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Comparative study of the endemic freshwater fauna of Lake Baikal—IV. Phospholipid and fatty acid compositions of two gastropod molluscs of the genus *Valvata*

Valery M. Dembitsky,* Tomas Rezanka† and Andrey G. Kashin‡

*Laboratory of Natural Products, Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem, 91904, Israel; †Institute of Microbiology, Prague, Videnska 1083, 142 20, Czech Republic; and ‡Institute of Ecology of the Volga River Basin, Russian Academy of Sciences, Togliatti 445003, Russia

Lipid and phospholipid compositions of endemic freshwater molluscs, belonging to the class Gastropoda *Valvata baicalensis* and *Valvata piligera* were investigated. Distribution of phospholipids was studied by qualitative and quantitative micro-thin-layer chromatography. Only major phospholipid classes, phosphatidylethanolamine and phosphatidylcholine, were found to contain plasmalogen forms. The fatty acids composition of total lipids, neutral, glyco- and phospholipid fraction was investigated by capillary gas chromatography–mass spectrometry. Ninety-five fatty acids were identified: 23 saturated (both *iso*- and *anteiso*-), 28 monoenoic, 14 dienoic and 30 polyenoic. Among polyenoic acids were identified a high percentage of tree main acids 18:3n-3, 18:3n-6 and 20:4n-6 in phospholipid and glycolipid fractions; among saturated acids were found a high percentage of *iso*-(3.56–4.25%) and *anteiso*-(2.02 and 2.75%) acids in neutral fractions. A number of unusual polyunsaturated fatty acids, such as 18:5n-3, 24:4n-6, 24:5n-6, 24:6n-3 and furanoid acids, were found.

Key words: Lake Baikal; Phospholipids; Fatty acids.

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Introduction

Lake Baikal is known as the blue eye of Siberia, and is the largest freshwater natural reserve in the world. It lies at the centre of the great Siberian internal drainage basin framed by rugged mountain ranges which are broken in only a few places by the broad valleys of its major affluents. Although it does not cover the greatest area it holds the most water of any lake in the world, over 20% of the world's resources of surface fresh water, which would be enough almost to fill all five of North America's Great Lakes.

From the palaeontological evidence the fauna of Lake Baikal is known to have lived through many geological and climatic events. Formation of the Baikalian fauna began in the warm Tertiary period and by the Pliocene, or even Miocene, had become ecologically distinct from the fauna of the nearby shallow Siberian lakes (Kozhov, 1962).

At present, 85 mollusc, including 56 endemic species are living in Lake Baikal (Kozhov, 1951; Martinson, 1959). The freshwater gastropods found in Lake Baikal are interesting in that their lipid biochemistry is very little studied.

Recent years have seen a period during which the analysis of marine organisms, including invertebrates, particularly their lipid compositions, received considerable attention (see a recently issued monograph of *Marine Biogenic Lipids, Fats and Oils*; Ackman, 1989a). Marine gastropod lipids have been studied rather

Correspondence to: V. M. Dembitsky, Laboratory of Natural Products, Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.
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Table 1. Lipid contents in the examined samples

Species	*V. basicalensis*	*V. piligera*
Neutral lipids (NL)	61.7	58.8
Glycolipids (GL)	7.9	16.8
Phospholipids (PL)	30.4	24.4
Total lipids (TL) (% of dry mass)	6.3	4.6

NL, GL and PL as percent of TL.

intensively with respect to some interesting components such as polyunsaturated, non-methylene-interrupted fatty acids, ether lipids and phospholipids (Ackman, 1989b; Dembitsky, 1979, 1985; Dembitsky *et al.*, 1992; Vaskovsky, 1989) which are abundant in this phylum. Although the lipid composition of molluscs is well-documented, there is only a limited amount of information on the lipid composition of the freshwater gastropods.

The present work continues our earlier one on the detailed study of fatty acid and lipid compositions of two endemic freshwater Gastropoda species living in Lake Baikal (Dembitsky *et al.*, 1993a).

Materials and Methods

Collection of samples

Valvata baicalensis Gerstf. and *Valvata piligera* Lingh., belonging to the class Gastropoda, were sampled in the south-western part of Lake Baikal, near Bolshye Koty (Biological Station of Irkutsk University) in July 1992, at depths of 0.5–1 m; the temperature of the water was 12°C. The animals were immersed in tanks at the site of collection and transported to the laboratory.

Lipid analysis

The species were treated immediately on arrival at the laboratory. Lipids were extracted, separated by column and micro-thin-layer chromatography, as described elsewhere (Dembitsky *et al.*, 1992).

Fatty acid ME esters were analysed by capillary gas–liquid chromatography—mass spectrometry using an Shimadzu QP-1000 instrument as described previously (Dembitsky *et al.*, 1993b).

Results and Discussion

The mollusc species examined for their total lipids are listed in Table 1. As seen from the table, the lipid content in gastropods varied from 4.6 to 6.3% of dry mass. According to Dembitsky *et al.* (1993a) the total lipid content in Baikalian freshwater gastropods varied from 2.1 in *Baicalia oviformis* to 7.3% of dry mass in *Benedictia baicalensis*.

In the examined gastropods, the major phospholipid classes were choline-containing lipids which constituted, in *V. baicalensis* and *V. piligera*, 49.4 AND 35.6% of the total phospholipids, respectively (Table 2). For instance, the total phospholipids in the other two freshwater species, *Limnaea stagnalis* and *Radix auricularia*, were dominated by amine-containing lipids making up 45.2 and 46.3%, respectively of the total phospholipids (Dembitsky *et al.*, 1992). Two of the examined species were found to posses CAEP in amounts varying from 4.8 to 9.8%. CAEP is widespread among marine molluscs and is detected in virtually all the examined species (De Koning, 1966, 1972; Simon and Rouser, 1969; Chelomin and Zhukova, 1981; Komai *et al.*, 1973; Matsubara, 1975; Kostetsky, 1982), with the maximum level at 16.8%, found in the muscle of *Hinnites giganteum* (Simon and Rouser, 1969) and at 15.1% in the bivalved *Crenomytilus grayanus* and the gastropod *Acmea paliida* (Kostetsky, 1982). The latter author showed that, apart from the basic form of CAEP, some molluscs contained two other forms of CAEP. According to the authors, CAEP-2 obviously contained oxyacids, while CAEP-X was supposed to be formed after reactive TLC during the detection of plasmalogens on plates. Possibly, some CAEP contained

Table 2. Phospholipid composition of molluscs

Species Phospholipid classes	*V. baicalensis*	*V. piligera*
Phosphatidylethanolamine (PE)	16.8 ± 0.5	13.6 ± 0.4
Phosphatidalethanolamine (PEP)	12.4 ± 0.6	13.9 ± 0.5
Ceramidaminoethylphosphonate (CAEP)	4.8 ± 0.2	8.3 ± 0.3
Phosphatidylserine (PS)	8.3 ± 0.4	9.8 ± 0.4
The sum of aminophospholipids (APL)	42.3	45.6
Phosphatidylcholine (PC)	46.7 ± 0.9	32.3 ± 0.7
Phosphatidalcholine (PCP)	2.7 ± 0.2	3.3 ± 0.4
The sum of cholinephospholipids (CPL)	49.9	35.6
Phosphatidylinositol (PI)	5.2 ± 0.3	12.4 ± 0.5
Diphosphatidylglycerol (DPG)	3.1 ± 0.1	6.4 ± 0.3

Table 3. Saturated fatty acids of total, neutral, glyco- and phospholipid fractions from two mollusc species

Species	*V. baicalensis*				*V. piligera*			
Fatty acids	TL	NL	GL	PL	TL	NL	GL	PL
13:0	0.13	0.27	0.07	0.06	0.11	0.29	0.09	0.07
14:0	2.09	10.93	0.66	0.28	2.28	6.96	0.95	0.46
15:0	0.41	1.33	0.16	0.10	0.63	0.76	0.15	0.13
16:0	6.58	10.79	4.14	6.14	8.13	12.32	5.48	5.65
17:0	0.49	1.34	0.19	0.09	0.67	1.70	0.13	0.23
18:0	3.07	7.89	0.52	0.17	1.65	5.96	0.43	0.62
19:0	0.31	0.83	0.09	0.07	0.34	0.85	0.09	0.06
20:0	0.64	1.77	0.26	0.24	0.79	1.90	0.22	0.17
22:0	0.22	0.68	0.12	0.10	0.47	1.09	0.13	0.13
24:0	0.12	0.27	0.08	0.06	0.29	0.56	0.07	0.07
i-13:0	0.07	0.18	0.06	0.06	0.07	0.16	0.06	0.06
i-14:0	0.10	0.19	0.07	0.06	0.12	0.25	0.09	0.08
i-15:0	0.25	1.16	0.12	0.07	0.26	0.84	0.10	0.09
i-16:0	0.28	0.87	0.09	0.07	0.44	1.12	0.17	0.17
i-17:0	0.17	0.53	0.09	0.07	0.21	1.34	0.10	0.13
i-18:0	0.15	0.29	0.07	0.06	0.17	0.19	0.07	0.06
i-19:0	0.17	0.34	0.07	0.06	0.17	0.35	0.07	0.08
ai-13:0	0.08	0.19	0.06	0.06	0.09	0.22	0.07	0.06
ai-15:0	0.31	0.73	0.09	0.09	0.38	1.54	0.17	0.11
ai-17:0	0.34	0.80	0.11	0.14	0.37	0.72	0.09	0.10
ai-19:0	0.16	0.30	0.06	0.06	0.12	0.27	0.06	0.06
4,8,12-triMe-13:0	0.26	1.14	0.06	0.06	0.18	0.87	0.06	0.06
Pristanic	0.33	1.11	0.07	0.06	0.24	0.90	0.09	0.08
The sum saturated	16.73	43.93	7.31	8.23	18.18	41.16	8.94	8.73
The sum of *iso*-	1.19	3.56	0.57	0.45	1.44	4.25	0.66	0.67
The sum of *anteiso*-	0.89	2.02	0.32	0.35	0.96	2.75	0.39	0.33

double bonds of a vinyl-bond type present in plasmalogens. CAEP was also detected in the freshwater snail *Limnaea stagnalis* at 7.5% (Liang and Strickland, 1969) and in the land snail *Eobania vermiculata* (Stavrakakis *et al.*, 1989). We found CAEP in all freshwater gastropod species collected in the Volga river basin (Dembitsky *et al.*, 1992), and Baikalian gastropods, too (Dembitsky *et al.*, 1993a). Three species of gastropods were found to contain, apart from CAEP, small amounts of sphingomyelin; thus *Acmaea pallida* yielded 2.3%, *Collisella radiata* 5.1%, and *Collisella verticollor*, 4.6% of this compound. The latter three species are littoral animals (Kostetsky, 1982).

As stated earlier (Dembitsky, 1979, 1985) plasmalogens are ubiquitous in marine invertebrates, including gastropod species. Nine distinct gastropod species sampled in the summer were shown to have percentages of plasmalogenous forms in PE varying within 61.5–77.4%, and within 0.0–20.9% in PC. High percentages of plasmalogen were found for freshwater molluscs: 2.6–50.5% in PE and 5.5–9.3% in PC of Baikalian gastropods, *V. baicalensis* and *V. piligera*, respectively. The data obtained by Liang and Strickland (1969) are in contrast to our data; the authors reported 3.4% plasmalogen in PE and 2.5% in PC. Such low levels of plasmalogen found in PE may be due, rather, to inappropriate determination techniques than to the true low plasmalogen content. The same authors determined the composition of alkylacyl forms contained in PE (15.4%) and PC (44.5%) and, in this case, their data are consistent with our own early results which suggested high percentages of alkylacyl compounds in molluscs (Dembitsky, 1985). We had not found plasmalogen forms in PS from two studied gastropods. The quantification of alkyl forms in the examined species was not attempted in the present study. DPG was identified in all the examined species, but no particular order emerged with regard to its distribution, which varied from 3.1 to 6.4% (Table 2). The content of DPG in marine molluscs is reportedly lower within 0.6–2.8% (Kostetsky and Sergeyuk, 1985; Lubet *et al.*, 1985; Simon and Rouser, 1969). DPG was found in two Baikalian gastropod, *Baicalia oviformis* (6.1%) and *Benedictia baicalensis* (2.7%). DPG was not found in *Limnaea stagnalis* (Liang and Strickland, 1969). No sphingomyelin was detected by us in the Baikalian endemic freshwater gastropods studied.

Fatty acid composition of Baikalian gastropods is interesting, too. We have identified 95 fatty acids, including 23 saturated, 28 monoenoic, 14 dienoic and 30 polyenoic acids.

The proportion of saturated fatty acids in the examined species varied from 43.93 to 41.16% of neutral lipids (Table 3). The major saturated acids were 14:0, 16:0 and 18:0. Among saturated acids, we identified a high percentage of *iso*-acids: *V. baicalensis*—3.56%; *V. piligera*—4.25%; and *anteiso*—2.02 and 2.75% in neutral fractions, respectively.

Most of the acids identified in the course of the present study were monoenoic, ranging from 14:1 to 24:1n-9. C18, C20 and C22 acids were represented by five isomers of each; 16:1 acids were also represented by four isomers, (n-5), (n-7), (n-9) and 3-*trans*-16:1. A wide range (28 acids, from C14 to C24) of monoenoic acids was detected in two species, in amounts generally less than 1%. Several acids, 16:1n-9, 18:1n-9, 20:1n-9 and 22:1n-9, were present in amounts exceeding 1%, and were characteristic of the studied species (Table 4).

Fourteen fatty acids were identified as dienoic acids, among which were non-methylene-interrupted (NMI) acids: 5,11–20:2, 5,13–20:0, 7,13–22:2 and 7,15–22:2, in amounts less than 0.4% (Table 5). The most interesting are NMI fatty acids which frequently occur in marine (Ackman, 1989a,b; Zhukova, 1991; Zhukova and Svetashev, 1986) and freshwater (Dembitsky *et al.*, 1992, 1993a,b) molluscs, including gastropod species.

Trienoic, tetra-, penta- and hexaenoic compounds are the most abundant fatty acids in the examined species (30 acids). The major acids among total lipids were 18:3n-3 (6.96 and 17.81%, 18:3n-6 (9.62 and 10.90%), 24:4n-6 (2.47 and 4.02%) and 22:6n-3 (2.57 and 1.22%) (Table 6). Small amounts of furanoid acids were found in both gastropod species, and high percentages in glycolipids of *V. biacalensis* (0.73%).

Small amounts of 18:4n-3 and 18:4 (possibly (n-6) isomer) acids were found in both gastropod species, mostly less than 1%. Earlier, we found a high percentage of 18:4n-6 in glycolipid fraction in two Baikalian gastropod species, in *Benedictia baicalensis* (13.7%) and in *Baicalia oviformus* (15.92%) and 18:4n-3 in phospholipid fraction of *Benedictia baicalensis* (19.35%) (Dembitsky *et al.*, 1993a).

The present results show that two gastropod species sampled in Lake Baikal, in their lipid and fatty acid compositions differ substantially from marine and other Baikalian gastropod species belonging to the same class, though the diversity of fatty acids present in the freshwater

Table 4. Monoenoic fatty acids of total, neutral, glyco- and phospholipid fractions from two mollusc species

Species	*V. baicalensis*				*V. piligera*			
Fatty acids	TL	NL	GL	PL	TL	NL	GL	PL
14:1	0.20	0.30	0.12	0.21	0.24	0.44	0.17	0.13
14:1n-5	0.12	0.15	0.08	0.09	0.12	0.18	0.10	0.08
16:1n-9	2.09	3.87	2.09	1.17	2.27	2.98	2.11	0.82
16:1n-7	0.79	2.15	0.28	0.22	0.32	2.20	0.36	0.29
3t-16:1[a]	0.09	0.27	0.06	0.06	0.11	0.29	0.06	0.06
16:1n-5	0.35	0.48	0.18	0.13	0.57	1.61	0.17	0.31
17:1n-8	0.11	0.15	0.08	0.06	0.10	0.12	0.09	0.09
18:1n-13	0.22	0.36	0.27	0.38	0.27	0.18	0.35	0.10
18:1n-11	0.62	0.87	0.60	0.64	0.77	0.71	0.75	0.29
18:1n-9	20.01	29.22	17.21	17.12	19.58	31.33	20.39	16.13
18:1n-7	0.16	0.25	0.29	0.73	0.71	1.64	0.24	0.53
18:1n-5	0.20	0.19	0.12	0.46	0.48	1.25	0.28	0.31
19:1n-[b]	0.07	0.06	0.06	0.06	0.07	0.06	0.06	0.06
19:1n-8	0.15	0.19	0.09	0.08	0.26	0.47	0.20	0.20
20:1n-15	0.12	0.29	0.19	0.07	0.21	0.60	0.11	0.18
20:1n-13	0.25	0.62	0.32	0.17	0.37	0.81	0.17	0.34
20:1n-11	0.83	1.00	0.59	0.25	0.77	1.25	0.30	0.49
20:1n-9	1.19	1.14	1.14	1.55	1.44	1.73	0.94	0.80
20:1n-7	0.16	0.19	0.26	0.14	0.18	0.11	0.13	0.11
21:1n-9	0.08	0.07	0.06	0.06	0.09	0.10	0.06	0.06
22:1n-15	0.07	0.11	0.06	0.06	0.09	0.14	0.07	0.06
22:1n-13	0.09	0.15	0.07	0.07	0.11	0.22	0.07	0.07
22:1n-11	0.80	0.62	0.44	0.39	0.56	0.99	0.34	0.32
22:1n-9	1.77	4.40	1.00	0.73	0.95	1.05	0.45	0.37
22:1n-7	0.33	0.48	0.36	0.43	0.56	1.27	0.31	0.28
23:1n-9	0.12	0.15	0.07	0.07	0.09	0.19	0.07	0.06
24:1n-11	0.15	0.22	0.10	0.07	0.18	0.25	0.14	0.30
24:1n-9	0.51	0.45	0.30	0.45	0.42	1.09	0.53	0.34
The sum of monoenoic	31.65	48.40	26.49	25.92	31.89	53.26	29.02	23.18

[a,b]See footnote in Table 6 for explanation.

Table 5. Dienoic fatty acids of total, neutral, glyco- and phospholipid fractions from two mollusc species

	V. baicalensis				*V. piliogera*			
Fatty acids	TL	NL	GL	PL	TL	NL	GL	PL
16:2n-4	0.35	0.07	0.47	0.42	0.18	0.10	0.30	0.41
18:2n-9	0.98	0.30	0.88	1.06	1.37	0.68	1.62	1.91
18:2n-6	5.67	2.87	6.47	8.40	5.32	1.59	8.65	9.42
18:2n-3	0.07	0.06	0.14	0.14	0.10	0.06	0.09	0.14
5,11-20:2	0.40	0.06	0.39	0.25	0.30	0.06	0.36	0.43
5,13-20:2	0.22	0.06	0.30	0.17	0.27	0.07	0.31	0.34
20:2n-9	0.33	0.06	0.32	0.32	0.77	0.06	0.33	0.68
20:2n-6	0.11	0.06	0.19	0.24	1.95	0.07	2.05	0.80
20:2n-3	0.10	0.06	0.17	0.22	0.32	0.06	0.45	0.70
7,13-22:2	0.23	0.06	0.24	0.29	0.18	0.06	0.32	0.34
7,15-22:2	0.14	0.06	0.12	0.24	0.12	0.07	0.12	0.17
22:2n-9	0.13	0.06	0.18	0.09	0.88	0.06	0.72	0.99
22:2n-6	0.12	0.06	0.12	0.10	0.74	0.06	1.15	1.52
24:2[b]	0.11	0.06	0.21	0.06	0.18	0.06	0.22	0.22
The sum of dienoic	8.96	3.90	10.20	12.00	12.68	3.06	16.69	18.07

[b]See footnote in Table 6 for explanation.

Table 6. Polyenoic fatty acids of total, neutral, glyco- and phospholipid fractions from two mollusc species

Species	*V. baicalensis*				*V. piligera*			
Fatty acids	TL	NL	GL	PL	TL	NL	GL	PL
16:3n-4	0.80	0.10	0.68	0.86	0.30	0.10	0.53	0.95
16:3n-6	0.36	0.07	0.98	1.42	1.23	0.07	1.46	2.26
18:3n-6	9.62	0.53	12.62	14.35	10.90	0.35	11.38	15.64
18:3n-3	17.81	1.21	19.03	18.70	6.96	0.31	7.05	7.12
20:3n-9	0.51	0.06	0.50	0.23	0.24	0.06	0.30	0.38
5,11,14-20:3	0.07	0.06	0.11	0.10	0.07	0.06	0.11	0.10
20:3n-6	0.13	0.06	0.38	0.20	0.32	0.06	0.36	0.30
20:3n-3	0.41	0.06	0.42	0.28	0.26	0.06	0.31	0.26
22:3n-9	0.17	0.06	0.21	0.17	0.23	0.06	0.30	0.26
22:3n-6	0.37	0.06	0.69	0.46	0.90	0.06	1.38	1.33
22:3n-3	0.23	0.06	0.31	0.27	0.16	0.06	0.19	0.18
16:4n-1	0.87	0.06	1.06	0.99	1.68	0.07	1.03	1.92
18:4[c]	0.05	0.06	0.06	0.06	0.10	0.06.	0.31	0.18
18:4n-3	0.51	0.06	0.46	0.97	0.85	0.06	1.44	0.74
19:4[a]	0.05	0.06	0.06	0.06	0.10	0.06	0.14	0.26
20:4n-6	2.47	0.06	5.28	4.87	4.02	0.14	8.03	9.95
5,11,14,17-20:4	0.13	0.06	0.46	0.15	0.32	0.06	0.34	0.31
20:4n-3	0.99	0.06	2.26	2.03	0.39	0.06	0.42	0.57
21:5n-3	0.07	0.06	0.09	0.06	0.12	0.06	0.10	0.08
22:4n-6	0.43	0.06	0.62	0.52	1.17	0.06	1.36	0.78
22:4n-3	0.49	0.06	0.69	0.81	1.65	0.06	2.34	1.55
24:4n-6	0.48	0.06	0.51	0.30	0.81	0.06	0.98	0.77
18:5n-3	0.12	0.06	0.27	0.06	0.21	0.03	0.33	0.06
20:5n-3	0.59	0.07	1.24	0.45	0.37	0.06	0.45	0.34
22:5n-6	0.70	0.06	0.90	0.68	0.26	0.06	0.49	0.30
22:5n-3	0.92	0.06	1.46	1.68	1.18	0.06	1.26	0.67
24:5n-6	0.43	0.06	0.63	0.82	0.66	0.06	0.67	0.60
22:6n-3	2.57	0.11	2.83	1.56	1.22	0.06	1.62	1.46
24:6n-3	0.26	0.06	0.46	0.68	0.51	0.06	0.61	0.63
F-acids	0.05	0.30	0.73	0.06	0.06	0.13	0.06	0.07
The sum of polyenoic	42.66	3.77	56.00	53.85	37.25	2.52	45.35	50.02

[a]Identified only on the basis retention time and mass spectrum of methyl ester.
[b]Unknown position of double bond(s), identified by retention time and value of molecular ion of methyl ester.
[c]Probably 18:4n-6, but this position of double bonds was not confirmed.
F-acids: the sum of furanoid fatty acids.

group is more modest than in marine species. This statement suggests that representatives of the relic endemic Baikalian fauna, whilst changing their habitat and experiencing adaptation to specific Baikalian freshwater conditions, preserve the basic principles of biosynthesis, at least with regard to their lipids and fatty acids.

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