Sponge Fatty Acids, 5. Characterization of Complete Series of 2-Hydroxy Long-Chain Fatty Acids in Phospholipids of Two Senegalese Marine Sponges from the Family Suberitidae: Pseudosuberites sp. and Suberites massa

Gilles Barnathan, Jean-Michel Kornprobst, Pierre Doumenq, Joseph Miralles, and Nicole Boury-Esnault

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SPONGE FATTY ACIDS, 5.[1] CHARACTERIZATION OF COMPLETE SERIES OF 2-HYDROXY LONG-CHAIN FATTY ACIDS IN PHOSPHOLIPIDS OF TWO SENEGALESE MARINE SPONGES FROM THE FAMILY SUBERITIDAE: *PSEUDOSUBERITES* SP. AND *SUBERITES MASSA*

GILLES BARNATHAN, JEAN-MICHEL KORNPROBST,*

Institut des Sciences et Organismes de la Mer (ISOMer), Groupe SMAB, 1, rue Gaston Veil, 44035 Nantes Cedex 01, France

PIERRE DOUMENQ,

Centre de Spectroscopie Moléculaire, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille III, 13397 Marseille Cedex 13, France

JOSEPH MIRALLES,

Ecole Normale Supérieure, Nouakchott, Mauritanie,

and NICOLE BOURY-ESNAULT

Centre d'Océanologie de Marseille, Station Marine d'Endoume, 13007 Marseille, France

ABSTRACT.—Phospholipid fatty acid composition was studied in two Senegalese sponges of the family Suberitidae. More than fifty acids were identified in either *Pseudosuberites* sp. or *Suberites massa*. A series of 2-hydroxy long-chain fatty acids (C_{22} to C_{27}) accounted for almost 50% of the total acid mixture of *Pseudosuberites* sp., including 2-hydroxyhexacosanoic acid (26%) and the unusual 2-hydroxy-heptacosanoic acid not yet reported in any sponge. A series of 3-hydroxy short-chain fatty acids was also detected in this sponge. Two new fatty acids were identified in these sponges, namely 4,8-dimethyldecanoic and 12-methyl-(*Z*)-6-tridecenoic, in addition to the rare 13-methyl-(*Z*)-4-tetradecenoic. *Su. massa* contained mainly 5,9-dienoic and trienoic demospongic acids. For the first time, 2-hydroxy long-chain fatty acid methyl esters were analyzed by gc-Ft-ir, and the corresponding *N*-acyl pyrrolidide derivatives by gc-ms.

As part of our ongoing comparative studies of fatty acids and sterols of sponges from Senegalese coastal waters (1–4), we investigated two sponges belonging to the family Suberitidae (Order Hadromerida): *Pseudosuberites* sp. Topsent (Hadromerida) and *Suberites massa* Nardo (Hadromerida). To our knowledge, no phospholipid fatty acids from any sponges of the family Suberitidae have yet been described.

Among the numerous phospholipid fatty acid compositions reported to date, the occurrence of 2-oxo-substituted fatty acids in sponge phospholipids has been described only a few times. The Senegalese sponge *Higginsia tethyoides* (Axinellida, Axinellidae) was the first shown to contain several unusual 2-methoxy fatty acids (up to 28 carbon atoms) (5,6). A series of 2-acetoxy fatty acids (up to 30 carbon atoms) was then encountered in *Polymastia gleneni* (Hadromerida, Polymastidae), (7). However it was only in 1989 that Carballeira and Lopez (8) identified 2-hydroxy fatty acids for the first time in phospholipids from *Amphimedon compressa* (Haposclerida, Niphatidae) and then from *Aplysina archeri* and *Verongula gigantea* (Verongida, Aplysinidae) (9). Two other marine sponges, *Tethya crypta* and *Spheciospongia cuspidifera* (Hadromerida, Tethyidae and Spirastrellidae, respectively), were shown to contain 2-hydroxy long-chain fatty acids (9,10). Recently, an interesting finding was the occurrence of six novel *iso* and *anteiso* branched 2-hydroxy fatty acids in a Caribbean sponge, *Smenospongia aurea* (Dictyoceratida, Thorectidae) (11).

In this paper, we report the occurrence of a complete series of 2-hydroxy long-chain

[1]For Part 4 of this series, see Barnathan *et al.* (4).

fatty acids, including the longest one ever identified in sponge phospholipids, namely 2-hydroxyheptacosanoic acid. In addition, we describe the first use of gc-Ft-ir for analysis of 2-hydroxy long chain fatty acid methyl esters and of gc-ms for the corresponding pyrrolidides. We also report here the identification of two new short-chain fatty acids, namely 4,8-dimethyldecanoic and 12-methyl-(*Z*)-6-tridecenoic.

RESULTS AND DISCUSSION

Tlc analysis showed that the main phospholipids from *Pseudosuberites* sp. and *Su. massa* are phosphatidylethanolamine and phosphatidylserine. As indicated in Table 1, more than 50 fatty acids were identified in each sponge, most being present in both organisms.

TABLE 1. Phospholipid Fatty Acids from Two Senegalese Marine Sponges of the Family Suberitidae.[a]

Fatty acids		ECL (OV-1)	Source	
			Pseudosuberites sp. (%)	*Suberites massa* (%)
Fatty acids				
4,8-Dimethyldecanoic[b]	4,8-DM-10:0	10.92[d]	—	0.4
12-Methyl-6-tridecenoic[b]	*i*-6-14:1	13.40	0.5	—
12-Methyltridecanoic	*i*-14:0	13.60	tr.	1.8
Tetradecanoic	14:0	14.00	0.6	3.1
13-Methyl-4-tetradecenoic[c]	*i*-4-15:1	14.40[d]	—	0.6
4,8,12-Trimethyltridecanoic	4,8,12-TM-13:0	14.47	1.8	11.8
13-Methyltetradecanoic	*i*-15:0	14.63	0.5	3.0
12-Methyltetradecanoic	*ai*-15:0	14.73	—	0.7
Pentadecanoic	15:0	15.00	0.5	2.5
14-Methylpentadecanoic	*i*-16:0	15.63	0.5	3.0
8-Hexadecenoic	8-16:1	15.74	1.6	—
9-Hexadecenoic	9-16:1	15.80	—	2.6
Hexadecanoic	16:0	16.00	6.5	5.1
7-Methyl-7-hexadecenoic	7-Me-6-16:1	16.34	0.4	1.0
15-Methylhexadecanoic	*i*-17:0	16.65	—	0.7
14-Methylhexadecanoic	*ai*-17:0	16.72	1.0	1.1
Heptadecanoic	17:0	17.00	0.5	1.0
9,12-Octadecadienoic	9,12-18:2	17.52	1.3	0.5
17-Methylheptadecanoic	*i*-18:0	17.66	1.7	tr.
9-Octadecenoic	9-18:1	17.76	1.6	1.4
11-Octadecenoic	11-18:1	17.84	—	2.8
Octadecanoic	18:0	18.00	3.6	6.2
16-Methyloctadecanoic	*ai*-19:0	18.73	—	0.3
11-Nonadecenoic	11-19:1	18.82	0.5	—
12-Nonadecenoic	12-19:1	18.85	—	1.0
Nonadecanoic	19:0	19.00	0.5	1.0
18-Methylnonadecanoic	*i*-20:0	19.64	0.6	0.8
Icosanoic	20:0	20.00	0.6	0.5
19-Methylicosanoic	*i*-21:0	20.63	0.8	0.4
18-Methylicosanoic	*ai*-21:0	20.72	—	0.6
Tricosanoic	23:0	23.00	0.5	tr.
21-Methyltricosanoic	*ai*-24:0	23.74	0.5	—
17-Tetracosenoic	17-24:1	23.80	1.0	tr.
Tetracosanoic	24:0	24.00	0.6	0.2
22-Methyltetracosanoic	*ai*-25:0	24.72	0.9	—
16-Pentacosenoic	16-25:1	24.72	—	0.5
Pentacosanoic	25:0	25.00	1.1	—
17-Hexacosenoic	17-26:1	25.69	0.5	—

TABLE 1. Continued.

Fatty acids		ECL (OV-1)	Source	
			Pseudosuberites sp. (%)	*Suberites massa* (%)
18-Hexacosenoic	18-26:1	25.78	—	5.5
19-Hexacosenoic	19-26:1	25.75	2.6	—
Hexacosanoic	26:0	26.00	1.1	tr.
2-Hydroxy fatty acids				
2-Hydroxytetradecanoic	2-OH-14:0	15.14	0.3	0.4
2-Hydroxypentadecanoic	2-OH-15:0	16.13	0.6	tr.
2-Hydroxy-14-methylpentadecanoic	2-OH-*i*-16:0	16.88	0.5	—
2-Hydroxyhexadecanoic	2-OH-16:0	17.10	0.6	0.4
2-Hydroxyheptadecanoic	2-OH-17:0	18.14	0.5	—
2-Hydroxyoctacosanoic	2-OH-18:0	19.12	—	0.5
2-Hydroxydocosanoic	2-OH-22:0	23.15	0.5	0.2
2-Hydroxytricosanoic	2-OH-23:0	24.22	0.9	0.2
2-Hydroxytetracosanoic	2-OH-24:0	25.26	9.7	0.4
2-Hydroxypentacosanoic	2-OH-25:0	26.28	9.2	0.5
2-Hydroxyhexacosanoic	2-OH-26:0	27.28	25.9	—
2-Hydroxyheptacosanoic[c]	2-OH-27:0	28.26	2.7	—
3-Hydroxy fatty acids				
3-Hydroxydodecanoic	3-OH-12:0	13.38	0.6	—
3-Hydroxytetradecanoic	3-OH-14:0	15.35	0.3	—
3-Hydroxypentadecanoic	3-OH-15:0	16.32	0.4	—
3-Hydroxyhexadecanoic	3-OH-16:0	17.32	0.2	—
3-Hydroxyoctadecanoic	3-OH-18:0	19.36	0.2	—
$\Delta^{5,9}$-Demospongic acids				
5,9-Pentacosadienoic	5,9-25:2	24.50	0.5	0.9
5,9-Hexacosadienoic	5,9,-26:2	25.52	7.8	21.3
5,9-Heptacosadienoic	5,9-27:2	26.50	0.6	3.3
25-Methyl-5,9-heptacosadienoic	*ai*-5,9-28:2	27.32	1.6	—
5,9,19-Octacosatrienoic	5,9,19-28:3	27.39	0.4	11.1
5,9,21-Octacosatrienoic	5,9,21-28:3	27.40	—	0.7
5,9-Octacosadienoic	5,9-28:2	27.52	1.6	—

[a]ECL=equivalent chain length. *i*=iso; *ai*=anteiso. Identified minor fatty acids (%<0.4): 9-19:1; *ai*-22:0; 22:0; 27:0; 28:0.

[b]Not previously found in nature.

[c]First reported in sponge phospholipids.

Many of the fatty acids reported in Table 1 were identified simply by comparing their gc mobilities as methyl esters with those of known compounds and by co-injection with commercial standards. Capillary gc analysis of a hydrogenated aliquot also provided useful information for detection and identification of unsaturated acids. Of particular interest in this respect was the presence of a series of gc peaks that remained unchanged upon catalytic hydrogenation. These peaks, each eluted just after a straight-chain saturated fatty acid methyl ester (FAME), were identified as 2-hydroxy FAMEs by gc-ms experiments. A plot of their retention time vs. carbon number is given in Figure 1. From these data we obtained three homologous series forming parallel lines: saturated straight-chain acids, $\Delta^{5,9}$-demospongic acids, and 2-hydroxy long-chain fatty acids (LCFAs).

Pseudosuberites sp. contained 51.4% 2-hydroxy fatty acids, 95.1% of these being long-chain (2-OH-LCFAs,$>C_{22}$) and 12.5% $\Delta^{5,9}$-demospongic acids. *Su. massa* contained only 2.6% 2-hydroxy fatty acids, 50% being long-chain ones, but had 37.3%

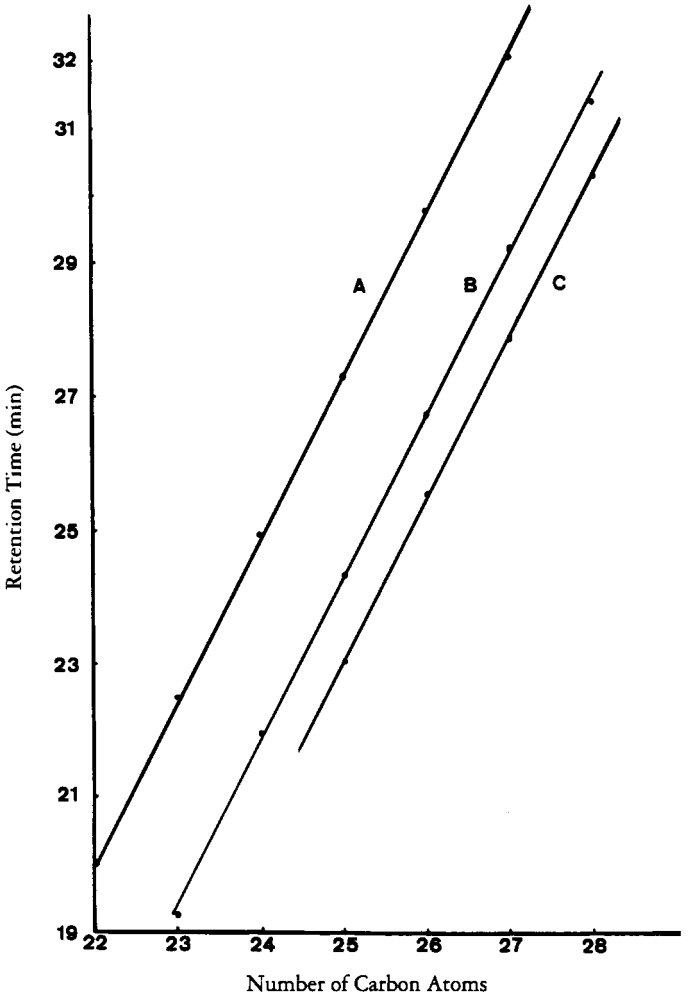

FIGURE 1. Plot of retention time (min) vs. number of carbon atoms for three complete series of fatty acid methyl esters from *Pseudosuberites* sp. A; 2-hydroxy acids; B: straight-chain saturated acids; C: straight-chain 5,9-dienoic acids.

typical $\Delta^{5,9}$-demospongic acids. 2-Hydroxyhexacosanoic acid and 2-hydroxyheptacosanoic acid accounted for 25.9% and 2.7%, respectively, in *Pseudosuberites* sp. but were lacking in *Su. massa*. The latter acid was identified for the first time here in sponge phospholipids, and the former one was previously encountered only in very small amounts (0.6%) in *Sm. aurea* (11). The mass spectra of these 2-hydroxy FAMEs exhibited molecular ion peaks and other diagnostic fragments as presented in Table 2.

All these ms contained a significant $[M-COOMe]^+$ fragmentation ion and a weak $[M-MeOH]^+$ fragmentation ion, suggesting α substitution. 2-Hydroxy substitution was indicated by the presence of two diagnostic fragmentation ions at *m/z* 90, arising from the McLafferty rearrangement (instead of the usual corresponding ion at *m/z* 74 for saturated methyl esters) and at *m/z* 103 (instead of the usual corresponding ion at *m/z* 87). The mass spectrum of 2-hydroxyheptacosanoic acid methyl ester is detailed as an example in the Experimental section, as this compound has not hitherto been described in any sponge. The ms of this methyl ester showed a molecular ion peak at *m/z* 440 and

TABLE 2. Diagnostic Fragments of 2-Hydroxy Long-chain Acid Methyl Esters of the Sponge *Pseudosuberites* sp. (base peak *m/z* 43).

Carbon	$[M]^+$ *m/z* (%)	$[M-MeOH]^+$ *m/z* (%)	$[M-COOMe]^+$ *m/z* (%)	McLafferty *m/z* 90 (%)	*m/z* 103 (%)
C-22	370 (10.0)	338 (0.7)	311 (11.5)	23.4	9.4
C-23	384 (13.1)	352 (1.4)	325 (13.9)	28.3	7.4
C-24	398 (9.5)	366 (1.3)	339 (8.7)	24.6	7.7
C-25	412 (11.8)	380 (2.2)	353 (7.9)	26.2	9.1
C-26	426 (9.8)	394 (0.8)	367 (5.3)	31.6	10.9
C-27	440 (7.8)	408 (0.7)	381 (5.7)	23.5	12.8

key fragments at *m/z* 90 and 103. Fragmentation ions at *m/z* 381 $[M-COOMe]^+$ and at 408 $[M-MeOH]^+$ were also present. Although the mass spectra of 2-hydroxy long-chain methyl esters seem sufficiently diagnostic, it was interesting to examine the mass spectra of the pyrrolidide derivatives. A previous study gave data just for isomeric hydroxyoctadecanoic acid pyrrolidides, including 2- and 3-hydroxy derivatives (12), but to our knowledge no data are available concerning 2-hydroxy long-chain fatty acid pyrrolidides. Results from our gc-ms experiments are summarized in Table 3.

As can be seen in Table 3, the base peak of all mass spectra was at *m/z* 129, arising from the McLafferty rearrangement, instead of the usual corresponding ion at *m/z* 113 for nonhydroxylated fatty acids. Two other major ions were also present in the mass spectra at *m/z* 98 and 100. However, two important fragmentation ions confirmed the presence of 2-hydroxy substitution at *m/z* 142 (a hydroxylated C_3 fragment, instead of the usual *m/z* 126) and at *m/z* 171 (a hydroxylated protonated C_5 fragment, instead of the usually weak peak at *m/z* 154). The mass spectrum of 2-hydroxyheptacosanoic acid pyrrolidide is detailed as an example in the Experimental section. The molecular ion peak $[M]^+$ was present at *m/z* 479, and ms showed the characteristic fragmentation ions at *m/z* 129, 100, and 98 and the homologous fragments at *m/z* 142, 156, 171, 184, etc.

This uncommon series of 2-hydroxylated long-chain fatty acids led us to perform gc-Ft-ir experiments. Previous reports showed that short-chain hydroxylated fatty acids could be readily identified from the position of the carbonyl band near 1746 cm^{-1} instead of 1758 cm^{-1} for non-functionalized fatty acid methyl esters (13,14). This frequency shift could be attributed to intramolecular hydrogen bonding. As can be seen in Figure 2, a frequency shift was also observed for the carbonyl band of 2-hydroxylated long-chain methyl esters: 1752 cm^{-1} instead of 1758 cm^{-1} (25:0).

Among twelve different demosponges harvested in the same area (see the Experimental section), only Suberitidae sponges contained 2-hydroxy fatty acids in their phospholipids (1,2, and unpublished results). Another exception from this same area was the sponge *Hi. tethyoides,* which contained a series of unusual 2-methoxy fatty acids (5,6). The occurrence of 2-hydroxylated long-chain fatty acids in phospholipids is a rather

TABLE 3. Ms Diagnostic Fragments of 2-Hydroxy Long-chain Acid Pyrrolidides of the Sponge *Pseudosuberites* sp.

Carbon	$[M]^+$ *m/z* (%)	$[M-OH]^+$ *m/z* (%)	McLafferty *m/z* 129 (%)	*m/z* 98 (%)	*m/z* 100 (%)	C_3 fragment *m/z* 142 (%)	C_5 fragment *m/z* 171 (%)
C-22	409 (0.9)	392 (0.5)	100	69.4	88.3	4.1	3.2
C-23	423 (1.2)	406 (0.6)	100	72.3	92.0	3.3	2.9
C-24	437 (1.3)	420 (0.5)	100	66.5	83.9	4.7	3.8
C-25	451 (0.8)	434 (0.2)	100	64.5	82.3	3.8	3.3
C-26	465 (0.8)	448 (0.4)	100	58.2	74.2	4.0	4.1
C-27	479 (0.6)	463 (0.3)	100	69.6	86.2	3.6	2.9

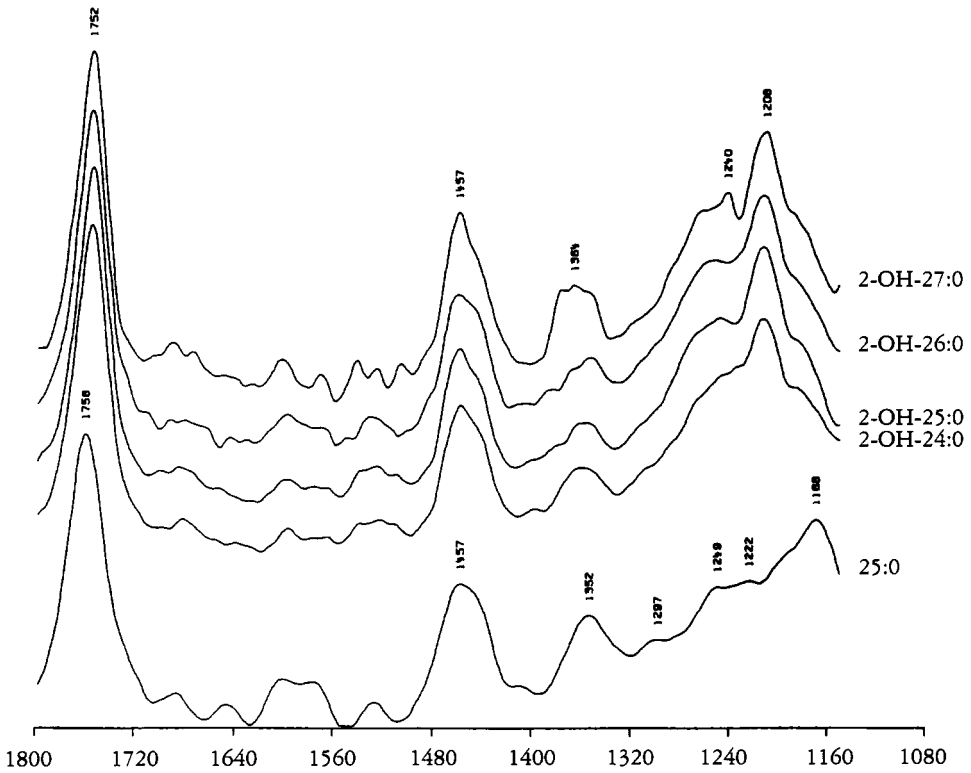

FIGURE 2. Gc-Ft-ir spectra of several 2-hydroxy long-chain fatty acid methyl esters.

unusual feature. Carballeira and coworkers have reported a few examples of such sponges, though not belonging to the family Suberitidae (8–10), and only one complete series of 2-hydroxyacids, from C_{17} to C_{26} including *iso* and *anteiso* acids, was found in the sponge *Sm. aurea* (11).

It is noteworthy that 2-hydroxy fatty acids are usually components of glycolipids such as cerebrosides from human brain (15), the sponges *Chondrilla nucula* (16) and *Halichondria japonica* (17), and starfishes (18,19). An important structural feature of cerebrosides is that 2-hydroxy acyl residues are usually linked in an amide bond. It is rather intriguing to find series of 2-hydroxy long-chain fatty acids both in sponges and human brain. At these high abundances (Table 1) the phospholipid 2-hydroxy fatty acids could not be of symbiotic origin, even though they are known to exist in microalgae (20) and sulfate-reducing bacteria (21). Therefore, it is very likely that these unusual phospholipid components play a specific role in cell membranes. In order to provide useful comparisons, we investigated the corresponding glycolipid fraction of *Pseudosuberites* sp. and found a complete series of 2-hydroxy acids accounting for 33% of the mixture, (Barnathan *et al.*, unpublished observations) compared to about 50% in phospholipids. In addition, 3-hydroxy short-chain fatty acids were readily identified in *Pseudosuberites* sp., since the mass spectra of their methyl esters and pyrrolidide derivatives showed typical base peaks at *m/z* 103 and 142, respectively. 3-Hydroxy short-chain acids are characteristic of bacteria and thus could be of symbiotic origin. These 3-hydroxy fatty acids have been rarely reported to occur in marine sponges (22).

Typical $\Delta^{5,9}$-demospongic acids were easily identified as methyl esters (base peak at *m/z* 81) and pyrrolidides (major peak at *m/z* 180). The large amount of the not-so-common 5,9,19-octacosatrienoic acid (11.1%, Table 1) in *Su. massa* probably indicates

the importance of biosynthetic elongation in the n-9 monoenoic series from oleic acid (23). Previously, significant amounts of this rare demospongic acid were found only in sponges from the genus *Xestospongia* (23,24). The first analysis of total lipids from *Suberites compacta* indicated that C_{28} fatty acids accounted for 17% of the total (25) but did not specify the molecular formula.

Furthermore, several short-chain fatty acids in our sponges were readily identified as *N*-acyl pyrrolidides. The first acid in question was detected in phospholipids of *Su. massa* (0.4%) and showed a very low equivalent chain length (ECL) value of 10.92 with a molecular ion peak at *m/z* 253 corresponding to a 12:0 fatty acid structure. The compound remained unchanged upon catalytic hydrogenation, indicating that no unsaturation was present. These results clearly point to methyl branching, as observed previously (1). The mass spectrum of the pyrrolidide showed an unusually diminished peak at *m/z* 140 flanked by a major ion peak at *m/z* 126 and an elevated peak at *m/z* 154, clearly indicating a methyl group at C-4 (Experimental). Similarly, the very diminished peak at *m/z* 210 (C_9 fragment, but C-8 position), flanked by a major peak at *m/z* 196 and an elevated peak at *m/z* 224, indicated a second methyl branch at C-8. These data corresponded to an isoprenoid acid, 4,8-dimethyldecanoic acid, not yet reported to our knowledge in any natural source. This acid has an *anteiso* structure instead of the *iso* structure present in known natural-isoprenoid acids (26). For example, 4,8-dimethylnonanoic acid was identified as a product of the catabolism of phytanic acid (26), whereas 4,8-dimethyldodecanoic acid was obtained as an oxidation product of 2,6,10-trimethyltetradecane by *Mycobacterium fortuitum* (27).

A second acid, from *Pseudosuberites* sp., was identified as a tetradecenoic acid (14:1, 0.5%) since its methyl ester presented an appreciable ion at *m/z* 280 $[MH]^+$. Its ECL value of 13.40 implied methyl branching. Positions of the double bond and the branching were determined from the gc-ms data of the pyrrolidide derivative (Figure 3).

There was a difference of 12 amu between the key fragmentation ions at *m/z* 154 (C_5) and *m/z* 166 (C_6), indicating Δ^6 unsaturation, and an *iso* methyl branch was indicated by a very low peak at *m/z* 250 (C_{12}), flanked by significant peaks at *m/z* 237 (C_{11}+H) and 265 (C_{13}+H). We thus identified the new acid as 12-methyl-6-tridecenoic acid, whose Ft-ir spectrum exhibited absorptions near 3010 cm^{-1} (valence vibration of the ethylenic bond) and 690 cm^{-1} (out-of-plane bending vibration), indicating a cis configuration (no absorption near 960 cm^{-1}) (13,14).

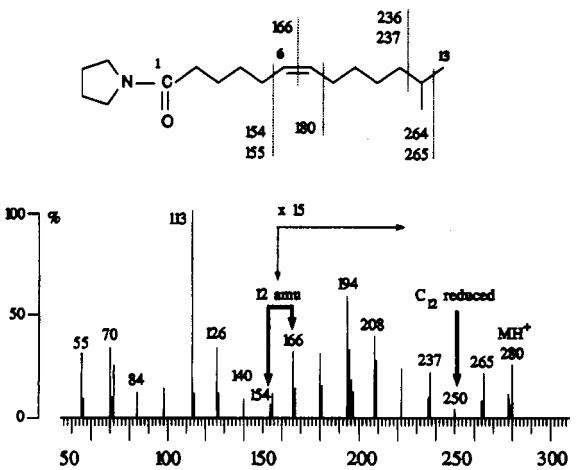

FIGURE 3. Mass spectrum of 12-methyl-6-tridecenoic acid pyrrolidide.

A third short-chain fatty acid was identified from *Su. massa* as a pentadecenoic acid (15:1, 0.6%) since its pyrrolidide derivative had a molecular ion peak at *m/z* 294 $[MH]^+$. The ECL value of 14.40 indicated a possible methyl branch. Positions of the double bond and the branching were determined from gc-ms data. The mass spectrum of the pyrrolidide (Experimental) showed key homologous fragments at *m/z* 126 (C_3) and 138 (C_4). The difference of 12 amu clearly indicated Δ^4 unsaturation. A diminished peak at *m/z* 264 (C_{13}) was consistent with methyl branching at the C-13 position. Thus, we were able to identify the very rare acid 13-methyl-4-tetradecenoic (*iso*-4-15:1), recently characterized for the first time in total lipids of the sponge *Dysidea fragilis* (28) as picolinyl ester. The double bond was of *Z* configuration , as the Ft-ir spectrum showed absorptions at 3012 and 690 cm^{-1} but no absorption at 960 cm^{-1}.

Another branched monoenoic acid was readily characterized as 7-methyl-6-hexadecenoic, already found in some marine organisms (29,30). These short-chain branched monoenoic acids probably arise from bacterial symbionts. In this respect, it should be noted that the corresponding *anteiso*-4-15:1 was reported from a plant pathogen *Corynebacterium* (31).

Several fatty aldehydes, characterized by their resulting dimethyl acetals, were also present in small amounts in the total mixture, arising from methanolysis of sponge phospholipids. Such long-chain aldehydes indicate the presence of plasmalogens (1-*O*-alk-1-enyl-2-acyl phospholipids). All mass spectra of dimethyl acetals displayed a typical base peak at *m/z* 75 $[C_3H_7O_2]^+$ and an appreciable fragmentation ion corresponding to the loss of the methoxy group $[M-31]^+$. *Pseudosuberites* sp. contained octadecanal (18:0) and nonadecanal (19:0), while *Su. massa* contained hexadecanal (16:0) and heneicosanal (21:0). An interesting result was the occurrence of a hexacosenal (26:1) in phospholipids of both Suberitidae sponges; its dimethyl acetal showed a base peak at *m/z* 75 and another peak at *m/z* 393 $[M-31]^+$. The position of the double bond was not determined due to the small amount available. (*Z*)-19-Hexacosenal was reported from *Amphimedon compressa* (32). *Aplysina lacunosa* has recently been shown to contain heptacosanal (27:0) but no hexacosanal (11).

In summary, these two Suberitidae sponges are of particular interest since they can synthesize and incorporate series of very long-chain 2-hydroxy fatty acids into phospholipids. Gc-ms and gc-Ft-ir are useful methods for their analysis. Biosynthetic pathways and membrane functions are still a matter of speculation. Work is in progress in our laboratory to examine phospholipids from other Senegalese sponges of the family Suberitidae.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The methyl esters were analyzed by glc using a Carlo-Erba 4130 chromatograph and a non-polar OV-1 silica capillary column (25 m×0.32 mm i.d., 0.40 μm phase thickness); H_2 was used as carrier gas (50 kPa, split 5/100). The temperature was programmed at 3°/min from 180° to 300°. Gc-ms was performed on a Hewlett-Packard HP-5890 chromatograph linked to a HP 9000/345 integrator. The gc column was a 30 m×0.32 mm i.d. fused silica capillary column coated with DB-1 (0.25 μm phase thickness). The carrier gas was He (0.5 bar, split 1/30). Column temperature was programmed from 180° to 310°, at 3°/min, for both derivatives (methyl esters and pyrrolidides). Gc-Ft-ir spectra were recorded with a 20S×B Nicolet spectrometer, interfaced with a 5300 Mega Carlo Erba FID chromatograph equipped with a fused silica capillary column (J&W; DB-1; 60 m, 0.32 mm i.d.; 0.25 μm film thickness). Oven temperature was programmed from 70° to 130° (15°/min) and from 130° to 290° (4°/min); isotherm steps of 2 min at 70° and 40 min at 290° were used. Carrier gas: He 140 kPa, H_2 50 kPa; air 100 kPa. The light-pipe (ir gas cell) was gold-coated with dimensions of 160 mm×1.2 mm; temperature was maintained at 293° for the experiments. Vapor phase ir spectra were performed with 8 cm^{-1} resolution; 16 data scans were collected and co-added per data file (2 sec each spectrum). Chromatogram reconstruction was performed with the Gram-Schmidt algorithm.

SPONGE MATERIAL.—*Su. massa* and *Pseudosuberites* sp. were collected near Joal (about 100 km south of Dakar, Senegal) on the seashore at low tide, in January and June 1991, respectively. *Pseudosuberites* sp. is a massive sponge, dark green on the top side and orange on the bottom side. The largest specimen had a surface area of 30×30 cm and was 4–5 cm thick. The surface is smooth and the oscules are visible at the top of each lobe of the upper surface. The choanosomal skeleton is composed of small ascending tracts linked by transversed spicules which give a confused appearance to the skeleton. The ectosomal skeleton is composed of a membrane in which the spicules are irregularly arranged. The spicules are of three types: tylostyles of two size classes and tylostrongyles. The main tylostyles are straight with an acerate or stepped extremity: 371–821/11–14 μm (mean 656/13μm). Small tylostyles are present especially in the ectosome: 122–371/3–5 μm (mean 269/3.5 μm). Tylostrongyles are short and thick: 69–122/20–2 μm (102/20.5 μm). A specimen is on file at the Centre d'Océanologie de Marseille, Station Marine d'Endoume, number SME-1991-M.

EXTRACTION AND ISOLATION OF PHOSPHOLIPIDS.—The sponges were washed in sea water, carefully cleaned, cut into small pieces, and lyophilized. Sponge pieces were ground in a Waring blender, using $CHCl_3$-MeOH (1:1), and steeped twice in this solvent for 24 h (room temperature). The combined extracts yielded the crude total lipids. Phospholipids were separated from other lipids by cc on Si gel (70–230 mesh) with hexane, $CHCl_3$, Me_2CO, and MeOH (phospholipids) as successive eluents. Phospholipids (182 mg) were recovered from 181 g (dry wt) of *Pseudosuberites* sp. (0.10%), while 95 g of *Su. massa* (dry wt) yielded 115 mg (0.12%). Phospholipid composition was analyzed by tlc [$CHCl_3$-MeOH-H_2O(65:25:4)] using standard phospholipid samples.

PREPARATION OF FATTY ACID DERIVATIVES.—Phospholipid fatty acids were converted to methyl esters by reaction (30 min under reflux) with methanolic HCl, then dissolved in hexane and purified by cc [Si gel, hexane-Et_2O (10:1)]. *N*-acyl pyrrolidide derivatives were prepared by direct treatment of methyl esters with pyrrolidine-Ac_2O (10:1) under reflux (2 h) and purified by tlc on 0.5 mm layers of Si gel with hexane-Et_2O (1:2). Fatty acid methyl esters were hydrogenated by stirring (4 h) at ambient pressure and temperature in MeOH with catalytic amounts of platinum (IV) oxide (Adam's catalyst). Mass spectral data for several interesting derivatives are presented below.

4,8-Dimethyldecanoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[M]^+$ 253 (18.9) 238 (4.0), 224 (22.1), 210 (2.0), 196 (9.0), 182 (8.8), 168 (16.8), 154 (27.9), 140 (6.0), 126 (34.7), 113 (100), 98 (6.2), 85 (7.1), 70 (27.9), 55 (27.5), 43 (27.6).

12-Methyl-6-tridecenoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[MH]^+$ 280 (1.6), 279 (0.7), 265 (1.4), 250 (0.1), 237 (1.6), 222 (1.4), 208 (2.7), 194 (3.1), 180 (3.2), 166 (2.8), 155 (8.1), 154 (2.8), 140 (6.5), 127 (21.0), 126 (46.8), 113 (100), 98 (14.5), 84 (6.5), 72 (21.0), 70 (29.0), 55 (17.7), 43 (21.0).

13-Methyl-4-tetradecenoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[MH]^+$ 294 (3.7), 293 (2.8), 278 (2.0), 264 (0.1), 250 (2.0), 236 (1.9), 222 (1.8), 210 (1.9), 208 (2.5), 196 (1.8), 194 (3.8), 180 (4.1), 167 (4.1), 166 (7.2), 154 (3.8), 153 (3.7), 152 (5.1), 138 (4.1), 126 (30.8), 113 (100), 98 (15.1), 85 (7.2), 70 (15.3), 55 (26.1), 43 (31.2).

2-Hydroxyheptacosanoic acid methyl ester.—Ms *m/z* (rel. int.) $[M]^+$ 440 (7.8), $[M-32]^+$ 408 (0.7) $[M-59]^+$ 381 (5.7), 362 (2.4), 349 (2.6), 337 (2.3), 281 (3.1), 252 (1.6), 223 (2.5), 211 (2.7), 199 (2.3), 195 (2.4), 181 (2.3), 171 (10.5), 157 (7.2), 145 (6.8), 127 (11.2), 111 (17.1), 109 (9.5), 103 (12.8), 97 (41.0), 90 (23.5), 83 (34.2), 69 (18.5), 57 (37.2), 43 (100).

2-Hydroxyhexacosanoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[M]^+$ 465 (0.8), $[M-OH]^+$ 448 (0.4), 434 (0.3), 420 (0.4), 406 (0.7), 380 (0.3), 366 (0.4), 352 (0.3), 338 (0.4), 296 (0.5), 282 (0.5), 268 (0.4), 254 (0.4), 240 (0.3), 226 (0.5), 212 (0.4), 184 (2.0), 171 (4.1), 156 (0.5), 142 (4.0), 129 (100), 100 (74.2), 99 (28.7), 98 (58.2), 84 (0.7), 70 (15.0), 55 (38.7), 43 (45.1).

2-Hydroxyheptacosanoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[M]^+$ 479 (0.6), $[M-OH]^+$ 463 (0.3), 448 (0.3), 434 (0.2), 420 (0.6), 406 (0.4), 364 (0.2), 350 (0.4), 340 (0.1), 338 (0.1), 322 (0.3), 282 (0.3), 268 (0.1), 254 (0.2), 240 (0.6), 226 (0.3), 212 (0.6), 198 (0.8), 184 (1.8), 171 (2.9), 156 (0.5), 142 (3.6), 129 (100), 100 (86.2), 99 (34.8), 98 (69.6), 85 (0.3), 83 (0.4), 70 (30.0), 55 (37.5), 43 (43.8).

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