



HAL
open science

New 17-Methyl-13-Octadecenoic and 3,16-Docosadienoic Acids from the Sponge *Polymastia penicillus*

Gaëtane Wielgosz-Collin, Nicolas Ruiz, Vony Rabesaotra, Nicole Boury-Esnault, Jean-Michel Kornprobst, Gilles Barnathan

► To cite this version:

Gaëtane Wielgosz-Collin, Nicolas Ruiz, Vony Rabesaotra, Nicole Boury-Esnault, Jean-Michel Kornprobst, et al.. New 17-Methyl-13-Octadecenoic and 3,16-Docosadienoic Acids from the Sponge *Polymastia penicillus*. *Lipids*, 2009, 44 (7), pp.655-663. 10.1007/s11745-009-3291-9 . hal-04050369

HAL Id: hal-04050369

<https://nantes-universite.hal.science/hal-04050369v1>

Submitted on 29 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

New 17-Methyl-13-Octadecenoic and 3,16-Docosadienoic Acids from the Sponge *Polymastia penicillus*

Claire Denis · Gaëtane Wielgosz-Collin · Anne Bretéché ·
Nicolas Ruiz · Vony Rabesaotra · Nicole Boury-Esnault ·
Jean-Michel Kornprobst · Gilles Barnathan

Received: 12 September 2008 / Accepted: 8 February 2009
© AOCS 2009

Abstract The phospholipid fatty acid composition of the North-East Atlantic sponge *Polymastia penicillus* (South Brittany, France) was investigated. Sixty fatty acids (FA) were identified as methyl esters (FAME) and *N*-acyl pyrrolidides (NAP) by gas chromatography–mass spectrometry (GC/MS), including eight $\Delta 5,9$ unsaturated FA and three long-chain 2-hydroxylated FA. The major phospholipid FA were palmitic (14.3% of the total FA mixture), vaccenic (12.7%), 15(*Z*)-docosenoic (13.4%) and 5(*Z*),9(*Z*)-hexacosadienoic (13.3%) acids. In addition to the *iso*- and *anteiso*-branched saturated FA, several unusual short-chain branched saturated FA were identified. In addition to the known $\Delta 5,9$ FA, and interestingly regarding their identification by GC–MS as *N*-acyl pyrrolidides, was the co-occurrence of unusual FA possessing a $\Delta 3$, $\Delta 4$ and $\Delta 5$ double bond such as *iso*-4-pentadecenoic, *iso*-5-heptadecenoic, *anteiso*-5-heptadecenoic and two new compounds, not hitherto found in nature, namely 17-methyl-13-octadecenoic (0.8%) and 3,16-docosadienoic (1.1%) acids.

Keywords 3,16-Docosadienoic acid · Fatty acids · Phospholipids · *Polymastia* · Sponge ·

17-Methyl-13-octadecenoic · *N*-Acyl pyrrolidides · Non-methylene-interrupted fatty acids

Abbreviations

amu	Atomic mass unit
FA	Fatty acid(s)
FAME	Fatty acid methyl ester(s)
GC/MS	Gas chromatography–mass spectrometry
MS	Mass spectrum (spectra)
NAP	<i>N</i> -Acyl pyrrolidide(s)
NMI	Non-methylene-interrupted
TLC	Thin layer chromatography

Introduction

Marine sponges are primitive invertebrates with a remarkable ability to adapt to environmental changes. Thus, they have given rise to intensive investigations into the basic lipid components of their cell membranes since the pioneer studies [1, 2]. The phospholipid fatty acid composition is important for the functions of the cell membranes [3, 4]. In addition, sponge phospholipid classes have also been examined [3–5]. Moreover, sponges have proved to be a rich source of many unusual sterols and fatty acids (FA). Common polyunsaturated FA from the n-6 and n-3 series, such as eicosapentanoic acid EPA and docosahexaenoic acid DHA, are quite rare in sponge phospholipids, that instead usually contain very long-chain (C_{22} – C_{34}) non-methylene-interrupted (NMI) FA including the particular unsaturation pattern $\Delta 5,9$ [3, 4, 6–11].

Sponges from the genus *Polymastia* are known to contain secondary metabolites with biological activity but there have been, in comparison, few investigations of

C. Denis · G. Wielgosz-Collin · A. Bretéché · N. Ruiz ·
V. Rabesaotra · J.-M. Kornprobst · G. Barnathan (✉)
Groupe MMS (Mer, Molécules, Santé) EA 2160, Equipe Lipides
Marins, Faculté de Pharmacie, Pôle Mer et Littoral,
Université de Nantes, 2 rue de La Houssinière, BP 92208,
44322 Nantes Cedex 03, France
e-mail: Gilles.Barnathan@univ-nantes.fr

N. Boury-Esnault
UMR 6540 DIMAR, Centre d'Océanologie de Marseille, Station
Marine d'Endoume, CNRS-Université de la Méditerranée,
rue Batterie des Lions, 13007 Marseille, France

their primary metabolites such as FA and sterols to date. Thus, organic extracts of *Polymastia janeirensis* demonstrated in vitro cytotoxicity against HT29 colorectal tumor cell lines [12]. *Polymastia croceus* has been farmed in order to produce quantities of bioactive compounds of medicinal interest [13, 14]. *Polymastia penicillus* is usually found in the North Atlantic in shallow waters and to depths of 600 m [15, 16]. The phospholipids of *P. gleneni*, collected around the Glenan Islands (South Brittany, France) were shown to contain a unique series of saturated 2-acetoxy long chain FA ranging from C₂₂ to C₃₀ chains [17].

As part of our ongoing investigations of sponge lipids [11, 18, 19], we present here the phospholipid FA composition of *P. penicillus* and the identification of several new and unusual Δ 3, Δ 4, Δ 5 and Δ 13 unsaturated FA.

Materials and Methods

Sponge Material

Samples of *Polymastia penicillus* were collected by hand (scuba) at a depth of 17 m, in the Etel River, Gulf of Morbihan, Brittany, France, in June 2005. Sponge identification was determined according to the recent classification [20, 21], and voucher specimens were deposited in the Marine Station of Endoume, Marseille, France. Like all Polymastiidae, this sponge is encrusting, hemispherical and cushion shaped, always with numerous papillae, but is characterized by the absence of a collagenous layer and by the absence of spicules in internal laminae, and spicules are tylostyles [15, 16].

Extraction, Isolation and Analysis of Phospholipids

The collected specimens were immediately transported to the laboratory for immediate lipid extraction. The sponges were washed in seawater, carefully cleaned, cut in small pieces and steeped twice in dichloromethane/methanol (1:1, v/v) for 24 h at room temperature. The combined extracts yielded the crude total lipids. Total lipid content (wt.%) was determined as the ratio total lipids versus sum of sponge dried residue plus total lipids. Total lipids were rapidly subjected to lipid class separation. Phospholipids were separated from other lipids by column chromatography on silica gel (70–230 mesh) successively eluted with dichloromethane (neutral lipids), acetone (glycolipids) and methanol (phospholipids) [10, 11]. The phospholipid classes were investigated by thin-layer chromatography on silica gel 60F₂₅₄ plates (0.2 mm thickness, SDS Carlo Erba, Val de Reuil, France) and dichloromethane/methanol

(70:30, v/v) was the eluent with phospholipid standards purchased from Sigma (Saint-Quentin Fallavier, France).

Preparation of Fatty Acid Methyl Esters and *N*-Acyl Pyrrolidides

Fatty acid methyl esters (FAME) were obtained by phospholipid transmethylation (40 min under reflux with 5% methanolic hydrogen chloride). *N*-Acyl pyrrolidide derivatives (NAP) were prepared by direct treatment of the FAME with pyrrolidine/acetic acid (10:1, v/v) for 2 h under reflux and purified by TLC on 0.5 mm silica gel layers using hexane/diethyl ether (1:2, v/v) [10, 22–26].

Fourier Transform Infrared Spectroscopy

Infrared spectra were recorded by means of a Bruker Vector-22 Fourier transform infrared spectrometer (Bruker Spectrospin, Karlsruhe, Germany) on liquid films between NaCl plates. Spectra were collected over the wavenumber range 4,000 to 600 cm⁻¹.

Gas Chromatography–Mass Spectrometry Analyses

Gas chromatography–mass spectrometry (GC/MS) coupling experiments were performed on a Hewlett-Packard HP-5890 chromatograph linked to a HP-5989-A mass spectrometer (70 eV) equipped with a HP-9000-345 integrator, and using a 30 m × 0.32 mm i.d. fused silica capillary column coated with HP-1 (0.25 μm phase thickness). The carrier gas was helium. The temperatures of injector and source were held at 240 °C, and the temperature of the GC–MS interface was held at 290 °C.

The column temperature was programmed for FAME and NAP from 180 to 310 °C at 3 °C/min. Equivalent chain length (ECL) were determined for FAME and for NAP in some cases since the observed ECL values have proved to be very similar for both derivatives except for highly branched FA [25].

Mass spectral data for the novel fatty acid structures are presented below.

17-Methyl-13-octadecenoic acid methyl ester MS *m/z* (rel. int.) [M⁺] 310 (6.5), 279 (13.7), 227 (2.3), 211 (8.7), 194 (17.4), 185 (6.5), 171 (19.5), 152 (2.5), 139 (28.3), 125 (21.7), 111 (13.0), 97 (32.6), 95 (21.2), 87 (8.7), 83 (50.0), 81 (20.6), 74 (16.3), 69 (100), 55 (76.1), 43 (23.7), 41 (30.4).

17-Methyl-13-octadecenoic acid pyrrolidide MS *m/z* (rel. int.) [M⁺] 349 (16.1), 334 (4.5), 320 (1), 306 (5.4), 292 (4.5), 278 (3.4), 264 (4.5), 252 (2.7), 238 (2.7), 224 (4.5), 210 (3.6), 196 (3.4), 182 (3.6), 168 (4.5), 154 (1.8), 140

(3.6), 126 (23.8), 113 (100), 98 (11.6), 85 (4.5), 71 (9.8), 55 (9.8).

3,16-Docosadienoic acid methyl ester MS *m/z* (rel. int.) [M⁺] 350 (4.8), 337 (1.0), 319 (14.8), 304 (3.7), 290 (3.7), 276 (12.1), 261 (5.1), 247 (5.5), 191 (3.7), 171 (2.8), 163 (7.4), 150 (14.8), 135 (20.4), 123 (20.4), 115 (19.7), 109 (31.5), 95 (53.7), 87 (11.1), 81 (63.0), 71 (40.7), 69 (46.3), 67 (48.4), 55 (100), 43 (37.0), 41 (64.8).

3,16-Docosadienoic acid pyrrolidide MS *m/z* (rel. int.) [M⁺] 389 (32.2), 374 (2.5), 360 (4.1), 346 (4.2), 332 (11), 318 (19.5), 304 (7.6), 292 (5.1), 278 (3.4), 264 (7), 250 (6.8), 236 (6.8), 222 (10.2), 208 (11.9), 194 (8.5), 180 (4.2), 166 (8.5), 152 (61.9), 139 (13.6), 126 (30.5), 124 (9.3), 113 (100), 98 (30.5), 81 (8.5), 72 (30.5), 55 (8.5).

Results and Discussion

Total lipids accounted for 5.3% of the dried sponge residues after extraction. Total phospholipids of *P. penicillus* accounted for 29.6% of the total lipids. As determined by thin layer chromatography, the most abundant phospholipids were phosphatidylserine, phosphatidylcholine and phosphatidylinositol, while phosphatidylethanolamine occurred at a significant level and phosphatidylglycerol was present at a lower amount. Acid methanolysis provided a complex phospholipid FA mixture. Sixty fatty acids (FA) were identified by gas chromatography–mass spectrometry (GC/MS) of their methyl esters (FAME) and *N*-acyl pyrrolidides (NAP) as shown in Table 1.

Many of the FA reported in Table 1 were identified by comparing their equivalent chain lengths (ECL) and their mass spectra (MS) with those of known standards. Unsaturated FA, mainly monoenes and dienes, accounted for almost 61% of the FA mixture. The Fourier transform infrared spectrum of the whole phospholipid FAME showed absorption at 3,004 cm⁻¹ (=C–H stretching), strong peaks at 2,923 and 2,852 cm⁻¹ (C–H stretching), 1,743 cm⁻¹ (C=O stretching, ester), significant peaks at 1,465 cm⁻¹ (CH₂ scissors deformation) and 720 cm⁻¹ (CH₂ rocking/C=C *cis*), and displayed no absorption in the region 940–980 cm⁻¹ (C=C–H out-of-plane), indicating the absence of (*E*)-unsaturation [27]. Thus, double bonds in major unsaturated phospholipid FA from *P. penicillus*, such as those at more than 1% have the (*Z*) configuration. It should be noted that the Δ^{5,9} FA have been generally reported to have the (*Z*) configuration [3, 4].

Five long-chain dimethylacetal derivatives occurred as minor compounds in the phospholipid FA mixture. All their MS displayed the fragmentation ion *m/z* 75 ([CH₃O)₂–CH]⁺ as the base peak, and a appreciable [M–MeO]⁺ ion [26, 28]. Unfortunately, complete identification

Table 1 Phospholipid fatty acids identified from the sponge *Poly-mastia penicillus*

Fatty acid (symbol)	ECL ¹ (HP-1)	Abundance (wt%)
Saturated fatty acids (SFA)		
Dodecanoic (12:0)	12.00	0.6
Tetradecanoic (14:0)	14.00	1.3
10-Methyltetradecanoic (br-15:0)	14.58	2.7
13-Methyltetradecanoic (<i>i</i> -15:0)	14.60	0.4
Pentadecanoic (15:0)	15.00	0.3
12-Methylpentadecanoic (br-16:0)	15.52	2.1
14-Methylpentadecanoic (<i>i</i> -16:0)	15.62	0.6
Hexadecanoic (16:0)	16.00	4.3
15-Methylhexadecanoic (<i>i</i> -17:0)	16.60	1.2
14-Methylhexadecanoic (<i>ai</i> -17:0)	16.71	1.8
Heptadecanoic (17:0)	17.00	0.2
Octadecanoic (18:0)	18.00	2.6
15-Methylnonadecanoic (br-20:0)	19.60	0.6
18-Methylnonadecanoic (<i>i</i> -20:0)	19.66	0.5
17-Methylnonadecanoic (<i>ai</i> -20:0)	19.72	0.3
Eicosanoic (20:0)	20.00	1.0
19-Methyleicosanoic (<i>i</i> -21:0)	20.63	0.6
Tricosanoic (23:0)	23.00	0.5
Hexacosanoic (26:0)	26.00	0.6
Total SFA: 32.2%		
2-Hydroxy fatty acids (HFA)		
2-Hydroxytetracosanoic (2-OH-24:0)	25.23	3.4
2-Hydroxypentacosanoic (2-OH-25:0)	26.22	0.8
2-Hydroxyhexacosanoic (2-OH-26:0)	27.14	2.8
Total HFA: 7.0%		
Monounsaturated fatty acids (MFA)		
13-Methyl-4-tetradecenoic (<i>i</i> -15:1)	14.28	0.4
(<i>Z</i>)-9-Hexadecenoic (16:1)	15.65	1.6
15-Methyl-5-hexadecenoic (<i>i</i> -17:1)	16.28	0.7
14-Methyl-5-hexadecenoic (<i>ai</i> -17:1)	16.40	0.4
(<i>Z</i>)-9-Heptadecenoic (17:1)	16.81	1.0
5-Octadecenoic (18:1)	17.49	0.6
(<i>Z</i>)-11-Octadecenoic (18:1)	17.82	12.7
17-Methyl-13-octadecenoic (<i>i</i> -19:1) ²	18.21	0.8
9-Nonadecenoic (19:1)	18.70	0.8
12-Nonadecenoic (19:1)	18.82	0.6
11-Eicosenoic (20:1)	19.76	0.5
13-Eicosenoic (20:1)	19.82	0.4
x ₁ -Heneicosenoic (21:1)	20.80	0.6
x ₂ -Heneicosenoic (21:1)	20.84	0.4
(<i>Z</i>)-15-Docosenoic (22:1)	21.87	13.4
16-Tetracosenoic (23:1)	22.82	0.3
(<i>Z</i>)-15-Tetracosenoic (24:1)	23.62	1.7
(<i>Z</i>)-17-Tetracosenoic (24:1)	23.81	1.5
19-Tetracosenoic (24:1)	23.88	0.7
(<i>Z</i>)-17-Hexacosenoic (26:1)	25.61	2.3

Table 1 continued

Fatty acid (symbol)	ECL ¹ (HP-1)	Abundance (wt%)
Total MFA: 41.4%		
Diunsaturated fatty acids (DFA)		
(5Z,9Z)-5,9-Octadecadienoic (18:2)	17.40	1.4
(9Z,12Z)-9,12-Octadecadienoic (18:2)	17.62	0.8
(5Z,9Z)-5,9-Eicosadienoic (20:2)	19.39	0.3
11,15-Eicosadienoic (20:2)	19.60	0.4
3,16-Docosadienoic (22:2) ²	21.55	1.1
(5Z,9Z)-5,9-Tetracosadienoic (24:2)	23.44	0.9
(5Z,9Z)-5,9-Pentacosadienoic (25:2)	24.50	0.7
(5Z,9Z)-5,9-Hexacosadienoic (26:2)	25.52	13.3
Total DFA: 18.9%		

Identified minor (<0.2%) fatty acids: *i*-14:0 (13.64), 10-Me-16:0 (16.52), 13-Me-16:0 (16.56), 14-Me-17:0 (17.55), Phytanic (17.74), 15-20:1 (19.88), 23:0 (23.00), 5,9,*x*-25:3 (24.20), br-5,9-25:2 (24.22), 5,9-27:2 (26.48)

¹ FAME

² Not previously found in nature

of these derivatives failed due to their very small amounts and that they were co-eluted with other compounds. It is known that they are obtained from particular phospholipids named plasmalogens (1-*O*-alk-1'-enyl-2-acyl-glycerophospholipids) of great biological interest, and were found in some sponges [29, 30]. Dimethylacetals are produced from fatty aldehydes, which are liberated from plasmalogen vinyl ether bonds on methanolic acid hydrolysis.

Saturated Fatty Acids

Twenty-five saturated FA of Table 1 were identified accounting for more than 32% of the total FA, including fourteen monobranched ones from C₁₄ to C₂₁ (almost 11%).

The highly branched phytanic acid present as trace was readily identified as methyl ester with an ECL value of 17.74 and diagnostic fragmentation ions including the molecular ion at *m/z* 326 and a base peak at *m/z* 101 (C-3 methyl branching), as previously described [25, 26]. It was the sole isoprenoid FA identified in the present mixture.

As usually, *iso*- and *anteiso*-branched FA were identified as FAME and NAP from their ECL values and their MS typical of saturated FA (*m/z* 74 base peak, McLafferty, *m/z* 87). The MS of their NAP displayed the corresponding molecular ion and a diminished peak for the ions [M-CH₃-CH₂]⁺ and [M-CH₃-2CH₂]⁺, respectively [10, 18, 19, 22, 24].

In addition to the *iso*- and *anteiso*-branched FA, seven various monomethyl-branched FA occurred in phospholipids of *P. penicillus*. Six of them with short-chains were

detected as FAME from their ECL values of 14.58, 15.52, 16.52, 16.56, 17.55 and 19.60, respectively, and from their MS that exhibited molecular ions at *m/z* 256, 270, 284 (two compounds) and 326, respectively. Thus, we dealt with branched pentadecanoic, hexadecanoic, heptadecanoic, eicosanoic acid structures. The MS of the 15:0 pyrrolidide showed a very low fragmentation ion at *m/z* 224 (C₁₀ fragment) and enhanced peaks at *m/z* 210 and 238 indicating a methyl branch at C-10 [10, 22, 24]. Thus, the unusual 10-methyltetradecanoic acid was identified. Another branched 17:0 acid was identified as FAME and as NAP. As FAME its MS showed the molecular ion at *m/z* 284 and an expected ion at *m/z* 227 of very low intensity, while the ions at *m/z* 213 and 241 were highly enhanced (methyl branch at C-13) [24, 29]. The branched 17:0 acid was then identified (FAME) as 13-methylhexadecanoic acid and this structure was confirmed by the MS of the NAP which showed a molecular ion at *m/z* 323. The expected ion *m/z* 266 was lacking, while the ions at *m/z* 252 and *m/z* 280 were highly enhanced (methyl branching at C-13). Mass spectra of NAP of 13-methyl- and 15-methyl-branched saturated FA were shown in a recent report [29]. The quite unusual 12-methylpentadecanoic, 14-methylheptadecanoic and 15-methylnonadecanoic acids were characterized as methyl esters since their MS showed the molecular ions at *m/z* 270, 298 and 326 (16:0, 18:0 and 20:0 structures) [24, 29]. Among these FA, the branched 20:0 acid (FAME) was identified as 15-methylnonadecanoic acid since the expected ion at *m/z* 255 was lacking, while the ions at *m/z* 241 and 269 were present (methyl branch at C-14) [22, 29].

Iso- and *anteiso* FA are typical for the occurrence of bacteria and the other monomethyl-branched short-chain FA probably have the same origin [31–34], although one of the most frequent bacterial phospholipids, namely phosphatidylglycerol, accounted here for a relatively low percentage [3, 4]. Branched-chain FA (*iso* and *anteiso*) are characteristic for gram-positive bacteria [34]. The 13-methylhexadecanoic acid was described in previous reports [22, 29]. Branched saturated FA (C₁₅–C₁₉) have been used as biomarkers for bacteria including anaerobic bacteria and the sulfate-reducing bacteria [33–37]. The 10-methyl-14:0, 10-methyl-15:0 and 10-methyl-16:0 acids were found in bacteria [33–35], and it was assumed that methyl branching on the tenth carbon atom in the acyl chain seems specific for actinomycetes [33, 38]. The 10-methylhexadecanoic acid was observed in a *Cinachyrella* sponge [10] and in a pioneering report on the sponge *Aplysina fistularis* [4]. The occurrence of bacteria in sponges has already been evidenced and studied in several reports [39, 40].

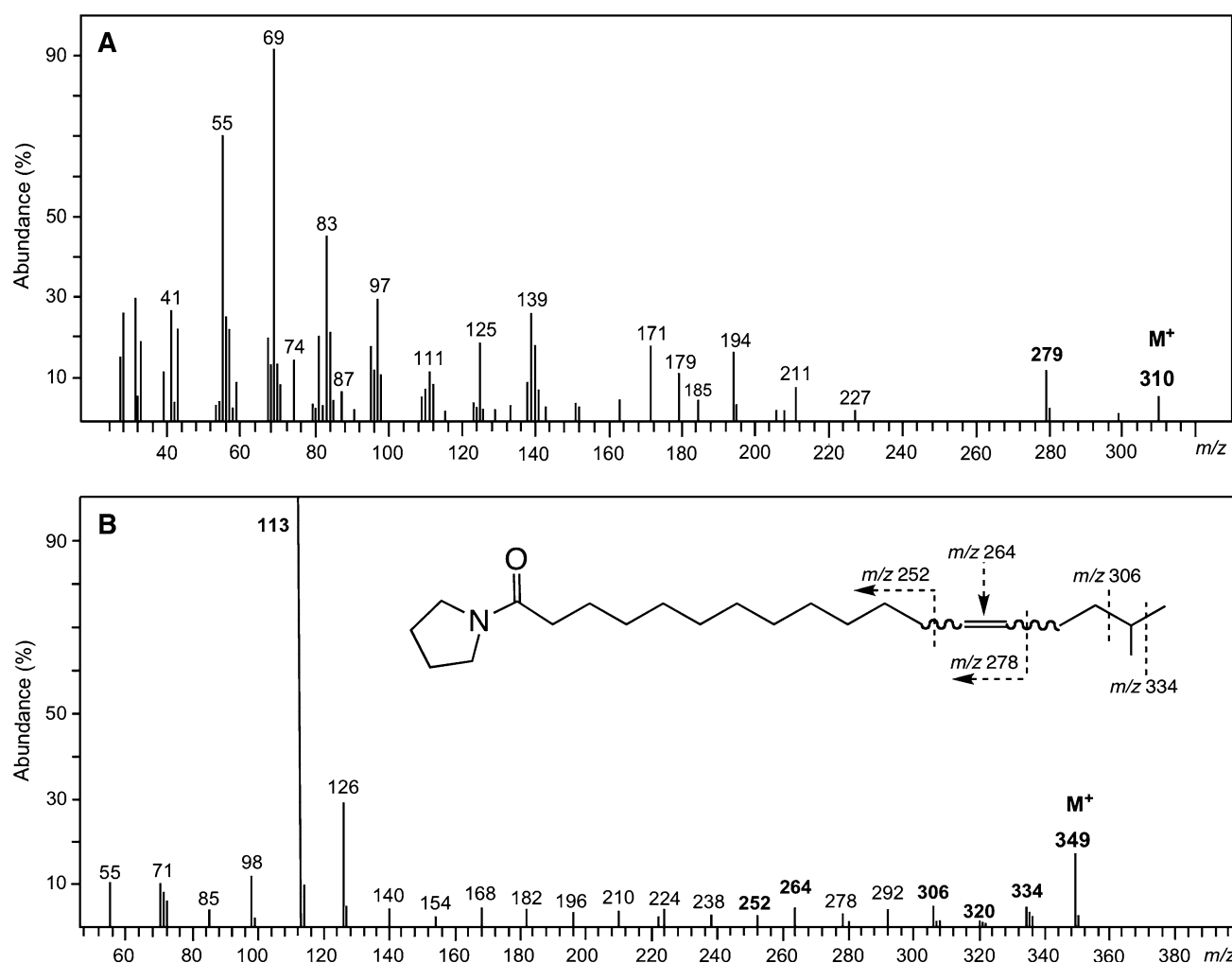


Fig. 1 Mass spectra of methyl ester (a) and pyrrolidide derivative (b) of 17-methyl-13 octadecenoic acid from *Polymastia penicillus*

2-Hydroxylated Fatty Acids

Three 2-hydroxylated FA (7.0% of FA mixture; C₂₄, C₂₅ and C₂₆) were readily identified by GC/MS as FAME since their MS displayed diagnostic fragmentation ions at *m/z* 90 (McLafferty) and 103, and the corresponding molecular ions at *m/z* 398, 412 and 426, respectively. Diagnostic ions of 2-hydroxy acids have been described in previous reports such as those on series of 2-hydroxylated FA found in two sponges of the family Suberitidae [29] and in two Caribbean sponges [31]. The M⁺ and *m/z* 90 and 103 ions were accompanied by the corresponding related ions [M–MeOH]⁺ at *m/z* 366, *m/z* 380 and *m/z* 394, respectively, and [M–COOMe]⁺ at *m/z* 339, *m/z* 353 and *m/z* 367, respectively. Mass spectra of NAP derivatives showed the corresponding molecular ions and the major ions at *m/z* 129 (McLafferty, base peak), *m/z* 113, *m/z* 100 and *m/z* 98, thus confirming the hydroxylated structures.

Monounsaturated Fatty Acids

Twenty-one monounsaturated FA listed in Table 1 were identified, accounting for more than 41% of the total FA, including four *iso*- and *anteiso*-branched FA. Three unusual short-chain monoenoic acids were readily identified in phospholipids of *P. penicillus*.

A short-chain acid was readily identified as a branched pentadecenoic acid (0.4%; ECL 14.28) since its NAP displayed a molecular ion at *m/z* 293 (15:1), and major fragment ions at *m/z* 126 and *m/z* 152, a peak at *m/z* 138 of low intensity, a very intense peak at *m/z* 166 (almost 70% of the base peak *m/z* 113; Δ4), and lacking the expected *m/z* 264, the ions at *m/z* 250 and *m/z* 278 being enhanced. Thus, we identified the *iso*-4-pentadecenoic acid, which was early observed in phospholipids from *Suberites massa* [29]. In addition, some examples of MS of NAP of Δ4 FA are shown in Christie's homepage "www.lipidlibrary.co.uk"

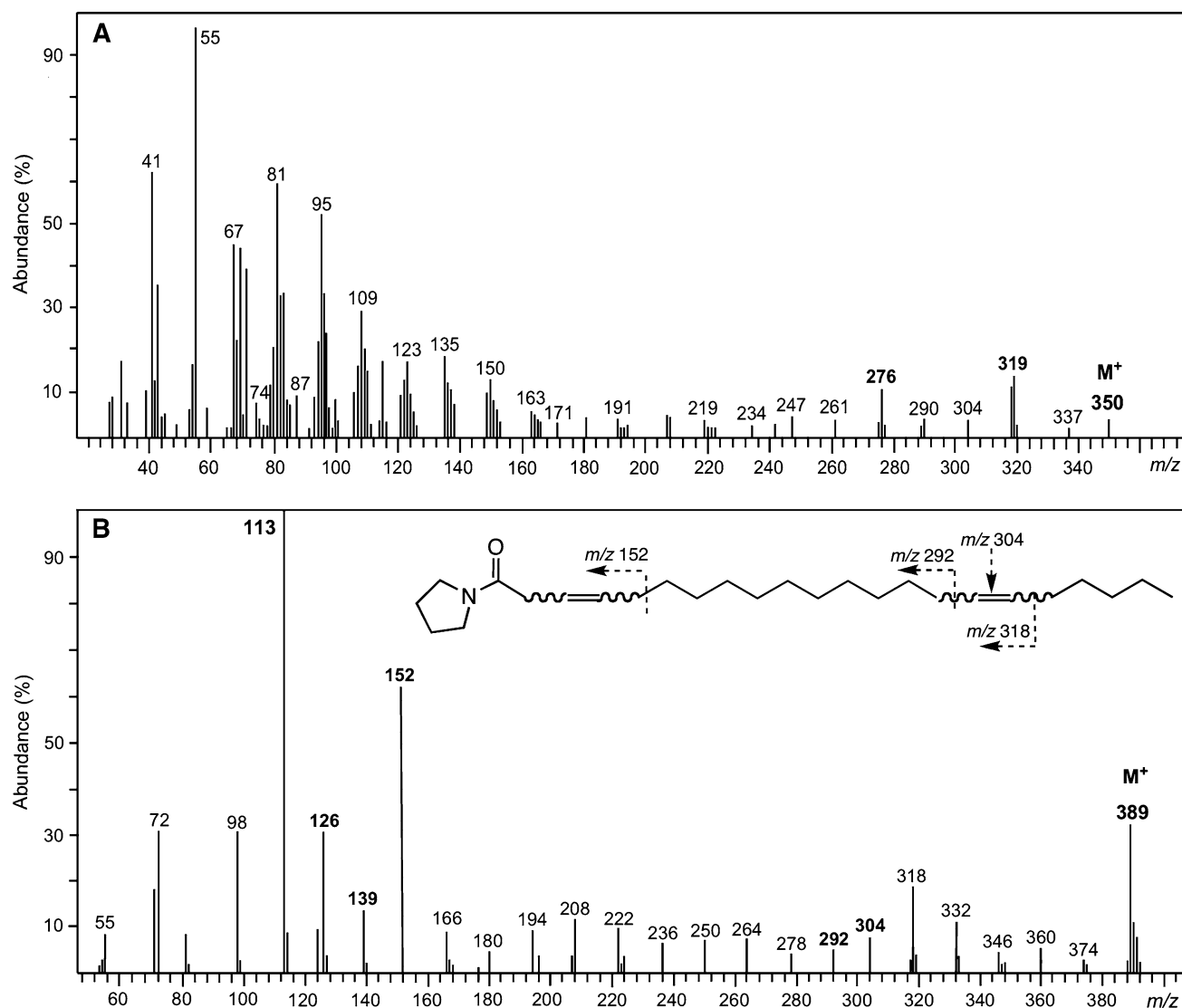


Fig. 2 Mass spectra of methyl ester (a) and pyrrolidide derivative (b) of 3,16-docosadienoic acid from *Polymastia penicillus*

[23, 24, 26]. Another $\Delta 4$ monounsaturated FA occurred in the freshwater sponge *Baicalospongia bacilifera*, namely *iso*-4-octadecenoic acid [41]. The latter sponge also contained $\Delta 5$ FA as shown with the use of the iodolactonization method [41].

Two closely related FA were identified as monounsaturated and monomethyl-branched FA since their MS showed a molecular ion (as FAME) at m/z 282, and that their ECL values were 16.28 and 16.40. Mass spectra of their NAP showed the expected molecular ion at m/z 321 and a gap of 12 amu between an appreciable m/z 140 and 152, while the ion at m/z 166 was enhanced, indicative of $\Delta 5$ unsaturation. The first br-17:1 acid was identified as 15-methylhexadecenoic (*iso*-17:1) acid because of the low relative intensity of the fragment ion at m/z 294 (C_{15}), while the ions at m/z 278 (C_{14}) and 306 (C_{16} ; M- CH_3) were enhanced. The second br-17:1 acid was identified as

14-methylhexadecenoic (*anteiso*-17:1) acid due to the low relative intensity of the fragment ion at m/z 278 (C_{14}), while the ions at m/z 264 (C_{13}) and 292 (C_{15}) were enhanced. Thus, we characterized two not-so-common FA, namely 15-methyl-5-hexadecenoic and 14-methyl-5-hexadecenoic acids. These *iso* and *anteiso* monounsaturated $\Delta 4$ or $\Delta 5$ FA probably originated from associated bacteria. Present at low growth temperatures in bacteria from the genera *Bacillus*, *Brevibacter* and *Streptomyces*, the *iso*- $\Delta 5$ -hexadecenoic, *anteiso*- $\Delta 5$ -pentadecenoic and *anteiso*- $\Delta 5$ -heptadecenoic acids were identified as methyl esters [42].

Another branched monoenoic FA was detected by GC-MS as FAME and characterized as NAP (Fig. 1).

This acid (FAME) showed a molecular ion at m/z 310, and a (M- CH_3O) $^+$ at m/z 279, corresponding to a 19:1 acid structure, and an ECL value of 18.21, implying methyl branching (Fig. 1a). The usual peaks for FA methyl esters

at m/z 74 and m/z 87 were present as minor fragment ions. Other abundant fragments were not relevant for the characterization of structure. The molecular ion peak of the corresponding NAP was present at m/z 349 confirming the 19:1 structure (Fig. 1b). The gap of 12 amu between homologous fragment ions at m/z 252 (C_{12}) and m/z 264 (C_{13}) indicated $\Delta 13$ unsaturation. A diminished peak at m/z 320 (C_{17}) flanked by an elevated peak at m/z 306 (C_{16}), and an appreciable peak at m/z 334 (C_{18}), indicated a methyl branch at C-17. Thus, we identified the new 17-methyl-13-octadecenoic acid, which to our knowledge, has not been reported from any natural source.

Diunsaturated Fatty Acids

Ten dienoic FA were identified accounting for about 19% and including the 9,12-octadecadienoic acid (0.8%), a series of eight FA possessing the 5,9-unsaturation pattern common in sponges, and two other interesting NMI FA.

The FA with the 5(*Z*),9(*Z*)-unsaturation, ranged from C_{18} to C_{27} , were readily characterized by GC-MS. Mass spectra of all their methyl esters displayed the corresponding molecular ion and the ion at m/z 81 (base peak), while spectra of their NAP displayed a significant ion at m/z 180 arising from the activated double allylic cleavage. Mass spectra of both derivatives showed corresponding molecular ions. They had been already found in sponges [4, 6–11, 18, 19, 26, 29, 31, 32]. It is now well known that lipids of most sponge species contain various $\Delta 5$ olefinic FA mainly including $\Delta 5,9$ FA. Interestingly, $\Delta 5,9$ FA displayed antiprotozoal activity, antitumor activity, and topoisomerase I inhibitory activity [43, 44]. Only one triunsaturated FA occurred as a minor component in the FA mixture and was identified as 5,9,*x*-pentacosatrienoic acid since its methyl ester (ECL of 24.20) showed the molecular ion at m/z 390 and the base peak at m/z 81.

A first additional NMI FA (0.3%) had an ECL value of 19.60 as methyl ester and a molecular ion at m/z 322 indicative of a 20:2 acid structure. The MS of the NAP showed a molecular ion at m/z 361 (20:2 acid structure). The two double bonds were located by the 12 amu differences between the peaks at m/z 224 (C_{10}) and m/z 236 (C_{11}), indicating of $\Delta 11$ unsaturation, and between the peaks at m/z 278 (C_{14}) and m/z 290 (C_{15}), indicating $\Delta 15$ unsaturation. Thus, we dealt with the rare 11,15-eicosadienoic acid, which was firstly reported from the sponge *Amphimedon complanata* [45] and observed in *Stylissa carteri* [19]. This FA has been considered as a biosynthetic intermediate of the unusual dienoic FA 17,21-hexacosadienoic and 19,23-octacosadienoic acids [10].

The second interesting dienoic NMI FA (1.1%) as a methyl ester had an ECL value of 21.55 and a molecular ion at m/z 350, and elevated ions at m/z 319 ($[M-74]^+$) and

m/z 276 ($[M-31]^+$), indicative of a 22:2 acid structure (Fig. 2a).

The usual peaks for FA methyl esters at m/z 74 and m/z 87 were present as minor fragment ions. The relatively abundant ions at m/z 304 (C_{16}), m/z 290 (C_{15}), and m/z 276 (C_{14}) confirmed a 22:2 FA structure. The MS of the NAP showed a molecular ion at m/z 389 (22:2 structure) (Fig. 2b). A first double bond was readily located by the 12 amu gap between the peaks at m/z 292 (C_{15}) and m/z 304 (C_{16}), indicating of $\Delta 16$ unsaturation. The second double bond was located with the sequence of m/z 113 (base peak), m/z 126, m/z 139, m/z 152 (the second-most prominent ion at more than 60% of the relative intensity) and m/z 166, indicating a $\Delta 3$ unsaturation according to Andersson [23, 24]. It should be noted that the ion at m/z 152 was the second-most prominent ion in the MS of the NAP of the novel 3(*Z*),9(*Z*),12(*Z*)-octadecatrienoic acid that also exhibited the two appreciable m/z 126 and m/z 139 [26, 46]. Also available on the Christie's homepage, MS of the NAP of the 3(*E*),9(*Z*)-octadecadienoic acid allows another useful comparison [26]. Indeed, the m/z 152 ion is also the second-most prominent ion but there is a prominent ion at m/z 124 suggesting a probable isomerization to 2,9-18:2 during the derivatization step. Thus, MS of the NAP of 2,6-18:2 shows intense ions at m/z 124 without the m/z 126, and at m/z 139 (base peak) [26]. In addition, MS of 2-16:1 and 2-18:1 show similar features with a major ion at m/z 152, a prominent ion at m/z 124 and a minor ion at m/z 126 [26].

In the MS of the 22:2 (NAP), from *P. penicillus*, the ion at m/z 126 is intense in accordance with a $\Delta 3$ double bond. Nevertheless, the presence of the ion at m/z 124 with a lower intensity than the ion at m/z 126 in the SM depicted in Fig. 2 suggested a possible partial isomerization $\Delta 3$ to $\Delta 2$ [26]. Thus, we identified the new 3,16-docosadienoic acid, which to our knowledge, has not been reported from any natural source.

In summary, phospholipids from the sponge *P. penicillus* contained a lot of monomethyl-branched monoenoic FA, several $\Delta 5,9$ FA, and two novel FA, namely 17-methyl-13-octadecenoic and 3,16-docosadienoic.

Acknowledgments We express our thanks to scientists from the MMS research group (EA 2160, University of Nantes), Prof. Jean-François Biard and Dr. Karina E. Petit, for their help in collecting specimens of *P. penicillus*, and Mr. Denis Loquet for his help for additional GC/MS measurements. Mrs. Maryvonne Luçon, Faculty of Sciences and Techniques of Nantes, Department of Chemistry, is greatly acknowledged for FTIR measurements.

References

1. Litchfield C, Morales RW (1976) Are Demospongiae membranes unique among living organisms? In: Harrison FW, Cowden RR

- (eds) Aspects of sponge biology. Academic Press, New York, pp 183–200
2. Litchfield C, Greenberg AJ, Noto G, Morales RW (1976) Unusually high levels of C₂₄–C₃₀ fatty acids in sponges of the class Demospongiae. *Lipids* 11:567–570
 3. Djerassi C, Lam WK (1991) Sponge phospholipids. *Acc Chem Res* 24:69–75
 4. Walkup RD, Jamieson GC, Ratcliff MR, Djerassi C (1981) Phospholipid studies of marine organisms: 2. Phospholipid-bound fatty acids and free sterols of the sponge *Aplysina fistularis* (Pallas) *forma fulva* (= *Verongia thiona*). Isolation and structure elucidation of unprecedented branched fatty acids. *Lipids* 16:631–646
 5. Genin E, Wielgosz-Collin G, Njinkoué JM, Kornprobst JM, Vacelet J, Barnathan G (2008) New trends of phospholipid class composition in marine sponges. *Comp Biochem Physiol B* 150:427–431
 6. Carballeira NM, Reyes ED (1990) Identification of the new 23-methyl-5, 9-pentacosadienoic acid in the sponge *Cribochalina vasculum*. *Lipids* 25:69–71
 7. Carballeira NM, Reyes ED, Shalabi F (1993) Identification of novel iso/anteiso nonacosadienoic acids from the phospholipids of the sponges *Chondrosia remiformis* and *Myrmekioderma styx*. *J Nat Prod* 56:1850–1855
 8. Barnathan G, Mirallès J, Gaydou EM, Boury-Esnault N, Kornprobst JM (1992) New phospholipid fatty acids from the marine sponge *Cinachyrella alloclada* Ulicza. *Lipids* 27:779–784
 9. Barnathan G, Doumenq P, Njinkoué JM, Debitus C, Levi C, Kornprobst JM (1994) Sponge fatty acids-3. Occurrence of complete series of n-7 monoenoic and iso-5,9 dienoic long-chain phospholipid fatty acids in the marine sponge *Cinachyrella* aff. *schulzei*. *Lipids* 29:297–303
 10. Barnathan G, Kornprobst JM, Doumenq P, Mirallès J (1996) Sponge new unsaturated long-chain fatty acids in the phospholipids from the Axinellida sponges *Trikenitron loeve* and *Pseudaxinella* cf. *lunaecharta*. *Lipids* 31:193–200
 11. Barnathan G, Genin E, Velosaotsy NE, Kornprobst JM, Al-Lihaibi S, Al-Sofyani A, Nongonierma R (2003) Phospholipid fatty acids and sterols of two *Cinachyrella* sponges from the Saudi Arabian Red Sea: comparison with *Cinachyrella* species from other origins. *Comp Biochem Physiol B* 135:297–308
 12. Monks NR, Lerner C, Henriques AT, Farias FM, Schapoval EES, Suyenaga ES, da Rocha AB, Schwartzmann G, Mothes B (2002) Anticancer, antichemotactic and antimicrobial activities of marine sponges collected off the coast of Santa Catarina, southern Brazil. *J Exper Mar Biol Ecol* 281:1–12
 13. Duckworth AR, Battershill CN (2003) Developing farming structures for production of biologically active sponge metabolites. *Aquaculture* 217:139–156
 14. Duckworth AR, Battershill CN, Schiel DR (2004) Effects of depth and water flow on growth, survival and bioactivity of two temperate sponges cultured in different seasons. *Aquaculture* 242:237–250
 15. Boury-Esnault N (1987) The *Polymastia* species (Demosponges, Hadromerida) of the Atlantic area. In: Vacelet J, Boury-Esnault N (eds) *Taxonomy of Porifera NATO*, vol G13. ASI Series Springer, Berlin, pp 29–66
 16. Plotkin A, Boury-Esnault N (2004) Alleged cosmopolitanism in sponges: the example of a common Arctic *Polymastia* (Porifera, Demospongiae, Hadromerida). *Zoosystema* 28:13–20
 17. Ayanoglu E, Kurtz K, Kornprobst JM, Djerassi C (1985) New natural 2-acetoxy fatty acids using chemical ionization and electron impact mass spectrometry. *Lipids* 20:141–144
 18. Barnathan G, Kornprobst JM (2000) Fatty acids from marine organisms: recent research developments. In: Baudimant G, Guezennec J, Roy P, Samain JF (eds) *Marine Lipids*. IFREMER Press, Brest, pp 35–43
 19. Velosaotsy NE, Genin E, Nongonierma R, Al-Lihaibi S, Kornprobst JM, Vacelet J, Barnathan G (2004) Phospholipid distribution and phospholipid fatty acids in four Saudi Red Sea sponges. *Boll Mus Ist Biol Univ Genova* 68:639–645
 20. Hooper JNA, van Soest RWM (2002) *Systema Porifera*. A guide to the classification of sponges, vol. 2. Kluwer/Plenum, New York, 1708 pp
 21. Boury-Esnault N (2002) Family Polymastiidae Gray, 1867. In: Hooper JNA, van Soest RWM (eds) *Systema Porifera*. A guide to the classification of sponges. Kluwer/Plenum, New York, pp 201–219
 22. Andersson BÅ, Holman RT (1975) Mass spectrometric localization of methyl branching in fatty acids using acylpyrrolidines. *Lipids* 10:716–718
 23. Andersson BÅ, Holman RT (1974) Pyrrolidides for mass spectrometric determination of the position of the double bond in monounsaturated fatty acids. *Lipids* 9:185–190
 24. Andersson B (1978) Mass spectrometry of fatty acid pyrrolidides. *Progr Chem Fats Lipids* 16:279–308
 25. Mirallès J, Barnathan G, Galonnier R, Sall T, Samb A, Gaydou EM, Kornprobst JM (1995) New branched-chain fatty acids from the Senegalese Gorgonian *Leptogorgia piccola* (white and yellow morphs). *Lipids* 30:459–466
 26. Christie WW The lipid library. www.lipidlibrary.co.uk, section updated 07.05.08
 27. Albuquerque MLS, Guedes I, Alcantara P Jr, Moreira SGC (2003) Infrared absorption spectra of Burity (*Mauritia flexuosa* L.) oil. *Vibr Spectrosc* 33:127–131
 28. Christiansen K, Mahadevan V, Viswanathan CV, Holman RT (1969) Mass spectrometry of long-chain aldehydes, dimethyl acetals and alk-1-enyl ethers. *Lipids* 4:421–427
 29. Barnathan G, Kornprobst JM, Doumenq P, Mirallès J, Boury-Esnault N (1993) 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*. *J Nat Prod* 56:2104–2113
 30. Nagan N, Zoeller RA (2001) Plasmalogens: biosynthesis and functions. *Prog Lipid Res* 40:199–229
 31. Carballeira NM, Shalabi F, Negron V (1989) 2-Hydroxy fatty acids from marine sponges 2. The phospholipid fatty acids of the Caribbean sponges *Verongula gigantea* and *Aplysina archeri*. *Lipids* 24:229–232
 32. Nechev J, Christie WW, Robain R, de Diego F, Popov S (2004) Chemical composition of the sponge *Hymeniacion sanguinea* from the Canary Islands. *Comp Biochem Physiol A* 137:365–374
 33. Ratledge C, Wilkinson G (1988) *Microbial lipids*. Academic Press, London, 963 pp
 34. Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria; biosynthesis, function, and taxonomic significance. *Microbiol Rev* 55:288–302
 35. Cronan JE (2003) Bacterial membrane lipids: where do we stand? *Annu Rev Microbiol* 57:203–224
 36. Haack SK, Garchow H, Odelson DA, Forney LJ, Klug MJ (1994) Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Appl Environ Microbiol* 60:2483–2493
 37. Cravo-Laureau C, Hirschler-Réa A, Matheron R, Grossi V (2004) Growth and cellular fatty-acid composition of a sulphate-reducing bacterium, *Desulfatibacillum aliphaticivorans* strain CV2803T, grown on n-alkenes. *C R Biologies* 327:687–694
 38. Saddler GS, O'Donnel AG, Goodfellow M, Minnikin DE (1987) SIMCA pattern recognition on the analysis of streptomycete fatty acids. *J Gen Microbiol* 133:1137–1147

39. Vacelet J (1975) Etude en microscopie électronique de l'association entre bactéries et Spongiaires du genre *Verongia* (Dictyoceratida). *J Microsc Biol Cell* 3:271–288
40. Gillan FT, Stoilov IL, Thompson JE, Hogg RW, Wilkinson CR, Djerassi C (1988) Fatty acids as biological markers for bacterial symbionts in sponges. *Lipids* 23:1139–1145
41. Imbs AB, Latyshev NA (1998) New $\Delta 5$ and $\Delta 4$ unsaturated medium- and long-chain fatty acids in the freshwater sponge *Baicalospongia bacilifera*. *Chem Phys Lipids* 92:117–125
42. Suutari M, Laasko S (1993) Signature GLC-MS ions in identification of $\Delta 5$ - and $\Delta 9$ -unsaturated *iso*- and *anteiso*-branched fatty acids. *J Microbiol Methods* 17:39–48
43. Bergé JP, Barnathan G (2005) Recent advances in fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically-active compounds and economical aspects. In: Le Gal Y, Ulber R (eds) *Marine Biotechnology*. *Adv Biochem Eng Biotechnol* 96:49–125
44. Carballeira NM (2008) New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. *Prog Lipid Res* 47:50–61
45. Carballeira NM, Restituyo J (1991) Identification of the new 11, 15-icosadienoic acid and related acids in the sponge *Amphimedon complanata*. *J Nat Prod* 54:315–317
46. Tsevegsuren N, Fujimoto K, Christie WW, Endo Y (2003) Occurrence of a novel *cis, cis, cis*-octadeca-3, 9, 12-trienoic (*Z,Z,Z*-octadeca-3,9,12-trienoic) acid in *Chrysanthemum (Tanacetum) zawadskii* Herb. (Compositae) seed oil. *Lipids* 38:573–578