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FATTY ACID AND PHOSPHOLIPID COMPOSITION OF FRESHWATER MOLLUSCS *ANADONTA PISCINALIS* AND *LIMNAEA FRAGILIS* FROM THE RIVER VOLGA

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Abstract—1. The composition of fatty acids from two fresh water molluscs, Bivalvia *Anadonta piscinalis* and Gastropoda *Limnaea fragilis* from the Volga river was investigated by capillary gas chromatography–mass spectrometry.

2. Ninety-five fatty acids were identified: 23 saturated (both iso- and anteiso-), 28 monoenoic, 14 dienoic, and 30 polyenoic. The composition of fatty acids in phospho-, glyco- and neutral fractions was studied.

3. A number of unusual 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 founded.

4. Phospholipid compositions, plasmalogen included, were examined.

INTRODUCTION

Among the world's animal types, the number of molluscs is the second greatest after the number of arthropods (Ekman 1953). Molluscs attract the attention of researchers by morphological variety and particular behaviour, physiology and biochemistry (Hoar, 1967; Vernberg and Vernberg, 1972). Of as much interest is molluscs' adaptability to environmental conditions (Berger, 1986; Hochachka and Somero, 1973). The marine fauna, as the most ancient and obviously initial, forms of life on the earth, is the most varied. The freshwater fauna, also formed in the very distant geological past (Birshtein, 1949) is, however, less rich compared to the marine one. Nevertheless, the freshwater fauna has a high endemic range (Khlebovich, 1969, 1974).

The freshwater molluscs found in the river Volga have great variety, but their biochemistry, in particular, their lipid composition, is very little studied.

The present work continues our earlier study on molluscs (Dembitsky *et al.*, 1992).

MATERIALS AND METHODS

Anadonta piscinalis Nils, from the class Bivalvia and *Limnaea fragilis* L., belonging to the class Gastropoda, were sampled in the Samara reserve near Togliatti. The molluscs species used for the analysis, were collected in August 1992, in the Volga river at depths of 0–2 m; the temperature of water was 25°C. The species were treated immediately after collection as described previously (Dembitsky *et al.*, 1992).

Lipids were extracted, separated by column and thin-layer chromatography, as described elsewhere (Dembitsky, 1979; Dembitsky *et al.*, 1992).

Fatty acids ME esters were analysed by capillary gas–liquid chromatography–mass spectrometry using an Shimadzu QP-1000 instrument as described previously (Dembitsky *et al.*, 1991).

RESULTS AND DISCUSSION

The general lipid compositions from the gastropod *L. fragilis* and the bivalve *A. piscinalis* are given in Table 1. Total lipids make up 102.6 and 104.8 mg/g dry wt in *L. fragilis* and *A. piscinalis*, respectively. In *L. fragilis*, phospholipids dominate and form 64.9% while glycolipids form only 5.3%. The main lipids in *A. piscinalis* are neutral and form 56.2%. The leader among phospholipids is PC (Table 2). Amine phospholipids are represented by PE, PS and CAEP. Among invertebrates, CAEP has been found in many freshwater and marine mollusc species (De Koning, 1966, 1972; Chelomin and Zhukova, 1981; Hori *et al.*, 1964, 1965; Komai *et al.*, 1973; Kostetsky, 1985; Matsubara, 1975); its content is observed to vary from 6% in soft tissues of *Haliotis midae* (De Koning,

Table 1. Lipid content in the examined samples

Class		Gastropoda	Bivalvia
species		*Limnaea fragilis*	*Anadonta piscinalis*
Neutral lipids	(NL)	29.8	56.2
Glycolipids	(GL)	5.3	12.2
Phospholipids	(PL)	64.9	31.6
Total lipids (mg/g dry wt)	(TL)	102.6	104.8

Table 2. Phospholipid composition of molluscs

Lipid classes	PE	CAEP	PS	APL	PA	DPG	PC	CPL	PI
Anadonta piscinalis	32.7 ± 0.9	7.3 ± 0.3	5.6 ± 0.2	45.6	0.2 ± 0.1	3.2 ± 0.2	46.9 ± 0.8	46.9	4.1 ± 0.2
Limnaea fragilis	27.6 ± 0.6	9.0 ± 0.3	10.8 ± 0.4	47.4	—	7.0 ± 0.3	38.6 ± 0.7	38.6	7.0 ± 0.3

Abbreviations—PE—phosphatidylethanolamine, CAEP—ceramidaminoethylphosphonate, PS—phosphatidylserine, APL—the sum of aminophospholipids, PA—phosphatidic acid, DPG—cardiolipin, PC—phosphatidylcholine, CPL—the sum of cholinephospholipids, PI—phosphatidylinositol.

Table 3. Major phospholipids and their content in the studied species

	Phosphatidylethanolamine		Phosphatidylcholine	
	PEA	PEP	PCA	PCP
Anadonta piscinalis	30.7	69.3	89.2	10.8
Limnaea fragilis	41.7	58.3	81.6	18.4

PEA and PCA, the sum of diacyl and alkylacyl forms; PEP and PCP, plasmalogen form. The data is percent from all forms in each phospholipid class.

Table 4. Saturated fatty acids of total, neutral, glyco- and phospholipids of molluscs

	Anadonta piscinalis				*Limnaea fragilis*			
	TL	NL	PL	GL	TL	NL	PL	GL
13:0	0.09	0.23	0.02	0.01	0.07	0.26	0.04	0.02
14:0	2.48	11.83	0.72	0.26	2.65	7.62	1.08	0.47
15:0	0.44	1.38	0.12	0.06	0.69	0.78	0.12	0.08
16:0	7.95	18.92	4.90	6.96	9.61	20.74	6.51	6.45
17:0	0.53	1.40	0.16	0.05	0.73	1.81	0.09	0.20
18:0	3.67	8.52	0.56	0.14	1.91	6.52	0.45	0.65
19:0	0.32	0.84	0.04	0.02	0.35	0.88	0.05	0.01
20:0	0.72	1.86	0.25	0.21	0.88	2.04	0.20	0.14
22:0	0.20	0.67	0.09	0.06	0.49	1.14	0.09	0.08
24:0	0.08	0.23	0.03	0.01	0.28	0.56	0.02	0.02
iso-13:0	0.01	0.13	—	—	0.02	0.11	—	—
iso-14:0	0.05	0.15	0.02	—	0.08	0.21	0.05	0.03
iso-15:0	0.24	1.20	0.09	0.02	0.24	0.86	0.06	0.04
iso-16:0	0.28	0.88	0.05	0.02	0.46	1.18	0.14	0.14
iso-17:0	0.15	0.52	0.04	0.02	0.19	1.42	0.06	0.08
iso-18:0	0.12	0.25	0.02	0.01	0.14	0.15	0.02	0.01
iso-19:0	0.15	0.31	0.02	0.01	0.13	0.32	0.02	0.03
anteiso-13:0	0.03	0.15	0.01	—	0.04	0.17	0.02	0.01
anteiso-15:0	0.32	0.73	0.05	0.04	0.39	1.64	0.13	0.07
anteiso-17:0	0.35	0.80	0.07	0.09	0.37	0.73	0.05	0.06
anteiso-19:0	0.13	0.27	0.01	—	0.08	0.24	0.01	—
4,8,12-triMe-13:0	0.26	1.17	—	—	0.15	0.90	—	—
Pristanic	0.34	1.15	0.02	0.01	0.23	0.93	0.04	0.03
Saturated	18.91	53.59	7.29	8.00	20.18	51.21	9.25	8.62
Iso-	1.00	3.44	0.24	0.08	1.26	4.25	0.35	0.33
Anteiso-	0.83	1.95	0.14	0.13	0.88	2.78	0.21	0.14

1966) to 20.2% in the erythrocytes of *Scapharca broughtoni* (Parker *et al.*, 1980). Studies of the subcellular particles of the scallop *Patinopecten yessoensis* showed that mitochondria are poor in CAEP (2.9%), though the other particles are relatively rich at 9.2–12.9% (Chelomin, 1979). DPG in marine molluscs is known to vary from 0.6% in *Haliotis corrugata* (Simon and Rouser, 1969) to 9.5% in gonads of *Mytilus platensis* (De Moreno *et al.*, 1980) and in freshwater ones, from 2 to 10% (Dembitsky *et al.*, 1992). Some marine molluscs are reported to possess sphingomyelin; this compound is not found in Bivalvia while its content in gastropods is not very high, being from 2 to 5% (Kostetsky, 1982). Sphingomyelin is reportedly present in all studied cephalopods with the maximum amount found in *Thysanotenthis* sp, being 13% (Kostetsky, 1982). Sphingomyelin is absent from our presently discussed samples.

Plasmalogens in PE and PC make up over 50% and less than 20%, respectively (Table 3). Our own data on plasmalogens contained in marine (Dembitsky and Vaskovsky, 1976; Dembitsky, 1979, 1985) and freshwater (Dembitsky *et al.*, 1992) molluscs show

Table 5. Monoenoic fatty acids of total, neutral, glyco- and phospholipids of molluscs

	Anadonta piscinalis				*Limnaea fragilis*			
	TL	NL	PL	GL	TL	NL	PL	GL
14:1(n–7)	0.17	0.27	0.09	0.17	0.23	0.43	0.13	0.08
14:1(n–5)	0.08	0.10	0.03	0.04	0.08	0.13	0.06	0.03
16:1(n–9)	2.48	4.15	2.44	1.27	2.63	3.23	2.47	0.88
16:1(n–7)	0.90	2.28	0.27	0.19	0.32	2.37	0.38	0.27
3-*trans*-16:1*	0.04	0.23	—	—	0.07	0.26	—	—
16:1(n–5)	0.36	0.46	0.15	0.08	0.61	1.72	0.14	0.30
17:1(n–8)	0.07	0.10	0.03	0.01	0.06	0.07	0.04	0.05
18:1(n–13)	0.20	0.33	0.26	0.38	0.26	0.13	0.36	0.06
18:1(n–11)	0.69	0.88	0.66	0.67	0.85	0.71	0.84	0.27
18:1(n–9)	13.48	21.60	12.18	10.92	10.03	22.67	10.36	9.21
18:1(n–7)	0.14	0.21	0.29	0.77	0.78	1.74	0.23	0.55
18:1(n–5)	0.17	0.15	0.09	0.47	0.51	1.33	0.27	0.30
19:1†	0.02	—	0.01	—	0.02	—	—	0.01
19:1(n–8)	0.12	0.15	0.05	0.03	0.24	0.45	0.17	0.16
20:1(n–15)	0.08	0.25	0.16	0.02	0.19	0.60	0.07	0.15
20:1(n–13)	0.24	0.61	0.33	0.14	0.38	0.84	0.13	0.33
20:1(n–11)	0.95	1.03	0.65	0.23	0.85	1.33	0.30	0.51
20:1(n–9)	1.39	1.17	1.31	1.71	1.65	1.85	1.06	0.86
20:1(n–7)	0.13	0.15	0.25	0.10	0.15	0.06	0.09	0.07
21:1(n–9)	0.03	0.02	—	—	0.04	0.04	—	—
22:1(n–15)	0.02	0.06	0.01	—	0.04	0.09	0.02	0.01
22:1(n–13)	0.04	0.10	0.02	0.02	0.07	0.17	0.02	0.02
22:1(n–11)	0.92	0.61	0.47	0.39	0.60	1.03	0.35	0.31
22:1(n–9)	2.09	4.73	1.14	0.77	1.07	1.10	0.48	0.36
22:1(n–7)	0.34	0.46	0.37	0.43	0.60	1.34	0.31	0.25
23:1(n–9)	0.08	0.10	0.02	0.02	0.05	0.15	0.02	0.01
24:1(n–11)	0.12	0.17	0.06	0.02	0.15	0.21	0.11	0.28
24:1(n–9)	0.56	0.42	0.30	0.46	0.44	1.14	0.57	0.33
Monoenoic	25.91	40.79	21.64	19.31	22.97	45.19	18.98	15.66

*†See footnote of Table 7 for explanation.

that the compounds in question are present in all studied species, their maximum amount in PE reaching 80%.

Fatty acid composition of molluscs is most interesting. Fatty acids have been studied in many mollusc species (Ackman, 1981, 1989).

We have identified 95 fatty acids, including 23 saturated, 28 monoenoic, 14 dienoic and 30 polyenoic acids. Fatty acid composition has been examined both from the total lipid extract and from neutral, glyco- and phospholipid fractions. Among saturated acids (Table 4) which show a considerable variation in fractions, palmitic acid is found to have the highest content. Neutral fractions are found to contain 18.92 and 20.74% palmitic acid in *A. piscinalis* and *L. fragilis*, respectively. Glyco- and phospholipid fractions have yielded low amounts of saturated acids varying from 7 to 9%. A large variety of iso- and anteiso- acids is found in neutral fractions in which 7 iso- and 4 anteiso-acids have been identified.

Among monoenoic acids (Table 5), we have identified four isomers, including a *trans* one, for 16-carbon acids; five isomers have been found for each of 18-, 20-, and 22-carbon acids. The greatest amount of monoenoic acid (over 40%) is found in neutral fractions.

Table 6. Dienoic fatty acids of total, neutral, glyco- and phospholipids of molluscs

	Anadonta piscinalis				*Limnaea fragilis*			
	TL	NL	PL	GL	TL	NL	PL	GL
16:2(n–4)	0.36	0.02	0.50	0.42	0.15	0.04	0.29	0.41
18:2(n–9)	1.12	0.27	0.99	1.16	1.56	0.69	1.88	2.14
18:2(n–6)	6.84	3.06	7.69	9.56	6.27	1.70	10.31	10.79
18:2(n–3)	0.02	—	0.11	0.09	0.06	—	0.05	0.10
5,11-20:2	0.42	—	0.41	0.23	0.29	—	0.37	0.43
5,13-20:2	0.20	—	0.30	0.14	0.25	0.02	0.31	0.33
20:2(n–9)	0.34	—	0.33	0.31	0.85	—	0.34	0.72
20:2(n–6)	0.07	—	0.16	0.21	2.26	0.02	2.40	0.87
20:2(n–3)	0.05	—	0.14	0.19	0.32	—	0.48	0.75
7,13-22:2	0.22	—	0.23	0.27	0.15	—	0.32	0.33
7,15-22:2	0.11	—	0.08	0.21	0.08	0.02	0.08	0.14
22:2(n–9)	0.10	—	0.15	0.05	0.98	—	0.80	1.07
22:2(n–6)	0.08	—	0.09	0.06	0.83	—	1.32	1.68
24:2*	0.07	—	0.19	—	0.15	—	0.20	0.19
Dienoic	10.00	3.35	11.37	12.90	14.20	2.49	19.15	19.95

*Unknown position of double bonds, identified by retention time and value of molecular ion of methyl ester.

Table 7. Polyenoic fatty acids of total, neutral, glyco- and phospholipids of molluscs

	Anadonta piscinalis				*Limnaea fragilis*			
	TL	NL	PL	GL	TL	NL	PL	GL
16:3(n–4)	0.91	0.04	0.75	0.92	0.29	0.04	0.57	1.04
16:3(n–6)	0.37	0.02	1.11	1.57	1.41	0.02	1.68	2.54
18:3(n–6)	11.65	0.52	15.06	16.37	12.97	0.32	13.59	17.95
18:3(n–3)	16.51	0.67	18.01	21.34	8.23	0.28	8.39	8.14
20:3(n–9)	0.56	—	0.54	0.20	0.23	—	0.30	0.38
5,11,14–20:3	0.02	—	0.07	0.06	0.02	—	0.07	0.06
20:3(n–6)	0.09	—	0.39	0.16	0.32	—	0.37	0.28
20:3(n–3)	0.44	—	0.44	0.25	0.24	—	0.31	0.24
22:3(n–9)	0.15	—	0.19	0.14	0.22	—	0.29	0.24
22:3(n–6)	0.38	—	0.76	0.47	1.01	—	1.59	1.47
22:3(n–3)	0.22	—	0.31	0.24	0.12	—	0.16	0.15
16:4(n–3)	1.00	—	1.21	1.07	1.94	0.02	1.17	2.15
18:4‡	—	—	—	—	0.06	—	0.31	0.15
18:4(n–3)	0.56	—	0.49	1.05	0.96	—	1.67	0.79
19:4*	—	—	—	—	0.06	—	0.11	0.24
20:4(n–6)	2.95	—	6.27	5.51	4.73	0.09	9.57	11.39
5,11,14,17–20:4	0.09	—	0.48	0.11	0.32	—	0.35	0.30
20:4(n–3)	1.14	—	2.64	2.26	0.40	—	0.44	0.60
22:4(n–6)	0.46	—	0.69	0.53	1.33	—	1.56	0.84
22:4(n–3)	0.53	—	0.77	0.87	1.91	—	2.74	1.73
24:4(n–6)	0.52	—	0.55	0.31	0.90	—	1.12	0.82
18:5(n–3)	0.08	—	0.26	—	0.21	—	0.33	0.01
20:5(n–3)	0.65	0.02	1.43	0.45	0.38	—	0.48	0.33
21:5(n–3)	0.02	—	0.05	0.01	0.08	—	0.06	0.03
22:5(n–6)	0.80	—	1.02	0.72	0.24	—	0.52	0.28
22:5(n–3)	1.06	—	1.68	1.85	1.34	—	1.57	0.70
24:5(n–6)	0.46	—	0.70	0.88	0.72	—	0.75	0.63
22:6(n–3)	3.07	0.06	3.34	1.73	1.39	—	1.88	1.62
24:6(n–3)	0.25	—	0.49	0.72	0.54	—	0.67	0.67
The sum of furanoid acids	0.24	0.94	—	—	0.08	0.34	—	—
Polyenoic	45.18	2.27	59.70	59.79	42.65	1.11	52.62	55.77

*Identified only on the basis retention time and mass spectrum of methyl ester.
†Unknown position of double bond(s), identified by retention time and value of molecular ion of methyl ester.
‡Probably 18:4(n–6), but this position of double bonds was not confirmed.

Four non-methylene-interrupted acids have been identified among dienoic acids while neutral fractions have been found to contain not more than 1% of such acids (Table 6). Marine species, however, are known to contain the mentioned acids in high amounts (Zhukova and Svetashev, 1986).

Polyenoic acids are basically concentrated in phospho-and glycolipid fractions at over a 50% level (Table 7). The amounts of arachidonic and eicosapentaenoic acids, presently found only in phospho- and glycolipid fractions, vary from 5.5 to 11%. An acid of rare occurrence, 19:4, has been identified in *L. fragilis*. Neutral fractions are found to contain furadoid acids as indicated in Table 7. Earlier, we reported 11 furanoid acids from Caspian invertebrates (Dembitsky *et al.*, 1993). The origin of furanoid acids remains unknown. One possible explanation of their presence is oil pollution.

Thus, our investigation into the lipids from two freshwater mollusc species has demonstrated that the animals in question contain a large variety of fatty acids, some of which belong to the number of rarely occurring ones, namely 19:4, 18:5(n–3), 24:5(n–6), 24:6(n–3) and furanoid acids.

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