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Comparative study of the endemic freshwater fauna of Lake Baikal—VI. Unusual fatty acid and lipid composition of the endemic sponge *Lubomirskia baicalensis* and its amphipod crustacean parasite *Brandtia (Spinacanthus) parasitica*

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Lipids and phospholipids (both plasmalogen and alkyl forms) of the freshwater sponge *Lubomirskia baicalensis* and the sponge's gammarid parasite *Brandtia (Spinacanthus) parasitica* were examined. Composition of alkenyl-acyl (plasmalogen), alkylacyl and diacyl forms of major phospholipid classes, phosphatidylethanolamine and phosphatidylcholine were determined. One hundred and eighty-three fatty acids were identified by GC–MS: 46 saturated, 55 monoenoic, 35 dienoic, 25 trienoic and 22 tetra-, penta- and hexaenoic. The freshwater sponges, belonging to the family Lubomirskiidae, were shown to contain unusual long-chain fatty acids: anteiso-5, 9–28:2, branched-5, 9–29:2, 5,9,23–29:3, 5,9,23–30:3, 15,18,21,24–30:4 and 15,18,21,24,27–30:5. Some from these fatty acids were found in lipids of the amphipod parasite.

Key words: Lake Baikal; Ether lipids; Fatty acids; Freshwater sponge; Sponge's gammarid parasite.

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Introduction

The biochemical composition of sponges belonging to the class Demospongiae has been carefully studied over the past 20–25 years (see reviews by Litchfield and Morales, 1976; Dembitsky and Chelomin, 1985; Joseph, 1989; Djerassi and Lam, 1991).

More than 200 spongal species have been examined for their unusual fatty acid composition (Bergquist *et al.*, 1984; Lawson *et al.*, 1984; Litchfield *et al.*, 1976), the majority of which are marine. Fatty acids from freshwater sponges (Hahn *et al.*, 1989; Dembitsky *et al.*, 1991), including certain Baikal species (Dembitsky, 1981a,b, 1982; Dembitsky *et al*, 1993; Latyshev *et al.*, 1992; Rezanka and Dembitsky, 1993), have also been studied.

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The amphipod crustacean *Brandtia* (*Spinacanthus*) *parasitica* is found only in the freshwater sponge, *Lubomirskia baicalensis*. It feeds only on sponge soft tissue (parenchyma) and avoids siliceous skeletal components (Bazikalova, 1945, 1948). Chemical composition and carotenoid content of some baicalian gammarids have been described (Bochkarev and Kurennych, 1964; Czeczuga, 1975; Koluaev and Krekeshyva, 1987; Kurennykh, 1970). Their lipid composition has not yet been reported.

Using GC–MS and micro-TLC methods, we have now attempted a detailed examination of fatty acid and lipid composition of the endemic sponge, *Lubomirskia baicalensis* and the sponge's endemic amphipod parasite, *Brandtia* (*Spinacanthus*) *parasitica*.

Materials and Methods

Samples

The freshwater sponge, *Lubomirskia baicalensis* Pallas (family Lubomirskiidae) was collected in July 1992, in Lake Baikal (Russia) at a depth of 10 m at 4°C. The animals were treated immediately after collection as described previously (Dembitsky *et al.*, 1991). To promote lipid extraction, tissues of sponges were thorougly cleaned of any impurities. *Brandtia* (*Spinacanthus*) *parasitica* Dyb., sponge's gammarid parasite, was isolated from the sponge, *Lubomirskia baicalensis*.

Lipid analysis

Lipids were extracted according to Dembitsky *et al.* (1991). The total lipid extract was separated by chromatography on a glass column with silica gel into neutral, phospho- and glycolipid fractions using the procedure of Rouser *et al.* (1976). Each fraction was measured gravimetrically. Total phospholipids in the extract and the content of phosphorus in individual phospholipid spots after chromatography on TLC plates were determined as described previously (Dembitsky, 1988). Quantitative determination of alkenyl-acyl (plasmalogens), alkyl–acyl and diacyl forms were carried out as described elsewhere (Dembitsky, 1988). Non-specific detection of lipids was performed by spraying the developed plates with 10% (v/v) H_2SO_4 in methanol followed by heating at 180°C. Different phospholipid classes were identified using specific reagents as described (Dembitsky *et al.*, 1989).

GC–MS analysis of fatty acid methyl esters

The HR–MS were determined by the Finnigan MAT 90 mass spectrometer with EI mode. All GC–MS separations were performed using a Finnigan MAT (San Jose, CA, U.S.A) 1020 B apparatus with electron impact. Injection temperature (splitless injection) was 100°C and a fused-silica capillary column (Supelcowax 10) (60 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The temperature programme was as follows: 100°C for 1 min, then increased at 20°C/min to 180°C and at 2°C/min to 280°C, which was maintained for 1 min. The carrier gas was hydrogen at a linear velocity of 60 cm/sec. All spectra were scanned within the range m/z 70–450.

Results and Discussion

We obtained extracts both from the freshwater (native) sample containing microalgae and from the sample with microalgae washed off in filtered Baikal water 10 times prior to lipid analysis. The native sponge samples had bright green surfaces because of numerous microalgae covering them. The washed sample was light grey in colour, indicating the absence of microalgae in tissue; light microscopy was used to further verify this. Recently, Berner and Titlyanov (1992) investigated the sponge *Lubomirskia baikalensis* and reported that it was the symbiotic system including the sponges, green-algae (zoochlorellae) and bacteria. The zoochlorellae occur in the ameboid archeocytes of the sponge, and each such host cell usully shows more than ten algal cells.

Kharchenko *et al.* (1989) reported isolation of a pure culture from microalgae living on the freshwater sponge *Spongilla lacustris*; the isolated microalgal species was demonstrated by the authors to be a typical *Chlorella* member. The same authors obtained cuts from spongal colonies and found the algae to form an active photosynthesizing layer within the body of the host sponge extending 1 cm from the surface. The microalgal concentration within a 3 mm surface layer was

72%. The authors reported to have distinguished three layers according to location and number of microalgae including: (1) the layer densely inhabited by algae from the surface to 3 mm depth; this layer was bright green in colour and contained the bulk of microalgae; (2) the light green layer moderately inhabited by algae from 3 mm to 10 mm depth; and (3) the light grey layer with a scarce algal population beyond 10 mm depth into the host sponge.

The results of lipid analysis showed that the total lipid content in the unwashed sample was 12.5 mg/g dry wt, while that from the washed one was 1.6 mg/g dry wt (see Table 1); thus, microalgae contained 87.2% (10.9 mg/g dry wt) total lipids of native sponge. Neutral lipids were found to constitute over 50%, and phospholipids made up almost equal amounts in each extract.

The phospholipid data for the sponge *L. baicalensis* obtained in the course of the present study is different from our earlier results for the same species (Dembitsky, 1982), mainly with respect to the composition of lyso-forms within PC and PE, as well as in PA composition. These compounds have greater amounts according to our present results (Table 2). It is probable that the observed dissimilarity is due to different environments from which the examined species were samnpled. Table 2 shows the phospholipid composition from the sponge specimen with microalgae preserved versus the specimen with microalgae washed off prior to analysis as described above. A considerable difference was found in the composition of nearly all phospholipids. The true phospholipid composition, in our opinion, is that observed in the washed sample. The content of lyso-forms of PC and PE, as well as PA in the latter sample is much lower. Whereas the native sample contains 5.3% PA, the washed one has only 2.2%. The contents of lyso-PC in the native and the washed samples are 19.8 and 10.8%, respectively; the content of lyso-PE is 13.3% and 7.5%, respectively. The observed differences may be due to the fact that lipolytic algal enzymes hydrolyse mostly diacyl forms of PC and PE, and in the washed sample, this process is minimal. In the chromatographic analysis, PE was found to form three spots on the chromatogram. The plasmalogen form was present only in PE1; no plasmalogen forms were observed in PE2. The separation of the PE into spots on the chromatogram is the result of different fatty radical compositions

Table 1. Lipid composition of sponge and sponge's parasite

Lipid classes		*Lubomirskia baicalensis*		*B. parasitica*
		Native	Washed	
Total lipids, mg/g dry wt	(TL)	12.5	1.6	11.3
NL, GL and PL as % of TL				
Neutral lipids	(NL)	64.9	59.3	56.5
Glycolipids	(GL)	11.8	18.7	7.8
Phospholipids	(PL)	23.3	22.0	35.7
Phosphatidylethanolamine	(PE)	23.5 ± 0.9 (100%)*	42.6 ± 1.1 (100%)	27.5 ± 0.7 (100%)*
alkenyl–acyl form		8.2 ± 0.9 (34.9%)	18.8 ± 0.7 (44.1%)	6.9 ± 0.4 (25%)
alkyl–acyl form		7.7 ± 0.4 (32.8%)	17.8 ± 0.6 (41.8%)	10.2 ± 0.9 (37%)
diacyl form		7.6 ± 0.3 (32.3%)	6.0 ± 0.3 (14.1%)	10.4 ± 0.6 (38%)
Lysophosphatidylethanolamine	(LPE)	13.3 ± 0.7	7.5 ± 0.4	—
Phosphatidylserine	(PS)	5.9 ± 0.2	4.8 ± 0.2	5.2 ± 0.2
The sum of amine-containing lipids		42.7	54.9	32.7
Phosphatidylcholine	(PC)	25.1 ± 0.4 (100%)	27.1 ± 0.9 (100%)*	48.1 ± 1.1 (100%)*
alkenyl–acyl form		1.6 ± 0.3 (6.4%)	3.7 ± 0.2 (13.6%)	6.4 ± 0.6 (13.3%)
alkyl–acyl form		5.2 ± 0.6 (20.7%)	9.9 ± 0.5 (36.5%)	15.9 ± 0.8 (33.1%)
diacyl form		18.3 ± 0.5 (72.9%)	13.5 ± 0.5 (49.9%)	25.8 ± 0.6 (53.6%)
Lysophosphatidylcholine	(LPC)	19.8 ± 0.4	10.8 ± 0.5	2.7 ± 0.1
Sphingomyelin	(SPH)	—	—	4.5 ± 0.2
The sum of choline-containing lipids		44.9	37.9	55.3
Phosphatidylinositol	(PI)	7.1 ± 0.3	5.0 ± 0.2	6.9 ± 0.4
Phosphatidic acid	(PA)	5.3 ± 0.4	2.2 ± 0.1	5.1 ± 0.3

‡The sum (100%) of alkenyl–acyl, alkyl–acyl and diacyl forms of the each phospholipid class, namely: PE and PC.

Table 2. Saturated fatty acid composition of sponge *Lubomirskia baicalensis*

Fatty acid	Washed				Native			
	TL	NL	GL	PL	TL	NL	GL	PL
i-12:0	0.05	0.08	0.04	0.03	0.05	0.07	0.04	0.03
12:0	0.35	0.15	0.51	0.40	0.36	0.15	0.51	0.41
i-13:0	0.05	0.00	0.08	0.06	0.05	0.00	0.08	0.06
ai-13:0	0.10	0.08	0.12	0.09	0.10	0.07	0.12	0.10
13:0	0.24	0.23	0.27	0.22	0.24	0.22	0.27	0.22
i-14:0	0.19	0.30	0.16	0.12	0.20	0.30	0.16	0.13
14:0	1.34	4.47	1.44	1.14	1.50	4.36	1.21	0.98
4,8,12-triMe-13:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
i-15:0	0.12	0.23	0.08	0.06	0.12	0.22	0.08	0.06
ai-15:0	0.15	0.38	0.04	0.03	0.15	0.37	0.04	0.03
15:0	0.14	0.61	0.35	0.28	0.14	0.59	0.35	0.29
i-16:0	0.42	0.91	0.19	0.15	0.42	0.89	0.20	0.16
Pristanic	0.15	0.45	0.00	0.00	0.15	0.44	0.00	0.00
16:0	6.78	11.29	3.30	4.57	7.00	11.69	3.21	4.59
i-17:0	0.12	0.30	0.04	0.03	0.12	0.30	0.04	0.03
ai-17:0	0.20	0.45	0.08	0.06	0.19	0.11	0.08	0.06
Phytanic	0.13	0.38	0.00	0.00	0.12	0.37	0.00	0.00
17:0	0.51	0.98	0.35	0.28	0.53	0.96	0.35	0.29
i-18:0	0.32	0.61	0.19	0.15	0.32	0.59	0.20	0.16
18:0	4.19	8.26	1.86	2.46	4.25	8.21	1.96	2.58
i-19:0	0.12	0.30	0.04	0.03	0.12	0.30	0.04	0.03
br_{11}-19:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
ai-19:0	0.08	0.23	0.00	0.00	0.07	0.22	0.00	0.00
19:0	0.27	0.61	0.12	0.09	0.27	0.59	0.12	0.10
i-20:0	0.10	0.30	0.00	0.00	0.10	0.30	0.00	0.00
20:0	1.53	3.48	0.62	0.49	1.51	3.40	0.63	0.51
i-21:0	0.05	0.15	0.00	0.00	0.05	0.15	0.00	0.00
ai-21:0	0.10	0.30	0.00	0.00	0.10	0.30	0.00	0.00
21:0	0.30	0.68	0.12	0.09	0.30	0.67	0.12	0.10
i-22:0	0.10	0.30	0.00	0.00	0.10	0.30	0.00	0.00
22:0	0.72	1.74	0.23	0.19	0.71	1.70	0.23	0.19
i-23:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
23:0	0.20	0.53	0.04	0.03	0.20	0.52	0.04	0.03
i-24:0	0.05	0.15	0.00	0.00	0.05	0.15	0.00	0.00
24:0	0.30	0.61	0.16	0.12	0.29	0.59	0.16	0.13
i-25:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
ai-25:0	0.05	0.15	0.00	0.00	0.05	0.15	0.00	0.00
25:0	0.10	0.30	0.00	0.00	0.10	0.30	0.00	0.00
c-19–26::0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
26:0	0.17	0.45	0.04	0.03	0.17	0.44	0.04	0.03
27:0	0.05	0.15	0.00	0.00	0.05	0.15	0.00	0.00
28:0	0.10	0.30	0.00	0.00	0.10	0.30	0.00	0.00
29:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
30:0	0.08	0.23	0.00	0.00	0.07	0.22	0.00	0.00
31:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
32:0	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
Total saturated	20.60	41.76	10.47	11.20	20.85	41.58	10.20	11.26

present in each of the PE spots. We also found two PE spots in the phospholipids of the marine sponge *Suberites domuncula* (Dembitsky and Nebylitsyn, 1980).

According to our results, phospholipids in native *L. baicalensis* amount to 23.3% of total lipids (Table 1), while in the previously studied Baikal and White and Japan Seas species, these were found to vary from 14.7 to 33.2% and from 8.2 to 34.6%, respectively (Dembitsky, 1982; Dembitsky *et al.* 1989; Dembitsky and Nebylitsyn, 1980). Previously, we examined the phospholipids of six species belonging to three genera, *Lubomirskia*, *Swartschewskia* and *Baicalospongia*, of the family Lubomirskiidae (Dembitsky, 1982). The major phospholipids, PE and plasmalogens of PC,

varied from 37.3 to 44.3%, and from 4.2 to 1.9%, respectively.

The changes occurring in lipid composition due to washing were identified. Alkenyl-acyl and alkyl-acyl forms increased in PE (from 34.9 to 44.1% and from 32.8 to 41.8%, respectively) and in PC (from 6.4 to 13.6% and 20.7 to 36.5%, respectively), but the diacyl forms decreased in the washed sponge (Table 1). The content of PS found for native *L. baicalensis* was 5.9 and 4.8% for washed sponge. PS in the previously studied marine sponges was 11–30% (Dembitsky and Nebylitsyn, 1980; Kostetsky, 1984; Ayanoglu *et al.*, 1983; Dembitsky *et al.*, 1989). Phospholipids of 19 marine species from northern seas, reported in our other work, contained small amounts of plasmologens in the three main phospholipid classes, PC, PE and PS (except few species) (Dembitsky *et al.*, 1989). Recently, we found that the freshwater sponge *Eusponsilla lacustris* contained plasmalogens in two main phospholipid classes, PE and PC (Dembitsky *et al.*, 1991).

The lipid of the amphipod parasite was 11.3% (as a per cent of dry weight of *Brandtia* (*Spinacanthus*) *parasitica*) and consisted of 56.5% neutral lipid, 7.8% glycolipid and 35.7% phospholipid (Table 1). The major phospholipid classes were choline-containing lipids which in the parasite constituted 55.3% of the total phospholipid (Table 1). These two phospholipids contained alkenyl–acyl and alkyl–acyl phosphoglycerides as well as diacyl compounds. Phosphatidylethanolamine consisted of 38% diacyl, 25% alkenyl–acyl and 37% alkyl–acyl forms, whereas 53.6% of the phosphatidylcholine was in diacyl form. Similarly, the ether-containing phospholipids of animal and plant parasites are also predominantly ethanolamine phosphoglycerides (Chitwood and Krusberg, 1981a,b).

The interesting lipid composition of the marine starfish *Henricia* sp. from the White Sea has been studied by Dembitsky (1987). Marine sponges are the major and single food of this starfish, *Henricia* sp. High levels of alkyl–acyl (28%) and alkenyl–acyl (72%) forms in PE were identified in digestive glands and total lipid extract.

Sugiura *et al.* (1992) studied the levels of alkenyl–acyl, alkyl–acyl and diacyl subclasses of PC and PE in 28 species of various invertebrates, including 15 arthropod species. The plasmalogen form in PE varied from a trace amount to 31.1% in prawn *Penaeus japonicus*, and the alkyl–acyl form from a trace amount to 23.2%.

The fatty acids from endemic sponges living in the Lake Baikal are most interesting. Our first study of fatty acid composition of Baikal freshwater sponges (Dembitsky, 1981a,b), showed that baikalian sponges contain 39 fatty acids. Latyshev *et al.* (1992) confirmed this result. Although GC–MS chromatography was not used for the analysis of fatty acids, the results obtained for six species of Baikal sponges from the class Demospongiae indicated that all the endemic sponges contained demospongic acids.

Our new collection of sponges was subjected to detailed analysis using capillary GC–MS. We identified 183 fatty acids, which is probably the greatest number of fatty acids yet found in sponges. Morales and Litchfield (1976) identified 126 fatty acids from the marine sponge *Microciona prolifera*. Among the identified fatty acids we found 46 saturated acids having 12–32 carbon atoms. We also observed the presence of the prystanic, phytanic, iso-, anteiso and branched acids of 4,8,12-tri-methyl-13:0—type as well as ringed (cyclo-19-26:0) ones, mainly within neutral lipids. Among saturated acids, 14:0, 16:0, 18:0 and 20:0 were the major ones (over 1%) (Table 2). No difference was found between the fatty acid composition in the lipid extracts from the native and the washed sponge samples (Table 2). Lawson *et al.* (1986, 1988) showed that the proportions of long-chain fatty acids in the membrane isolate (51%) were similar to the proportions from unfractionated tissue (51%) of sponge *Halichondria moori*.

Fifty-five monoenoic fatty acids were identified, more than any other class of fatty acids. Among the monoenoic fatty acids, the major ones were 7–16:1, 9–16:1, 9–18:1, 11–20:1, 11–22:1 and 13–22:1 (Table 3). We identified four isomers for 23 and 26 carbon acids; five isomers for 16, 20 and 24-carbon acids; six isomers for 18 and 22-carbon acids; and seven isomers, including iso-, anteiso-and branched, for 17:1

acid. The greatest amount of monoenoic (over 50%) is found in neutral fractions in the native and the washed samples.

Thirty-five dienoic fatty acids were identified, mainly in glyco- and phospholipid fractions (Table 4). The major dienoic

Table 3. Monoenoic fatty acid composition of sponge *Lubomrskia baicalensis*

	Washed				Native			
Fatty acid	TL	NL	GL	PL	TL	NL	GL	PL
7–12:1	0.15	0.38	0.04	0.03	0.15	0.37	0.04	0.03
8–13:1	0.05	0.08	0.01	0.03	0.05	0.07	0.04	0.03
7–14:1	0.22	0.45	0.12	0.09	0.22	0.44	0.12	0.10
9–14:1	0.26	0.30	0.27	0.22	0.26	0.30	0.27	0.22
5–15:1	0.10	0.23	0.01	0.03	0.10	0.22	0.01	0.03
7–15:1	0.10	0.08	0.12	0.09	0.10	0.07	0.12	0.10
5–16:1	0.22	0.38	0.16	0.12	0.22	0.37	0.16	0.13
7–16:1	1.01	1.36	0.93	0.74	0.99	1.33	0.90	0.73
9–16:1	5.12	10.61	2.64	2.10	5.23	11.09	2.54	2.06
3t–16:1	0.10	0.08	0.12	0.09	0.10	0.07	0.12	0.10
11–16:1	0.31	0.38	0.31	0.25	0.31	0.37	0.31	0.25
i-9–17:1	0.05	0.08	0.04	0.03	0.05	0.07	0.04	0.03
i-12–17:1	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
ai-9–17:1	0.08	0.23	0.00	0.00	0.07	0.22	0.00	0.00
br_7-12–17:1	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
br_9-6–17:1	0.05	0.15	0.00	0.00	0.05	0.15	0.00	0.00
7–17:1	0.10	0.15	0.08	0.06	0.10	0.15	0.08	0.06
9–17:1	0.24	0.30	0.23	0.19	0.24	0.30	0.23	0.19
5–18:1	0.29	0.23	0.35	0.28	0.29	0.22	0.35	0.29
7–18:1	0.12	0.30	0.54	0.43	0.41	0.30	0.51	0.41
9–18:1	7.19	8.33	5.75	7.50	7.50	8.88	5.48	4.49
11–18:1	0.93	0.98	1.01	0.80	0.86	0.96	0.90	0.73
13–18:1	0.85	1.36	0.66	0.52	0.80	1.33	0.59	0.48
15–18:1	0.44	0.61	0.39	0.31	0.43	0.59	0.39	0.32
19:1	0.05	0.08	0.04	0.03	0.05	0.07	0.04	0.03
11–19:1	0.32	0.68	0.16	0.12	0.32	0.67	0.16	0.13
5–20:1	0.24	0.45	0.16	0.12	0.24	0.44	0.16	0.13
7–20:1	0.26	0.23	0.31	0.25	0.26	0.22	0.31	0.25
9–20:1	0.42	1.06	0.12	0.09	0.42	1.04	0.12	0.10
11–20:1	1.32	3.33	0.35	0.28	1.20	2.96	0.35	0.29
13–20:1	0.60	0.83	0.51	0.43	0.58	0.81	0.51	0.41
11–21:1	0.17	0.45	0.04	0.03	0.17	0.44	0.04	0.03
5–22:1	0.26	0.23	0.31	0.25	0.26	0.22	0.31	0.25
7–22:1	0.29	0.53	0.19	0.15	0.29	0.52	0.20	0.16
9–22:1	0.59	1.06	0.39	0.31	0.58	1.04	0.39	0.32
11–22:1	1.38	2.88	0.70	0.56	1.34	2.81	0.67	0.54
13–22:1	0.95	0.91	1.09	0.86	1.00	0.89	1.17	0.95
15–22:1	0.53	0.76	0.47	0.37	0.53	0.74	0.47	0.38
5–23:1	0.12	0.30	0.04	0.03	0.12	0.30	0.04	0.03
13–23:1	0.27	0.68	0.08	0.06	0.27	0.67	0.08	0.06
14–23:1	0.10	0.23	0.04	0.03	0.10	0.22	0.04	0.03
17–23:1	0.10	0.15	0.08	0.06	0.10	0.15	0.08	0.06
9–24:1	0.22	0.38	0.16	0.12	0.22	0.37	0.16	0.13
13–24:1	0.80	1.36	0.58	0.46	0.77	1.33	0.55	0.44
15–24:1	0.82	1.06	0.78	0.62	0.79	1.04	0.74	0.60
17–24:1	0.67	1.52	0.27	0.22	0.66	1.48	0.27	0.22
19–24:1	0.30	0.68	0.12	0.09	0.30	0.67	0.12	0.10
15–25:1	0.05	0.08	0.04	0.03	0.05	0.07	0.04	0.03
17–25:1	0.20	0.53	0.04	0.03	0.20	0.52	0.04	0.03
5–26:1	0.27	0.68	0.08	0.06	0.29	0.74	0.08	0.06
9–26:1	0.30	0.68	0.12	0.09	0.30	0.67	0.12	0.10
15–26:1	0.17	0.23	0.16	0.12	0.17	0.22	0.16	0.13
17–26:1	0.30	0.61	0.16	0.12	0.29	0.59	0.16	0.13
27:1	0.03	0.08	0.00	0.00	0.02	0.07	0.00	0.00
28:1	0.05	0.15	0.00	0.00	0.05	0.15	0.00	0.00
Total monoenoic	30.49	50.35	21.46	19.90	30.57	50.14	20.81	16.90

Table 4. Dienoic fatty acid composition of sponge *Lubomirskia baicalensis*

Fatty acid	Washed				Native			
	TL	NL	GL	PL	TL	NL	GL	PL
6,9–14:2	0.33	0.15	0.19	0.15	0.17	0.15	0.20	0.16
9,12–16:2	0.56	0.08	0.89	0.71	0.59	0.07	0.94	0.76
5,9–16:2	0.12	0.23	0.08	0.06	0.12	0.22	0.08	0.06
6,9–18:2	2.21	1.74	2.72	2.16	2.34	1.70	2.94	2.38
6,11–18:2	0.05	0.00	0.08	0.06	0.05	0.00	0.08	0.06
9,12–18:2	5.00	3.71	4.66	6.63	5.34	3.70	5.71	6.62
12,15–18:2	2.06	0.61	3.11	2.47	2.04	0.59	3.05	2.47
6,11–20:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
8,11–20:2	0.30	0.00	0.51	0.40	0.28	0.00	0.47	0.38
11,14–20:2	0.21	0.00	0.35	0.28	0.21	0.00	0.35	0.29
11,15–20:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
14,17–20:2	0.14	0.00	0.23	0.19	0.14	0.00	0.23	0.19
10,13–22:2	0.07	0.00	0.12	0.09	0.07	0.00	0.12	0.10
13,16–22:2	0.11	0.00	0.19	0.15	0.12	0.00	0.20	0.16
5,9–24:2	0.23	0.00	0.39	0.31	0.24	0.00	0.39	0.32
15,18–24:2	0.16	0.00	0.27	0.22	0.16	0.00	0.27	0.22
17,20–24:2	0.19	0.00	0.31	0.25	0.19	0.00	0.31	0.25
24:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9–25:2	0.11	0.00	0.19	0.15	0.12	0.00	0.20	0.16
16,19–25:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
i-5,9–26:2	0.14	0.00	0.23	0.19	0.14	0.00	0.23	0.19
ai-5,9–26:2	0.19	0.00	0.31	0.25	0.19	0.00	0.31	0.25
5,9–26:2	0.60	0.00	1.01	0.80	0.59	0.00	0.98	0.79
17,20–26:2	0.09	0.00	0.16	0.12	0.10	0.00	0.16	0.13
19,22–26:2	0.19	0.00	0.31	0.25	0.19	0.00	0.31	0.25
i-5,9–27:2	0.07	0.00	0.12	0.09	0.07	0.00	0.12	0.10
ai-5,9–27:2	0.14	0.00	0.23	0.19	0.14	0.00	0.23	0.19
5,9–27:2	0.21	0.00	0.35	0.28	0.21	0.00	0.35	0.29
i-5,9–28:2	0.53	0.00	0.89	0.71	0.50	0.00	0.82	0.67
ai-5,9–28:2	0.09	0.00	0.16	0.12	0.10	0.00	0.16	0.13
5,9–28:2	0.39	0.00	0.66	0.52	0.40	0.00	0.67	0.54
br_{22}-5,9–29:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9–29:2	0.21	0.00	0.35	0.28	0.21	0.00	0.35	0.29
5,9–30:2	0.42	0.00	0.70	0.56	0.40	0.00	0.67	0.54
5,9–31:2	0.09	0.00	0.16	0.12	0.10	0.00	0.16	0.13
Total dienoic	15.31	6.52	20.13	18.99	15.62	6.43	21.26	19.22

acids were in glyco- and phospholipid fractions (over 1%): 9,12–16:2, 6,9–18:2, 9,12–18:2 and 12,15–18:2. The range of isomers was the greatest for acids 26:2, 20:2 and 24:2, 18:2 with five and four isomers, respectively.

Trienoic fatty acids are the most interesting of all the fatty acids (Table 5). Earlier studies of fatty acids from the genus Baikalospongia, revealed only *cis* isomers of 26:2 and 26:3 acids (Dembitsky, 1981b). Recently, Vysotskii *et al.* (1990) reported that three Baikalian sponges contain *trans* isomers of 26:3 acid from 9.0 to 10.2% of total acids. We have found 14 novel polyisoprenoid fatty acids, including 13,17,21,25-tetramethyl-5–26:1 and 13,17,21,25-tetramethyl-5,9–26:2 in amounts from 0.012% to 0.042% of total fatty acids (Rezanka and Dembitsky, 1993).

Tetra-, penta- and hexaenoic acids were not found in neutral fractions (Table 5). The major polyenoic fatty acids in phospholipids were arachidonic (5,8,11,14–20:4, 4.72% in the washed and 4.73% in the native samples), eicosapentaenoic (5,8,11,14,17–20:5, 2.87 and 2.84%) and docosahexaenoic (4,7,10,13,16,19–22:6, 6.07 and 6.10%, respectively). The content of polyenoic acids was found to vary from 20.43 to 20.55% in phospholipids, and from 15.41 to 15.97% in glycolipids.

The fatty acid composition of total fatty acids of symbiotic microalgae *Zoochlorella* sp. was calculated from the data of the native and the washed sponge samples (Tables 2–5); 14:0—0.16% (percentage calculated, 6.2%—percentage of total fatty

acids of microalgae), 16:0—0.22% (8.5%), 18:0—0.06% (2.3%), 9–16:1—0.11% (4.3%), 9–18:1—0.31% (12.0%), 9,12–16:2—0.03% (1.2%), 6,9–18:2—0.13% (5.0%), 9,12–18:2—0.34% (13.2%), 9,12,-15–18:3—1.01% (39.2%) and 6,9,12–18:3—0.21% (8.1%). Latyshev *et al.* (1992) reported the fatty acid composition of intracellular algae *Zoochlorella sp.* isolated from the sponge *L. baicalensis*. Our calculated data of total fatty acids of symbiotic microalgae is similar to that of Latyshev *et al.* (1992) data.

We have identified 182 fatty acids in the baikalian amphipod-parasite, including 46 saturated, 55 monoenoic, 35 dienoic, 25 trienoic and 21 polyenoic acids. The proportion of saturated fatty acids in the

Table 5. Polyenoic fatty acid composition of sponge *Lubomirskia baicalensis*

	Washed				Native			
Fatty acid	TL	NL	GL	PL	TL	NL	GL	PL
6,9,12–16:3	0.32	0.00	0.54	0.43	0.33	0.00	0.55	0.44
4,7,10–16:3	0.14	0.08	0.19	0.15	0.44	0.07	0.20	0.16
7,10,13–16:3	0.39	0.00	0.66	0.52	0.38	0.00	0.63	0.51
6,9,12–18:3	2.79	0.00	4.66	3.70	3.00	0.00	4.97	4.02
9,12,15–18:3	5.82	0.30	7.38	9.77	6.83	0.30	7.83	12.37
5,8,11–20:3	1.13	1.29	1.17	0.93	1.18	1.48	1.44	0.92
8,11,14–20:3	0.47	0.00	0.78	0.62	0.45	0.00	0.74	0.60
11,14,17–20:3	0.39	0.00	0.66	0.52	0.36	0.00	0.59	0.48
7,10,13–22:3	0.49	0.00	0.82	0.65	0.52	0.00	0.86	0.70
10,13,16–22:3	0.44	0.00	0.74	0.59	0.42	0.00	0.70	0.57
13,16,19–22:3	0.32	0.00	0.54	0.43	0.31	0.00	0.51	0.41
24:3	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
15,18,21–24:3	0.35	0.00	0.58	0.46	0.36	0.00	0.59	0.48
25:3	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9,17–26:3	3.41	0.00	4.97	3.95	3.07	0.00	5.09	4.12
5,9,19–26:3	1.07	0.00	1.79	1.42	1.01	0.00	1.68	1.36
17,20,23–26:3	0.07	0.00	0.12	0.09	0.07	0.00	0.12	0.10
5,9,19–27:3	0.28	0.00	0.47	0.37	0.26	0.00	0.43	0.35
5,9,20–27:3	0.42	0.00	0.70	0.56	0.40	0.00	0.67	0.54
27:3	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9,19–28:3	0.88	0.00	1.48	1.17	0.95	0.00	1.57	1.27
5,9,21–28:3	1.07	0.00	1.79	1.42	0.95	0.00	1.57	1.27
5,9,23–28:3	0.39	0.00	0.66	0.52	0.36	0.00	0.59	0.48
5,9,23–29:3	0.44	0.00	0.74	0.59	0.42	0.00	0.70	0.57
5,9,23–30:3	0.30	0.00	0.51	0.40	0.28	0.00	0.47	0.38
4,7,10,13–16:4	0.21	0.00	0.35	0.28	0.21	0.00	0.35	0.29
6,9,12,15–16:4	0.19	0.00	0.31	0.25	0.19	0.00	0.31	0.25
18:4	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
6,9,12,15–18:4	0.63	0.00	1.05	0.83	0.61	0.00	1.02	0.82
19:4	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
3,6,9,12,15–18:5	0.44	0.00	0.74	0.59	1.41	0.00	0.78	0.63
5,8,11,14–20:4	2.32	0.00	2.25	4.72	1.29	0.00	2.15	4.73
6,9,12,15–20:4	0.56	0.00	0.93	0.74	0.59	0.00	0.98	0.79
8,11,14,17–20:4	0.32	0.00	0.54	0.43	0.33	0.00	0.55	0.44
5,8,11,14,17–20:5	1.73	0.00	2.33	2.87	1.70	0.00	2.27	2.84
6,9,12,15,18–21:5	0.16	0.00	0.27	0.22	0.16	0.00	0.27	0.22
7,10,13,16–22:4	0.28	0.00	0.47	0.37	0.28	0.00	0.47	0.38
10,13,16,19–22:4	0.19	0.00	0.31	0.25	0.19	0.00	0.31	0.25
4,7,10,13,16–22:5	0.37	0.00	0.62	0.49	0.31	0.00	0.51	0.41
7,10,13,16,19–22:5	0.28	0.00	0.47	0.37	0.28	0.00	0.47	0.38
4,7,10,13,16,19–22:6	2.93	0.00	2.72	6.07	2.91	0.00	2.62	6.10
9,12,15,18–24:4	0.21	0.00	0.35	0.28	0.21	0.00	0.35	0.29
6,9,12,15,18–24:5	0.19	0.00	0.31	0.25	0.19	0.00	0.31	0.25
6,9,12,15,18,21–24:6	0.28	0.00	0.47	0.37	0.28	0.00	0.47	0.38
15,18,21,24–30:4	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
15,18,21,24,27–30:5	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
Unknown (from total)	0.79	0.00	1.32	1.05	0.64	0.00	1.06	0.86
Total polyenoic	33.60	1.67	48.03	49.90	32.97	1.85	47.72	52.62

Table 6. Saturated fatty acids of sponge's parasite *B. parasitica*

Fatty acid	TL	NL	GL	PL
i-12:0	0.05	0.08	0.04	0.12
12:0	0.34	0.15	0.49	1.51
i-13:0	0.05	0.00	0.08	0.22
ai-13:0	0.10	0.08	0.12	0.35
13:0	0.23	0.23	0.26	0.82
i-14:0	0.19	0.31	0.15	0.47
14:0	1.86	4.57	1.29	2.01
4,8,12-triMe-13:0	0.03	0.08	0.00	0.00
i-15:0	0.12	0.23	0.08	0.22
ai-15:0	0.15	0.39	0.04	0.12
15:0	0.41	0.62	0.34	1.05
i-16:0	0.42	0.93	0.19	0.58
Pristanic	0.15	0.46	0.00	0.00
16:0	5.95	11.16	2.93	5.93
i-17:0	0.13	0.31	0.04	0.12
ai-17:0	0.20	0.46	0.08	0.22
Phytanic	0.13	0.39	0.00	0.00
17:C	0.53	1.01	0.34	1.05
i-18:0	0.32	0.62	0.19	0.58
18:0	3.58	8.10	1.79	3.45
i-19:0	0.13	0.31	0.04	0.12
br_{11}-19:0	0.03	0.08	0.01	0.03
ai-19:0	0.08	0.23	0.01	0.03
19:0	0.27	0.62	0.12	0.35
i-20:0	0.10	0.31	0.00	0.00
20:0	1.51	3.56	0.59	1.84
i-21:0	0.05	0.15	0.00	0.00
ai-21:0	0.10	0.31	0.00	0.00
21:0	0.30	0.70	0.12	0.35
i-22:0	0.10	0.31	0.00	0.00
22:0	0.73	1.78	0.22	0.70
i-23:0	0.03	0.08	0.00	0.00
23:0	0.21	0.54	0.04	0.12
i-24:0	0.05	0.15	0.00	0.00
24:0	0.30	0.62	0.15	0.47
i-25:0	0.03	0.08	0.00	0.00
ai-25:0	0.05	0.15	0.00	0.00
25:0	0.10	0.31	0.00	0.00
c-19–26:0	0.03	0.08	0.00	0.00
26:0	0.18	0.46	0.04	0.12
27:0	0.05	0.15	0.00	0.00
28:0	0.10	0.31	0.00	0.00
29:0	0.03	0.08	0.00	0.00
30:0	0.08	0.23	0.00	0.00
31:0	0.03	0.08	0.00	0.00
32:0	0.03	0.08	0.00	0.00
Total saturated	19.64	41.94	9.79	22.95

examined fractions varied from 9.79% in glycolipids to 41.94% in neutral lipids (Table 6). The major saturated acids were 14:0, 16:0, 18:0 and 20:0. Among saturated acids, there were high percentages of *iso*-acids in neutral lipids (3.87%), and *anteiso*- (1.62%). We have isolated long-chain fatty acids from C24:0 to C32:0, including the cyclic fatty acid (c-19–26:0).

Most of the acids identified in the course of the present study were monoenoic ranging in carbon number from 12:1 to 28:1. C18 and C22 acids were represented by six isomers each, C16, C20, and C24 have five isomers; acid C26 has four isomers and 17:1 acids have seven isomers. A wide range (55 acids from C12 to C28) of monoenoic acids was detected in three actions, in amounts generally less than 1%. Several acids, 7–16:1, 9–16:1, 9–18:1, 11–20:1, 11–22:1 and 13–22:1, were present in amounts exceeding 1% in total lipids, and were characteristic of the studied species (Table 7).

Thirty-five fatty acids were identified as dienoic acids, among which were very long-chain acids: 5,9–30:2 and 5,9–31:2 in amounts less than 0.4% in total lipids (Table 8).

Among 25 trienoic acids, five major fatty acids were identified, namely: 6,9,12–18:3 (3.22% in total lipids), 9,12,15–18:3 (6.44%), 5,8,11–20:3 (1.08%), 5,9,17–26:3 (2.82%) and 5,9,21–28:3 (1.08%). High levels of 26:3 and 28:3 (all isomers) were found in the glyco- and phospholipid fractions (Table 9). Tetra-, penta- and hexaenoic compounds are the least abundant fatty acids in the species examined (21 acids). The major fatty acids of the total lipid were arachidonic (2.02%), eicosapentaenoic (1.80%) and docosahexaenoic (3.84%) (Table 9). Small amounts of fatty acids (15,18,21,24–30:4 and 15,18,21,24,27–30:5) were found in both glyco- and phospholipid fractions. These fatty acids were found in lipids of *Lubomirskia baicalensis* too (see Table 5).

The biosynthesis of very long-chain fatty acids in the marine sponge *Microciona prolifera* was studied earlier by Morales and Litchfield (1976) who reported the presence in sponges of a well-developed fermentation system of elongases and desaturases with the capability to synthesize very long-chain fatty acids. Recently, Hahn *et al.* (1989) studied, for the first time, the biosynthesis of very long-chain fatty acids in the freshwater sponge, *Ephydatia fluviatilis*. These authors concluded that, whereas the typical marine sponge *Microciona Prolirera* biosynthesizes the 5,9,19–26:3 acid solely by homologation of exogenous palmitooleic acid (9–16:1) and subsequent

Table 7. Monoenoic fatty acids of sponge's parasite *B. parasitica*

Fatty acid	TL	NL	GL	PL
7–12:1	0.15	0.39	0.04	0.03
8–13:1	0.05	0.08	0.04	0.03
7–14:1	0.22	0.46	0.11	0.09
9–14:1	0.26	0.31	0.25	0.21
5–15:1	0.10	0.23	0.04	0.03
7–15:1	0.10	0.08	0.11	0.09
5–16:1	0.22	0.39	0.15	0.12
7–16:1	1.17	1.39	0.90	0.77
9–16:1	4.54	9.21	2.36	1.93
3t–16:1	0.10	0.08	0.11	0.09
11–16:1	0.31	0.39	0.29	0.23
i-9–17:1	0.05	0.08	0.04	0.03
i-12–17:1	0.03	0.08	0.00	0.00
ai-9–17:1	0.08	0.23	0.00	0.00
br_7-12–17:1	1.03	0.08	0.00	0.00
br_9-6–17:1	0.05	0.15	0.00	0.00
7–17:1	0.10	0.15	0.07	0.06
9–17:1	0.24	0.31	0.22	0.18
5–18:1	0.28	0.23	0.33	0.26
7–18:1	0.43	0.31	0.53	0.43
9–18:1	8.15	8.67	5.32	6.88
11–18:1	0.97	1.01	0.99	0.84
13–18:1	0.79	1.39	0.51	0.43
15–18:1	0.41	0.62	0.35	0.27
19:1*	0.05	0.08	0.04	0.03
11–19:1	0.32	0.70	0.15	0.12
5–20:1	0.24	0.46	0.15	0.12
7–20:1	0.25	0.23	0.29	0.23
9–20:1	0.43	1.08	0.11	0.09
11–20:1	1.45	3.76	0.33	0.26
13–20:1	0.64	0.85	0.54	0.46
11–21:1	0.18	0.46	0.04	0.03
5–22:1	0.25	0.23	0.29	0.23
7–22:1	0.29	0.54	0.18	0.15
9–22:1	0.58	1.08	0.37	0.29
11–22:1	1.32	2.87	0.62	0.48
13–22:1	1.07	0.93	1.17	1.00
15–22:1	0.52	0.77	0.43	0.35
5–23:1	0.13	0.31	0.04	0.03
13–23:1	0.28	0.70	0.07	0.06
14–23:1	0.10	0.23	0.04	0.03
17–23:1	0.10	0.15	0.07	0.06
9–24:1	0.22	0.39	0.15	0.12
13–24:1	0.81	1.39	0.55	0.46
15–24:1	0.86	1.08	0.69	1.01
17–24:1	0.67	1.55	0.25	0.21
19–24:1	0.30	0.70	0.11	0.09
15–25:1	0.05	0.08	0.04	0.03
17–25:1	0.21	0.54	0.04	0.03
5–26:1	0.40	1.07	0.07	0.06
9–26:1	0.30	0.70	0.11	0.09
15–26:1	0.17	0.23	0.15	0.12
17–26:1	0.30	0.62	0.15	0.12
27:1*	0.03	0.08	0.00	0.00
28:1*	0.05	0.15	0.00	0.00
Total monoenoic	31.40	50.33	20.00	19.36

*Identified only on the basis of retention time and mass spectrum of methyl (unknown position of double bond(s)).

Table 8. Dienoic fatty acids of sponge's parasite *B. parasitica*

Fatty acid	TL	NL	GL	PL
6,9–14:2	0.21	0.15	0.18	0.15
9,12–16:2	0.67	0.08	1.01	0.85
5,9–16:2	0.12	0.23	0.07	0.06
6,9–18:2	2.08	1.80	2.51	1.95
6,11–18:2	0.05	0.00	0.07	0.06
9,12–18:2	4.91	3.26	5.21	6.52
12,15–18:2	2.11	0.62	2.95	2.50
5,11–20:2	0.02	0.00	0.04	0.03
8,11–20:2	0.32	0.00	0.50	0.43
11,14–20:2	0.20	0.00	0.33	0.26
11,15–20:2	0.02	0.00	0.04	0.03
14,17–20:2	0.13	0.00	0.22	0.18
10,13–22:2	0.07	0.00	0.11	0.09
13,16–22:2	0.11	0.00	0.18	0.15
5,9–24:2	0.21	0.00	0.36	0.27
15,18–24:2	0.16	0.00	0.25	0.21
17,20–24:2	0.18	0.00	0.29	0.23
24:2*	0.02	0.00	0.04	0.03
5,9–25:2	0.11	0.00	0.18	0.15
16,19–25:2	0.02	0.00	0.04	0.03
i-5,9–26:2	0.13	0.00	0.22	0.18
ai-5,9–26:2	0.18	0.00	0.29	0.23
5,9–26:2	0.65	0.00	1.03	0.86
17,20–26:2	0.09	0.00	0.15	0.12
19,22–26:2	0.18	0.00	0.29	0.23
i-5,9–27:2	0.07	0.00	0.11	0.09
ai-5,9–27:2	0.13	0.00	0.22	0.18
5,9–27:2	0.20	0.00	0.33	0.26
i-5,9–28:2	0.55	0.00	0.84	0.73
ai-5,9–28:2	0.09	0.00	0.15	0.12
5,9–28:2	0.37	0.00	0.62	0.50
br_{22}-5,9–29:2	0.02	0.00	0.04	0.03
5,9–29:2	0.20	0.00	0.33	0.26
5,9–30:2	0.37	0.00	0.61	0.49
5,9–31:2	0.09	0.00	0.15	0.12
Total dienoic	15.04	6.14	19.96	18.58

*Identified only on the basis of retention time and mass spectrum of methyl ester (unknown position of double bond(s)).

desaturation at positions 5 and 9, it was found that the freshwater sponge could further desaturate the 5,9–26:2 acid to the triene, indicating for the first time the existence of 19-desaturase activity in a living organism.

This study has demonstrated that Baikal sponges contain more total fatty acids than those reported for both marine and freshwater sponges. These present results show, that the composition of ether lipids and demospongic acids of the amphipod crustacean parasite *Brandtia* (*Spinacanthus*) *parasitica* does not differ substantially from the freshwater host sponge *Lubomirskia baicalensis*. Fatty acid diversity present in the parasite and in the host is also very similar.

Table 9. Polyenoic fatty acids of sponge's parasite *B. parasitica*

Fatty acid	TL	NL	GL	PL
6,9,12–16:3	0.29	0.00	0.50	0.39
4,7,10–16:3	0.14	0.08	0.19	0.15
7,10,13–16:3	0.46	0.00	0.77	0.60
6,9,12–18:3	3.22	0.00	5.28	4.63
9,12,15–18:3	6.44	0.31	7.91	1.31
5,8,11–20:3	1.08	1.20	1.14	0.89
8,11,14–20:3	0.49	0.00	0.82	0.64
11,14,17–20:3	0.37	0.00	0.62	0.48
7,10,13–22:3	0.56	0.00	0.94	0.73
10,13,16–22:3	0.38	0.00	0.64	0.51
13,16,19–22:3	0.36	0.00	0.60	0.47
24:3*	0.02	0.00	0.04	0.03
15,18,21–24:3	0.34	0.00	0.58	0.45
25:3*	0.02	0.00	0.04	0.03
5,9,17–26:3	2.82	0.00	5.09	3.98
5,9,19–26:3	0.99	0.00	1.67	1.30
17,20,23–26:3	0.07	0.00	0.12	0.09
5,9,19–27:3	0.30	0.00	0.51	0.40
5,9,20–27:3	0.38	0.00	0.64	0.50
27:3*	0.02	0.00	0.04	0.03
5,9,19–28:3	0.90	0.00	1.51	1.18
5,9,21–28:3	1.08	0.00	1.82	1.42
5,3,23–28:3	0.41	0.00	0.70	0.54
5,9,23–29:3	0.40	0.00	0.68	0.53
5,9,23–30:3	0.33	0.00	0.56	0.43
4,7,10,13–16:4	0.20	0.00	0.34	0.26
6,9,12,15–16:4	0.18	0.00	0.30	0.23
18:4*	0.02	0.00	0.04	0.03
6,9,12,15–18:4	0.60	0.00	1.01	0.78
19:4*	0.02	0.00	0.04	0.03
3,6,9,12,15–18:5	0.54	0.00	0.93	0.73
5,8,11,14–20:4	2.02	0.00	2.21	4.61
6,9,12,15–20:4	0.61	0.00	1.02	0.80
8,11,14,17–20:4	0.30	0.00	0.50	0.39
5,8,,11,14,17–20:5	1.80	0.00	2.36	3.05
6,9,12,15,18–21:5	0.16	0.00	0.26	0.21
7,10,13,16–22:4	0.27	0.00	0.46	0.30
10,13,16,19–22:4	0.18	0.00	0.30	0.23
4,7,10,13,16–22:5	0.39	0.00	0.65	0.50
7,10,13,16,19–22:5	0.26	0.00	0.44	0.34
4,7,10,13,16,19–22:6	3.84	0.00	4.84	4.03
9,12,15,18–24:4	0.20	0.00	0.34	0.26
6,9,12,15,18–24:5	0.17	0.00	0.30	0.23
9,12,15,18,21–24:6	0.25	0.00	0.42	0.33
15,18,21,24–30:4	0.02	0.00	0.04	0.03
15,18,21,24,27–30:5	0.02	0.00	0.04	0.03
Total polyenoic	33.92	1.60	50.25	39.09

*Identified only on the basis of retention time and mass spectrum of methyl ester (unknown position of double bond(s)).

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