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LIPID COMPOSITION OF THREE MACROPHYTES FROM THE CASPIAN SEA

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Key Word Index—*Acrosiphonia* sp.; Acrosiphoniaceae; *Chara vulgaris*; Characeae; *Nitellopsis obtusa*; Nitellaceae; macrophytes; Caspian Sea; lipids; phospholipids; fatty acids.

Abstract—Lipid compositions of three macrophytic species from the Caspian Sea were examined. Lipid contents varied from 32.5 to 73.2 $mg\,g^{-1}$ dry wt. The main classes found were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol. The compositions of the fatty acids in the neutral, glycolipid and phospholipid fractions were also studied. The neutral fraction was composed mainly of palmitic, oleic, linoleic and α-linolenic acids. Essential fatty acid representatives in the glycolipid and phospholipid fractions were oleic, linoleic and α-linolenic acids. Besides the common fatty acids, more than 40 other acids, including very long-chain ones in minor amounts, were identified.

INTRODUCTION

Lipid compositions of more than 200 macrophytic species are presently known [1, 2]; the majority of the examined species are marine. At the same time, there is little information concerning the macrophytes living in brackish waters and it seems likely that their lipids will differ from the lipids found in marine species [3–6]. Macrophytic species living in such habitats could be described as marine species adapted to new conditions of lower salt concentrations or freshwater macrophytes likewise adapted to conditions of higher salt concentrations. Both groups should, obviously, have certain lipid similarities, preserving, at the same time, the principal fatty acids and phospholipids inherited from their respective ancestors.

The present work describes an examination of the lipids from three macrophytic species inhabiting the Caspian Sea where the level of water salinity is almost three times less than that in the open sea.

RESULTS AND DISCUSSION

The three species for the present study were collected along the south eastern coast of the Caspian Sea where the concentration of salts can reach 13.4%. The species belonging to the order Acrosiphonales are typical marine algae widespread in the coastal waters. They are, taxonomically, one of the hardest to identify among the marine green algae. However, they belong to a young genus, in evolutionary terms, where the species formation processes are still in progress and the various species possess certain biological peculiarities [7–10].

The mentioned peculiarities considered, it is interesting to compare lipids from this genus with those from other green algae. Lipids from an *Acrosiphonia* sp. are shown in Table 1. The total lipid content found in marine species varied from 0.8 to 6 $mg\,g^{-1}$ [11–13]. The total lipid content from freshwater species is known to have a wider range, i.e. from 39 to 241 $mg\,g^{-1}$ dry wt [14]. The *Acrosiphonia* sp. having 38.2 $mg\,g^{-1}$ dry wt total lipid (Table 1) is closer to freshwater than to marine species.

Marine and freshwater green algal species also show different phospholipid compositions. Phospholipids vary from 6 to 22% in marine species, and from 14 to 24% in freshwater species [12–14]. The *Acrosiphonia* sp. is again closer to freshwater representatives than to marine ones.

Chara vulgaris and *Nitellopsis obtusa* belonging to the Charophyta occur mainly in freshwater ponds, particularly in those with hard lime water; some species are known to occur in saltish continental pools and in sea bays [15]. Charophytes, in their total lipid and phospholipid compositions, are closer to freshwater than to marine species.

The three examined macrophytic species contain similar amounts of glycolipids, from 39.8% in *N. obtusa* to 46.3% in *C. vulgaris*. Glycolipids found in marine green algae, as a rule, vary from 16 to 32%; glycolipids in freshwater species vary from 39 to 81% [14–16].

Phospholipid compositions of the three examined species are given in Table 2. The *Acrosiphonia* sp. differed from *C. vulgaris* and *N. obtusa* by a higher content of PG, 17.4% vs 4% and 9.7%, respectively. PG is the major

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Table 1. Lipid composition of some macrophytes from the Caspian Sea

	Acrosiphonia sp.	*Chara vulgaris*	*Nitellopsis obtusa*
Total lipids (TL)			
$mg\,g^{-1}$ fresh wt	11.6	18.1	16.7
$mg\,g^{-1}$ dry wt	38.2	73.2	32.5
Neutral lipids (NL)	33.9	26.6	35.1
Glycolipids (GL)	40.6	46.3	39.8
Phospholipids (PL)	25.5	27.1	25.1

NL, GL and PL are given as percentages of TL.

Table 2. Phospholipid composition of some Caspian macrophytes*

	Acrosiphonia sp.	*C. vulgaris*	*N. obtusa*
Phosphatidylglycerol (PG)	17.4 ± 0.5	4.0 ± 0.2	9.7 ± 0.3
Diphosphatidylglycerol (DPG)	—	—	4.0 ± 0.1
Phosphatidylinositol (PI)	31.6 ± 0.8	15.7 ± 0.6	11.2 ± 0.2
Phosphatidylethanolamine (PE)	3.9 ± 0.1	20.7 ± 0.7	13.6 ± 0.5
Phosphatidylserine (PS)	5.6 ± 0.2	0.5 ± 0.1	2.9 ± 0.1
Phosphatidic acid (PA)	7.9 ± 0.3	6.8 ± 0.2	5.7 ± 0.2
Phosphatidylcholine (PC)	33.6 ± 0.9	47.6 ± 1.3	39.7 ± 1.2
Lyso-phosphatidylcholine (LPC)			10.0 ± 0.4
X, unidentified phospholipid		4.8 ± 0.2	3.2 ± 0.1

*Percentage of total lipid phosphorus, see Experimental.

phospholipid among marine green algae and its content can vary from 30 to 52% [12, 13]. Freshwater algal species have lower PG contents, ranging from 6 to 18% [14]. From our own data, the *Acrosiphonia* sp. resembles a marine species while *C. vulgaris* and *N. obtusa* are closer to freshwater species.

The most important point of difference between marine and freshwater green algal species appears to be the presence of diacylglycerotrimethylhomoserine (DGTS) in all the marine green macrophytes and its absence from freshwater species [13, 17]. Our results from an examination of the phospholipids from *Acrosiphonia* showed that it contains a polar lipid with an R_f value similar to that of DGTS. Comparison with DGTS previously isolated by us from the marine microseaweed *Nephrochloris salina* [18] and the behaviour of this polar lipid in various chromatographic systems suggest that the polar lipid is indeed DGTS (no detailed physicochemical analysis has been undertaken). The intensity of the relevant chromatographic spot is comparable with that of PG, i.e. *ca* 10–15%. This polar lipid was not detected in *N. obtusa* whereas it was present in *C. vulgaris* in concentration of *ca* 5% judging by the spot intensity. It follows from the analyses of phospholipids and polar lipids of the Caspian macrophytes that the *Acrosiphonia* sp. is a typical marine species adapted to low salinity conditions. However, despite the presence of a high percentage PG, a low content of PE or the presence of a polar lipid similar to DGTS, the species in question has preserved the ratio between phospholipid classes and DGTS, characteristic of marine green macrophytic algae. *Nitellopsis obtusa* and *C. vulgaris*, from their lipid, glyco- and phospholipid characteristics, can be considered to be freshwater macrophytes adapted to a brackish environment.

Evolutionarily, fatty acids reflect the taxonomic particularities of algae to a lesser extent than polar lipids [2]; the latter are more conservative in this respect. Nevertheless, certain principles of the distribution of fatty acids among various taxonomic groups do exist, as reported by us earlier [1].

We have examined fatty acid compositions of neutral, glyco- and phospholipid fractions from three algal species. The neutral lipids from each differ considerably in their respective fatty acid compositions. Thus, the *Acrosiphonia* sp. contains 63.88% saturated fatty acids; *C. vulgaris* 54.81% and *N. obtusa* 40.42% (Table 3). Monoenoic acids comprise 19.46%, 26.85% and 15.98%, respectively. Polyunsaturation also differs between species; the greatest degree of unsaturation is found in *N. obtusa* (43.6%). *Chara vulgaris* and the *Acrosiphonia* sp. have almost equal amounts of unsaturated fatty acids. The relationship between saturated, monoenoic and polyenoic fatty acids is similar for all three species in the glyco- and phospholipid fractions (Tables 4 and 5).

The polyunsaturated C_{18} fatty acids of 18:2 (n-6), 18:3 (n-3), 18:4 (n-3) are the major ones in green marine algae [11, 19–22]. The acid 18:4 (n-3) was not detected in any lipid fraction of the three examined species. Green marine algae are known to possess high polyunsaturation in their C_{16} fatty acids, namely 16:1, 16:3, 16:4 [19–21, 23, 24].

Table 3. Fatty acid composition of neutral lipids from some macrophytes

	Acrosiphonia sp.	*C. vulgaris*	*N. obtusa*
n-12:0	2.31	2.22	1.65
n-14:0	5.42	5.22	3.88
n-15:0	2.31	2.22	1.65
n-16:0	30.58	22.71	16.58
n-17:0	2.81	2.71	2.01
n-18:0	9.84	9.47	7.04
n-19:0	0.70	0.68	0.50
n-20:0	3.71	3.57	2.66
n-21:0	0.60	0.58	0.43
n-22:0	0.60	0.58	0.43
n-23:0	0.10	0.10	0.07
n-24:0	0.40	0.39	0.29
n-26:0	0.10	0.10	0.07
iso-14:0	0.50	0.48	0.36
iso-15:0	0.70	0.68	0.50
iso-16:0	0.60	0.58	0.43
iso-17:0	0.50	0.48	0.36
anteiso-15:0	0.60	0.58	0.43
anteiso-17:0	0.70	0.68	0.50
Pristanic	0.40	0.39	0.29
Phytanic	0.40	0.39	0.29
Saturated	63.88	54.81	40.42
Iso-	2.30	2.22	1.65
Anteiso-	1.30	1.26	0.93
12:1	0.30	0.29	0.22
14:1 (n-5)	0.20	0.19	0.14
15:1 (n-6)	0.20	0.19	0.14
16:1 (n-9)	0.20	0.19	0.14
16:1 (n-7)	5.62	2.61	6.60
trans-			
16:1 (n-13)	0.20	0.19	0.14
17:1 (n-9)	0.10	1.10	0.07
17:1 (n-7)	0.20	0.19	0.14
18:1 (n-11)	0.20	0.19	0.14
18:1 (n-9)	8.53	19.13	5.60
18:1 (n-7)	0.50	0.48	0.36
19:1 (n-9)	0.50	0.48	0.36
20:1 (n-11)	0.20	0.19	0.14
20:1 (n-9)	1.31	1.26	0.93
21:1 (n-9)	0.10	0.10	0.07
22:1 (n-11)	0.50	0.48	0.36
22:1 (n-9)	0.40	0.39	0.29
24:1 (n-9)	0.10	0.10	0.07
26:1 (n-11)	0.10	0.10	0.07
Monoenoic	19.46	26.85	15.98
16:2 (n-4)	0.90	0.87	0.65
18:2 (n-6)	12.55	9.36	25.95
16:3 (n-3)	0.10	0.10	0.07
18:3 (n-6)	0.20	0.19	0.14
18:3 (n-3)	2.71	7.63	16.65
16:4 (n-3)	0.20	0.19	0.14
Di-, tri- and tetraenoic	16.66	18.34	43.60

Table 4. Fatty acid composition of glycolipids from some macrophytes

	Acrosiphonia sp.	*C. vulgaris*	*N. obtusa*
n-12:0	0.21	0.21	0.21
n-14:0	0.63	0.63	0.62
n-15:0	0.42	0.42	0.41
n-16:0	7.52	6.40	5.85
n-17:0	0.14	0.14	0.14
n-18:0	2.23	2.25	2.20
n-19:0	0.07	0.07	0.07
n-20:0	0.84	0.84	0.83
n-22:0	0.07	0.07	0.07
iso-14:0	0.07	0.07	0.07
iso-15:0	0.21	0.21	0.21
iso-16:0	0.14	0.14	0.14
anteiso-15:0	0.14	0.14	0.14
anteiso-17:0	0.07	0.07	0.07
Pristanic	0.07	0.07	0.07
Phytanic	0.07	0.07	0.07
Saturated	12.90	11.80	11.17
Iso-	0.42	0.42	0.42
Anteiso-	0.21	0.21	0.21
12:1	0.07	0.07	0.07
14:1 (n-5)	0.63	0.63	0.62
15:1 (n-6)	0.49	0.49	0.48
16:1 (n-9)	3.34	3.38	3.30
16:1 (n-7)	6.62	5.28	12.31
trans-			
16:1 (n-13)	0.77	0.77	0.76
17:1 (n-9)	0.28	0.28	0.28
17:1 (n-7)	0.63	0.63	0.62
18:1 (n-11)	1.11	1.13	1.10
18:1 (n-9)	24.95	24.91	11.14
18:1 (n-7)	2.93	2.96	2.89
20:1 (n-11)	1.25	1.27	1.24
20:1 (n-9)	3.76	3.80	3.71
22:1 (n-11)	0.70	0.70	0.69
22:1 (n-9)	1.18	1.20	1.17
24:1 (n-9)	0.07	0.07	0.07
Monoenoic	48.77	47.57	40.45
16:2 (n-4)	2.93	2.96	2.89
18:2 (n-6)	19.65	18.86	10.11
16:3 (n-6)	1.60	1.62	1.58
16:3 (n-3)	1.18	1.20	1.17
18:3 (n-6)	0.63	0.63	0.62
18:3 (n-3)	11.36	14.37	31.05
16:4 (n-3)	0.98	0.99	0.96
Di-, tri- and tetraenoic	38.33	40.63	48.37

The marine species *Acrosiphonia arcta* collected near the coasts of Canada, is reported to yield 16:3, 16:4 and 18:4 in amounts of 3%, 7.9% and 11.4%, respectively [21]. According to Johns *et al.* [22], one of these acids may be regarded as taxonomically important for all green marine macrophytes, as species from other divisions are found to have only trace amounts of the said acid. However, 16:4 (n-3) is reported to be absent from a number of green marine algae [25]. High percentages of 16:4 (n-3) have been found in many species from the genus *Enteromorpha* in various amounts ranging from 6 to 23% [11, 19, 20, 23, 24]. At the same time, this acid is reportedly absent from the genus *Codium* [23, 24, 26, 27] while some species from the genus *Ulva* possess the acid in relatively high amounts up to 18% [20]; other species from the same genera are not reported to contain it [25]. The reason for such inconsistency of knowledge concerning polyunsaturated C_{16} fatty acids is probably in the difficulty of identification [21]. The distribution of the C_{16} acids both in marine and freshwater algae as well as their taxonomic importance are worthy of careful attention and further investigation.

Table 5. Fatty acid composition of phospholipids from some macrophytes

	Acrosiphonia sp.	*C. vulgaris*	*N. obtusa*
n-12:0	0.44	0.56	0.53
n-14:0	0.69	0.88	0.84
n-15:0	0.50	0.64	0.61
n-16:0	10.33	4.54	4.10
n-17:0	0.25	0.32	0.30
n-18:0	1.31	1.67	1.59
n-19:0	0.13	0.16	0.15
n-20:0	0.56	0.72	0.68
n-21:0	0.06	0.08	0.08
n-22:0	0.13	0.16	0.15
iso-14:0	0.19	0.24	0.23
iso-15:0	0.19	0.24	0.23
iso-16:0	0.19	0.24	0.23
iso-17:0	0.06	0.08	0.08
anteiso-15:0	0.25	0.32	0.30
anteiso-17:0	0.13	0.16	0.15
Phytanic	0.06	0.08	0.08
Saturated	15.47	11.09	10.33
Iso-	0.63	0.80	0.77
Anteiso-	0.38	0.48	0.45
12:1	0.06	0.08	0.08
13:1	0.06	0.08	0.08
14:1 (n-5)	0.50	0.64	0.61
15:1 (n-6)	0.38	0.48	0.46
16:1 (n-9)	2.19	2.79	2.66
16:1 (n-7)	10.21	5.42	12.72
trans-			
16:1 (n-13)	0.81	1.04	0.99
17:1 (n-9)	0.56	0.72	0.68
17:1 (n-7)	0.50	0.64	0.61
18:1 (n-11)	0.88	1.12	1.06
18:1 (n-9)	25.17	25.02	13.86
18:1 (n-7)	2.13	2.71	2.58
20:1 (n-11)	1.69	2.15	2.05
20:1 (n-9)	3.01	3.82	2.64
22:1 (n-11)	0.75	0.96	0.91
22:1 (n-9)	0.81	1.04	0.99
24:1 (n-9)	0.13	0.16	0.15
Monoenoic	49.84	48.87	43.13
16:2 (n-4)	2.19	2.79	2.66
18:2 (n-6)	20.23	18.73	9.34
16:3 (n-6)	1.13	1.43	1.39
16:3 (n-3)	0.81	1.04	0.99
18:3 (n-6)	0.50	0.64	0.61
18:3 (n-3)	9.20	14.61	30.79
16:4 (n-3)	0.63	0.80	0.76
Di-, tri- and tetraenoic	34.69	40.04	46.54

We have demonstrated high percentages of 16:1 (n-7) in phospho- and glycolipid fractions (Tables 4 and 5). The acids 16:3 (n-6) and 16:3 (n-3) are found in all fractions but in small amounts. The content of 16:4 (n-3) did not exceed 1% in all the species examined. Green marine algae are known to contain 20:4 and 20:5 in total amounts ranging from 0.5 to 24% [1]. The presence of the latter acids is characteristic of green marine macrophytes but the Caspian species do not appear to contain them.

The adaptation of macrophytes, both of marine and freshwater origin, to brackish water would appear to influence the lipid composition and, to a greater extent, the fatty acid composition of macrophytes. Phospholipids are more conservative components of biomembranes, preserving the qualitative and quantitative characteristics of polar lipids inherited from the ancestral forms, particularly from marine algae.

EXPERIMENTAL

The macrophytes, *Acrosiphonia* sp., *C. vulgaris* L. and *N. obtusa* Gr. were sampled during September 1991 at a site located 70 km south of Krasnovodsk on the western coast of the Caspian Sea (Republic of Turkmenia) from a depth of 2–3 m; the temp. of the H_2O was 20°. The macrophytes were analysed immediately after sampling as described in refs [13, 14].

Qualitative and quantitative determination of lipids from extracts was done by micro TLC as described in refs [13, 28]. Phospholipid classes were isolated on silica gel TLC plates and detected as described elsewhere. The data shown in Table 2 were obtained from 4 parallel expts [13, 28].

Fatty acids as trimethylsilyl-oxazolines were prepd and analysed by capillary GC-MS as described in refs [29, 30] (data shown in Tables 3–5 were obtained from 3 experiments).

REFERENCES

1. Dembitsky, V. M. (1993) *Biochem. Syst. Ecol.* (in press).
2. Dembitsky, V. M., Rozenberg, G. S. and Kashin, A. G. (1993) *Biochem. Syst. Ecol.* (in press).
3. Gapochka, L. D. (1981) *The Adaptation of Algae.* p. 78. Moscow University Press.

4. Spektrov, K. S. and Stroganov, B. P. (1979) *Physiol. Plant. (U.S.S.R.)* **26**, 5.
5. Schwenke, H. (1958) *Kieler Meeresforsch.* **14**, 29.
6. Brown, L. M. (1977) *J. Phycol.* **13**, 2.
7. Vinogradova, K. L. (1977) in *The Life of Plants*, Vol. 3, pp.291–296. Prosveschenie Press, Moscow.
8. Vinogradova, K. L. (1979) *The Algae of Far East Seas of the U.S.S.R. (Green Algae)*, p.143. Nauka Press. Leningrad.
9. Jonsson, S. (1959) *Compt. Rend. Acad. Sci., Paris, Ser.* D **248**, 1565.
10. Jonsson, S. (1962) *Ann. Sci. Nat. Bot., Paris*, Ser. 2, **3**, 25.
11. Ackman, R. G. (1981) in *New Sources of Fats and Oils*, pp. 189–225. Champaign, Amer. Oil Chem. Soc.
12. Khotimchenko, S. V., Klochkova, N. G. and Vaskovsky, V. E. (1991) *Biochem. Syst. Ecol.* **18**, 93.
13. Dembitsky, V. M. and Rozentsvet, O. A. (1989) *Phytochemistry* **28**, 3341.
14. Dembitsky, V. M., Rozentsvet, O. A., Zuikova, V. S., Vasilenko, R. F. and Kashin, A. G. (1992) *Phytochemistry* **31**, 3259.
15. Gollerbach, M. M. (1977) in *The Life of Plants*, Vol. 3, pp.338–350. Prosvischenie Press, Moscow.
16. Khotimchenko, S. V. (1985) Dissertation, Vladivostok, p.141.
17. Khotimchenko, S. V., Vysotskii, M. V., Svetashev, V. I. and Vaskovskii, V. E. (1985) *Bioorgan. Khim.* **11**, 108.
18. Dembitsky, V. M. (1988) *Khim. Prir. Soed.* **4**, 589.
19. Dembitsky, V. M., Pechenkina-Shubina, E. E. and Rozentsvet, O. A. (1991) *Phytochemistry* **30**, 2279.
20. Jamieson, G. R. and Reid, E. H. (1972) *Phytochemistry* **11**, 1423.
21. Ackman, R. G. and Mclachlan, J. (1977) *Proc. Nat. Scot. Inst. Sci.* **28**, 47.
22. Johns, R. B., Nichols, P. D. and Perry, G. J. (1979) *Phytochemistry* **18**, 799.
23. Klenk, K., Knipprath, W., Eberhagen, D. and Koof, H. P. (1963) *Hoppe-Seyler's Z. Physiol. Chem.* **334**, 44.
24. Kato, M. and Ariga, N. (1982) *Kyoyobu Kenkyu Hakoku (Gifu Daigaku)* **18**, 53.
25. Hayashi, K., Kida, K., Kato, K. and Yayada, M. (1974) *Bull. Jap. Soc. Sci. Fish.* **40**, 609.
26. Sato, S. (1975) *Bull. Jap. Soc. Sci. Fish.* **41**, 1177.
27. Khotimchenko, S. V. and Svetashev, V. I. (1983) *Biol. Morya (Vladivostok)* **5**, 45.
28. Dembitsky, V. M., Bychek, I. A., Rozentsvet, O. A. and Shustov, M. V. (1992) *Cryptogamic Bot.* **2**, 391.
29. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Shustov, M. V. (1991) *Phytochemistry* **30**, 4015.
30. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Shustov, M. V. (1992) *Phytochemistry* **31**, 841.