Sponge Fatty Acids. 3. Occurrence of Series of n-7 Monoenoic and *iso*-5,9 Dienoic Long-Chain Fatty Acids in the Phospholipids of the Marine Sponge *Cinachyrella* aff. *schulzei* Keller[1]

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The fatty acid composition of phospholipids from the New Caledonian sponge *Cinachyrella* aff. *schulzei* Keller was studied. More than 60 fatty acids were identified as methyl esters and *N*-acyl pyrrolidides by gas chromatography and gas chromatography/mass spectrometry. Two isoprenoid fatty acids also were shown to be present, namely 4,8,12-trimethyltridecanoic and 5,9,13-trimethyltetradecanoic acids. The unusual 6-tetradecenoic, 6-pentadecenoic, 12-nonadecenoic and 26-methylheptacosanoic (*iso*-28:0) acids were found for the first time in sponge phospholipids. A series of six n-7 monoenoic long-chain fatty acids (C_{23} to C_{28}) were identified, including the rare 16-tricosenoic, 18-pentacosenoic and 21-octacosenoic acids. Fifteen fatty acids possessing the typical 5,9 dienoic moiety accounted for 30% of the total fatty acid mixture. Two new fatty acids were identified, namely 5(*Z*)-octacosenoic and 27-methyl-5(*Z*),9(*Z*)-octacosadienoic (*iso*-5,9-29:2). Based on gas chromatography/Fourier transform infrared experiments, the double bonds were assigned the (*Z*) configuration.
***Lipids* 29, 297–303 (1994).**

Marine sponges are rich sources of long-chain phospholipid fatty acids possessing unique branched or substituted carbon chains probably arising from unique biosynthetic pathways (1–4). Many aspects of sponge phospholipid chemistry and biochemistry have been reviewed by Djerassi and Lam (5). We recently described the phospholipid fatty acid composition of *Cinachyrella alloclada* from the Senegalese coast (6), and have continued our investigations on sponge species belonging to the *Cinachyrella* genus collected in different geographical locations to provide comparative data that also may serve as chemotaxonomic criteria. Some isoprenoid fatty acids have been reported to occur in sponges, especially 4,8,12-trimethyltridecanoic acid (4,8,12-TMTD), which rarely occurs in abundance; however, it amounted to about 20% of total fatty acids in *C. alloclada* and *Spheciospongia vesparium* (6,7). It was in the phospholipids of *C. alloclada* that two different isoprenoid fatty acids were first found, namely the 4,8,12-TMTD (6) and the unusual 5,9,13-trimethyltetradecanoic acid (5,9,13-TMTD). *Iso*- and *anteiso*-saturated long-chain fatty acids are not common in nature, but some have been reported to be associated with sponge phospholipids (8). Straight-chain monoenoic fatty acids with Δ5, Δ6 or Δ8 unsaturation are also not very common in the phospholipids of sponges. Thus, only a few examples of Δ5 monoenoic long-chain fatty acids (LCFA) have been reported (9,10) even though they are known to be intermediates in the biosynthesis of 5,9-dienoic demospongic acids (11,12).

Our search of novel fatty acids in sponge phospholipids is also intended to find potential biosynthetic intermediates of sponge fatty acid metabolism. The biosynthetic route operative in marine sponges involves an elongation process in the n-7 monoenoic series from short-chain homologues, which extends only to C_{26} unsaturated fatty acids (12,13). The Senegalese sponge *Higginsia tethyoides* contains a complete series of such n-7 monoenoic LCFA, but all are α-methoxy-substituted analogues (2). 16-Tricosenoic acid was found first in *C. alloclada* (6). This acid and several other monoenoic LCFA have been found by Carballeira *et al.* (14) in *Amphimedon compressa* and *Mycale laevis*. In this respect, our findings of naturally occurring 23-triacontenoic and 9,23-triacontadienoic acids in *Trikentrion loeve* would seem to be of considerable interest (1), as these acids have been postulated to be intermediates in the biosynthesis of the relatively abundant 5,9,23-triacontatrienoic acid (15). One feature common to many sponge fatty acids is the occurrence of the 5,9-dienoic system in fatty acids, with even and odd long carbon chains. However, very few sponges studied to date have been shown to contain many of these acids (16,17). Brominated demospongic fatty acids are rare and, to our knowledge, only three have been described to date (18,19).

We report here the phospholipid fatty acid composition of the sponge *Cinachyrella* aff. *schulzei* obtained from the lagoon of Noumea, New Caledonia.

EXPERIMENTAL PROCEDURES

Specimens of *Cinachyrella* aff. *schulzei* Keller (1891) (Demospongia, Tetractinomorpha, Spirophorida, Tetillidae) were collected by hand in Canal Woodin, Noumea, New Caledonia, at a depth of 25–30 m in late 1990. The sponge, referenced as MNHN R 1467 New Caledonia, is

[1]For part 2 of this series, see Reference 1.

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Abbreviations: ECL, equivalent chain length; FAME, fatty acid methyl ester(s); GC/MS, gas chromatography/mass spectrometry; GC/FT-IR, gas chromatography/Fourier transform infrared; LCFA, long-chain fatty acid(s); MS, mass spectrum; TLC, thin-layer chromatography; 4,8,12-TMTD, 4,8,12-trimethyltridecanoic acid; 5,9,13-TMTD, 5,9,13-trimethyltetradecanoic acid.

80 × 70 × 60 mm with porocalices 6 × 3 to 13 × 11 mm; its skeleton has an hispidation, 5 mm; sigmaspires, 22 µm; oxeas, 4.3 mm; small oxeas, 200–260 µm; anatriaenes, 5 mm, with studded or knobby clades, 25 µm, or with normal clades, 35–50 µm; protriaenes like anatriaenes; several prodiaenes. A similar species is *C. hirsuta* Dendy (1889).

The sponges were washed in sea water, carefully cleaned, cut into small pieces and lyophilized. Sponge pieces were ground in a Waring blender, using chloroform/methanol (1:1, vol/vol), 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 column chromatography on silica gel (70–230 mesh) using hexane, chloroform, acetone and methanol (phospholipids) as successive eluents. About 55 mg of phospholipids was recovered from 90 g of sponge (dry weight). Phospholipid composition was analyzed by thin-layer chromatography (TLC) using chloroform/methanol/water (65:25:4, by vol) as eluent, and fractions were identified by comparison with phospholipid standards. Phospholipid fatty acids were converted to methyl esters by reaction (30 min under reflux) with methanolic hydrogen chloride (20), and the residue was dissolved in hexane and purified by column chromatography (silica gel; hexane/diethyl ether, 10:1, vol/vol). The resulting methyl esters were analyzed by gas–liquid chromatography using a Carlo Erba 4130 chromatograph (Milano, Italy) and a nonpolar OV-1 silica capillary column (A.M.L.-Chromato, Limoges, France) (25 m × 0.32 mm i.d., 0.40 µm film thickness); hydrogen was used as carrier gas (0.5 bar; split ratio, 5:100). Standard fatty acid methyl esters (from C_{20} to C_{31}) and standard phospholipid samples were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-Acyl pyrrolidide derivatives were prepared by treatment of methyl esters with pyrrolidine/acetic acid (10:1, vol/vol) under reflux (2 h) and were purified by TLC on 0.5-mm layers of silica gel with hexane/diethyl ether (1:2, vol/vol) as developing solvent. Fatty acid methyl esters were hydrogenated by stirring (4 h) at ambient pressure and temperature dissolved in methanol in the presence of catalytic amounts of platinum (IV) oxide (Adam's catalyst). Combined gas chromatography/mass spectrometry (GC/MS) was performed on a Hewlett-Packard HP-5890 instrument linked to an HP 9000/345 integrator (Palo Alto, CA). The GC column was a 0.32 mm × 30 m fused silica capillary column coated with DB-1 (0.25 µm film thickness). The carrier gas was helium. Column temperature was programmed from 180 to 310°C, at 3°C/min, for methyl esters and pyrrolidides.

Gas chromatography/Fourier transform infrared (GC/FT-IR) spectra were measured in Marseille, France, on a 20SxB Nicolet spectrometer (Madison, WI), interfaced with a 5300 Mega Carlo Erba chromatograph (flame-ionization detector additional detection) fitted with an on-column injector. The GC/IR light pipe is a gold-coated borosilicate cylinder (1 mm × 16 cm) with KBr windows. A medium range Hg-Cd-Te liquid-nitrogen cooled detector was used. The GC capillary column was a 60 m × 0.32 mm i.d. fused silica (J & W Scientific, Folsom, CA) coated with DB-1 phase (0.25 µm film thickness). The flow rate of the helium carrier gas (140 kPa) was about 2 mL/min. The column was then directed to the GC/IR light pipe through an inlet stainless steel glass lined transfer line maintained at 300°C. The oven was initially set at 70°C with a 2 min isotherm and then programmed to reach 130°C at 15°C/min, followed by a rate of 15°C/min to reach 290°C, and then maintained at this temperature for 40 min. For the experiment, the light pipe was heated to 293°C. Vapor phase IR spectra were recorded with a resolution of 8 cm^{-1} over the range of 4,000–650 cm^{-1}; 16 data scans were collected, and co-added per data file (2.03 for each spectrum). The IR reconstructed chromatogram was done using the Gram-Schmidt algorithm.

RESULTS

The major phospholipid classes were shown to be phosphatidylethanolamine and phosphatidylserine as judged by TLC analysis. Many of the fatty acids were identified by comparing their equivalent chain length (ECL) values as fatty acid methyl esters (FAME) with those of known compounds and with fatty acids from mixtures of known composition, as well as by co-injection with commercial standards (normal LCFA up to 31:0). Capillary GC analysis of a hydrogenated aliquot also provided information useful for identification (*iso* and *anteiso* compounds) of unsaturated fatty acids. The complete list of phospholipid fatty acids from *C.* aff. *schulzei* is given in Table 1.

LCFA with more than 22 carbons accounted for more than 50% of all acids. The major LCFA were 25-methyl-5,9-hexacosadienoic (*iso*-27:2; 7.2%), 19-hexacosenoic (26:1; 6.8%), 5,9,21-octacosatrienoic (28:3; 5.8%) and 26-methyl-5,9-heptacosadienoic (*iso*-28:2; 3.6%) acids. Unsaturated LCFA comprised about 90% of the total LCFA. Fifteen LCFA containing the 5,9 diene system accounted for 30% of total; this is the greatest number of Δ5,9 dienoic acids found to date in a marine sponge. However, for many acids, GC/MS data on pyrrolidide derivatives were obtained to confirm the structures and to determine the double bond and branching positions (21). The mass spectral data of some of the most interesting fatty acids are as follows:

8-Heptadecenoic acid pyrrolidide. MS *m/z* (rel. intensity), 321 (M^+, 2.0), 292 (1.3), 278 (1.8), 266 (1.5), 264 (2.0), 251 (2.2), 250 (4.4), 249 (3.1), 237 (1.3), 236 (2.9), 223 (1.8), 222 (1.6), 208 (1.5), 196 (0.7), 195 (1.8), 194 (2.2), 182 (4.1), 168 (4.3), 167 (2.0), 155 (2.6), 154 (2.8), 140 (5.7), 126 (39.6), 113 (85.4), 98 (46.5), 70 (45.8), 55 (100).

12-Nonadecenoic acid pyrrolidide. MS *m/z* (rel. intensity), 349 (M^+, 2.0), 334 (0.3), 320 (0.9), 306 (1.6), 292 (1.6), 278 (2.2), 264 (1.8), 252 (0.8), 250 (1.2), 239 (0.9), 238 (1.1), 224 (1.2), 222 (0.9), 210 (1.3), 196 (2.5), 182 (3.1), 168 (3.5), 154 (1.5), 141 (1.4), 140 (4.1), 126 (37.1), 113 (100), 98 (17.6), 70 (37.2), 55 (74.5).

18-Pentacosenoic acid pyrrolidide. MS *m/z* (rel. intensity), 433 (M^+, 3.1), 418 (0.3), 404 (0.8), 390 (1.7), 376 (1.6), 363 (3.5), 362 (3.1), 348 (2.2), 334 (1.2), 322 (1.3),

TABLE 1

Major Phospholipid Fatty Acids from *Cinachyrella* aff. *schulzei*[a]

Fatty acid	Symbol	ECL (FAME)	Abundance (%)
Dodecanoic	12:0	12.00	0.7
Tridecanoic	13:0	13.00	0.5
6-Tetradecenoic[b]	14:1	13.70	0.3
Tetradecanoic	14:0	14.00	2.6
4,8,12-Trimethyltridecanoic	16:0	14.49	8.8
14-Methyltetradecanoic	15:0	14.63	0.5
6-Pentadecenoic[b]	15:1	14.72	0.2
Pentadecanoic	15:0	15.00	0.8
5,9,13-Trimethyltetradecanoic	17:0	15.39	0.5
6-Hexadecenoic	16:1	15.74	0.7
9-Hexadecenoic	16:1	15.79	2.4
Hexadecanoic	16:0	16.00	10.3
10-Methylhexadecanoic	17:0	16.42	0.5
8-Heptadecenoic	17:1	16.82	0.6
Heptadecanoic	17:0	17.00	0.6
9,12-Octadecadienoic	18:2	17.52	0.6
16-Methylheptadecanoic	*i*-18:0	17.66	1.0
9-Octadecenoic	18:1	17.75	2.1
11-Octadecenoic	18:1	17.78	1.6
Octadecanoic	18:0	18.00	6.9
12-Nonadecenoic[b]	19:1	18.84	1.2
Heneicosanoic	21:0	21.00	0.5
Docosanoic	22:0	22.00	0.6
16-Tricosenoic	23.1	22.79	0.3
Tricosanoic	23:0	23.00	0.5
21-Methyltricosanoic	*a*-24:0	23.72	1.1
17-Tetracosenoic	24:1	23.79	2.2
Tetracosanoic	24:0	24.00	0.8
23-Methyl-5,9-tetracosadienoic	*i*-25:2	24.12	0.4
18-Pentacosenoic	25:1	24.81	1.0
24-Methyl-5,9-pentacosadienoic	*i*-26:2	25.08	0.8
5,9-Hexacosadienoic	26:2	25.43	2.6
17-Hexacosenoic	26:1	25.68	2.2
19-Hexacosenoic	26:1	25.79	6.8
Hexacosanoic	26:0	26.00	0.8
25-Methyl-5,9-hexacosadienoic	*i*-27:2	26.06	7.2
24-Methyl-5,9-hexacosadienoic	*a*-27:2	26.17	1.5
5,9-Heptacosadienoic	27:2	26.40	2.3
20-Heptacosenoic	27:1	26.80	0.5
Heptacosanoic	27:0	27.00	0.5
26-Methyl-5,9-heptacosadienoic	*i*-28:2	27.05	3.6
5,9,21-Octacosatrienoic	28:3	27.15	5.8
5,9,23-Octacosatrienoic	28:3	27.25	0.7
5,9-Octacosadienoic	28:2	27.40	2.6
26-Methylheptacosanoic[b]	*i*-28:0	27.63	1.2
5-Octacosenoic[c]	28:1	27.75	0.6
21-Octacosenoic	28:1	27.83	0.8
27-Methyl-5,9-octacosadienoic[c]	*i*-29:2	28.08	0.5
Bromo-5,9-heptacosadienoic	Br-5,9-27:2	28.18	0.5
5,9-Nonacosadienoic	29:2	28.38	1.1
5,9,23-Tricontatrienoic	30:3	29.10	0.9
5,9,x-Tricontatrienoic	30:3	29.20	trace
Tricontanoic	30:0	30.00	0.6

[a]Several common minor (<0.5%) fatty acids have also been identified, including *i*-14:0, *i*-15:0, *a*-15:0, *i*-16:0, *i*-17:0, *a*-17:0, *i*-19:0, 19:0, *a*-20:0, 20:0, *a*-23:0, *br*-24:0, 25:0, *a*-27:0, 28:0. *i, iso; a, anteiso; br,* branched; Br, bromine. ECL, equivalent chain length; FAME, fatty acid methyl esters.

[b]Not previously found in sponges.

[c]Not previously found in nature.

308 (1.1), 294 (1.5), 280 (2.3), 266 (1.6), 252 (1.7), 238 (1.9), 224 (1.7), 210 (1.1), 208 (0.9), 196 (0.8), 182 (3.0), 168 (3.9), 155 (1.6), 154 (1.8), 140 (5.3), 126 (56.5), 113 (100) 98 (38.5), 70 (100).

5-Octacosenoic acid pyrrolidide. MS *m/z* (rel. intensity), 476 (MH^+, 1.6), 446 (0.4), 432 (0.3), 431 (0.4), 418 (1.8), 404 (0.4), 390 (0.3), 376 (3.2), 362 (0.4), 348 (0.8), 334 (0.5), 322 (0.5), 320 (0.3), 308 (0.6), 292 (0.7), 278 (1.8), 266 (1.7), 264 (1.4), 252 (1.3), 250 (1.2), 236 (1.7), 222 (1.6), 210 (1.8), 209 (2.9), 208 (2.1), 195 (3.8), 194 (4.5), 180 (2.0), 168 (1.1), 166 (1.4), 152 (7.3), 140 (4.7), 126 (20.9), 113 (67.3), 98 (30.4), 70 (78.4), 55 (100).

21-Octacosenoic acid pyrrolidide. MS *m/z* (rel. intensity), 475 (M^+ 3,4), 446 (0.3), 432 (0.4), 430 (0.5), 418 (1.2), 404 (1.2), 390 (0.6), 376 (0.8), 364 (0.3), 350 (0.5), 340 (0.3), 336 (0.5), 322 (0.6), 308 (1.4), 295 (0.7), 294 (1.0), 280 (2.7), 266 (1.3), 252 (0.7), 238 (0.6), 224 (0.7), 210 (1.3), 198 (1.4), 196 (1.6), 182 (2.2), 180 (2.0), 168 (1.8), 154 (1.6), 140 (3.5), 126 (19.8), 113 (100), 98 (17.5), 70 (30.2), 55 (59.3).

27-Methyl-5,9-octacosadienoic acid pyrrolidide. MS *m/z* (rel. intensity), 487 (M^+, 2.3), 472 (0.8), 444 (1.7), 435 (0.8), 417 (0.8), 375 (1.7), 360 (2.2), 346 (1.8), 318 (1.6), 300 (0.7), 276 (2.5), 262 (2.4), 248 (1.2), 235 (2.2), 234 (1.2), 221 (2.0), 207 (4.6), 192 (2.0), 180 (20.5), 168 (2.5), 166 (2.4), 154 (2.0), 152 (2.4), 140 (5.2), 126 (17.9), 113 (100), 98 (21.8), 85 (12.3), 71 (14.5).

Bromo-5,9-heptacosadienoic acid pyrrolidide. MS *m/z* (rel. intensity), 458 ($[M - Br]^+$, 5.5), 260 (4.2), 258 (4.0), 210 (1.2), 180 (6.3), 126 (8.3), 113 (100), 98 (18.4), 85 (11.6), 72 (23.0), 70 (23.0).

Four homologous series of fatty acids were recognized (Fig. 1) when the retention times of the LCFA (as methyl esters) were plotted vs. the carbon numbers. These were the straight-chain acids, n-7 monoenoic acids, normal Δ5,9 dienoic acids and *iso*-5,9-dienoic acids.

Saturated fatty acids. We readily identified two saturated isoprenoid acids, 4,8,12-TMTD (8.8%) and 5,9,13-TMTD acids (0.5%). GC mobilities and GC/MS data for the methyl esters and pyrrolidides of these compounds were described in a recent paper (6). 26-Methylheptacosanoic acid (*iso*-28:0) was also present (1.2%) and readily identified as its methyl ester. It had an ECL of 27.63, and the molecular ion peak was at *m/z* 438, with additional fragments at *m/z* 74 (base peak), 87 and 395

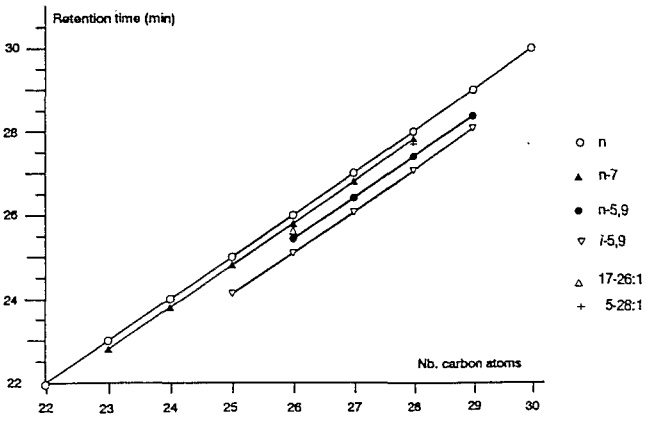

FIG. 1. Plot of retention time (min) vs. number of carbon atoms for four series of fatty acid methyl esters from *Cinachyrella* aff. *schulzei*. The monoenoic acids 5-28:1 and 17-26:1 are located outside the n-7 monoenoic straight line.

(M – 43). The GC peak did not change upon catalytic hydrogenation.

Monoenoic fatty acids. Three interesting monoenoic fatty acids were identified in *C.* aff. *schulzei* (14:1, 15:1, 16:1). Although they were present in small amounts, these acids could readily be identified based on GC mobility and GC/MS data. On the other hand, they were converted to 14:0, 15:0 and 16:0 derivatives, respectively, upon catalytic hydrogenation. Mass spectra of the pyrrolidide derivatives showed the key fragments at m/z 154 and 166 (with a difference of 12 amu occurring between the C-5 and C-6 fragments), clearly indicating Δ6 unsaturation, and molecular ions at m/z 279 (14:1), 293 (15:1) and 307 (16:1), respectively. No diminished homologous fragment was observed, thus excluding branching. Another rare fatty acid identified, namely 8-heptadecenoic acid (0.6%), had an ECL value as methyl ester (FAME) of 16.82 and a molecular ion peak at m/z 282. The mass spectrum of the corresponding pyrrolidide derivative showed a 12 amu difference between peaks m/z 182 and 194 (Δ8 unsaturation). The unusual 12-nonadecenoic acid (1.2%) was readily identified as its methyl ester had an ECL value of 18.84 and a molecular ion peak at m/z 310. Upon catalytic hydrogenation, this compound was converted to the nonadecanoic acid methyl ester. The mass spectrum of the pyrrolidide derivative exhibited a molecular ion peak at m/z 349 and key fragments at m/z 238 and 250 (Δ12 unsaturation). An interesting monoenoic LCFA was found in *C.* aff. *schulzei*. Its methyl ester had an ECL value of 27.75 and a molecular peak at m/z 436, indicating that it was an octacosenoic acid methyl ester. The MS of the pyrrolidide exhibited a molecular ion peak at m/z 476 (MH^+) and key fragments at m/z 140 and 152, indicating desaturation at Δ5 (Fig. 2). This 5-octacosenoic acid (5-28:1, 0.6%) had not been found previously in any natural source.

In addition to the usual 17-tetracosenoic and 19-hexacosenoic acids of the n-7 monoenoic series, we identified the 16-tricosenoic acid that we recently found in *C. alloclada* (6), as well as three other very rare fatty acids lately found for the first time in Caribbean sponges (14) and characterized by GC/MS of the dimethyl disulfide adducts, namely 18-pentacosenoic, 20-heptacosenoic and 21-octacosenoic.

The first acid (18-25:1, 1.0%) showed and ECL value as FAME of 24.81 and a molecular ion peak at m/z 394. It was converted to pentacosanoic acid methyl ester upon catalytic hydrogenation. The mass spectrum of its pyrrolidide showed a molecular peak at m/z 433 and key fragments at m/z 322 and 334, indicating Δ18 unsaturation. We were thus able to identify the 18-pentacosenoic acid previously described to occur in *M. laevis* (14). The second acid, 21–28:1, (0.8%) was identified in the same way; its methyl ester had an ECL value of 27.83 and a molecular peak at m/z 436 and, converted to octacosanoic acid methyl ester upon catalytic hydrogenation, was characterized by comparison with an authentic sample. The MS of its pyrrolidide derivative showed a molecular ion peak at m/z 475 and key fragments at m/z 364 and 376, indicating Δ21 unsaturation. This fatty acid was the 21-octacosenoic acid already identified in *A. compressa* (14). The third fatty acid was identified as a heptacosenoic acid (27:1, 0.5%); its methyl ester had an ECL value of 26.80 and a molecular ion peak at m/z 422. The corresponding peak disappeared upon catalytic hydrogenation. Unfortunately, a suitable MS of the pyrrolidide derivative was not obtained owing to the small amount available and the fact that the spectrum was obscured by fragments derived from the stationary silicone phase. When retention time vs. carbon number was plotted, this compound fell exactly in line with the other n-7 monoenoic FAME (Fig. 1), especially the homologous 19–26:1 and 21–28:1. In Figure 1 the second line includes the heptacosenoic acid in question, but not closely related monoenoic acids, such as 17-hexacosenoic and 5-octacosenoic (as FAME). Thus, the 20-heptacosenoic structure was only tentatively assigned to this fatty acid, which was previously identified in *A. compressa* (14). The FT-IR spectra of the n-7 monoenoic methyl ester (Fig. 3) showed absorptions at 3013 cm^{-1} (stretching frequency of the ethylenic bond) and 703 cm^{-1} (out-of-plane bending vibration) character-

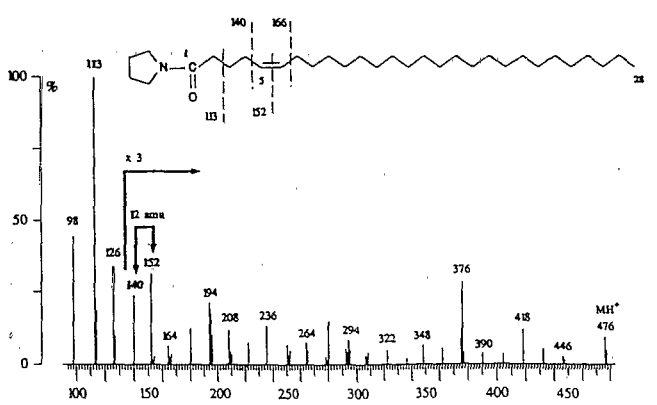

FIG. 2. Mass spectrum of 5-octacosenoic acid pyrrolidide.

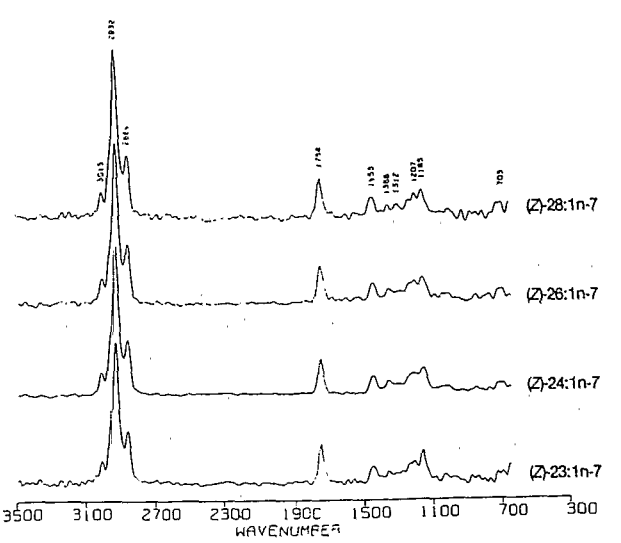

FIG. 3. Gas chromatography/Fourier transform infrared spectra of several n-7 monoenoic fatty acid methyl esters.

FIG. 4. Mass spectrum of 27-methyl-5,9-octacosadienoic acid pyrrolidide showing the typical fragmentation peak at *m/z* 180 and lack of a peak at *m/z* 458 (C_{27} fragment).

istic of a *cis* configuration (no absorption near 960 cm^{-1}) (22,23).

Long-chain Δ5,9 dienoic fatty acids. One main feature of *C.* aff. *schulzei* is the presence of fifteen long-chain fatty acids possessing typical sponge Δ5,9 unsaturation. This pattern was readily identified (MS), as all these fatty acids afforded the characteristic peaks at *m/z* 81 and 180 as methyl esters and pyrrolidides, respectively. Catalytic hydrogenation yielded the corresponding saturated FAMEs that had a normal *iso* or *anteiso* hydrocarbon chain for the two latter fractional chain lengths of 0.62–0.65 and 0.72–0.75, respectively. Most of them have been already found in sponges. In addition to the rare 23-methyl-5,9-tetracosadienoic acid (24,25), we also identified another compound belonging to the series of *iso*-5,9-dienoic fatty acids for which the MS of the FAME (ECL 28.08) exhibited a molecular ion peak at *m/z* 448, indicating a 29:2 structure. The base peak was the typical peak at *m/z* 81, indicating a 5,9-dienoic structure. This FAME gave the 27-methyloctacosanoic acid methyl ester (*iso*-29:0, ECL 28.64) upon catalytic hydrogenation. The MS of the corresponding pyrrolidide derivative (Fig. 4) showed a molecular ion peak at *m/z* 487 (29:2) and the typical major ion at *m/z* 180 (5,9-29:2). Methyl branching at the C-27 position was indicated by significant peaks at *m/z* 444 (C_{26}) and *m/z* 472 (C_{28}) and by absence of the C_{27} fragment at *m/z* 458. Thus a novel fatty acid was identified, namely 27-methyl-5,9-octacosadienoic (*iso*-5,9-29:2).

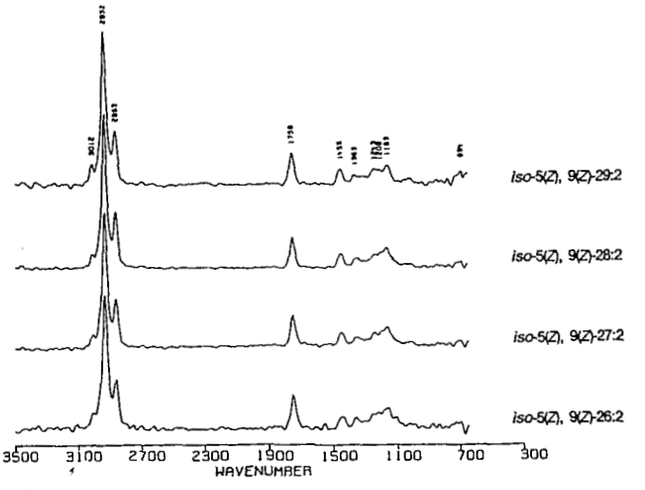

FIG. 5. Gas chromatography/Fourier transform infrared spectra of several *iso*-5,9 long-chain fatty acid methyl esters.

A complete series of *iso*-5,9 long-chain fatty acids (C_{25}–C_{29}) occurs in *C.* aff. *schulzei.* In Figure 5, the double bonds are in *cis* configuration as all infrared spectra exhibited absorptions at 3012 cm^{-1} and 694 cm^{-1} (out-of-plane bending vibration) (22,23). No absorption band was present near 960 cm^{-1}. Finally, it is noteworthy that this novel FAME fell in line with the *iso*-5,9-dienoic methyl esters in Figure 1. Moreover, an interesting 5,9-dienoic fatty acid with an ECL value of 28.18 was also detected. The MS of the methyl ester showed a peak at *m/z* 74 (McLafferty rearrangement) and a major peak at *m/z* 81 characteristic of the 5,9-dienoic moiety. In addition, the MS showed a series of peaks at *m/z* 331, 345, 355, 373 and 404, which closely corresponded to those obtained with the 5,9-heptacosadienoic acid methyl ester (*m/z* 332, 346, 356, 374 and 406). These data suggest the presence of a labile substituent that can be easily lost under electron impact, according to the observations of Lam *et al.* (19). The MS of the pyrrolidide derivative was very simple, with the usual base peak at *m/z* 113 and the typical peak at *m/z* 180, confirming the presence of a 5,9-dienoic pattern. However, the usual series of homologous fragments was absent. There was a significant peak at *m/z* 458 and a doublet of equal intensity at *m/z* 258 and 260, suggesting that the labile substituent was, in fact, a bromine atom. Thus, the double allylic fragmentation between C-7 and C-8 (typical of 5,9-dienoic acid pyrrolidides) gave the usual ion at *m/z* 180 after bromine loss and peaks at *m/z* 258 and 260 with the bromine substituent intact, so that the peak at *m/z* 458 corresponded to the molecular ion peak after bromine loss. These data imply that the point of bromine attachment was between C-2 and C-7. The bromine substituent is at a vinylic position in all known brominated fatty acids (18,19,26), especially in brominated 5,9-dienoic long-chain fatty acids (18,19). Unfortunately, we were unable to identify the precise position of bromine attachment by nuclear magnetic resonance experiments, due to the small amount of sample available. The acid could be identical to the LCFA (5*E*,9*Z*)-6-bromo-5,9-heptacosadienoic recently described in *Petrosia* sp. (27).

Δ5,9 Trienoic LCFA. We identified three Δ5,9-trienoic acids (MS of FAME and *N*-acyl pyrrolidides) already known to occur in sponges, particularly the major one (5.8%), 5,9,21-octacosatrienoic acid, in addition to the common 5,9,23-tricontatrienoic acid. Traces of another tricontatrienoic acid were also detected. This compound was readily identified as methyl ester possessing the typical Δ5,9 unsaturation pattern (ECL 29.20, M^+ at *m/z* 460 and prominent peak at *m/z* 81), but the exact position of the third double bond could not be assigned due to the very small amount of pyrrolidide available.

DISCUSSION

The co-occurrence of two saturated isoprenoid fatty acids in a marine sponge was an interesting finding. It had been previously suggested (7) that it would be unlikely that the two isoprenoid fatty acids would occur in the same sponge. We initially found two such acids, namely 4,8,12-TMTD and 5,9,13-TMTD acids, in the Senegalese sponge *C. alloclada* (6), and two other isoprenoid fatty acids, 4,8,12-trimethyl-13:0 and phytanic acid, have recently been identified in *Dysidea fragilis* (25). It is surprising that *C.* aff. *schulzei*, from a quite different location, also contains these two isoprenoid fatty acids. The former acid is frequently found, though not in all sponges, whereas the latter is a very rare compound identified here only for the second time in a sponge. In addition, all specimens studied of the Senegalese sponge *C. kükenthali*, either from deep or shallow waters, contained both these isoprenoid acids (28). The co-occurrence of these two isoprenoid fatty acids would thus seem to characterize a sponge belonging to the genus *Cinachyrella*. Work is in progress in our laboratory to verify this feature for other *Cinachyrella* species collected from different geographical locations and to elucidate the biosynthetic pathways. The very rare 26-methylheptacosanoic acid (*iso*-28:0) was identified here for the first time in a sponge. It had already been mentioned as a hydrogenation product of *iso*-5,9-28:2 acid found in some sponges (16,17). Only a few natural sources of *iso*-28:0 were previously known, and none was associated with natural phospholipids, although it was recently reported to occur as a minor component of the total lipid fatty acids from sulfate-reducing bacteria (29).

Two unusual monoenoic fatty acid patterns were found in *C.* aff. *schulzei*. The identification of the rare 8-heptadecenoic acid is the second report of this acid in a sponge after its recent identification by Carballeira and Restituyo in *A. complanata* (30). We reported the rare 8-hexadecanoic acid in *C. alloclada* (6). These acids could have a dietary or a symbiotic origin. We also identified small amounts of 6-tetradecenoic, 6-pentadecenoic and 6-hexadecenoic acids. The latter acid had earlier been found in the sponge *A. complanata* (30), but we have not been aware of a report of 6-tetradecenoic and 6-pentadecenoic acids in any marine sponge. The 6-monoenoic acids were recently found to be biomarkers of planktonic input (31). 6-Heptadecenoic and 6-nonadecenoic acids were identified in the marine sponges *Strongylophora durissima* (10) and *Calyx niceaensis* (32), respectively, and 14-methyl-6-pentadecenoic acid was detected in *Tethya aurantia* (33). Several marine organisms, such as turtles (34), sunfish (35), whales (36), sea anemones (37), jellyfish and gorgonians (38), have been shown to contain *trans*- or *cis*-6-hexadecanoic acid. These 6-monoenoic fatty acids may also be of bacterial origin. As previously suggested by Carballeira and Maldonado for *Euryspongia rosea* (39), we think that a Δ6 desaturase may be operative in *C.* aff. *schulzei*.

One of the main findings of the present study is the occurrence in *C.* aff. *schulzei* of a series of n-7 LCFA possessing even- and odd-numbered carbon chains. The odd monoenoic series contains the 12-nonadecenoic, 13-tricosenoic, 18-pentacosenoic and the tentatively assigned 20-heptacosenoic acids. The rare 12-nonadecenoic acid, previously identified from *Thiobacillus* strains (40), is reported here for the first time as being present in sponge phospholipids. In fact, few nonadecenoic acids are known to occur in nature, the most common examples being 9-19:1 and 11-19:1. The 6-nonadecenoic acid was found in sponges (27), and we reported the 13-nonadecenoic acid, probably of bacterial origin, in *C. alloclada* (6). 16-Tricosenoic acid was previously identified in the Senegalese sponge *C. alloclada* (6), together with 10-heptadecenoic acid and an uncharacterized pentacosenoic acid. The 18-pentacosenoic acid described here was already found in *M. laevis* (14). Thus, from *Cinachyrella* sponges, we isolated a complete series of odd n-7 monenoic fatty acids (17–27 carbon atoms) with the exception of 14-21:1, which would also be expected to be present in sponge phospholipids. A complete series of n-7 monoenoic LCFA is known to occur in human brain lipids (41). The phospholipids of the Senegalese sponge *Higginsia tethyoides* (2) contain acids 2-methoxy-18-pentacosenoic, 2-methoxy-20-heptadecenoic and 2-methoxy-21-octacosenoic. This seems to suggest that in *H. tethyoides* methoxyl is introduced in a last step. In addition to the known 17-24:1 and 19-26:1 acids, *C.* aff. *schulzei* was shown to contain the 21-28:1 acid, which was encountered before only in *A. compressa*. The acid was recently synthesized (42). An interesting octacosenoic isomer was also characterized, namely 5-octacosenoic acid, not previously found in a sponge. The co-occurrence of 21-octacosenoic, 5-octacosenoic and 5,9,21-octacosatrienoic acids suggests a role for the first of these acids as a biosynthetic intermediate toward the 5,9,21-octacosatrienoic acid *via* elongation from palmitoleic or vaccenic acid. The 21-28:1 acid was postulated as such an intermediate (43), but in *C.* aff. *schulzei* Δ9 desaturation appears to be the last biosynthetic step. In addition to the well known 5,9,23-30:3, we detected another distinct 30:3 possessing typical Δ5,9 desaturation. As we found both 5,9,21-28:3 and 5,9,23-28:3 acids in *C.* aff. *schulzei*, it is likely, and in accordance with our GC data, that this tricontatrienoic fatty acid is the 5,9,25-30:3 that we recently identified in *T. loeve* (1). Work is in progress in our laboratory on other *Cinachyrella* sponge specimens to identify this new tricontatrienoic acid. Only one of the tricontatrienoic acids has been reported to date in marine sponges, the relatively abundant 5,9,23-30:3 acid. The finding of a brominated demospongic acid also seems of interest. Lam *et al.* (19) recently demonstrated that biological bromination occurs at the final step in the biosynthesis of such fatty acids.

ACKNOWLEDGMENT

We wish to thank Gilbert NOurrisson (CNRS, Laboratoire de Synthèse Organique, Faculté des Sciences, Nates) for GC/MS measurements.

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[Received March 3, 1993, and in revised form January 11, 1994; Revision accepted January 11, 1994]