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FATTY ACIDS FROM THE SPONGE *Tedania dirhaphis*

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The fatty acid (FA) composition of total lipids from the marine sponge Tedania dirhaphis *from the Sea of Okhotsk was studied. GC and GC-MS identified 50 acids, particular attention being paid to components with 14-22 C atoms. Acids 16-Me-19:0, 10,14-Me$_2$-15:1(Δ6), 18:1(Δ6), 18:1(Δ8), and 22:1(Δ16) were observed and identified for the first time in sponges. The main FA in lipids from* T. dirhaphis *was 28:3(Δ5,9,21), the relative content of which reached 63.3%.*

Key words: sponges, *Tedania dirhaphis*, long-chain fatty acids, gas chromatography, mass spectrometry.

Marine sponges are the most primitive multicelled organisms. The unique ability of these organisms to adapt to any ecosystem may be due to biochemical factors, in particular, the structural features of their membranes. It is known that the main components of sponge membranes, phospholipids, consist of fatty acids (FA) of the usual length (16-22 C atoms) in addition to a large amount of unusual FA of longer chain length [1, 2]. These were identified using modern research methods. They include brominated FA [3-5], unsaturated FA with double bonds in unusual positions [6], branched FA [7], and acids with unusual substituents such as cyclopropane [8], methoxyl [9], acetoxyl [10], and others in the C chain. It was also shown that many unusual FA isolated from sponges have biological activity [11-13].

We investigated for the first time the FA composition of the marine sponge *Tedania dirhaphis* from the Sea of Okhotsk. Using modern GC-MS methods we identified new saturated and monoenoic acids that had not been previously observed in other sponges: 16-Me-19:0, 10,14-Me$_2$-15:1(Δ6), 18:1(Δ6), 18:1(Δ8), 22:1(Δ16). Information on the FA composition of sponges broadens our understanding of the diversity of biological molecules.

According to GC, the FA mixture of total lipids from *T. dirhaphis* contained more than 70 components, 50 of which (98.4%) were identified (Table 1).

The saturated FA made up 8.3% of the total FA from *T. dirhaphis*; total saturated branched FA, 3.2%. The principal saturated acids were 16:0, 18:0, and 16-Me-19:0. The last has not been observed previously in other sponges.

The pyrrolidide of 16-Me-19:0 has a molecular ion $[M]^+$ with *m*/*z* 365 but differs in chromatographic mobility and peak intensities of characteristic ions from the pyrrolidide of linear 20:0 acid. It is known that the intensity of the peak characterizing the branching position is minimal whereas those of the neighboring peaks increase, i.e., a gap of 28 amu forms, if branching is present [14]. In the spectrum of the pyrrolidide of 16-Me-19:0, the intensity of the peak with *m*/*z* 308 (0.0%) is less whereas those of the neighboring peaks with *m*/*z* 294 (1.1%) and 322 (1.5%) are greater than those of the pyrrolidide of 20:0. This indicates that the methyl group is located on the 16th C atom.

Other saturated FA with branched C chains that were present included *i*/*ai*, 15:0; *i*, 16:0, and *i*/*ai* 17:0; *i*/*ai* 19:0; *i*/*ai* 21:0, which have been observed previously in sponges and other organisms [15-17]. Such acids are usually considered to originate from bacteria [18, 19]. Saturated FA with one branching in the middle of the chain, for example, 10-Me-16:0, 10-Me-18:0, 11-Me-18:0, and 13-Me-20:0 were previously found in other sponges [20-23]. We assume that such acids are synthesized by bacteria associated with the sponges and are very characteristic just for demosponges bacterial symbionts [24, 25].

The total monoenoic FA made up 13.1% of the total FA from *T. dirhaphis*. The principal ones were 16:1(Δ9), 18:1(Δ11), 10,14-Me$_2$-15:1(Δ6), 24:1(Δ15), and 26:1(Δ9). Acids 10,14-Me$_2$-15:1(Δ6), 18:1(Δ6), 18:1(Δ8), and 22:1(Δ16) were found for the first time in sponges.

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TABLE 1. Fatty-Acid Composition of Marine Sponge *Tedania dirhaphis* (% of Total FA)

FA	Content, %		Content, %	FA	Content, %
14:0	0.3	*i*-19:0	0.2	22:1 (Δ16)	0.2
i-15:0	0.3	*ai*-19:0	0.2	22:6 (Δ4,7,10,13,16,19)	4.6
ai-15:0	0.2	19:0	0.2	24:0	0.1
15:0	0.2	19:1 (Δ11)	0.4	24:1 (Δ15)	1.1
i-16:0	0.3	16-Me-19:0	0.7	24:1 (Δ17)	0.4
16:0	1.7	20:0	0.5	26:1 (Δ9)	0.7
16:1 (Δ9)	0.8	20:1 (Δ9)	0.5	26:1 (Δ15)	0.4
16:1 (Δ11)	0.4	20:1 (Δ11)	0.4	26:1 (Δ17)	0.3
10,14-Me2-15:1 (Δ6)	1.8	20:1 (Δ14)	0.2	26:1 (Δ19)	0.2
i-17:0	0.5	20:1 (Δ17)	0.2	26:2 (Δ5,9)	1.4
ai-17:0	0.8	20:4 (Δ5,8,11,14)	0.6	26:3 (Δ5,9,19)	0.5
17:0	0.2	20:5 (Δ5,8,11,14,17)	1.6	27:2 (Δ5,9)	0.7
17:1 (Δ9)	1.4	*i*-21:0	0.1	28:1	0.2
18:0	1.4	*ai*-21:0	0.2	28:3 (Δ5,9,19)	0.8
18:1 (Δ6)	0.2	22:0	0.2	28:3 (Δ5,9,21)	63.3
18:1 (Δ8)	0.2	22:1 (Δ13)	0.6	28:3 (Δ5,9,23)	3.5
18:1 (Δ11)	1.9	22:1 (Δ15)	0.6		

The positions of the double bonds in the FA were determined using mass spectra of the corresponding pyrrolidides. The pyrrolidide decomposes into two fragments with charge retention in the amide after it ionizes and forms the molecular ion [26]. Thus, the mass spectrum of FA pyrrolidide contains peaks only for fragments containing the carboxyl. The interval between neighboring peaks in the mass spectrum for an aliphatic C chain is 14 amu (one methylene) whereas it is 12 amu between peaks for fragments with C atoms n-1 and n with a double bond in the starting molecule between C atoms n and n+1 [26].

The pyrrolidides of both 18:1 isomers had molecular ions $[M]^+$ with *m*/*z* 335. An interval with 12 amu in the mass spectrum of the pyrrolidide of 18:1(Δ6) was located between fragments C-5 (*m*/*z* 154) and C-6 (*m*/*z* 166) whereas it occurred between fragments C-7 (*m*/*z* 182) and C-8 (*m*/*z* 194) in the mass spectrum of the pyrrolidide of 18:1(Δ8). This indicates that the double bond is located at C atoms 6 and 8, respectively.

Other monoenoic FA with a double bond at the sixth C atom were observed previously in several sponges. Thus, acids 14:1(Δ6), 15:1(Δ6), and 16:1(Δ6) were found in *Cinachyrella schulzei* [23]; 17:1(Δ6), in *Strongylophora durissima* [27]; 19:1(Δ6), in *Calyx niceaensis* [8]. Δ6-Desaturase has been observed in algae, insects, and many animals [28]. It is probable that such acids are ingested with food or synthesized by the sponge itself. Monoenoic Δ8-unsaturated acids 16:1 and 17:1 were previously found in *C. alloclada* and *Amphimedon complanata*, respectively [29, 30]. Δ8-Desaturase was found in certain protozoa [31]. These acids are consideed to have a dietary or symbiotic origin in sponges [23].

The mass spectrum of the pyrrolidide of acid 22:1(Δ16) had a molecular ion $[M]^+$ with *m*/*z* 391 and a gap of 12 amu between ions for fragments C-15 (*m*/*z* 294) and C-16 (*m*/*z* 306), indicating that the double bond was located on C-16. Other monoenes with a straight chain and a C-16 double bond were not observed in the FA from *T. dirhaphis* investigated by us. Therefore, we assume that this acid is formed by elongation of 20:1(Δ14), which also occurs in *T. dirhaphis* lipids. However, according to the literature, lipids of certain sponges contain 20:1(Δ16) (*Trikentnon loeve*) [32], 23:1(Δ16) (*A. compressa*) [33], and 25:1(Δ16) (*Pseudaxinella cf. lunaecharta*) [32]. However, possible precursors of them are lacking.

The mass spectrum of the pyrrolidide of 10,14-Me_2-15:1(Δ6) had a molecular ion $[M]^+$ with *m*/*z* 321 and a gap of 12 amu between ions with *m*/*z* 154 and 166. Peaks for ions with *m*/*z* 222 and 292 had the minimal intensity (0.6 and 0.2%, respectively) whereas the intensities of the neighboring peaks with *m*/*z* 208 (4.2%) and 236 (2.0%) and 278 (0.9%) and 306 (0.7%) are noticeably greater. This is consistent with branching at the 10th and 14th C atoms. This acid is probably of bacterial origin.

The polyenoic FA made up 77.0% of the total from *T. dirhaphis*. The principal ones without methylene separation were 26:2(Δ5,9), 26:3(Δ5,9,19), 27:2(Δ5,9), 28:3(Δ5,9,19), 28:3(Δ5,9,21), and 28:3(Δ5,9,23), which are typical for sponges (Table 1). Other polyunsaturated FA included arachidonic 20:4(Δ5,8,11,14), eicosapentaenoic 20:5(Δ5,8,11,14,17), and

docosahexaenoic 22:6(Δ4,7,10,13,16,19). The principal FA in *T. dirhaphis* phospholipids was 28:3(Δ5,9,21), the relative content of which reached 63.3%. According to the literature, the greatest content of this acid was found in *Tethya aurantia* (37.7%). However, other isomers of 28:3 were not observed in *T. aurantia* [17]. Earlier all three isomers 28:3(Δ5,9,19), 28:3(Δ5,9,21), and 28:3(Δ5,9,23) were found in several freshwater sponges (*Ephydatia syriaca*, *Nudospongilla* sp., *Cortispongilla barroisi*, *Baicalospongia bacillifera*, and *B. intermedia*). However, their total content was less than 2.5% of the FA mass [34, 35]. Because the content of long-chain acids in lipids of *T. dirhaphis* was more than half of the total FA, it is obvious that namely these unusual components are responsible for the unique structure and function of the cellular membranes.

EXPERIMENTAL

Colonies of *T. dirhaphis* were collected in July 2003 from a depth of 150 m in the Sea of Okhotsk (Kuril Islands, Onekotan) and thoroughly purified of epibionts. Total lipids were extracted by $CHCl_3$:CH_3OH (2:1) [36]. Methyl esters of FA (MEFA) of total lipids were prepared by the Carreau and Dubacq method [37] and purified by preparative TLC on Sorbifil (Russia) plates in benzene. Pyrrolidides of FA for mass spectrometric analysis were prepared by the Andersson method [26] and purified by preparative TLC on Sorbifil plates in ethylacetate.

GC was performed on a Shimadzu GC-17A chromatograph (Japan) equipped with a vaporizer with a regulated flow-gas divider, capillary quartz columns (0.25 mm × 30 m) with Supelcowax-10 or SPB-5 phases (Supelco Inc., USA), a flame-ionization detector, and He carrier gas.

The temperatures of the vaporizer, column thermostat, and detector were regulated separately. Data were collected and processed using ATsP (Z-Lab) and PO (Z-Chrom) (NPO "Binar," Russia). GC of MEFA was carried out on a capillary column (0.25 mm × 30 m) with Supelcowax-10 (0.25 μm) at 210°C. The vaporizer and detector temperatures were 250°C. The flow in the vaporizer was divided 1:30 at 2 atm. MEFA were additionally identified using a capillary column (0.25 mm × 30 m) with SPB-5 (0.25 μm) at 205°C. Short-chain MEFA were additionally separated using a column temperature gradient from 160 to 210°C at 2°C/min with subsequent constant temperature of 210°C for 20 min. Components were identified by comparing retention times of unknowns with authentic standard MEFA and by calculating the equivalent carbon length, ECL, [38] for an isothermal regime. The relative contents of the components in total FA were calculated using GC data for MEFA.

GC-MS was performed on a Shimadzu QP-5050A instrument (Japan) with He carrier gas. GC-MS of FA pyrrolidides was performed on a capillary column (25 mm × 30 m) with MDN-5S (0.25 μm) and a temperature gradient from 210 to 270°C at 3°C/min and subsequent constant temperature at 270°C for 40 min. The vaporizer and detector temperatures were 300°C. The vaporizer flow was divided 1:18. The linear carrier-gas flow rate was 30 cm/s. Mass spectra were obtained by electron impact at 70 eV ionization energy, detector potential 1 kV, scan range 50-500 amu, scan rate 0.3 s. Mass spectra were averaged over the whole peak width with background subtraction and H-isotope compensation.

16-Me-19:0, *m/z* (I_{rel}, %): 365 (1.3) $[M]^+$, 113 (100), 126 (17.8), 140 (2.0), 154 (0.8), 168 (1.9), 182 (1.4), 196 (0.7), 210 (0.4), 224 (0.4), 238 (0.5), 252 (0.3), 266 (0.3), 280 (0.2), 294 (1.1), 308 (0.0), 322 (1.5), 336 (0.8), 350 (0.4).

18:1(Δ6), *m/z* (I_{rel}, %): 335 (2.4) $[M]^+$, 113 (100), 126 (9.8), 140 (0.9), 154 (0.6), 166 (1.4), 180 (2.1), 194 (1.3), 208 (0.7), 222 (0.2), 236 (0.4), 250 (0.3), 264 (0.1), 278 (0.1), 292 (0.2), 306 (0.2), 320 (0.1).

18:1(Δ8), *m/z* (I_{rel}, %): 335 (6.2) $[M]^+$, 113 (100), 126 (52.4), 140 (5.9), 154 (1.9), 168 (4.3), 182 (5.8), 194 (2.1), 208 (2.1), 222 (1.3), 236 (3.0), 250 (3.3), 264 (1.7), 278 (0.9), 292 (1.0), 306 (0.7), 320 (0.2).

22:1(Δ16), *m/z* (I_{rel}, %): 391 (14.1) $[M]^+$, 113 (100), 126 (40.9), 140 (3.9), 154 (1.2), 168 (3.8), 182 (3.1), 196 (1.5), 210 (1.2), 224 (1.6), 238 (2.1), 252 (1.7), 266 (2.0), 280 (0.9), 294 (1.1), 306 (0.7), 320 (1.6), 334 (2.0), 348 (4.4), 362 (2.3), 376 (0.2).

10,14-Me$_2$-15:1(Δ6), *m/z* (I_{rel}, %): 321 (3.7) $[M]^+$, 113 (100), 126 (48.4), 140 (4.7), 154 (3.5), 166 (4.1), 180 (5.6), 194 (6.0), 208 (4.2), 222 (0.6), 236 (2.0), 250 (0.9), 264 (1.4), 278 (0.9), 292 (0.2), 306 (0.7).

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