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ORIGINAL ARTICLE

Novel Cyclopropane Fatty Acids from the Phospholipids of the Caribbean Sponge *Pseudospongosorites suberitoides*

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Abstract The cyclopropane fatty acids 17-methyl-*trans*-4,5-methyleneoctadecanoic acid, 18-methyl-*trans*-4,5-methylenenonadecanoic acid, and 17-methyl-*trans*-4,5-methylenenonadecanoic acid were characterized for the first time in nature in the phospholipids (mainly PE, PG and PS) of the hermit-crab sponge *Pseudospongosorites suberitoides*. Pyrrolidine derivatization was the key in identifying the position of the cyclopropyl and methyl groups in the acyl chains and ^{1}H NMR was used to determine the *trans* stereochemistry of the cyclopropane ring. The phospholipids from the sponge also contained an interesting series of *iso-anteiso* $\Delta^{5,9}$ fatty acids with chain-lengths between 17 and 21 carbons, with the fatty acids (5*Z*,9*Z*)-18-methyl-5,9-nonadecadienoic acid and the (5*Z*,9*Z*)-17-methyl-5,9-nonadecadienoic acid being described for the first time in sponges. The *anteiso* α-methoxylated fatty acid 2-methoxy-12-methyltetradecanoic acid was also identified for the first time in nature in the phospholipids of this interesting marine sponge. The novel cyclopropyl fatty acids could have originated from the phospholipids of a cyanobacterium living in symbiosis with the sponge.

Keywords Cyanobacteria · Cyclopropane · Fatty acids · Phospholipids · *Pseudospongosorites suberitoides* · Pyrrolidides · Sponges

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Abbreviations

ATCC	American type culture collection
ECL	Equivalent chain length
FAME	Fatty acid methyl ester
GC–MS	Gas chromatography–mass spectrometry
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PS	Phosphatidylserine

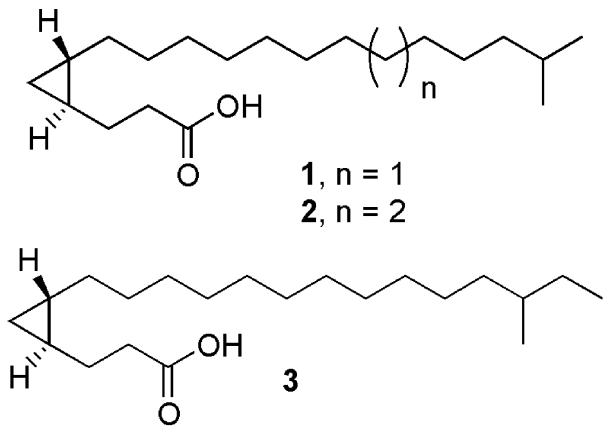
Introduction

Cyclopropane fatty acids (FA) are quite ubiquitous in seed oils, bacteria, and other microorganisms [1]. Since the discovery of lactobacillic acid (*cis*-11,12-methyleneoctadecanoic acid) in 1951, several interesting cyclopropyl FA have been identified in both Gram-negative and Gram-positive bacteria [2]. For example, the acid 9,10-methylenehexadecanoic acid was reported in the Gram-negative bacterium *Pseudomonas cepacia* [3]. The protozoan *Herpetomonas megaseliae* synthesizes de novo the *iso*-branched FA 17-methyl-*cis*-9,10-methyleneoctadecanoic acid [4], while the acids 9,10-methylene-5-hexadecenoic acid and 11,12-methylene-5-octadecenoic acid are present in the slime mould *Polysphondylium pallidum* [5]. The *cis*-9,10-methyleneoctadecanoic acid has been identified in the seeds of *Litchi chinensis*, in longan seed oil, and in trypanosomatids and is preferentially located at the *sn*-2 position of triglycerides [6–7]. At present there is no known role for these cyclopropane FA.

Although less ubiquitous, cyclopropane FA have also been reported in marine organisms. Sponges have provided some interesting examples. For example, the acid

19,20-methylenehexacosanoic acid was identified in the phospholipids of the sponge *Calyx niceansis* [8], while the sponge *Amphimedon* sp., collected in Australia, contains the acid 10,11-methyleneheptacosa-5,9-dienoic acid, a topoisomerase I inhibitor with an IC_{50} = 1.2 μM [9]. More recently, majusculoic acid, a brominated diunsaturated cyclopropyl fatty acid with a *trans* cyclopropane ring between carbons 4 and 5, was isolated from a cyanobacterial mat assemblage [10]. Majusculoic acid exhibited antifungal activity against *Candida albicans* ATCC 14503 with a MIC of 8 μM [10].

Aimed at discovering other unusual cyclopropyl FA in marine sponges, we investigated herein, for the first time, the phospholipid FA composition of the hermit-crab sponge *Pseudospongosorites suberitoides* (Class Demospongiae, Family Suberitidae) [11] and report on the occurrence of the novel cyclopropane FA **1**–**3** with an uncommon *trans* cyclopropyl group between carbons 4 and 5 of the acyl chain [12]. In addition, other previously unidentified phospholipid FA containing either the α-methoxy substitution or the Δ5,9 diunsaturation were also identified for the first time in nature in *P. suberitoides*.



Materials and Methods

Instrumentation

Fatty acid methyl esters (FAME) and pyrrolidides were analyzed by direct ionization using GC–MS (Hewlett-Packard 5972A MS ChemStation) at 70 eV equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS). The GC-temperature program was: 130 °C for 1 min, increased at a rate of 3 °C/min to 270 °C, and maintained for 30 min at 270 °C. ^{1}H-NMR spectra were recorded on a Bruker DPX-300 spectrometer. ^{1}H-NMR chemical shifts are reported with respect to internal Me_4Si, and chemical shifts are given in parts per million (ppm).

Sponge Collection

The sponge *Pseudospongosorites suberitoides* (Class Demospongiae, Family Suberitidae) (Díaz, van Soest and Pomponi 1993) (Sandford and Kelly-Borges 1997) was collected from Mona Island (near Monito), Puerto Rico in July 2006 at 26 m depth by scuba. The sponge was freeze-dried and stored at –20 °C until extraction. A voucher specimen (IM06-06) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras campus.

Extraction and Isolation of Phospholipids

The sponge (74 g of dry weight) was carefully cleaned and cut into small pieces. Extraction with 2 × 200 ml of $CHCl_3$/MeOH (1:1) yielded the total lipids (8.4 g). The neutral lipids, glycolipids, and phospholipids (3.9 g) were separated by column chromatography on Si gel (60–200 mesh) using the procedure of Privett et al. [13]. The phospholipid classes were identified by R_f values using thin-layer chromatography using Si gel H plates and $CHCl_3$/MeOH/NH_4OH (65:30:5) as developing solvent. The main phospholipids identified were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylserine (PS).

Derivatives

The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction of the phospholipids with methanolic HCl followed by column chromatography on Si gel eluting with hexane/ether (9:1). An aliquot of the methyl esters were hydrogenated in 10 ml methanol with catalytic amounts of Pd/C (10%). The double-bonds, methyl-branching, and cyclopropane positions in these compounds were determined by pyrrolidide derivatization of an aliquot of the methyl esters following the preparation procedure previously described [14]. Mass spectral data for the novel compounds follows and/or are presented in Table 1.

Methyl 2-methoxy-12-methyltetradecanoate

ECL = 15.93; GC–MS *m/z* (relative intensity) M^+ 286 (2), 228 (17), 227 (100), 139 (4), 138 (2), 125 (15), 123 (3), 111 (34), 109 (8), 104 (7), 99 (5), 97 (63), 95 (18), 87 (9), 85 (17), 83 (71), 81 (26), 79 (5), 75 (9), 71 (79), 70 (10), 69 (72), 67 (27), 59 (12), 58 (24), 57 (91), 56 (13), 55 (77).

Methyl 18-methyl-5,9-nonadecadienoate

ECL = 19.09; GC–MS *m/z* (relative intensity) M^+ 322 (6), 199 (3), 181 (4), 164 (4), 154 (4), 150 (10), 143 (7), 141

Table 1 Mass spectral data (70 eV) for the novel cyclopropane compounds and their pyrrolidide derivatives

Compound	ECL	*m/z* (relative intensity)
Methyl 17-methyl-*trans*-4,5-methyleneoctadecanoate	19.15	M^+ 324 (1), 293 (2), 292 (9), 250 (16), 237 (2), 208 (3), 194 (3), 180 (1), 166 (2), 165 (2), 155 (3), 152 (3), 151 (3), 141 (5), 139 (4), 137 (6), 128 (16), 127 (6), 125 (8), 123 (9), 114 (8), 111 (19), 110 (15), 109 (14), 101 (54), 97 (39), 96 (36), 95 (25), 87 (23), 85 (29), 83 (43), 82 (29), 81 (36), 74 (66), 71 (28), 69 (60), 67 (51), 59 (65), 57 (61), 55 (100)
N-17-methyl-*trans*-4,5-methyleneoctadecanoylpyrrolidine		M^+ 363 (9), 349 (3), 348 (12), 321 (2), 320 (7), 307 (1), 306 (3), 292 (3), 279 (1), 278 (5), 265 (1), 264 (5), 251 (1), 250 (5), 237 (2), 236 (6), 223 (2), 222 (10), 210 (1), 209 (3), 208 (11), 195 (3), 194 (12), 181 (4), 180 (12), 167 (10), 166 (31), 154 (7), 152 (7), 139 (5), 138 (34), 126 (15), 114 (13), 113 (100), 98 (59), 91 (8), 85 (19), 79 (11), 70 (38), 67 (21), 57 (19), 55 (82)
Methyl 18-methyl-*trans*-4,5-methylenenonadecanoate	20.14	M^+ 338 (1), 306 (9), 264 (13), 263 (3), 141 (6), 139 (4), 137 (5), 128 (14), 127 (5), 125 (8), 123 (7), 114 (6), 111 (17), 110 (14), 109 (16), 101 (41), 97 (36), 96 (33), 95 (25), 87 (22), 85 (26), 83 (40), 82 (26), 81 (40), 74 (57), 71 (29), 69 (54), 67 (52), 59 (52), 57 (57), 55 (100)
N-18-methyl-*trans*-4,5-methylenenonadecanoylpyrrolidine		M^+ 377 (8), 363 (4), 362 (13), 335 (2), 334 (8), 320 (3), 306 (3), 292 (4), 278 (5), 264 (6), 250 (6), 237 (2), 236 (7), 223 (3), 222 (13), 209 (3), 208 (14), 195 (4), 194 (16), 181 (7), 180 (21), 167 (14), 166 (41), 153 (7), 152 (10), 139 (8), 138 (44), 127 (5), 126 (17), 114 (11), 113 (94), 98 (72), 85 (17), 83 (15), 72 (22), 70 (38), 57 (31), 55 (100)
Methyl 17-methyl-*trans*-4,5-methylenenonadecanoate	20.24	M^+ 338 (1), 306 (6), 264 (10), 141 (4), 139 (4), 137 (6), 128 (15), 127 (5), 125 (9), 123 (10), 114 (7), 111 (18), 110 (14), 109 (16), 101 (41), 97 (36), 96 (30), 95 (28), 87 (21), 85 (24), 83 (40), 82 (26), 81 (41), 74 (53), 71 (32), 69 (51), 67 (51), 59 (51), 57 (88), 55 (100)
N-17-methyl-*trans*-4,5-methylenenonadecanoylpyrrolidine		M^+ 377 (7), 363 (3), 362 (11), 348 (5), 321 (2), 320 (7), 307 (2), 306 (3), 292 (4), 278 (4), 264 (5), 250 (5), 237 (2), 236 (5), 223 (3), 222 (11), 209 (3), 208 (11), 195 (4), 194 (15), 181 (5), 180 (17), 167 (14), 166 (36), 153 (7), 152 (10), 139 (7), 138 (39), 127 (5), 126 (15), 114 (11), 113 (100), 98 (68), 97 (11), 85 (17), 83 (10), 81 (10), 72 (18), 70 (37), 57 (52), 55 (92)

ECL Equivalent chain-length

(21), 136 (10), 135 (9), 123 (7), 121 (10), 110 (18), 109 (45), 107 (8), 99 (17), 97 (21), 95 (24), 87 (23), 85 (10), 83 (29), 81 (100), 79 (27), 74 (41), 71 (16), 69 (51), 67 (73), 57 (50), 55 (85).

N-18-methyl-5,9-nonadecadienoylpyrrolidine

GC–MS *m/z* (relative intensity) M^+ 361 (3), 348 (3), 320 (2), 306 (1), 292 (1), 278 (2), 264 (2), 250 (2), 236 (2), 234 (3), 222 (3), 208 (4), 194 (4), 182 (2), 180 (19), 166 (10), 154 (1), 152 (3), 140 (3), 126 (20), 113 (100), 98 (31), 85 (14), 72 (15), 71 (14), 57 (16), 55 (53).

Methyl 17-methyl-5,9-nonadecadienoate

ECL = 19.21; GC-MS *m/z* (relative intensity) M^+ 322 (1), 237 (3), 163 (5), 150 (9), 141 (17), 136 (12), 135 (9), 123 (12), 121 (12), 110 (19), 109 (40), 107 (10), 99 (16), 97 (24), 95 (31), 87 (14), 85 (15), 83 (29), 81 (100), 79 (31), 74 (30), 71 (21), 69 (48), 67 (72), 57 (60), 55 (89).

Results and Discussion

Pseudospongosorites suberitoides presented a typical sponge phospholipid profile where PE, PG, and PS were the most abundant phospholipids. Acid methanolysis of the total phospholipids provided a rather complex and unusual phospholipid FA composition of around 51 identifiable FA as shown in Table 2. FA chain lengths ranged between 14 and 27 carbons, mainly consisting of methyl-branched fatty acids (75% of the total FA mixture). The Δ5,9 FA were particularly abundant in this sponge (25% of the total FA) but their chain lengths were atypical with respect to other sponge phospholipid Δ5,9 FA inasmuch as here the most abundant Δ5,9 FA had chain lengths between 17 and 20 carbons. Among the Δ5,9 FA the even-chain (5*Z*,9*Z*)-5,9-octadecadienoic acid was particularly abundant (7.8% relative abundance), a most unusual finding for sponges [15]. Around 66% of the total FA contained odd carbon chains. The isoprenoid FA 4,8,12-trimethyltridecanoic acid was also present in *P. suberitoides*. It is important to mention that this FA is a common constituent of the phospholipids of the sponge families Spirastrellidae and Clionidae [16].

The most interesting series of FA from *P. suberitoides* were the cyclopropane fatty acids 17-methyl-*trans*-4,5-methyleneoctadecanoic acid (**1**), 18-methyl-*trans*-4,5-methylenenonadecanoic acid (**2**), and 17-methyl-*trans*-4,5-methylenenonadecanoic acid (**3**), all FA with an unusual *trans* cyclopropane ring between carbons 4 and 5. The relative GC retention times and mass spectra of the methyl esters, the mass spectra of the corresponding pyrrolidide derivatives, catalytic hydrogenation, and ^{1}H NMR of the whole FAME mixture provided the basis for their characterization. For

Table 2 Identified phospholipid FA from *Pseudospongosorites suberitoides*

FA	Relative abundance (wt %)
Tetradecanoic (14:0)	0.6
4,8,12-Trimethyltridecanoic (16:0)	4.3
6-Methyltetradecanoic (*br*-15:0)	2.0
13-Methyltetradecanoic (*i*-15:0)	3.3
12-Methyltetradecanoic (*ai*-15:0)	3.2
Pentadecanoic (*n*-15:0)	0.7
14-Methylpentadecanoic (*i*-16:0)	1.3
(*Z*)-9-Hexadecenoic (16:1n-7)	1.0
2-Methoxy-12-methyltetradecanoic (2-OMe-*ai*-15:0)[a]	1.5
Hexadecanoic (*n*-16:0)	3.2
(5*Z*,9*Z*)-15-Methyl-5,9-hexadecadienoic (*i*-17:2n-7)	0.9
(*Z*)-15-Methyl-9-hexadecenoic (*i*-17:1n-7)	3.8
(5*Z*,9*Z*)-14-Methyl-5,9-hexadecadienoic (*ai*-17:2n-7)	0.5
10-Methylhexadecanoic (*br*-17:0)	4.6
15-Methylhexadecanoic (*i*-17:0)	2.5
14-Methylhexadecanoic (*ai*-17:0)	1.2
(*Z*)-9-Heptadecenoic (17:1n-8)	0.8
(5*Z*,9*Z*)-5,9-Octadecadienoic (18:2n-9)	7.8
(*Z*)-9-Octadecenoic (18:1n-9)	2.1
(*Z*)-11-Octadecenoic (18:1n-7)	1.7
Octadecanoic (*n*-18:0)	2.3
(5*Z*,9*Z*)-17-Methyl-5,9-octadecadienoic (*i*-19:2n-9)	2.2
11-Methyloctadecanoic (*br*-19:0)	7.6
12-Methyloctadecanoic (*br*-19:0)	7.0
(5*Z*,9*Z*)-16-Methyl-5,9-octadecadienoic (*ai*-19:2n-9)	3.5
17-Methyloctadecanoic (*i*-19:0)	1.7
16-Methyloctadecanoic (*ai*-19:0)	0.7
(5*Z*,9*Z*)-18-Methyl-5,9-nonadecadienoic (*i*-20:2n-10)[a]	1.0
17-Methyl-*trans*-4,5-methyleneoctadecanoic (*i*-cy-20:0)[a]	5.5
(5*Z*,9*Z*)-17-Methyl-5,9-nonadecadienoic (*ai*-20:2n-10)[a]	0.6
5,8,11,14-Eicosatetraenoic (20:4n-6)	1.9
(5*Z*,9*Z*)-Eicosadienoic (20:2n-11)	3.8
Eicosanoic (*n*-20:0)	1.3
(5*Z*,9*Z*)-19-Methyl-5,9-eicosadienoic (*i*-21:2n-11)	1.5
18-Methyl-*trans*-4,5-methylenenonadecanoic (*i*-cy-21:0)[a]	0.9
(5*Z*,9*Z*)-18-Methyl-5,9-eicosadienoic (*ai*-21:2n-11)	0.6
17-Methyl-*trans*-4,5-methylenenonadecanoic (*ai*-cy-21:0)[a]	0.9
19-Methyleicosanoic (*i*-21:0)	0.5
18-Methyleicosanoic (*ai*-21:0)	0.5
Heneicosanoic (*n*-21:0)	0.4
Methylheneicosanoic (*br*-22:0)	0.2
Docosanoic (*n*-22:0)	0.1
21-Methyldocosanoic (*i*-23:0)	0.6
20-Methyldocosanoic (*ai*-23:0)	0.1
Methyltricosanoic (*br*-24:0)	0.3
Tetracosanoic (24:0)	0.2
23-Methyltetracosanoic (*i*-25:0)	0.7
22-Methyltetracosanoic (*ai*-25:0)	0.2
(5*Z*,9*Z*)-25-Methyl-5,9-hexacosadienoic (*i*-27:2n-17)	1.6
(5*Z*,9*Z*)-24-Methyl-5,9-hexacosadienoic (*ai*-27:2n-17)	0.4
Heptacosanoic (27:0)	0.1

[a] Unprecedented in nature

example, methyl 17-methyl-*trans*-4,5-methyleneoctadecanoate exhibited unusual chromatographic properties, namely an equivalent chain length (ECL) value of 19.15, for which the fractional value is well in agreement with a highly branched FA structure. The mass spectrum of methyl 17-methyl-*trans*-4,5-methyleneoctadecanoate contained a molecular ion peak at *m/z* 324 ($C_{21}H_{40}O_2$) and an intense peak at *m/z* 101 (54%) as well as the classical McLafferty rearrangement peak at *m/z* 74 (66%). Upon mild catalytic hydrogenation (Pd/C) the molecular ion of this methyl ester remained the same, while other monoenes and dienes in the FAME mixture incorporated hydrogen. Therefore, this experiment excluded the possibility of a monounsaturated methyl ester and suggests the presence of a cyclopropyl ring in the fatty acyl chain.

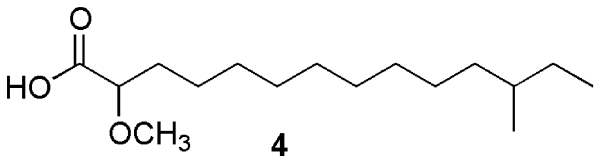
Pyrrolidide derivatization was instrumental in elucidating the basic structure of this FA. For example, in the mass spectrum of *N*-17-methyl-*trans*-4,5-methyleneoctadecanoylpyrrolidine the presence of a cyclopropyl group between carbons 4 and 5 was indicated from a difference of 12 amu between fragments at *m/z* 126 (C_3) and *m/z* 138 (C_4), and confirmed by a strong fragmentation peak at *m/z* 166 (C_6) arising from cleavage between carbons 5 and 6 at the other end of the cyclopropane ring. Previous work by Andersson on the mass spectra of pyrrolidides of FA containing cyclopropyl groups indicates that if an interval of 12 amu, instead of the regular 14, is observed between the most intense peaks of clusters of fragments containing *n-1* and *n* carbon atoms of the acid moiety, a cyclopropane ring occurs between carbon atoms *n* and *n + 1* in the molecule [17]. However, caution should be exercised when dealing with pyrrolidides since the cyclopropane ring can

be located within one carbon position of its true location if the ring lies between carbons 6,7 and 16,17, but in our case such an exception does not apply since the ring lies between carbons 4,5 [17]. The presence of the *iso* terminal methyl branching in this compound was also clearly observed in the pyrrolidide mass spectrum since a diminished peak (and/or absent peak) at *m/z* 334 with the corresponding intense peaks at *m/z* 320 and 348 were also observed.

In order to corroborate the presence of the cyclopropyl group as well as to assign its *trans* stereochemistry ^{1}H NMR was used [18]. Since the ^{1}H NMR signals of a cyclopropane ring normally resonate at high-field it was possible to observe these signals in the ^{1}H NMR spectrum of the whole FAME mixture without interference from other peaks. Proton signals were indeed observed as multiplets at δ 0.29 ppm and at δ 0.66 ppm, while no absorption was observed at –0.3 ppm. This observation is indicative of a *trans* stereochemistry for the cyclopropane rings, since FA with a cyclopropane ring in *cis* configuration normally show absorptions at 0.6 ppm and at –0.3 ppm for the two methylene cyclopropane hydrogens [18]. In the case of a *trans* cyclopropane ring these two methylene hydrogens are similar, due to a pseudo-C_{2V} symmetry, and both resonate at around 0.2 ppm [10, 18]. Therefore, we can unequivocally assign as methyl 17-methyl-*trans*-4,5-methyleneoctadecanoate our methyl ester in question.

The other cyclopropane fatty acids **2** and **3**, as well as their corresponding derivatives, displayed similar spectral characteristics as **1** that allowed their characterization as isomeric C_{21} members of the same family of cyclopropane FA. A slight difference in the spectral data was observed for methyl 17-methyl-*trans*-4,5-methylenenonadecanoate, inasmuch as an ECL value of 20.24 indicated the presence of an *anteiso* C_{21} isomer. This structural assignment was further confirmed in the mass spectrum of *N*-17-methyl-*trans*-4,5-methylenenonadecanoylpyrrolidine (M^+ = 377) where a diminished peak (and/or absent peak) at *m/z* 334 confirmed the C-17 methyl substitution.

Among the studied FA from *P. suberitoides* a novel α-methoxylated fatty acid, namely **4,** was also characterized using GC–MS as well as gas chromatographic ECL values as compared to synthetic standards [14, 19]. The mass spectrum of the methyl ester of **4** displayed a molecular ion peak at *m/z* 286 and a strong M^+–59 peak at *m/z* 227 (100%), together with a small peak at *m/z* 104 (McLafferty rearrangement), all typical mass fragments for α-methoxylated saturated methyl esters [14, 19]. The α-methoxylation was further confirmed by ^{1}H NMR spectroscopy since it was also possible to observe the characteristic signals of the α-methoxy methyl ester in the ^{1}H NMR spectrum of the whole FAME mixture without interference from other peaks. In this case, the methoxy protons resonated as a singlet at δ 3.66 ppm, while the methine α-hydrogen resonated as a multiplet at δ 3.75 ppm, all chemical shift values in agreement with other α-methoxylated methyl esters [20]. The GC retention time of the methyl ester of **4** (ECL value of 15.93) suggested it to be an *anteiso* methyl-branched fatty acid [14]. A normal chain α-methoxylated C_{15} FA methyl ester displays an ECL value of 16.20, while an *iso* α-methoxylated C_{15} FA methyl ester displays an ECL value of 15.86. Therefore, based on this evidence the most logical structural assignment for the original acid is that of 2-methoxy-12-methyltetradecanoic acid (**4**), which has not been identified before in sponges. It should be pointed out that pyrrolidide derivatization has limited usefulness in locating methyl branching in α-methoxylated fatty acids [14].



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As we mentioned before *P. suberitoides* also contained a complete series (11% of the total phospholipid fatty acid composition) of *iso* and *anteiso* C_{17}–C_{21} FA with the typical Δ5,9 diunsaturation pattern of ''demospongic'' acids [21]. Among these Δ5,9 fatty acids the 18-methyl-5,9-nonadecadienoic acid (*i*-20:2Δ5,9) and the 17-methyl-5,9-nonadecadienoic acid (*ai*-20:2 Δ5,9) appear not to have been identified before in sponges. In mass spectrometry the methyl esters of *i*-20:2Δ5,9 and *ai*-20:2 Δ5,9 displayed the same molecular ion (M^+) at *m/z* 322. In addition, the Δ5,9 diunsaturation was readily recognized from the characteristic base peak at *m/z* = 81, the allylic cleavage fragmentation between C-7 and C-8 at *m/z* 141, and the fragmentation at *m/z* 109 which results from the loss of methanol from the *m/z* 141 fragment [22]. Pyrrolidides were also informative in characterizing these FA [22]. For example, in the mass spectrum of *N*-18-methyl-5,9-nonadecadienoylpyrrolidine the characteristic allylic cleavage between C-7 and C-8 was observed at *m/z* 180 as well as cleavage between C-11 and C-12 at *m/z* 234 [22]. Catalytic hydrogenation of methyl 18-methyl-5,9-nonadecadienoate and methyl 17-methyl-5,9-nonadecadienoate afforded methyl 18-methylnonadecanoate and methyl 17-methylnonadecanoate, respectively, as confirmed by their GC retention times and mass spectrometry, thereby confirming the terminal *iso-anteiso* methyl branching in these compounds. The mass spectrum of *N*-18-methyl-5,9-nonadecadienoylpyrrolidine also confirmed the *iso* methyl branching whereby a diminished (or absent peak) at *m/z* 334 was also observed.

The results obtained for *P. suberitoides* are interesting for several reasons. The phospholipid FA composition of *P. suberitoides* is unusual in the sense that the main Δ5,9 fatty acids present in the sponge have chain lengths between 17 and 21 carbons, in contrast to the typical FA composition of many sponges belonging to the Demospongiae where very long-chain Δ5,9 FA with chain lengths between 25 and 29 carbons predominate [21, 23]. This finding could have significance in the chemotaxonomy of sponges belonging to the Suberitidae family.

The characterization of the novel cyclopropyl FA **1**–**3** in the phospholipids of *P. suberitoides* is also a rare finding, inasmuch as these fatty acids possess a *trans* cyclopropyl ring attached to carbons 4 and 5. This unusual 4,5 cyclopropane substitution seems to be specific for marine cyanobacteria since it has only been identified before in complex FA containing metabolites from the marine cyanobacterium *Lyngbya majuscula* and from other cyanobacterial communities [10, 12]. Our findings indicate that the more classical *iso-anteiso* FA can also exist with this type of 4,5 cyclopropyl substitution. Moreover, based on the highly methyl-branched nature of these FA it is very likely that they also originate from symbiotic microorganisms (bacteria or cyanobacteria) within *P. suberitoides*. If the latter is true, then we could be dealing with a novel marine bacterial strain previously unidentified since, to the best of our knowledge, there are no known marine bacteria with *iso-anteiso* FA with a 4,5 cyclopropyl substitution. More interesting could be the biological potential of these cyclopropyl FA as antimycobacterial agents (in particular against *Mycobacterium tuberculosis*) [24] or even against pathogenic fungi such as *Candida albicans* [10]. Further research is thus needed to put in perspective the pharmacological potential of these compounds.

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