Comp. Biochem. Physiol. Vol. 106B, No. 4, pp. 819–823, 1993
Printed in Great Britain

0305-0491/93 \$6.00 + 0.00

COMPARATIVE STUDY OF THE ENDEMIC FRESHWATER FAUNA OF LAKE BAIKAL—I. PHOSPHOLIPID AND FATTY ACID COMPOSITION OF TWO MOLLUSC SPECIES, *BAICALIA OVIFORMUS* AND *BENEDICTIA BAICALENSIS*

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(*Received* 19 *April* 1993; *accepted* 17 *June* 1993)

Abstract—1. Lipid and phospholipid compositions of endemic freshwater molluscs belonging to the class Gastropoda, *Baicalia oviformus* and *Benedictia baicalensis*, were studied.

2. The fatty acids composition of total lipids, neutral, glyco- and phospholipid fraction was investigated by capillary gas chromatography–mass spectrometry.

3. Ninety-five fatty acids were identified: 23 saturated (both iso- and anteiso-), 28 monoenoic, 14 dienoic and 30 polyenoic.

4. High percentage of the two main acids, 18:4 and 18:4(n-3) in phospholipid and glycolipid fractions were identified.

5. A number of unusual polyunsaturated fatty acids, such as 19:4, 18:5(n-3), 24:4(n-6), 24:5(n-6), 24:6(n-3), and furanoid acids, were found.

INTRODUCTION

Baikal is the largest freshwater lake in the world. Its origins date back to the days of dinosaurs and giant ferns, the foundations of the modern relief of the trans-Baikal area being laid back in the Mesozoic era. As early as the Upper Jurassic and Lower Cretaceous periods, large lakes are thought to have filled the intermountain depressions in the area. The embryo lakes, which were to become Baikal, began to expand and deepen during the early-to-middle Tertiary period and eventually merged sometime between the Tertiary and Quaternary periods (Belova *et al.*, 1983). Such a time-scale makes Lake Baikal the world's oldest lake. The lake is a natural museum of living endemic fossils; of the approximate 900 animal species inhabiting its open waters less than 20% are found anywhere else (Kozhov, 1947, 1962).

At present, 85 mollusc, including 56 endemic species, are living in Lake Baikal (Kozhov, 1951; Martinson, 1959). The freshwater molluscs found in the lake are interesting objects in terms of biochemistry, but their lipid biochemistry, and in particular, their lipid composition, has been very little studied.

Marine Mollusca lipids have been studied rather intensively with respect to some interesting components such as polyunsaturated, non-methylene-interrupted fatty acids, ether lipids and phospholipids (Ackman, 1989; Dembitsky, 1979, 1985; Vaskovsky, 1989) which are abundant in this phylum. Although the lipid composition of the marine mollusc is well-documented, there is only a limited amount of information on the lipid composition of the freshwater mollusc.

In this paper, we present results of a detailed study of the fatty acid and lipid compositions of two freshwater Gastropoda species living in Lake Baikal.

MATERIAL AND METHODS

Collection of samples

Baicalia oviformus Dyb. and *Benedictia baicalensis* Gerstf., 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 put into tanks under water at the site of collection and transported to the laboratory.

Lipid analysis

The species were treated immediately in the laboratory as described previously (Dembitsky *et al.*,

Table 1. Lipid contents in the examined samples

		Baicalia oviformis	*Benedictia baicalensis*
Neutral lipids	(NL)	57.4	70.3
Glycolipids	(GL)	8.0	7.9
Phospholipids	(PL)	34.6	21.7
Total lipids (% of dry wt)	(TL)	2.1	7.3

Table 2. Phospholipid composition of molluscs

Phospholipid classes		*Baicalia oviformus*	*Benedictia bicalensis*
Phosphatidylethanolamine	(PE)	10.7 ± 0.4	15.9 ± 0.6
Phosphatidalethanolamine	(PEP)	15.7 ± 0.6	15.7 ± 0.5
Lysophosphatidylethanolamine	(LPE)	3.6 ± 0.2	—
Ceramidaminoethylphosphonate	(CAEP)	6.7 ± 0.3	3.9 ± 0.1
Phosphatidylserine	(PS)	4.3 ± 0.2	3.4 ± 0.1
The sum of amino phospholipids	(APL)	41.0	38.9
Phosphatidylcholine	(PC)	35.8 ± 0.9	44.6 ± 1.1
Phosphatidalcholine	(PCP)	2.6 ± 0.2	4.4 ± 0.4
Lysophosphatidylcholine	(LPC)	6.2 ± 0.3	2.7 ± 0.2
The sum of choline phospholipids	(CPL)	44.6	51.7
Phosphatidylinositol	(PI)	8.3 ± 0.4	6.7 ± 0.5
Diphosphatidylglycerol	(DPC)	6.1 ± 0.4	2.7 ± 0.2

1992a). Lipids were extracted and separated by column and thin-layer chromatography, as described elsewhere (Dembitsky *et al.*, 1992a,b).

Fatty acids ME esters were analysed by capillary gas–liquid chromagraphy–mass spectrometry using a Shimadzu QP-1000 instrument as described previously (Dembitsky *et al.*, 1992b, 1993b).

RESULTS AND DISCUSSION

The general lipid compostions from the two gastropod species *Baicalia oviformus* and *Benedicta baicalensis* are given in Table 1. Total lipids make up 2.1 and 7.3% dry wt in *B.oviformus* and *B.baicalensis*, respectively. In the examined gastropod, the major phospholipid classes were choline-containing lipids which constituted, in *Baicalia oviformus* and *Benedictia baicalensis*, 44.6 and 51.7% of the total phospholipid, respectively (Table 2). The studied species were found to possess CAEP in amounts varying from 3.9% in *B. baicalensis* to 6.7% in *B. oviformus*. CAEP is widespread among marine (Vaskovsky, 1989) and freshwater molluscs (Dembitsky *et al.*, 1992). High percentages of plasmalogen were found for freshwater gastropods: 59.3% in PE and 6.9% in PC in *B. oviformus*; 49.8% in PE and 9.0% in PC in *B. baicalensis*. The major phospholipids were PC and PE.

The fatty acid composition of the freshwater gastropod species examined by capillary GC–MS is shown in Tables 3–6. We identified 95 fatty acids, including 23 saturated. The proportion of saturated fatty acids in the examined species varied from 17.14 to 18.20% of total lipids (Table 3). The major saturated acids were 14:0, 16:0 and 18:0. Most of the acids identified in the course of the present study were monoenoic, ranging from 14:1 to 24:1(n-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, 16:1(n-9), 18:1(n-9), 20:1(n-9), and 22:1(n-9), were present in amounts exceeding 1%, and were characteristic of the studied species.

Fourteen fatty acids were identified as dienoic acids, among which were non-methylene-interrupted (NMI) acids: 5,11–20:2, 5,13–20:2, 7,13–22:2 and 7,15–22:2, in amounts less than 0.8% (Table 5). The most interesting are NMI fatty acids which frequently occur in marine (Ackman, 1989; 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 them were

Table 3. Saturated fatty acids of TL, NL, PL and GLs

	Benedictia baicalensis				*Baicalia oviformus*			
	TL	NL	PL	GL	TL	NL	PL	GL
i-13:0	0.07	0.18	0.05	0.06	0.07	0.15	0.06	0.06
ai-13:0	0.08	0.19	0.05	0.06	0.09	0.21	0.07	0.06
13:0	0.13	0.26	0.06	0.06	0.11	0.28	0.09	0.07
i-14:0	0.10	0.19	0.06	0.06	0.12	0.25	0.09	0.08
14:0	2.14	10.67	0.62	0.27	2.28	6.86	0.95	0.47
4,8,12-triMe-13:0	0.27	1.11	0.5	0.06	0.18	0.86	0.06	0.06
i-15:0	0.26	1.13	0.12	0.07	0.26	0.82	0.10	0.09
ai-15:0	0.32	0.71	0.08	0.09	0.38	1.52	0.17	0.12
15:0	0.42	1.30	0.15	0.10	0.63	0.75	0.16	0.13
i-16:0	0.29	0.85	0.08	0.07	0.44	1.11	0.17	0.18
pristanic	0.33	1.09	0.06	0.06	0.24	0.88	0.09	0.08
16:0	6.74	10.72	3.90	5.90	8.14	10.52	5.51	5.76
i-17:0	0.18	0.52	0.08	0.07	0.21	1.32	0.10	0.13
ai-17:0	0.34	0.78	0.10	0.13	0.37	0.71	0.09	0.11
17:0	0.50	0.31	0.18	0.09	0.67	1.67	0.13	0.23
i-18:0	0.16	0.28	0.06	0.06	0.17	0.19	0.07	0.06
18:0	3.14	7.69	0.49	0.17	1.66	5.88	0.43	0.63
i-19:0	0.18	0.33	0.06	0.06	0.17	0.34	0.07	0.08
ai-19:0	0.17	0.29	0.05	0.06	0.12	0.27	0.06	0.06
19:0	0.32	0.81	0.08	0.07	0.34	0.84	0.09	0.06
20:0	0.66	1.72	0.24	0.23	0.79	1.87	0.22	0.18
22:0	0.22	0.66	0.12	0.10	0.47	1.07	0.13	0.13
24:0	0.12	0.26	0.07	0.06	0.29	0.55	0.07	0.07
Total	17.14	43.05	6.81	7.98	18.20	38.92	8.98	8.90

Table 4. Monoenoic fatty acids of TL, NL, PL and GLs

	Benedictia baicalensis				*Baicalia oviformus*			
	TL	NL	PL	GL	TL	NL	PL	GL
14:1n	0.20	0.29	0.12	0.20	0.24	0.44	0.17	0.13
14:1n-5	0.12	0.14	0.07	0.09	0.12	0.18	0.10	0.08
16:1n-9	2.14	3.78	1.97	1.12	2.27	2.93	2.12	0.83
6:1n-7	0.81	2.10	0.26	0.21	0.32	2.17	0.37	0.29
3t-16:1*	0.09	0.26	0.05	0.06	0.11	0.28	0.06	0.06
16:1n-5	0.36	0.47	0.17	0.12	0.57	1.59	0.17	0.32
17:1n-8	0.11	0.14	0.07	0.06	0.10	0.12	0.09	0.09
18:1n-13	0.22	0.35	0.25	0.37	0.27	0.18	0.35	0.11
18:1n-11	0.63	0.85	0.57	0.61	0.77	0.70	0.75	0.29
18:1n-9	22.40	30.06	20.57	20.34	19.43	33.34	20.03	14.59
18:1n-7	0.17	0.25	0.27	0.70	0.71	1.61	0.24	0.54
18:1n-5	0.20	0.19	0.12	0.44	0.48	1.24	0.28	0.32
19:1†	0.07	0.06	0.05	0.06	0.07	0.06	0.06	0.06
19:1n-8	0.16	0.19	0.08	0.08	0.26	0.46	0.20	0.20
20:1n-15	0.12	0.28	0.18	0.07	0.21	0.59	0.11	0.19
20:1n-13	0.26	0.60	0.30	0.17	0.37	0.80	0.17	0.35
20:1n-11	0.86	0.98	0.56	0.24	0.77	1.24	0.30	0.50
20:1n-9	1.22	1.11	1.08	1.49	1.45	1.71	0.94	0.82
20:1n-7	0.17	0.19	0.24	0.13	0.18	0.11	0.13	0.12
21:1n-9	0.08	0.07	0.05	0.06	0.09	0.09	0.06	0.06
22:1n-15	0.07	0.11	0.05	0.06	0.09	0.14	0.07	0.06
22:1n-13	0.09	0.14	0.06	0.07	0.11	0.21	0.07	0.07
22:1n-11	0.82	0.60	0.42	0.38	0.56	0.98	0.34	0.33
22:1n-9	1.81	4.29	0.94	0.70	0.96	1.04	0.45	0.37
22:1n-7	0.18	0.25	0.80	0.03	0.01	0.09	0.01	0.04
23:1n-9	0.33	0.47	0.34	0.41	0.56	1.25	0.31	0.28
24:1n-11	0.12	0.14	0.06	0.07	0.09	0.19	0.17	0.06
24:1n-9	0.16	0.21	0.09	0.07	0.18	0.25	0.14	0.30
Total	33.97	48.57	29.79	28.41	35	53.99	28.16	21.46

*Identified only on the bases of retention time and mass spectrum of methyl ester.
†Unknown position of double-bond(s), identified by retention time and the value of molecular ion of methyl ester.

18:4 (my be isomer (n-6), 18:4(n-3), 20:3(n-3) and 24:4(n-6) (Table 6). Small amounts of arachidonic and eicosapentaenoic acids were found in both gastropod species.

High percentages of the two main acids 18:4(n-6?) in glycolipid fractions of *Benedictia baicalensis* (13.70%) and *Baicalia oviformus* (15.92%), and 18:4(n-3) in phospholipid fractions of *Benedictia baicalensis* (19.35%) were identified. Traces of 18:4(n-3) acid were found at an early stage in marine fish and copepods, mainly in wax esters and triglycerides (Ackman, 1989; Ackman *et al.*, 1974; Hooper and Ackman, 1971). Recently, Morris (1984) obtained interesting results in research on fatty acids of the copepod *Epischura* and the freshwater fish *Comephorus dybowski* and *Comephorus baikalensis* collected in Baikal, and showed that these animal contain high percentages of this fatty acid, in triacylglycerides, of 12.8, 10.3 and 8.8%, respectively. Webster and Lovell (1991) examined fatty acids from three geographical sources of brine shrimp nauplii (*Artemia* sp.), and reported that studied species contained percentages of 18:4(n-3) acid of from 1.37 to 6.55% in phospholipid fractions. Hayashi and Yamada (1974, 1975) analysed the acetone-soluble lipids (essentially triacylglycerides) of four species of car-

Table 5. Dienoic fatty acids of TL, NL, PL and GLs

	Benedictia baicalensis				*Baicalia oviformus*			
	TL	NL	PL	GL	TL	NL	PL	GL
16:2n-4	0.52	0.44	0.28	0.43	0.42	1.07	0.53	0.35
18:2n-9	0.36	0.07	0.44	0.40	0.18	0.09	0.30	0.42
18:2n-6	1.00	0.29	0.83	1.02	1.37	0.67	1.63	1.94
18:2n-3	5.81	2.80	6.10	8.08	5.33	1.57	8.69	9.59
5,11-20:2	0.07	0.06	0.14	0.13	0.10	0.06	0.09	0.14
5,13-20:2	0.41	0.06	0.37	0.24	0.30	0.06	0.37	0.43
20:2n-9	0.22	0.06	0.28	0.17	0.27	0.07	0.31	0.35
20:2n-6	0.33	0.06	0.30	0.31	0.77	0.06	0.33	0.69
20:2n-3	0.11	0.06	0.18	0.23	1.96	0.07	2.06	0.82
7,13-22:2	0.10	0.06	0.16	0.21	0.32	0.06	0.45	0.71
7,15-22:2	0.23	0.06	0.23	0.28	0.18	0.06	0.32	0.35
22:2n-9	0.14	0.06	0.12	0.23	0.12	0.07	0.12	0.18
22:2n-6	0.13	0.06	0.17	0.09	0.88	0.06	0.72	1.01
24:2*	0.12	0.06	0.12	0.10	0.75	0.06	1.15	1.54
Total	9.55	4.20	9.72	11.90	12.95	4.03	17.07	18.52

*See footnote of Table 4 for explanation.

Table 6. Polyenoic fatty acids of TL, NL, PL and GLs

	Benedictia baicalensis				*Baicalia oviformus*			
	TL	NL	PL	GL	TL	NL	PL	GL
16:3n-4	0.11	0.06	0.20	0.06	0.18	0.06	0.22	0.22
16:2n-6	0.82	0.09	0.64	0.82	0.30	0.09	0.53	0.97
16:4n-1	0.37	0.07	0.92	0.37	0.23	0.07	0.46	2.30
18:3n-6	0.89	0.06	1.00	0.96	1.68	0.07	1.03	1.95
18:4*	9.85	0.52	11.89	13.70	10.97	0.34	11.43	15.92
18:3n-3	0.06	0.06	0.05	0.06	0.10	0.06	0.31	0.19
18:4n-3	13.94	1.88	19.35	17.97	6.97	0.91	7.08	7.25
19:4†	0.52	0.06	0.43	0.93	0.86	0.06	1.45	0.75
18:5n-3	0.06	0.06	0.05	0.06	0.10	0.06	0.14	0.27
20:3n-9	0.12	0.06	0.25	0.06	0.23	0.06	0.33	0.06
5,11,14-20:3	0.52	0.06	0.47	0.22	0.24	0.06	0.30	0.39
20:3n-6	0.07	0.06	0.10	0.10	0.07	0.06	0.11	0.11
20:4n-6	0.13	0.06	0.36	0.19	0.32	0.06	0.37	0.30
20:3n-3	2.53	0.6	4.98	4.68	4.03	0.14	8.07	10.13
5,11,14,17-2-:4	0.32	0.06	0.40	0.27	0.26	0.06	0.31	0.27
20:4n-3	0.13	0.06	0.43	0.14	0.32	0.06	0.34	0.32
20:5n-3	1.01	0.06	2.13	1.95	0.39	0.06	0.42	0.59
21:5n-3	0.60	0.07	0.17	0.43	0.37	0.06	0.45	0.35
22:3n-9	0.07	0.06	0.08	0.06	0.12	0.06	0.10	0.08
22:3n-6	0.18	0.06	0.20	0.17	0.23	0.06	0.30	0.27
22:4n-6	0.38	0.06	0.65	0.44	0.90	0.06	1.38	1.36
22:3n-3	0.44	0.06	0.59	0.50	1.17	0.06	1.36	0.80
22:4n-3	0.23	0.06	0.29	0.26	0.16	0.06	0.19	0.19
22:5n-6	0.50	0.06	0.65	0.78	1.66	0.06	2.35	1.58
22:5n-3	0.72	0.06	0.85	0.66	0.26	0.06	0.49	0.30
22:6n-3	0.94	0.06	1.37	1.61	1.18	0.06	1.36	0.68
24:4n-6	2.63	0.11	2.67	1.50	1.22	0.06	1.63	1.49
24:5n-6	0.49	0.06	0.48	0.31	0.81	0.06	0.99	0.78
24:6n-3	0.44	0.06	0.60	0.79	0.66	0.06	0.68	0.61
F-acids‡	0.27	0.06	0.43	0.66	0.51	0.06	0.61	0.64
Total	9.34	4.13	53.68	51.71	37.58	3.86	45.79	51.12

*Probably 18:4(n-6), but this position of double-bonds was not confirmed.
†See footnote of Table 4 for explanation.
‡F-acids: the sum of furanoid fatty acids.

nivorous gastropods and demonstrated that the level of 18:4 acid varied from 1.5 to 7.9%.

Small amounts of furanoid fatty acids were detected in all lipid fractions of two gastropod species.

The present results show that two freshwater molluscs, sampled in Lake Baikal, differ essentially in their fatty acid compositions from marine species. The study of the lipids of Lake Baikal fauna has given a deeper insight into some of the most puzzling aspects of the lipid chemistry of aquatic organisms, generally. Roughly speaking, the similarity in fatty acid composition between the major endemic old fauna of Baikal and typical marine organisms may point to the origin of Baikalian fauna as relic marine fauna rather than ancient freshwater fauna.

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