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FATTY ACIDS FROM THE OKHOTSK SEA SPONGE *Forcepia uschakowi*

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The fatty-acid (FA) composition of total lipids from the Okhotsk sea marine sponge Forcepia uschakowi *was studied. A total of 56 acids were identified by GC and GC—MS. The principal saturated acids were 16:0 and 18:0. The main monoene acid was 15-Me-24:1(14), which was observed for the first time in sponge lipids. Polyunsaturated acids represented of 64.1% of the total FA of* F. uschakowi. *Of these, the principal ones were non-methylene-separated acids 26:2(5,9) and 26:3(5,9,19), which are typical of sponges, and bromo-acid 6-Br-26:2(5,9).*

Key words: sponges, *Forcepia uschakowi*, super-long-chain fatty acids, GC, MS.

The search for new fatty acids, the elucidation of their structures and biosynthetic pathways, and the study of their properties and roles in organisms are currently of great biochemical interest. From this viewpoint, some of the most interesting subjects are representatives of the phylum *Porifera*. Of all marine animals, sponges have the largest variety of sterols, lipids, and fatty acids (FA) with unusual and sometimes unique structures [1]. Certain unusual sponge lipids possess biological activity [2].

According to the literature, lipid and FA compositions of tropical and subtropical sponges are most studied whereas sponges from boreal regions are practically unstudied. Herein the FA composition of total lipids from the Okhotsk Sea marine sponge *Forcepia uschakowi* is studied for the first time.

According to GC analysis, FA from total lipids of *F. uschakowi* contained more than 70 components. Of these, 56 components (94.8%) were identified (Table 1).

Saturated FA made up 10.2% of the total FA from *F. uschakowi*. The principal saturated acids were 16:0 and 18:0. Saturated FA with a branched carbon skeleton made up 4.3%. These were basically acids with a methyl group in the (*n*-2) and (*n*-3) position. Saturated $C_{15\text{-}20}$ FA of i/ai-structure were observed previously in many sponges and other organisms [3]. It is commonly thought that such acids are of bacterial origin [4]. Saturated FA of isoprenoid structure were observed in small quantities in lipids of *F. uschakowi*. It is known that acids of isoprene structure are formed by degradation of phytol, which in turn is produced by destruction of chlorophyll [5]. It was shown earlier that isoprene acids in sponges occurred in plasma membranes [6].

Monoene FA of linear structure totalled 13.3% of total FA in *F. uschakowi*. Of these, the principal ones were 16:1(9), 18:1(11), and 22:1(15). Two different monoene FA with one branch in the middle of the chain were observed in the studied sponge. These were 7-Me-16:1(6) (1.6%) and 15-Me-24:1(14) (6.1%). Acid 7-Me-16:1(6) was observed earlier in sponges *Trikentnon loeve* and *Pseudaxinella af. lunaecharta* [7] whereas acid 15-Me-24:1(14) was found for the first time in sponge lipids and is probably synthesized in *F. uschakowi* by elongation of 7-Me-16:1(6).

Mass spectra of the pyrrolidide of 15-Me-24:1(14) contained a molecular ion $[M]^{\cdot +}$ with *m*/*z* 433. A gap of 12 amu occurred between ions of C-13 (*m*/*z* 266) and C-14 (*m*/*z* 278) fragments. The peak for the C-15 ion (*m*/*z* 292) was almost completely absent (0.1%), i.e., a gap of 28 amu occurred between peaks for the C-14 (*m*/*z* 278) and C-16 (*m*/*z* 306) ions. This was consistent with a 14-double bond and a methyl on C-15.

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TABLE 1. Fatty-Acid Composition of Marine Sponge *Forcepia uschakowi*, % of Total FA

FA	Content, %	FA	Content, %	FA	Content, %
14:0	0.5	18:0	1.5	22:1(15)	2.5
4,8,12-Me3-13:0	0.1	18:1(9)	0.5	22:6(4,7,10,13,16,19)	1.2
13-Me-14:0	0.4	18:1(11)	4.6	24:1(15)	0.8
12-Me-14:0	0.2	18:1(13)	0.2	24:1(17)	0.4
15:0	0.2	17-Me-18:0	0.1	15-Me-24:1(14)	6.1
14-Me-15:0	0.1	16-Me-18:0	0.1	24:2(5,9)	0.8
13-Me-15:0	0.3	18:2(9,12)	0.5	24:2	0.3
16:0	2.5	19:0	0.3	24:3(5,9,20)	0.2
16:1(5)	0.1	11,12-Methylene-18:0	0.2	25:2(5,9)	2.3
16:1(9)	1.7	19:2	0.5	25:3(5,9,18)	0.4
16:1(11)	0.1	18-Me-19:0	0.4	26:1(15)	1.7
15-Me-16:0	0.3	17-Me-19:0	0.4	26:2(5,9)	23.9
14-Me-16:0	0.4	20:0	0.4	26:3(5,9,19)	16.1
7-Me-16:1(6)	1.6	20:1(11)	0.2	27:2(5,9)	0.7
17:0	0.3	20:1(13)	0.2	27:3(5,9,20)	0.3
17:1(9)	0.1	20:1(14)	0.2	28:3(5,9,19)	0.5
16-Me-17:0	0.6	20:2	0.3	28:3(5,9,21)	0.9
15-Me-17:0	0.4	20:4(5,8,11,14)	3.6	6-Br-26:2(5,9)	3.5
3,7,11,15-Me4-16:0	0.5	20:5(5,8,11,14,17)	8.1		

Polyunsaturated FA (PUFA) made up 64.1% of the total FA from *F. uschakowi*. Of these, the main ones were non-methylene-separated acids 26:2(5,9) and 26:3(5,9,19), which are typical of sponges, and bromo-acid 6-Br-26:2(5,9) (Table 1). Furthermore, 24:2, 25:2, and 27:2, which contain the 5,9-diene structure, were observed in total lipids of this sponge in smaller quantities. Several triene FA, 24:3(5,9,20), 25:3(5,9,18), 27:3(5,9,20), 28:3(5,9,19), and 28:3(5,9,21) were also observed. Mass spectra of polyene FA pyrrolidides showed a molecular ion $[M]^{\cdot +}$ and ions characteristic of fragments with 14 and 12 amu spacing that corresponded to fragments in the starting molecule with single or double bonds between neighboring C atoms [8].

The mass spectrum of the pyrrolidide of 6-Br-26:2(5,9) contained a peak for $[M - Br]^{\cdot +}$ with *m*/*z* 444 and peaks characteristic of brominated ions with *m*/*z* 258 and 260 of equal strength [9, 10]. The peak for the ion with *m*/*z* 180 became very strong for pyrrolidides of acids containing a 5,9-diene structure. This was due to the fact that decomposition of the parent molecular ion at the center of the dimethylene-separated diene fragment is energetically favorable because the electronic structure of the two resulting fragments is effectively stabilized by the terminal allyl double bonds. In general, the presence of a strong peak with *m*/*z* 180 in the mass spectrum of a FA pyrrolidide indicates the presence of the 5,9-diene structure in the parent molecule.

Because the content of super-long-chain acids in lipids from *F. uschakowi* was more than half of the total amount of FA, it is obvious that namely these unusual components were responsible for the unique structure and functions of the cell membranes. The FA composition of sponges from a single species can vary depending on the habitat, reflecting the adaptation of these animals to the environmental conditions [11]. From this viewpoint, it is interesting to discuss the presence of 6-Br-26:2(5,9) in the investigated *F. uschakowi*.

Many unusual brominated FA were observed previously in lipids from marine sponges [9, 10, 12]. It was hypothesized that these FA are produced by the action of bromoperoxidase on FA precursors of analogous structure that do not contain Br and are usually always present in sponges together with those that contain Br [13]. Experiments carried out with radioactively tagged precursors showed that biological bromination occurs in the final biosynthetic stages of these unusual FA [14]. The role of these FA is not yet clear. It is assumed that brominated FA may be part of the protective strategy of these animals or adaptation mechanisms to changing environmental conditions [12, 15].

Some researchers point out the taxonomic significance of these FA because brominated FA were observed previously almost exclusively in specimens of the two orders *Haplosclerida* and *Halichondrida*. Furthermore, these same researchers proposed that namely boreal species of *Halichondrida*, in contrast with tropical ones, are rich sources of these components [12]. However, we note that brominated FA were not found in lipids from the sponge *Halichondria panicea*, a representative of

Halichondrida from the Japan and Okhotsk Seas [16] whereas 6-Br-26:2(5,9) was observed in lipids from *F. uschakowi* of the order *Poecilosclеridae*. Thus, our results expand the list of species that contain brominated FA. The taxonomic significance of brominated FA remains controversial because the majority of species from genera, families, or orders of sponges from various habitats should be studied and compared in order to assign lipid markers to them.

Other observed polyunsaturated FA included arachidonic [20:4(5,8,11,14)], eicosapentaenoic [20:5(5,8,11,14,17)], and docosahexaenoic [22:6(4,7,10,13,16,19)] acids, the total content of which was 12.9%. The almost complete absence of C_{20-22} PUFA of the *n*-3 and *n*-6 series in tropical sponges should be noted. Arachidonic acid has been observed only in a few species of tropical sponges. Microalgae produce PUFA of the *n*-3 and *n*-6 series [17]. The Okhotsk Sea is highly productive of and rich with microalgae that provide food for sponges. The high content of C_{20-22} *n*-3 and *n*-6 PUFA in lipids from *F. uschakowi* can be explained by the significant inclusion of microalgae in the diet of sponges.

Investigations of FA and other lipids from sponges and the elucidation of their structures and biosynthetic pathways can assist in explaining several phenomena connected with the metabolism of sponges. In particular, research on the chemistry and biochemistry of lipids can provide a new paradigm for the unique adaptive capabilities of sponges that continue to flourish after such a long time (starting with the pre-Cambrian) in highly varied aqueous ecosystems, in all geographical regions from shallow to deep waters, and over wide ranges of salinity and temperature.

EXPERIMENTAL

Colonies of *F. uschakowi* were collected in July 2003 at 150 m in the Okhotsk Sea (Kuril Islands, Onekotan) and thoroughly cleaned of epibionts. Total lipids were extracted by $CHCl_3$:CH_3OH (2:1) [18]. Methyl esters of FA of total lipids were prepared by the literature method [19] and purified by preparative TLC on Sorbifil plates (Russia) in benzene. FA pyrrolidides for mass spectrometric analysis were prepared as before [20] and purified by preparative TLC on Sorbifil plates in ethylacetate.

GC was carried out in a Shimadzu GC-17A (Japan) chromatograph equipped with a vaporizer with a regulated gas divider, capillary columns (0.25 mm × 30 m) with Supelcowax-10 or SPB-5 (Supelco Inc., USA) phases, and a flame-ionization detector. The carrier gas was He. The vaporizer, column thermostat, and detector temperatures were regulated independently. Data were collected and processed on ATsP Z-Lab and PO Z-Chrom (NPO Binar, Russia) computers. GC analysis of FA methyl esters was performed 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 ratio in the vaporizer was 1:30; pressure, 2 atm. FA methyl esters were also identified using a capillary column (0.25 mm × 30 m) with SPB-5 (0.25 μm) at 205°C. Components were identified by comparing retention times of unknown components and authentic standards and by calculating the equivalent carbon length, ECL [21] for an isothermal regime. The relative content of components in total FA was calculated using GC data for FA methyl esters.

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

15-Me-24:1(14). (EI, 70 eV, *m*/*z*, I_{rel}, %): 433 (22.7) $[M]^+$, 113 (100), 126 (50.2), 140 (4.2), 154 (12), 168 (3.9), 182 (4.1), 196 (2.2), 210 (1.1), 224 (2.2), 238 (2.1), 252 (4.3), 266 (4.8), 278 (2.6), 292 (0.1), 306 (1.7), 320 (8.3), 334 (2.8), 348 (3.4), 362 (1.1), 376 (0.8), 390 (0.7), 404 (0.3), 418 (0.9).

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