12), 83 (39), 81 (44), 71 (17), 69 (31), 67 (51), 57 (14), 55 (78).

N-*Nonadeca-5,13-dienoylpyrrolidine.*—Ms *m/z* [M]$^+$ 347 (2), 332 (0.05), 318 (0.1), 304 (0.4), 290 (0.4), 276 (0.2), 262 (0.1), 250 (0.2), 236 (1.8), 222 (0.4), 208 (0.6), 194 (1.3), 180 (1.8), 166 (1.3), 152 (0.6), 140 (1), 126 (9.7), 113 (100), 98 (8), 95 (3), 85 (9), 70 (9), 67 (5), 55 (15).

Methyl 15,16-bis(methylthio)heneicosanoate.—Ms *m/z* [M]$^+$ 432 (7), 401 (6), 302 (18), [C$_{17}$H$_{33}$SO$_2$]$^+$ 301 (100), 270 (13), [C$_{16}$H$_{29}$SO]$^+$ 269 (75), 257 (1), [C$_7$H$_{15}$S]$^+$ 131 (84), 104 (35), 76 (17), 61 (78).

N-*Heneicos-15-enoylpyrrolidine.*—Ms *m/z* [M]$^+$ 377 (10), 348 (2), 334 (4), 320 (3), 306 (2), 292 (1), 280 (2), 266 (3), 252 (2), 238 (3), 224 (5), 210 (2), 196 (2), 182 (2), 168 (3), 154 (3), 140 (4), 126 (28), 113 (100), 98 (13), 85 (25), 71 (27), 57 (29).

ACKNOWLEDGMENTS

LITERATURE CITED

1.   V.M. Dembitsky, T. Rezanka, and A.G. Kashin, *Comp. Biochem Physiol.,* **106B**, 825 (1993).
2.   T. Rezanka and V.M. Dembitsky,*J. Nat. Prod.,* **56**, 1898 (1993).
3.   N. Carballeira, V. Negrón, and E.D. Reyes,*J. Nat. Prod.,* **55**, 333 (1992).
4.   N. Carballeira and J. Restituyo,*J. Nat. Prod.,* **54**, 315 (1991).
5.   J.C. Pascal and R.G. Ackman, *Lipids,* **10**, 478 (1975).
6.   Y. Sano, *Yukagaku,* **16**, 606 (1967).
7.   N. Carballeira and M. Maldonado, *Lipids,* **23**, 690 (1988).
8.   B.A. Andersson, *Prog. Chem. Fats Lipids,* **16**, 279 (1978).
9.   I.A. Wolff and T.K. Miwa,*J. Am. Chem. Soc.,* **42**, 212 (1965).
10. M. Geiger, B.S. Mohammed, S. Sankarappa, and H. Sprecher, *Biochim. Biophys. Acta,* **1170**, 137 (1993).
11. B.A. Kulkarni, A. Chattopadhyay, and V.R. Mamdapur, *Synth. Commun.,* **22**, 2921 (1992).
12. W.W. Christie, E.Y. Brechany, K. Stefanov, and S. Popov, *Lipids,* **27**, 640 (1992).
13. O.S. Privett, K.A. Dougherty, W.L. Erdahl, and A. Stolyhwo,*J. Am. Oil Chem. Soc.,* **50**, 516 (1973).
14. J.P. Carreau and J.P. Dubacq,*J. Chromatogr.,* **151**, 384 (1978).
15. G.W. Francis and K. Veland,*J. Chromatogr.,* **219**, 379 (1981).