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Hydrocarbons, Fatty Acids, and Lipids of Freshwater Grasses of the Potamogetonaceae Family

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Abstract—The composition of hydrocarbons, fatty acids, and of total, polar, and neutral lipids was studied in freshwater Potamogetonaceae grasses collected in two different regions of the Volga river. More than 40 fatty acids and hydrocarbons were separated and identified by chromato-mass spectrometry. The variability of lipid characteristics of plants of the same family is discussed.

Key words: fatty acids, neutral lipids, polar lipids, hydrocarbons, freshwater plants

The study was performed on freshwater grasses of the *Potamogeton* genus. These are higher freshwater plants widely distributed in Russia and the Middle Volga regions, which grow in stagnant and slow fresh and brackish waters [1, 2]. Higher water plants are known to have a stable homeostasis mechanism that promotes their occupation of large territories and wide geographical distribution [3, 4]. The resistance of living organisms to external influences significantly depends on their ability for adaptive rearrangements. On the cell level, these adaptive abilities are thought to be associated with the state of cell membranes. The membranes of resistant plants differ from the membranes of nonresistant ones by increased stability and by better maintenance of integrity under unfavorable conditions [4]. The main components of cell membranes are lipids that should be in a liquid state to provide normal functions of the membrane. And the normal functions are provided by the qualitative composition and quantitative ratio of individual lipids. Therefore, studies on lipid composition of resistant plants are of interest.

The present work is an extension of our comparative studies on lipids of water plants depending on their systematic and ecological status [5-9]. Because the comparison of unrelated species could significantly complicate the understanding of findings, in the present work lipids of more closely related plants of the same family were studied. The composition of lipids of various chemical structures and variability of lipid characteristics in the limits of this taxon were analyzed.

MATERIALS AND METHODS

Plant material. Representatives of the *Potamogeton* genus (*Potamogeton compressus* L., *Potamogeton friesii* Rupr., *Potamogeton pectinatus* L., *Potamogeton pusillus* L., *Potamogeton perfoliatus* L., *Potamogeton trichoides* Cham. et Schlect) are of the Potamogetonaceae family, the order of Najadales, the Liliopsida class [2]. The plants were collected according to methods for water plants [10]. For analysis, averaged samples of the biomass were taken which were represented by some whole plants, i.e., including leaves, stalks, and roots. The plants were collected in those reservoirs where they were predominant. Point 1 was a gulf of the Volga in the protected territory of the Samarskaya Luka reservation located 30 km downstream from the Kuibyshev hydroelectric power station; point 2 was another gulf of the Volga 130 km further downstream. The plants were collected in the end of July and early August, 1998.

Extraction and analysis of lipids. Specimens washed from epiphytes by running water were minced with a high-

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Abbreviations: DGDG) digalactosyldiacylglycerol; MGDG) monogalactosyldiacylglycerol; MEFA) methyl esters of fatty acids; SQDG) sulfoquinovosyldiacylglycerol; PG) phosphatidylglycerol; PI) phosphatidylinositol; PA) phosphatidic acid.

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speed homogenizer, and lipids were extracted by the method described in [11]. The total amount of lipids was determined gravimetrically. Neutral lipids were separated by one-dimensional thin-layer chromatography (TLC) on 10 × 10 cm plates with a fastened silica gel level (Khaapsalu, Estonia), successively using the following systems: toluene–hexane–formic acid (140 : 60 : 1) and hexane–diethyl ether–formic acid (60 : 40 : 1). Glycolipids were analyzed using the system acetone–benzene–water (91 : 30 : 8). Phospholipids were analyzed on 6 × 6 cm microplates. Quantitative determinations of phospholipids and of neutral lipids were performed by the methods of Vaskovsky [12] and of Kabara and Chen [13], respectively; the amount of glycolipids was determined by the content of galactose using the anthrone reagent [14].

Fatty acids were converted into methyl esters by reaction with 5% HCl in methanol for 1 h [11]. Methyl esters of fatty acids were purified by preparative TLC using the systems for neutral lipids [11]. Methyl esters of fatty acids were separated by gas–liquid chromatography with a Hewlett-Packard 5890 device (USA) (series 2) using a selective 5971B MSD mass-detector. Methyl esters were analyzed using successively connected capillary columns of 60 m in length. Column 1: RTX-1 (Restek, USA), 100% dimethyl polysiloxan, 30 m in length, internal diameter 0.32 mm, stationary phase of 1 µm in thickness; column 2: RTX-1701 (Restek), 14% cyanopropyl phenyl, 86% methyl polysiloxan, length 30 m, internal diameter 0.32 mm, the sorbent of 0.25 µm in thickness. A programmed regimen of the chromatograph was used: 2 min at 40°C, then an increase in the temperature at the rate of 2°/min up to 300°C. The injector temperature was 180°C, and the rate of the gas-carrier (helium) 25 cm/sec. MEFA were scanned in the range of 30-650 m/z. Fatty acids were identified using the mass-spectrum library (NBS49, NBS75, Wiley 138 & 275) as described in [15].

The findings are presented in Tables 1-4 and in the figure. The tables present the data of three repeated determinations.

RESULTS AND DISCUSSION

Depending on chemical structures, lipids have different functions and different variability corresponding to the organism's place in systematics and ecology [16]. Therefore, three classes of lipids (neutral, glyco- and phospholipids) were comparatively analyzed; the qualitative and quantitative relations of fatty acids and hydrocarbons were also compared.

The amount of total lipids in the plants studied varied from 5.9 to 15.7 mg/g wet weight (Table 1). Thus, there was a significant difference in the lipid accumulation by various species. Being of the same family, all plants studied were phylogenetically close. According to ecological classification, they all were hydrophytes [17]. By morphology, they were subdivided into two subgroups: platyphyllous emergent plants (*P. lucens*, *P. perfoliatus*) and stenophyllous emergent plants (*P. compressus*, *P. friesii*, *P. pectinatus*, *P. pusillus*, *P. trichoides*) [10]. The content of total lipids in macrophyllous pondweeds (5.9-6.5 mg/g wet weight) was lower than in narrow-leaved pondweeds (7.9-15.7 mg/g wet weight). The composition of fatty acids is the best studied in both adaptive and chemotaxonomic aspects. Thus, an increase in the content of unsaturated fatty acids or a decrease in the mean length of acyl chains under conditions of temperature stress are well

Table 1. Total lipids (mg/g wet weight) and neutral lipids (% of total neutral lipids) from freshwater Potamogetonaceae grasses

Lipids	*P. lucens*	*P. perfoliatus*	*P. pectinatus*	*P. trichoides*	*P. compressus*	*P. friesii*	*P. pusillus*
	1	1	2	1	2	2	2
Total lipids	6.5 ± 0.6	5.9 ± 0.5	9.6 ± 0.8	8.0 ± 0.8	15.7 ± 1.0	7.9 ± 0.7	12.3 ± 1.1
Monoacylglycerol	21.4 ± 1.0	22.3 ± 1.1	9.9 ± 0.5	10.3 ± 0.4	19.8 ± 0.8	16.0 ± 0.8	7.9 ± 0.3
Diacylglycerol	15.4 ± 0.7	13.9 ± 0.7	8.7 ± 0.3	35.5 ± 1.7	22.0 ± 1.5	16.7 ± 0.9	15.7 ± 0.9
Triacylglycerol	20.5 ± 0.8	20.0 ± 1.0	28.6 ± 1.0	19.6 ± 1.0	20.6 ± 0.8	26.0 ± 0.7	20.9 ± 0.9
Free acids	17.3 ± 0.6	17.5 ± 0.8	11.1 ± 0.5	12.1 ± 0.6	12.4 ± 0.5	20.0 ± 1.2	25.6 ± 1.2
Free sterols	7.3 ± 0.3	7.2 ± 0.3	7.4 ± 0.3	9.3 ± 0.4	7.0 ± 0.2	8.0 ± 0.1	5.6 ± 0.1
Sterol esters	3.0 ± 0.4	2.3 ± 0.1	3.5 ± 0.1	6.0 ± 0.3	2.4 ± 0.2	—	4.5 ± 0.2
Waxes	5.6 ± 0.2	7.9 ± 0.4	4.9 ± 0.1	4.0 ± 0.1	4.4 ± 0.4	4.0 ± 0.3	5.0 ± 0.1
Alcohols	4.5 ± 0.2	4.0 ± 0.2	5.5 ± 0.3	—	—	5.3 ± 0.2	—
Hydrocarbons	5.0 ± 0.2	4.8 ± 0.2	20.4 ± 1.0	3.0 ± 0.1	11.4 ± 0.9	4.0 ± 0.2	14.8 ± 0.7

Note: 1, 2 in the Tables 1-4 present the regions of collecting the specimens.

known. Such changes decrease the density of lipid packing in the membrane and thus provide its fluidity, which is required for multiple functions of the corresponding membrane-bound proteins [18-20]. Because fatty acids and their derivatives are involved in the regulation of growth and morphogenesis of all organisms including plants, fatty acids are often used as biological markers for taxonomy of plants [21]. Distinctions between large taxons are especially clear. However, the fatty acid composition of plants is very variable and markedly depends on conditions of their existence. It is still not clear how much the distinctions between the closely related species can be manifested. Therefore, new data on the fatty acid composition of a whole family, in particular, of Potamogetonaceae, are interesting for chemotaxonomy. Using chromato-mass spectrometry, we detected and identified more than 30 fatty acids (Table 2). However, the main fatty acids are 16:0, 18:1, 18:2, and 18:3, which are characteristic for most higher plants [21]. The total amount of these main acids varies from 75.7 (*P. pusillus*) to 92% (*P. compressus*). Palmitic acid (16:0) prevails among saturated acids in virtually all species studied, and it is the main acid in *P. lucens*, *P. pectinatus*, and *P. friesii*. Methyl-branched long-chain fatty acids (26-*methyl*-27:0) (*P. pectinatus*, *P. compressus*, *P. friesii*, *P. pusillus*) and polyunsaturated acids 20:3 and 20:4 are unusual for higher plants, although their relative content is rather low.

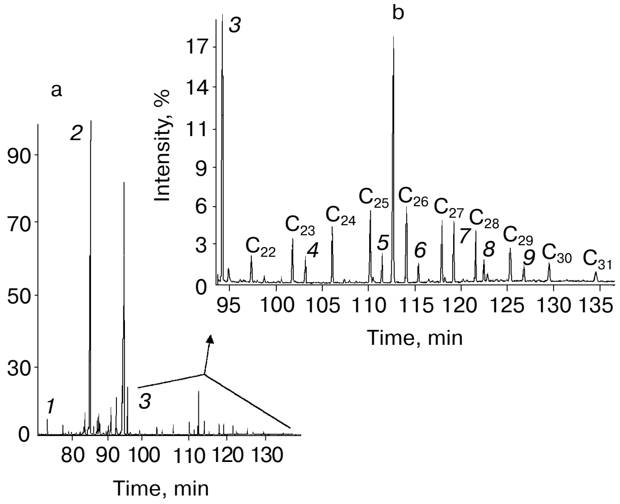
Chromato-mass spectrometry also allowed us to concurrently determine contents of fatty acids and hydrocarbons and to study them more in detail. The chromatogram (figure) clearly presents methyl esters of fatty acids and hydrocarbons. Note, that natural hydrocarbons are also very interesting as chemical markers in biogeochemistry [22, 23]. A definite class of hydrocarbons can be a specific feature only for certain groups of living organisms or plants [24]. C_{15}-C_{17} are the main hydrocarbons for many marine macrophytes [25], C_{26} or C_{24} are such for some species of green algae of the *Chlorella* genus [26]. Note that hydrocarbons of water plants of the Volga basin had not been studied earlier. Table 3 shows that all hydrocarbons are normal alkanes with even and odd numbers of carbon atoms in the chain. In all pondweeds studied four C_{24}-C_{28} hydrocarbons prevailed. Their quantitative characteristics varied depending on the species, but they remained dominant. Thus, the amount of C_{27} changed from 10.1% in *P. pectinatus* to 14.9% in *P. compressus*, and the content of C_{23} varied from 6.3% in *P. compressus* to 10.8% in *P. pectinatus*. The ratio of even and odd chains was approximately the same in all plant species. The total content of hydrocarbons in different species of the Potamogetonaceae family fluctuated in wide limits from 3.0 to 20.4% (Table 1).

Thus, the qualitative set of fatty acids and hydrocarbons is rather similar in all plants studied, except minor acids. But the quantitative ratio between them is a species-specific value in the limits of the taxon studied.

In addition to the hydrocarbons mentioned, neutral lipids in all pondweed species studied contained mono-, di-, and triacylglycerols, free fatty acids, free sterols and their esters, alcohols and waxes (Table 1). The sum of glycerol esters was 44.5-65.6%, and they prevailed in all pondweeds studied. The ratio between mono-, di-, and tri-substituted glycerol esters was species-specific. Thus, the contents of the three acylglycerols in *P. compressus* are rather similar: 19.8, 22.0, and 20.6% of mono-, di-, and triacylglycerols, respectively. In other species the content of triacylglycerols is higher than the contents of mono- and diacylglycerols, except *P. trichoides*, which contains 35.5% of diacylglycerols against 10.3 and 19.6% of mono- and triacylglycerols, respectively.

Free fatty acids act as lipid-soluble anions and play roles of transmembrane transporters of various physiologically active cations. Their contents can vary regardless of the phospholipid contents [27]. In our studies the content of fatty acids varied from 11.1% in *P. pectinatus* to 25.6% in *P. pusillus*. The pool of fatty acids can be contributed due to hydrolysis of membrane phospholipids as a result of activation of membrane phospholipases or of lipid peroxidation. Changes in the level of free fatty acids are thought to be a mechanism responsible for the maintenance of microviscosity of the plasmalemma in certain limits [28].

The level of sterols varies from 5.6 to 9.3% of the total neutral lipids (Table 1). Notwithstanding a rather low level of free sterols, their influence on the structure and functions of membranes is very great. Most (75%) of sterols are bound to plasmalemma [29]. First of all, plant



Chromatogram of hydrocarbons and methyl esters of fatty acids from *P. lucens*. Hydrocarbons and MEFA were separated by gas chromatography using paired capillary columns of different polarity: *1-9*) methyl esters of fatty acids; C_{22}-C_{31}) hydrocarbons. The identification of the peaks is presented in Tables 2 and 3

Table 2. Fatty acids (% of total fatty acids) from freshwater Potamogetonaceae grasses

Acid	*P. lucens*	*P. perfoliatus*	*P. pectinatus*	*P. trichoides*	*P. compressus*	*P. friesii*	*P. pusillus*
	1	1	2	1	2	2	2
12 : 0	—	0.04	—	0.12	0.15	0.1	—
14 : 0	1.2	1.5	0.33	0.65	0.76	1.0	2.2
9-(Me)-14 : 0	—	—	—	0.17	0.19	0.35	—
15 : 0	0.3	0.04	0.08	0.06	0.06	0.2	0.21
16 : 0	43.4	24.4	48.53	18.1	18.96	33.6	31.07
14-(Me)-16 : 0	—	0.21	—	1.06	0.59	—	0.63
17 : 0	0.4	0.01	0.46	0.72	0.77	0.71	3.95
18 : 0	4.1	3.3	0.8	3.03	1.19	4.1	—
11-(Me)-18 : 0	—	—	—	—	—	0.12	1.0
12-(Me)-18 : 0	—	—	—	—	—	—	0.13
19 : 0	—	0.1	0.04	0.07	0.09	0.15	0.5
20 : 0	0.4	0.5	0.37	0.34	0.37	0.89	—
22 : 0	0.4	0.2	0.15	—	0.47	—	6.82
23 : 0	0.3	1.0	0.67	0.44	0.24	1.42	0.34
24 : 0	0.9	0.32	0.26	0.21	0.44	0.66	1.03
25 : 0	0.5	0.73	0.66	0.39	0.07	1.65	0.16
26 : 0	0.3	0.06	0.06	0.07	0.07	0.19	0.42
27 : 0	—	0.16	0.16	0.12	0.02	0.11	—
26-(Me)-27 : 0	—	—	0.02	—	0.02	0.1	0.25
28 : 0	—	0.13	0.12	0.13	—	0.4	—
30 : 0	—	0.05	0.02	—	—	—	—
Saturated	52.2	32.99	52.01	25.68	24.46	45.75	48.71
7(Z)-16 : 1	1.0	1.6	0.56	0.69	0.94	0.37	0.93
9(Z)-16 : 1	2.0	4.67	10.53	0.61	0.59	1.44	5.74
9(Z)-18 : 1	9.2	13.96	8.28	19.03	29.36	24.46	25.59
11(Z)-20 : 1	—	0.1	—	—	—	—	—
Monounsaturated	12.2	20.33	19.37	20.33	30.89	26.47	32.18
7,10 (Z,Z)-16 : 2	—	—	0.72	—	—		—
9,12 (Z,Z)-18 : 2	9.5	4.32	12.96	16.39	15.06	17.08	7.04
11,14 (Z,Z)-20 : 2	—	0.1	—	—	—	—	—
9,12,15-18 : 3	26.1	41.9	14.3	37.5	29.59	10.68	11.99
5,8,11-20 : 3	—	0.09	—	—	—	—	—
5,8,11,14-20 : 4	—	0.26	0.64	—	—	—	—
Polyunsaturated	35.6	46.67	28.61	53.89	44.65	27.76	19.03

Note: Z) double-*cis* bond; Me) CH_3 or methyl group.

sterols influence the structure and permeability of membranes. Moreover, they can also act as plant hormones or their precursors. Along with free sterols, the plant species studied can synthesize sterol esters, and this is a specific character of higher plants. The amount of sterol esters is 2.3-6.0% of total neutral lipids.

Alcohols have not been found in every representative of the pondweed family. They are suggested to be intermediates in the synthesis of other lipid components.

Thus, the composition of neutral lipids had certain specific taxonomic features. In particular, glycerol esters are the main contributors to the neutral lipids, the next are sterols and their esters, and then waxes and alcohols follow. As to a rather high level of fatty acids and to wide limits of their changes, it is more associated not with taxonomic characteristics of the plants studied but with their abilities for adaptive rearrangements.

Glyco- and phospholipids are polar lipids. The qualitative composition of polar lipids is less affected by changes in the environment. However, the ratio of these lipids is also responsible for the maintenance of the phase state required for normal functions of the membrane [30, 31]. In most photosynthesizing plants glyco- and phospholipids are 40 and 20%, respectively, of the total lipids, and more than 50% of glycolipids are located in chloroplasts [32, 33]. Glycolipids of the plants studied include

Table 3. Hydrocarbons (% of total hydrocarbon) from freshwater Potamogetonaceae grasses

Hydrocarbons	*P. lucens*	*P. perfoliatus*	*P. pectinatus*	*P. trichoides*	*P. compressus*	*P. friesii*	*P. pusillus*
	1	1	2	1	2	2	2
C_{20}	1.5	1.8	1.1	1.1	0.4	0.3	1.3
C_{21}	2.8	2.3	2.3	2.5	1.8	1.6	2.9
C_{22}	5.4	4.8	5.1	4.9	3.3	4.6	6.1
C_{23}	8.3	6.7	10.8	7.7	6.3	9.9	8.9
C_{24}	11.5	10.7	13.2	11.6	9.9	10.1	11.9
C_{25}	14.2	13.9	14.8	14.0	15.2	13.7	14.6
C_{26}	15.1	17.9	13.4	14.3	13.8	13.5	15.0
C_{27}	12.3	14.4	12.1	13.1	14.9	12.8	10.4
C_{28}	10.2	11.3	9.3	10.1	9.6	11.6	9.4
C_{29}	7.9	6.8	6.2	9.2	8.7	7.6	6.8
C_{30}	5.6	4.9	5.0	5.3	6.3	6.9	6.4
C_{31}	3.2	2.8	4.3	3.7	5.7	5.1	2.9
C_{32}	2.0	1.7	2.4	3.0	4.1	2.3	3.4

Table 4. Polar lipids of freshwater Potamogetonaceae grasses

Lipids	*P. lucens*	*P. perfoliatus*	*P. pectinatus*	*P. trichoides*	*P. compressus*	*P. friesii*	*P. pusillus*
	1	1	2	1	2	2	2
Glycolipids*							
monogalactosyldiacylglycerol	56.1 ± 3.0	54.3 ± 1.8	51.9 ± 0.7	53.7 ± 0.8	60.6 ± 0.4	53.5 ± 1.0	60.0 ± 2.8
digalactosyldiacylglycerol	31.6 ± 1.7	28.6 ± 1.4	31.7 ± 0.6	36.6 ± 0.8	27.3 ± 0.7	28.7 ± 0.7	26.6 ± 0.9
sulfoquinovosyldiacylglycerol	12.3 ± 0.5	17.1 ± 0.7	16.4 ± 0.3	9.8 ± 0.3	12.1 ± 0.3	17.8 ± 1.1	13.4 ± 0.3
Phospholipids**							
phosphatidylcholine	48.2 ± 0.3	58.2 ± 1.7	40.4 ± 0.2	45.7 ± 2.7	40.4 ± 2.9	40.1 ± 0.3	49.0 ± 2.3
phosphatidylethanolamine	14.6 ± 0.4	19.9 ± 1.4	18.4 ± 0.9	16.9 ± 0.3	21.9 ± 0.4	18.4 ± 0.7	25.6 ± 0.7
phosphatidylglycerol	20.4 ± 0.6	14.0 ± 0.7	22.0 ± 0.7	18.1 ± 0.9	20.8 ± 0.8	14.8 ± 0.3	13.9 ± 0.9
diphosphatidylglycerol	2.8 ± 0.1	1.5 ± 0.2	4.0 ± 0.7	4.1 ± 0.2	2.4 ± 0.3	3.7 ± 0.2	4.4 ± 0.6
phosphatidylinositol	9.1 ± 0.4	5.9 ± 1.3	10.3 ± 0.3	4.9 ± 0.7	6.4 ± 0.8	8.9 ± 0.6	4.7 ± 0.7
phosphatidic acid	4.9 ± 0.5	0.4 ± 0.0	4.9 ± 0.3	10.3 ± 0.8	8.1 ± 1.7	14.0 ± 1.5	2.4 ± 0.5

* Glycolipids, % of total glycolipids.

** Phospholipids, % of total phospholipids.

two galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), and a sulfolipid, sulfoquinovosyldiacylglycerol (SQDG). Independently of the ecological or systematic status, MGDG prevailed in all pondweeds studied (51.9-60.6%), the fraction of DGDG being 26.6-36.6%. SQDG also found in all pondweeds studied was the lowest contributor to glycolipids (9.8-17.8%). The MGDG/DGDG ratio has an adaptive significance [33, 34]. It can vary depending on changes in the environment. We have found the MGDG/DGDG ratio to change from 1.5 to 2.3.

The ratio of individual phospholipids (Table 4) shows phosphatidylcholine to prevail, as in all photosynthesizing plants. Its amount varies from 40.1 to 58.2% depending on the plant species. Depending on the systematic position, the second place in the contribution to phospholipids belongs either to phosphatidylglycerol or to phosphatidylethanolamine. Thus, phosphatidylglycerol prevails compared to phosphatidylethanolamine in *P. lucens*, *P. pectinatus*, and *P. trichoides*, whereas the second compound prevails in *P. compressus*, *P. pusillus*, and *P. pectinatus*. The relative contributions of other phospholipids are lower: the content of diphosphatidylglycerol, which was found in all plants studied, was 1.5-4.4% and the content of phosphatidylinositol together with phosphatidylserine was 4.7-10.3%.

Thus, our findings significantly extend concepts on the composition of hydrocarbons, fatty acids, and on the lipid profile of water plants. The qualitative composition of neutral lipids, fatty acids, and polar lipids is shown to be typical for both a taxon of the family level and all higher plants. Despite some quantitative variations in polar lipids, MGDG prevails in glycolipids and phosphatidylcholine prevails in phospholipids, and this is also specific for the majority of higher plants. The ratios of individual components in the class of neutral lipids indicate that such features as contents of glycerol esters, of sterols and their esters, of waxes are also rather similarly distributed in the plants of the taxon studied and can be used as an additional taxonomic character.

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