

Phospholipid Fatty Acids Compositions of Sponges from Qatar

I - Haplosclerida

Jean-Michel Njinkoué¹, Gilles Barnathan¹, Jean-Michel Kornprobst¹,
Hala Sultan Saif Al-Easa², Abdulrahman Al-Muftah²
and Jean Vacelet³

¹Institut de Recherche sur les Substances et Organismes de la Mer (ISOMer), Groupe SMAB, Laboratoire de Chimie Marine, 2 rue de la Houssinière, 44322 Nantes Cedex 3, France.

²Chemistry and Earth Sciences Department, College of Art and Science, University of Qatar, P.O. Box 2713, Doha, Qatar.

³Centre d'Océanologie de Marseille, Station Marine d'Endoume, rue de la Batterie des Lions, 13007 Marseille, France.

الأحماض الدهنية الفسفوليبيدية في الاسفنجيات التي تنمو في قطر

أ- هالوسكاليدا

جين ميشيل نينكو¹ وجيل بارناتان¹ وجين ميشيل كورنبروبست¹ وهالة سلطان سيف العيسى²
و عبدالرحمن المفتاح² وجين فاساليه³

¹مجموعة سماب - معهد المنتجات البحرية - نانت فرنسا

²قسم الكيمياء وعلوم الأرض - كلية الآداب والعلوم - جامعة قطر

³مركز علوم البحار - مرسيليا - فرنسا

تم التعرف على الأحماض الدهنية الفسفوليبيدية لستة أنواع من اسفنج هالوسكاليدا الذي ينمو في قطر باستخدام كروماتوجرافيا الغاز وطيف الكتلة. وتم فصل أكثر من مائة حمض دهني مشبع وغير مشبع. وأوضحت الدراسة وجود فارق كبير في بنية الأحماض الدهنية المشبعة وغير المشبعة بين ثلاثة فصائل هي: كاليبونجيا وجليوديس ونيفاتيس كما تم التعرف على حمض ديانويك جديد 14-Me-5,9-7 وبعض الأحماض الدهنية النادرة. كما أن الدراسة أوضحت اختلاف واضح في مكونات الأحماض الدهنية التي تنتمي إلى عائلة n-7 لأسفنج جليوديس حيث تراوحت نسبتها من صفر إلى 18%.

Key words: Marine Sponges, Haplosclerida, Callyspongia, Gelliodes, Niphates, Phospholipid fatty acids, Gas Chromatography/Mass Spectroscopy, Biogenesis.

ABSTRACT

The phospholipid fatty acid contents of six Haplosclerida sponges collected along Qatari coasts, Arabian Gulf, were identified by coupling GC-MS experiments. More than hundred of saturated and unsaturated fatty acids have been identified. Strong variations were observed for both saturated and unsaturated fatty acids within the three studied genera: *Callyspongia*, *Gelliodes*, and *Niphates*. A new dienoic fatty acid: 14-Me-5,9-17:2 and several rare or uncommon fatty acids were identified from GC/MS coupling experiments. Striking differences between both *Gelliodes* species were observed for fatty acids belonging to the n-7 family with total content ranging from 0 to 18%.

Introduction

Thirteen families are included within Haplosclerida Order, seven of which contain freshwater sponges. Marine Haplosclerida are included within two suborders and six families as detailed in Table 1 according to the recent *Systema Porifera* [1].

Table 1. Classification of marine Haplosclerida [1]

Suborders	Families	Main genus
Haplosclerina	Callyspongiidae	<i>Callyspongia</i> , <i>Siphonochalina</i>
	Chalinidae	<i>Chalinula</i> , <i>Cladocroce</i> , <i>Haliclona</i>
	Niphatidae	<i>Amphimedon</i> , <i>Cribochalina</i> , <i>Gelliodes</i> , <i>Niphates</i>
Petrosina	Calcifibrospongiidae	<i>Calcifibrospongia</i>
	Petrosiidae	<i>Acanthostrongylophora</i> , <i>Petrosia</i> , <i>Xestospongia</i>
	Phloeodictyidae	<i>Aka</i> , <i>Calyx</i> , <i>Oceanapia</i>

Extensive chemical studies of Haplosclerida sponges, mainly non-lipidic compounds are regularly updated but most of know compounds isolated from marine Haplosclerida sponges are non-lipidic [2]. Structural analysis of lipids and especially phospholipid fatty acids are scarce in literature and concern almost a third of the marine genera (8 out of 22). Of the 27 genera of marine Haplosclerida [1], only the lipids of five genera have been reported *viz.* *Haliclona* [3, 4], *Amphimedon* [5-8], *Cribochalina* [9], *Petrosia* [10-14] and *Xestospongia* [3, 15, 16]. As part of our continuing investigation of phospholipids from marine sponges [17-25] we present here the first publication on phospholipid fatty acids for six species of marine Haplosclerida sponges collected around Qatari coasts, Arabian Gulf. All of them belong to the suborder Haplosclerina: *Callyspongia* (Callyspongiidae), *Gelliodes* and *Niphates* (Niphatidae), three studied genera still not reported for their fatty acid contents.

Experimental

All Sponge species were collected by hand or by Scuba diving during March 1996 and March 1997 along Qatari coasts. *Gelliodes cf. incrustans* (Al-Wakrah, shallow water); *Callyspongia cf. siphonella* (Khor-Al-Udeid, Scuba, 10-15m); *Callyspongia sp. 1* (Hallul Island, Scuba, 18-20m); *Callyspongia sp. 2* (Hallul Island, Scuba, 18-20m); *Niphates sp.* (Khor-Al-Udeid, Scuba, 10-15m) and *Gelliodes cf. incrustans ssp.1* (Khor-Al-Udeid, Scuba, 10-15m). All specimens were identified by Dr. Jean Vacelet, CNRS Oceanographic Research Center, Endoume, France where holotypes are deposited. The sponges were washed in sea water, cleaned and cut into small pieces and lyophilized, then were ground in a Waring blender, using chloroform-methanol (1:1, v/v), and steeped twice in this solvent for 24 hours at 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. The phospholipid fatty acids were converted to methyl esters by refluxing with methanolic hydrogen chloride, and the residue was dissolved in hexane and purified on a silica gel column chromatography using hexane/ether (10:1, v/v). The resulting methyl esters were analyzed by gas-liquid chromatography using Carlo Erba 4130 chromatograph (Milano, Italy) and a nonpolar OV-1 silica capillary column (A. M. L.-Chromato, Limoges, France) (25m x 0.32mm i.d., 0.40µm film thickness); hydrogen was used as a carrier gas (0.5 bar; split ratio, 5:100). Standard fatty acid methyl esters and phospholipids were purchased from Sigma Chemical Co (St. Louis, MO). *N*-Acyl pyrrolidine derivatives were prepared by refluxing the methyl esters with pyrrolidine/acetic acid (10:1) for 2 hours and were purified by TLC (silica gel, 0.5mm) with hexane/ether (1:2, v/v) as developing solvent. Fatty acid methyl esters were hydrogenated by stirring for 4 hours at ambient pressure and temperature dissolved in methanol in the presence of catalytic amount of Pt(IV) oxide (Adam's catalyst). GC/MS chromatography was performed on Hewlett-Packard HP-5890 instrument linked to a HP 9000/345 integrator (Palo Alto, CA). Fused silica gel capillary column was used for GC (0.32mm x 30m) coated with DB-1 (0.25 µm film thickness) and helium was used as carrier gas. The column temperature was 180-310°C at 3°C/minute for methyl esters and *N*-acyl pyrrolidide derivatives.

Results and Discussion

More than 100 fatty acids were identified in the six studied sponges (Table 2). The majority of them, of a known structure, are usually found in Demospongiae but few were identified at trace levels (< 0.1%) such as 6-18:1; *i*-19:0; *ai*-19:0.

Table 2. Phospholipid⁽¹⁾ fatty acids of six Haplosclerida sponges from Qatar

Fatty acids	ECL ⁽²⁾	<i>Callyspongia</i> sp.			<i>Gelliodes</i> sp.		<i>Niphates</i> sp.
		A	B	C	D ^a	E ^b	F
12:0	12.00	0.9	-	-	0.2	2.4	0.2
13:0	13.00	0.2	-	-	-	0.4	tr.
<i>i</i> -14:0	13.63	1.6	-	-	0.1	-	0.2
5-14:1	13.70	-	-	-	-	-	0.2
6-14:1	13.72	-	-	-	0.2	-	-
9-14:1	13.81	-	-	-	-	-	0.2
14:0	14.00	17.5	3.2	1.5	1.2	6.6	1.7
4,8,12-TM-13:0	14.50	-	20.0	13.6	-	3.2	tr.
<i>i</i> -15:0	14.65	24.8	5.2	1.2	1.0	9.2	3.4
<i>ai</i> -15:0	14.73	4.6	tr.	-	0.4	-	0.9
15:0	15.00	2.0	1.5	0.5	-	1.8	1.0
Me-5-16:1 ⁽³⁾	15.10	-	-	-	0.6	-	-
5,9-16:2	15.40	-	-	-	-	-	0.3
5-16:1	15.50	-	-	-	5.1	-	-
6-16:1	15.56	-	-	-	1.6	-	-
7-16:1	15.58	-	1.3	-	-	-	-
<i>i</i> -16:0	15.70	4.8	-	-	-	-	-
8-16:1	15.72	-	-	-	-	1.5	1.7
9-16:1	15.75	3.0	-	3.3	2.3	-	6.3
11-16:1	15.80	-	1.2	-	-	-	-
16:0	16.00	13.4	10.8	6.0	6.8	16.4	8.0
<i>i</i> -6-17:1	16.10	-	-	-	2.8	-	0.6
5,9-17:2	16.20	-	-	5.9	-	-	-
<i>ai</i> -6-17:1	16.28	-	-	-	2.5	-	tr.
9-Me-16:0	16.40	-	-	-	3.5	-	-
6-17:1	16.60	-	-	-	2.5	-	-
<i>i</i> -17:0	16.62	3.3	1.0	-	-	3.0	0.7
9-17:1	16.65	-	-	-	1.1	-	-
11-17:1	16.70	-	-	-	0.6	-	-
<i>ai</i> -17:0	16.72	-	2.1	-	0.6	0.5	1.3

Fatty acids	ECL ⁽²⁾	<i>Callyspongia</i> sp.			<i>Gelliodes</i> sp.		<i>Niphates</i> sp.
		A	B	C	D	E	F
7-17:1	16.75	-	-	-	-	-	0.5
17:0	17.00	-	0.9	-	1.0	2.2	1.8
3,7,11,15-TM-16:0	17.22	-	2.4	-	-	-	-
14-Me-5,9-17:2 ⁽⁴⁾	17.28	2.2	-	-	-	-	-
5,9-18:2	17.30	-	-	-	-	-	1.9
6,11-18:2	17.40	-	-	-	2.6	-	0.9
7,13-18:2	17.45	-	-	-	-	-	1.0
9,12-18:2	17.48	-	-	-	-	-	2.2
5-18:1	17.50	-	-	-	6.2	-	-

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8,13-18:2	17.56	-	-	-	-	-	1.0
9-18:1	17.70	0.8	1.0	2.1	1.8	-	2.9
11-18:1	17.74	1.1	-	2.0	10.1	-	4.6
12-18:1	17.76	-	1.0	-	-	-	-
18:0	18.00	3.2	4.1	10.2	3.2	16.8	15.6
Me-6-19:1 ⁽³⁾	18.12	-	-	-	0.8	-	-
Me-7-19:1 ⁽³⁾	18.50	-	-	-	0.7	-	-
19:1 ⁽³⁾	18.80	-	-	2.2	-	-	-
5,8,11,14-20:4	18.82	-	-	-	0.4	-	2.0
6-19:1	18.83	-	-	-	1.2	-	-
7-19:1	18.90	-	-	-	0.7	-	-
19:0	19.00	1.0	0.2	1.5	-	1.6	1.3
5,9-20:2	19.32	-	-	-	0.7	-	-
6,11-20:2	19.23	-	-	2.0	0.3	-	0.6
7,13-20:2	19.48	-	-	-	0.6	-	-
5,8,11,14,17,20-22:6	19.52	-	-	-	-	-	0.3
6-20:1	19.62	-	-	1.0	0.7	-	0.5
20:1 ⁽³⁾	19.76	-	tr.	0.6	-	-	0.3
9-20:1	19.78	-	-	-	0.2	-	-
20:0	20.00	1.2	0.8	0.6	0.4	0.8	-
6-21:1	25.50	-	-	-	0.1	-	-
7-21:1	20.63	-	-	-	0.2	-	-
21:0	21.00	0.4	-	0.6	-	0.8	-

Fatty acids	ECL ⁽²⁾	<i>Callyspongia</i> sp.			<i>Gelliodes</i> sp.		<i>Niphates</i> sp.
		A	B	C	D	E	F
5,9-22:2	21.32	-	-	-	0.4	-	-
7,13-22:2	21.42	-	-	-	0.5	-	-
7,15-22:2	21.47	-	-	-	0.2	-	-
6-22:1	21.62	-	-	-	0.3	-	-
<i>i</i> -22:0	21.64	0.4	-	-	-	-	-
13-22:1	21.70	-	-	-	0.3	-	0.4
14-22:1	21.80	-	-	-	-	-	0.3
22:0	22.00	3.0	1.6	0.4	4.5	15.7	2.8
<i>i</i> -23:0	22.63	0.7	-	-	0.6	-	-
<i>ai</i> -23:0	22.72	0.3	0.2	-	tr	-	-
15-23:1	22.76	-	-	-	-	-	0.5
16-23:1	22.85	-	-	-	0.4	-	-
18-23:1	22.94	-	-	-	-	-	1.0
23:0	23.00	0.4	0.4	0.5	0.4	3.0	-
5,9-24:2	23.34	-	-	-	0.4	-	0.3
15-24:1	23.72	-	-	-	-	-	0.7
17-24:1	23.80	0.3	1.1	0.4	1.2	-	-
18-24:1	23.84	-	-	-	-	1.6	-
19-24:1	23.86	-	-	-	-	-	2.2
24:0	24.00	1.2	1.9	1.2	2.6	8.6	-
5,9-25:2	24.38	0.4	2.2	-	2.1	-	-
5-25:1	24.78	-	0.2	-	0.2	-	-
25:0	25.00	-	-	-	-	0.5	0.4
5,9,19-26:3	25.10	-	-	-	1.2	-	-
5,9-26:2	25.42	1.9	32.4	1.8	7.7	2.1	5.3
7-26:1	25.68	-	0.8	-	-	-	-
9-26:1	25.78	-	-	-	0.4	-	-
18-26:1	25.80	-	-	0.7	-	-	-
19-26:1	25.84	-	-	-	1.8	-	-
26:0	26.00	-	-	-	-	0.5	-
5,9,19-27:3	26.20	-	1.5	-	-	-	-

5,9-27:2	26.30	0.5	0.3	3.1	6.1	-	5.5
5,9,21-27:3	26.35	-	0.7	-	-	-	-
5,9,21-28:3	26.80	-	-	2.8	0.3	-	-
5,9-28:2	27.20	-	-	26.4	1.1	-	14.6
5,9,21-29:3	27.40	-	-	-	-	-	0.7
6-Br-5,9-26:2	27.88	-	-	-	2.3	-	-
5,9-29:2	28.10	-	-	0.3	-	0.8	0.5
5,9-30:2	28.30	-	-	3.6	-	-	-
6-Br-5,9-27 :2	28.54	-	-	-	-	-	0.3
□ % PFAs ⁽⁵⁾	95.1	100.0	96.0	100.0	100.0	100.0	99.6
□ % SFAs ⁽⁶⁾	84.9	56.3	37.8	26.5	94.0	94.0	39.3
□ % UFAs ⁽⁷⁾	10.2	43.7	58.2	73.5	6.0	6.0	60.3
□ % □5,9	5.0	37.1	43.9	22.3	2.9	2.9	29.4
□5,9/UFAs ratio	49.0	84.9	75.4	30.3	48.3	48.3	48.7
□ % (n-7) FAs	4.4	1.1	8.5	18.1	-	-	11.2
□ % VLCFAs ⁽⁸⁾ (≥ 24C)	4.3	41.1	40.3	27.4	14.1	14.1	30.5

A: *Callyspongia* cf. *siphonella*; **B:** *Callyspongia* ssp. 1; **C:** *Callyspongia* ssp. 2; **D:** *Gelliodes* cf. *incrusters* (collected by hand in shallow waters (less than 1 m depth) near Al Wakrah); **E:** *Gelliodes* cf. *incrusters* ssp.1 (collected by SCUBA at Khor-Al-Udeid, 10-15m depth); **F:** *Niphates* sp.⁽¹⁾ For each sponge 5 to 10 unidentified fatty acids account for less than 5% of the total fraction; ⁽²⁾DB1, *N*-acyl pyrrolidides; ⁽³⁾ the position of the methyl group or the double bond is still ambiguous; ⁽⁴⁾ unprecedented as natural compound; ⁽⁵⁾ PFAs: phospholipid fatty acids; ⁽⁶⁾ SFAs: saturated fatty acids; ⁽⁷⁾ UFAs: unsaturated fatty acids; ⁽⁸⁾ VLCFAs: very long-chain fatty acids (≥ 24C)

br: branched; *i*: iso; *ai*: anteiso; tr. (traces): % < 0.1

It is clear from Table 2 that species of the same genus exhibited different phospholipid fatty acid (PFAs) compositions. The largest variation is shown within the order Haplosclerida. On the other hand, unsaturated fatty acid (UFAs) content from PFAs varied from 73% (**D**) to 6% (**E**) for the two *Gelliodes* species, but within UFAs the percentages of □5,9 varied in the same way (22.3 and 2.9% respectively), which made, the □5,9/UFAs ratio approximately constant (30.5 and 48% respectively). This is not the case for the three studied *Callyspongia* species (**A**), (**B**) and (**C**); for which the □5,9/UFAs ratio ranged between 19.1 and 84.9. Such variations could have biological significations because in marine organisms the presence of □5,9 fatty acids is usually considered as a characteristic of sponges [3]. Even though, some of these fatty acids were recently identified in Cnidaria [26] and in some terrestrial plants [27]. In general, □5,9 fatty acids are biosynthesized by Demospongiae through unsaturated fatty acids, mainly from exogenous palmitoleic acid (9-16:1) [28]. Thus, the □5,9/UFAs ratio could be considered as the ability index to synthesize □5,9 fatty acids from unsaturated ones. Another interesting index is the level of demospongiac acids, *i.e.* the fatty acids in the range of C₂₄-C₃₄ are usually polyunsaturated [28-29]. For the six Haplosclerida studied species in this work, demospongiac acid contents ranged from 9.2 to 41.1% which is noticeably less than those published by Litchfield *et al.* [4] for three marine Haplosclerida (47 to 66%). Therefore, the Arabian Gulf Haplosclerida species could be different from the Atlantic species ones collected in Massachusetts and Florida [4].

From a quantitative point of view, the most striking difference appeared within *Callyspongia* genus. In this case, the average content of saturated (37.4 to 56.3%) and unsaturated (43.7 to 62.6%) fatty acids remained approximately constant for the three studied species (48.3/51.7 and 37.4/62.6% respectively), strong differences were observed for □5,9 (9.9, 37.1 and 43.9 respectively for *Callyspongia* **A**, **B**, and **C** in Table 2) and □5,9/UFAs ratios (19.1, 84.9 and 70.1% respectively for *Callyspongia* **A**, **B**, and **C** in Table 2).

Interesting differences were observed for demospongiac acid content (9.2, 41.1 and 40.3% respectively for *Callyspongia* **A**, **B**, and **C** in Table 2). Another difference concerning short-chain fatty acids (C₁₂-C₁₆), is their content which was high for sponge species (**A**) (73.4%), and low for species (**B**) and (**C**) (43.2 and 26.1% respectively). Another interesting observation was the presence of only one isoprenoid fatty acid, namely 4,8,12-

trimethyltridecanoic acid (4,8,12-Me₃-13:0). This branched fatty acid was found in the two *Callyspongia* sp. (B) and (C) in high content viz. 20.0 and 13.6% respectively. Such amounts are difficult to explain by exogenous intake but more likely by the sponge itself, or by symbiotic associations between the sponge and halophilic bacteria, due to the very high salinity of the Arabian Gulf. A new unsaturated fatty acid 14-Me- Δ 5,9-17:2 was identified only in a *Callyspongia* sp. (A, 2.2%) ; with a molecular peak (M+1)⁺ at *m/z* 333 (10%), this acid clearly appeared as 18:2. The base peak at *m/z* 113 and a prominent ion peak at *m/z* 180 (45%) confirmed the Δ 5,9 system. The peak at *m/z* 319 (M-15, 30%) implied a methyl branching thus, this acid is a Me- Δ 5,9-17:2. The location of the methyl is given by the usual observation for branched-chain fatty acids, a very low ion peak at *m/z* 276 (C-14) associated with the two ion peaks at *m/z* 262 (C-13) and *m/z* 290 (C-15) confirmed the location of the methyl group on C-14. As regards the two studied *Gelliodes* species (D) and (E), the most striking observation was the range of saturated phospholipid fatty acids that varied from 26.9 to 94.0% respectively. Demospongiac acid contents were generally low: 14.2 and 27.4% for (E) and (D) respectively. The Δ 5,9/UFA ratio were even lower than those observed for *Callyspongia* genus (30.5 and 48.0% compared to 70.1 and 84.9% respectively). Another interesting observation concerned the monounsaturated fatty acids (Table 2) was that the majority were mainly encountered in *Gelliodes* sp. and rarely in *Callyspongia* or *Niphates* species. Furthermore, the rare 7,15-22:2 fatty acid was identified in a *Gelliodes* sp. The base peak at *m/z* 389 implied a 22:2 fatty acid, a difference of 12 amu between the peaks at *m/z* 168 and *m/z* 180 implied the first double bond on C-7 and second difference of 12 amu between the peaks at *m/z* 278 and *m/z* 290 implied the presence of a second double bond on C-15 hence, the structure is unambiguously 7,15-22:2. This rare non-methylene interrupted fatty acid belongs to the n-7 family and another striking difference between both *Gelliodes* sp. must be mentioned. For the *Gelliodes* sp. collected in shallow waters near Al Wakrah the total content of n-7 fatty acids is 18.1 % but for *Gelliodes* sp. collected by SCUBA at about 10-15 meters depth at Khor-Al-Udeid, no traces of n-7 fatty acids were identified. This strange observation could be explained by strong differences in marine environment between the shallow waters near Al Wakrah and the deeper waters in Khor-Al-Udeid.

The sponge *Niphates* sp. is the sole species to contain the brominated fatty acid 6-Br-5,9-27:2. This observation could be of taxonomic significance due to the fact that within Haplosclerida order, brominated demospongiac acids have only been encountered so far in Niphatidae family. Along with *Niphates*, brominated demospongiac acids were found in *Amphimedon terpenensis* [8] and it was shown that the long-chain brominated fatty acids occur only in sponge cells and not in the symbionts [30].

Finally it should be noticed that over the six studied species only two have phospholipid fatty acids up to 28 carbon atoms. *Callyspongia* ssp. 1 and *Niphates* sp. have respectively 33.1% and 15.8% of (C₂₈ + C₂₉ + C₃₀) fatty acids. Promising results are still to be found with sponges from Qatar and researches are in progress in our laboratories to continue these first investigations on Haplosclerida.

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