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ACETYLENIC ACIDS AND LIPID COMPOSITIONS OF SOME MOSSES FROM RUSSIA

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Key Word Index—*Dicranum polysetum*; *Pleurozium schreberi*; *Polytrichum juniperinum*; mosses; acetylenic acids; lipids; phospholipids; fatty acids.

Abstract—The composition of lipids, phospholipids and fatty acids from three moss species was studied. The major phospholipid classes were phosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine. Fatty acid composition was examined by capillary GC-MS in the neutral, glyco- and phospholipid fractions; eighty-five fatty acids were identified. The identification of acetylenic acids was achieved using an original technique. The neutral fraction from *Dicranum polysetum* yielded 72% acetylenic acids amongst which some unusual rarely occurring acids were identified, i.e. 9a,12-18:2, 6a,9,12,15-18:4, 8a,11,14-20:3 and 5a,8,11,14-20:4. The presence of 16:4 (*n*-3) in mosses was identified for the first time.

INTRODUCTION

The group of species bearing the general name bryophytes includes mosses and liverworts and forms the third largest group, after angiosperms and fungi, in the plant kingdom [1]. Mosses are distinctive in many ways; they have a peculiar lipid composition. Some species are reported to contain arachidonic and eicosapentaenoic acids in high concentrations [2–8] compared to those in red [9], brown [10] and some green [9, 11, 12] seaweeds and in other algal groups [13]. Other compounds detected in mosses include epoxyhydroxy, hydroxy, dicarboxylic acids, saturated normal fatty alcohols [14–18], hydroxy and epoxy fatty alcohols [19], phytols [3, 16, 17, 20] and acetylenic fatty acids (AFA) [21–26]. Mosses are highly responsive to environmental pollution and are known to store chlorinated hydrocarbons [27–30]. Phospholipids from mosses have not been extensively studied.

The present work deals with phospholipid and fatty acid compositions of mosses growing in the European part of Russia.

RESULTS AND DISCUSSION

Table 1 shows the lipid composition of the three mosses examined. Total lipids varied from 27.9 to 53.7 mg g^{-1} dry wt. Neutral lipids make up more than half of the total lipid composition while the quantity of glycolipids is twice that of phospholipids. Total lipids showed a strong seasonal variation, e.g. in *Dicranum elongatum* they made up 50 mg g^{-1} dry wt from a January collection in contrast to a June one where they comprised 112 mg g^{-1} dry wt [31]. The latter figure is similar to that obtained by other authors and ourselves, namely 30–280 mg g^{-1} dry wt [7, 8, 23, 32–34].

Neutral lipids are present in the greatest amount (Table 1) according to various authors and our own data [7, 23, 32, 34]. Triacylglycerols can reach as high as 66% among the neutral lipids. Some moss species are known to posses fairly high amounts of glycolipids; in *Hygrohypnum luridum* they make up 73% in gametophores [23].

Amongst the polar lipids, phospho- and glycolipids, the latter predominate (Table 1). *Ceratodon purpureus* [35] possesses 36.7% MGDG, 24% DGDG and 20.9% PC. Individual polar lipids contained in various moss species are known to be highly varied but their distribution patterns seem hard to define. Thus, the major glycolipid in *Ctenidium molluscum* was found to be DGDG (30%) followed by MGDG (19.7%) and trace

Table 1. Lipid composition of mosses

Genera/species	TL	NL	GL	PL
Dicranaceae				
Dicranum polysetum	48.9	60.14	25.90	13.96
Entodontaceae				
Peurozium schreberi	27.9	59.13	28.80	12.07
Polytrichaceae				
Polytrichum juniperinum	53.7	50.62	32.33	17.05

TL, total lipids (the sum of acyl and unsaponifiable lipids), mg g^{-1} dry wt; NL, neutral lipids; GL, glycolipids; PL, phospholipids. NL, GL and PL as percentage of TL.

Table 2. Phospholipid composition of mosses (% of total lipid)

Species	PG	PC	PE	PI+LPE	PS
Dicranum polysetum	8.8±0.5	58.9±1.1	17.7±0.8	11.7±0.4	2.9±0.2
Pleurozium schreberi	15.5±0.6	55.6±1.3	20.0±0.9	8.9±0.3	—
Polytrichum juniperinum	20.8±0.8	33.4±0.7	25.0±0.5	16.6±0.7	4.2±0.1

PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; LPE, liso-PE; PS, phosphatidylserine; PI+LPE, the sum of PI and LPE.

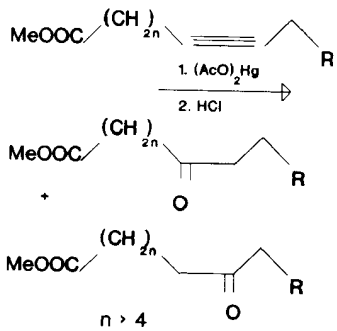
amounts of SQDG. PG was the major class among the phospholipids and amounted to 65.8% while PC was practically absent [32]. In the moss *Tortella tortuosa*, MGDG made up 20.6% and was the major one while DGDG amounted to 12.2%; noticeable among phospholipids was PE while PG and PC were detected only in trace amounts [32].

Among the phospolipid classes only six were found in the mosses examined; they were PC, PE, PG, PS, PI and LPE (Table 2). PC in two moss species exceeded 55%; the second in amount was PE followed by PG. PS was found in small amounts only in *D. polysetum* and *P. juniperinum* at 2.9 and 4.2%, respectively.

All the examined species contained a polar lipid having R_f 0.75 in the first direction and R_f 0.45 in the second direction when subjected to two-dimensional TLC on silica gel. The content of the polar lipid, judging by the spot intensity after treatment with H_2SO_4 in MeOH, varied according to species and could be defined as levels of PS or the sum of PI and LPE, i.e. 2–10%. The quantitative analysis of the polar lipid was not attempted because the lipid did not respond to phosphorus. The position of the lipid in its R_f on a TLC plate strongly suggested diacylglycerotrimethylhomoserine (DGTS). A polar lipid having an R_f similar to DGTS is widespread among green macrophytic algae [36, 37]; in the class Ulotrichophyceae its content can be as high as 56.5% of the total polar lipids including phospholipids [36], while PC is absent from the species of this class. The data obtained from the examination of a greater number of species could help to throw light on the role of DGTS. PC is absent from some moss species [36, 38] as well as Ulotrichophyceae algae [36].

Moss lipids possessing fatty acids characteristic of plants also have fatty acids having four or five double bonds, such as arachidonic (20:4) or eicosapentaenoic (20:5) [2–8, 31, 34]. Certain species also contain acetylenic acids, mainly 9,12,15-octadecatrien-6-ynoic acid, with smaller amounts of 9,12-octadecadien-6-ynoic and 11,14-eicosadien-8-ynoic acids [21–26]. We have developed a method for identifying acetylenic fatty acids based on the interaction between the triple bond and mercuric acetate, followed by HCl treatment to give two isomers (Scheme 1).

GC-MS analysis of the neutral fraction from mosses showed the domination of acetylenic fatty acids in *D. polysetum* (Table 3). The total neutral lipid fraction was transmethylated (5% HCl in MeOH) and divided into two parts. The first part was analysed on polar capillary



Scheme 1. Reaction scheme for identification of acetylenic fatty acids.

columns as methyl esters (FAME). The second part was refluxed with $(MeCO)_2$ Hg and the total adduct was regenerated by HCl in MeOH [39, 40]. The original double bond(s) is regenerated from the triple bond(s) by reaction with 2 mol of mercuric acetate; the original double bond(s) cannot be regenerated and keto (oxo)-derivatives are formed by acid hydrolysis.

The mixture was separated by prep. TLC on silica gel G (hexane–diethyl ether, 9:1). Two bands were obtained (R_f 0.9 unchanged FAME and R_f 0.7, keto FAME). The keto FAME was dissolved in MeOH and reduced for 3 hr with $NaBH_4$. After acidification (HCl) the hydroxy-FAME was extracted and trimethylsilyl-oxazolines were prepared and analysed by GC-MS as described elsewhere [41]. This sequence of reactions was excellent, but had one drawback. Addition of mercuric acetate to a triple bond is controlled by the Markovnikov rule and two isomers are obtained (in a ratio of *ca* 1:1). These positional isomers could not be separated by capillary GC, although in the mass spectrum two hydroxy-isomers were identified in every peak (as trimethylsilyl ethers).

Three AFAs were identified among the monoenes, i.e. 6a–18:1, 9a–18:1 and 12a–18:1, and three AFAs among the dienes, 6a,9–18:2, 9a,12–18:2, 9,12a–18:2. AFAs among polyenoic acids comprised 90% (Tables 3–5). The other examined species yielded smaller percentages. High amounts (*ca* 40%) of 18:3 and 18:4 acetylenic acids were noteworthy in *D. polysetum*; the same acids were detected in the other species. Total unsaturation in the individual species varied from 58% in *Polytrichum juniperium* to 73% in *Pleurozium schreberi* and up to 90% in *D. polysetum*.

Table 3. Fatty acid composition of neutral lipids from mosses

Fatty acids (% of total)	*D. polysetum*	*P. juniperinum*	*P. schreberi*
n-12:0	0.56	0.89	0.88
n-13:0	0.17	0.26	0.25
n-14:0	0.32	0.52	0.50
n-15:0	0.25	0.39	0.38
n-16:0	5.11	22.01	12.58
Phytanic	—	0.26	0.25
n-17:0	0.48	0.77	0.75
n-18:0	2.13	11.39	5.53
n-19:0	0.09	0.39	0.50
n-20:0	0.39	1.65	0.88
n-21:0	0.25	0.77	1.13
n-22:0	0.17	0.52	0.88
n-23:0	0.09	0.14	0.13
n-24:0	0.17	0.51	0.63
n-25:0	0.09	0.13	—
anteiso-13:0	0.09	0.13	0.13
anteiso-17:0	—	0.13	0.13
anteiso-19:0	—	0.25	0.38
iso-12:0	0.17	0.25	0.25
iso-13:0	0.17	0.25	0.25
iso-14:0	0.24	0.38	0.38
iso-17:0	—	0.25	0.25
Saturated	10.86	42.24	27.04
anteiso-	0.09	0.51	0.64
iso-	0.58	1.13	1.13
12:1 (*n*-5)	0.24	0.38	0.38
13:1 (*n*-5)	0.16	0.25	0.25
14:1 (*n*-5)	0.31	0.51	0.50
15:1 (*n*-7)	0.24	0.38	0.38
16:1 (*n*-9)	0.94	7.21	12.33
16:1 (*n*-7)	0.63	1.64	4.40
17:1 (*n*-9)	0.24	0.38	0.38
iso-17:1 (*n*-9)	0.16	0.38	0.38
anteiso-17:1 (*n*-9)	—	0.63	0.63
17:1 (*n*-7)	0.16	0.25	0.25
18:1 (*n*-11)	0.47	1.52	1.01
18:1 (*n*-9)	5.02	25.03	11.95
18:1 (*n*-7)	0.16	0.63	0.88
6a-18:1*§	5.02	—	—
9a-18:1*§	6.99	—	—
12a-18:1*§	2.98	—	—
19:1 (*n*-8)	—	0.51	0.25
20:1 (*n*-11)	0.08	1.14	0.38
20:1 (*n*-9)	0.16	0.13	0.25
21:1 (*n*-8)	0.08	0.13	—
22:1 (*n*-11)	0.08	0.13	0.38
22:1 (*n*-9)	0.16	—	0.25
24:1 (*n*-11)	0.08	0.25	—
Monoenoic	24.36	41.35	35.23
Acetylenic	14.99	—	—
18:2 (*n*-6)	2.90	5.94	15.47
18:2 (*n*-3)	—	0.25	0.38
6a,9-18:2‡§	12.09	0.25	0.63
9,12a-18:2‡§	0.94	—	—
9a,12-18:2†§	1.18	—	—
Dienoic	17.11	6.44	16.48
Acetylenic	14.21	0.25	0.63
18:3 (*n*-6)	1.41	1.90	2.14
18:3 (*n*-3)	3.38	1.52	6.54
18:4 (*n*-3)	—	1.01	—
6a,9,12-18:3‡§	15.70	1.26	11.31
8a,11,14-20:3‡§	1.28	—	—
6a,9,12,15-18:4	23.47	4.28	1.26
5a,8,11,-14-20:4‡	2.43	—	—
Polyenoic	47.67	9.97	21.25
Acetylenic	42.88	5.54	12.57
The sum of acetylenic acids	72.08	5.79	13.20

*Known in nature.
†Prepared only by synthesis.
‡Known in some mosses and/or liverworts.
§Structure was confirmed by GC-MS of trimethyl-oxazolines after preseparation, see refs [41, 59].

Using our technique for triple bond determination we found common acetylenic acids having the triple bond in position 6; among monoenoic acetylenic acids, we identified those with the triple bond in positions 6, 9 and 12 (Table 3). Among the dienes, apart from the common acid 6a, 9–18:2, we identified acids having the triple bond in positions 9 and 12. Among the polyenoic acids, of interest were 18:4, 20:3 and 20:4. The 20:2 acid with the triple bond in position 8 had been previously identified [22, 42], while we identified acid 20:3 with the acetylenic bond also in position 8. The acid 20:4 had the triple bond in position 5. Arachidonic acid from algae and living organisms characteristically possesses double bonds in positions 5, 8, 11 and 14. The order Dicranales is unusual in containing rather large amounts of acetylenic acids [26] which are completely absent from or present only in small amounts in other families [5].

A few known techniques for the quantitative determination of acetylenic bonds in aliphatic chains are: interaction with methyl magnesium iodide [43–45], precipitation in the form of acelenide of metals [46] and the method of Hanna and Siggia [47]. Water solutions (even acid ones) of mercuric salts act to form salts. The mechanism of such formation is not completely clear. An acid acts on salts to release water adducts, in particular, ketones [48].

An isolated triple bond combines with $AgNO_3$ about as strongly as does an isolated *cis*-ethylenic double bond and allows comparable separations between saturated and unsaturated fatty acids [49]. Conjugated triple bonds and conjugated ethylenic and triple bonds form a complex with $AgNO_3$ less readily and become less retarded

Table 4. Fatty acid compositions of glycolipids from mosses

Fatty acids (% of total)	*D. polysetum*	*P. juniperinum*	*P. schreberi*
n-12:0	0.96	1.07	1.02
n-13:0	0.05	0.06	0.06
n-14:0	0.59	0.66	0.63
n-15:0	0.27	0.30	0.28
Pristanic	0.43	0.48	0.46
n-16:0	10.61	5.01	15.42
Phytanic	0.43	0.48	0.46
n-17:0	0.54	0.60	0.57
n-18:0	3.05	6.50	1.82
n-19:0	0.27	0.06	—
n-20:0	0.21	0.12	0.51
n-21:0	—	0.18	0.40
n-22:0	0.11	—	0.28
n-23:0	0.11	0.06	0.23
n-24:0	—	—	0.06
n-25:0	—	—	0.28
n-26:0	—	—	0.34
anteiso-15:0	0.05	0.06	0.06
anteiso-13:0	0.11	0.12	0.11
anteiso-19:0	0.11	—	—
iso-13:0	0.05	0.06	0.06
iso-14:0	0.27	0.30	0.28
iso-15:0	0.16	0.18	0.17
iso-16:0	0.11	0.12	0.11
iso-17:0	0.16	0.18	0.17
Saturated	18.65	16.60	23.78
anteiso-	0.27	0.18	0.17
iso-	0.75	0.84	0.79
12:1 (*n*-5)	0.64	0.72	0.68
13:1 (*n*-5)	0.16	0.18	0.17
14:1 (*n*-5)	0.54	0.60	0.57
15:1 (*n*-9)	0.16	0.18	0.17
15:1 (*n*-7)	0.48	0.54	0.51
16:1 (*n*-9)	6.43	5.72	5.69
16:1 (*n*-7)	0.75	5.43	2.11
trans-16:1 (*n*-13)	0.37	0.42	0.40
17:1 (*n*-9)	0.64	0.72	0.68
iso-17:1 (*n*-9)	0.16	0.18	0.17
anteiso-17:1 (*n*-9)	0.11	0.12	0.11
17:1 (*n*-7)	0.32	0.36	0.34
18:1 (*n*-11)	0.48	1.43	0.85
18:1 (*n*-9)	5.73	14.73	8.76
18:1 (*n*-7)	0.48	1.61	0.80
19:1 (*n*-8)	0.16	0.12	0.06
20:1 (*n*-11)	0.32	0.12	0.23
20:1 (*n*-9)	0.11	0.18	0.28
21:1 (*n*-8)	0.05	0.06	0.06
22:1 (*n*-11)	0.05	0.12	0.06
22:1 (*n*-9)	—	0.06	0.17
24:1 (*n*-11)	—	—	0.28
24:1 (*n*-9)	—	—	0.40
25:1 (*n*-9)	—	—	0.11
26:1 (*n*-11)	—	—	0.06
26:1 (*n*-9)	—	—	0.11
Monoenoic	18.09	33.60	23.83
16:2 (*n*-4)	0.27	0.30	0.28
18:2 (*n*-6)	9.86	9.42	14.23
18:2 (*n*-3)	1.02	1.01	1.25
20:2 (*n*-6)	0.91	0.83	1.54
22:2 (*n*-6)	0.15	0.48	0.97
Dienoic	12.21	12.04	18.27
16:3 (*n*-6)	0.59	0.66	0.63
16:3 (*n*-3)	0.37	0.42	0.40
18:3 (*n*-6)	13.98	1.25	1.65
18:3 (*n*-3)	22.92	13.95	8.54
20:3 (*n*-9)	0.27	1.13	1.76
20:3 (*n*-6)	0.70	1.61	0.80
20:3 (*n*-3)	0.43	0.42	0.97
22:3 (*n*-6)	0.27	0.36	1.31
22:3 (*n*-3)	0.16	0.30	1.02
16:4 (*n*-3)	1.29	1.43	1.37
18:4 (*n*-3)	3.96	1.73	1.42
20:4 (*n*-6)	2.52	7.77	6.68
20:4 (*n*-3)	0.48	0.83	1.76
22:4 (*n*-3)			0.57
20:5 (*n*-3)	3.11	5.90	5.24
Polyenoic	51.05	37.76	34.12

on aggregation TLC. This method, therefore, cannot provide the total resolution of mixtures of fatty acid esters containing ethylenic and acetylenic bonds [50]. GC of simple acetylenic esters on both polar and non-polar liquid phases and conventional columns is effective for their tentative identification provided that proper reference compounds are available [51–53].

FAME with triple bonds yield mass spectra with prominent peaks resulting from cyclic elimination with hydrogen transfer [54]. Esters with unsaturation at 2, 3, 4 or terminal positions have spectra different from those having the triple bond at the central positions of the molecule. Because of secondary fragmentations and shift of the triple bond to other positions, it is not possible to determine the exact location of the triple bond. The position of the triple bond in the MEFA can be located after deuteration by way of pyrolysis, or by any of the other methods applicable to olefinic esters after a partial reduction of the triple bond to a double bond [55].

All the above-mentioned techniques have a number of drawbacks; therefore, they are of little use for the identification of acetylenic bonds in aliphatic acids. The mercuric acetate procedure for triple bond(s) identification in fatty acids is much more useful.

We did not find acetylenic acids either in the glyco- or in the phospholipid fraction of the mosses examined (Tables 4, 5). All acetylenic acids were contained in the neutral lipids.

The glycolipid fractions of the mosses had similar amounts of unsaturation above 75% (Table 4). The contents of arachidonic and eicosapentaenoic acids (6%) were low in *D. polysetum* but were 14% in *P. schreberi*.

Table 5. Fatty acid compositions of phospholipids from mosses

Fatty acids (% of total)	*D. polysetum*	*P. juniperinum*	*P. schreberi*
n-12:0	0.47	0.39	0.45
n-13:0	0.13	0.11	0.12
n-14:0	0.72	0.60	0.69
n-15:0	0.38	0.32	0.37
n-16:0	12.01	8.47	5.31
n-17:0	0.72	0.60	0.69
n-18:0	4.09	1.76	1.14
n-19:0	0.34	0.07	0.08
n-20:0	0.43	0.18	0.29
n-21:0	0.04	0.07	0.37
n-22:0	0.12	0.04	0.12
n-23:0	—	0.07	0.04
n-24:0	0.13	0.04	0.08
n-26:0	—	0.04	—
anteiso-13:0	0.21	0.18	0.20
anteiso-15:0	0.09	0.07	0.08
anteiso-17:0	0.09	0.07	0.08
anteiso-19:0	0.21	—	—
iso-12:0	0.04	0.04	0.04
iso-13:0	0.17	0.14	0.16
iso-14:0	0.51	0.42	0.49
iso-15:0	0.26	0.21	0.24
iso-16:0	0.47	0.39	0.45
iso-17:0	0.21	0.18	0.20
Saturated	21.84	14.46	11.69
anteiso-	0.60	0.32	0.36
iso-	1.66	1.38	1.58
12:1 (*n*-5)	0.34	0.28	0.33
13:1 (*n*-5)	0.30	0.25	0.29
14:1 (*n*-5)	0.68	0.56	0.65
15:1 (*n*-9)	0.26	0.21	0.24
15:1 (*n*-7)	0.77	0.63	0.73
16:1 (*n*-9)	1.66	5.20	9.43
16:1 (*n*-7)	3.19	0.42	4.08
17:1 (*n*-9)	0.77	0.63	0.73
iso-17:1 (*n*-9)	0.38	0.32	0.37
anteiso-			
17:1 (*n*-9)	0.21	0.18	0.20
17:1 (*n*-7)	0.47	0.39	0.45
18:1 (*n*-11)	1.19	1.30	0.78
18:1 (*n*-9)	6.43	11.07	8.86
18:1 (*n*-7)	1.32	0.74	1.27
19:1 (*n*-8)	0.47	0.11	0.20
20:1 (*n*-11)	0.30	0.11	0.08
20:1 (*n*-9)	0.13	0.07	0.12
21:1 (*n*-8)	0.09	0.04	0.04
22:1 (*n*-11)	0.09	0.11	0.04
22:1 (*n*-9)	0.04	0.07	0.08
24:1 (*n*-11)	—	0.07	—
24:1 (*n*-9)	—	0.04	—
Monoenoic	19.09	22.80	28.97
anteiso-	0.21	0.18	0.20
iso-	0.38	0.32	0.37
14:2 (*n*-5)	0.13	0.11	0.12
16:2 (*n*-4)	0.81	0.67	0.78
18:2 (*n*-6)	10.05	10.40	13.02
18:2 (*n*-3)	0.98	1.16	1.22
20:2 (*n*-6)	0.38	0.67	0.94
22:2 (*n*-6)	0.26	0.32	0.78
Dienoic	12.61	13.33	16.86
16:3 (*n*-6)	0.98	0.81	0.94
16:3 (*n*-3)	1.02	0.84	0.98
18:3 (*n*-6)	13.12	0.88	1.84
18:3 (*n*-3)	14.22	14.58	10.20
20:3 (*n*-9)	0.51	1.19	1.39
20:3 (*n*-6)	0.77	0.49	0.65
20:3 (*n*-3)	0.72	0.81	0.49
22:3 (*n*-6)	0.34	0.39	0.57
22:3 (*n*-3)	0.21	0.40	1.18
24:3 (*n*-3)	0.26	0.11	—
16:4 (*n*-3)	1.49	1.23	1.43
18:4 (*n*-3)	4.22	3.30	1.71
20:4 (*n*-6)	4.05	13.14	10.41
20:4 (*n*-3)	1.15	1.65	0.94
22:4 (*n*-3)	0.72	0.81	1.67
20:5 (*n*-3)	2.68	8.78	8.08
Polyenoic	46.46	49.41	42.48

The phospholipid fraction (Table 5) was found to contain the greatest variety of iso- and anteiso- acids. The summed content of archidonic and eicosapentaenoic acids in the phospholipid fraction was somewhat higher than that in the neutral or glycolipid ones and reached 23% in *P. juniperinum*.

Glycolipid and phospholipid fractions contained 16:4 (*n*-3) at 1.29–1.49%. The presence of this acid has not been reported before for mosses; it is characteristically present in green seaweeds and found in many species [11–13], comprising 4.9% in *Briopsis hypnoidea* [9] to 20.3% in *Enteromorpha intestinalis* [56] and 23.1% in *E. linza* [9]. However, the acid is absent from some green seaweeds [11, 12, 57, 58].

The main fatty acids in *D. polysetum* were 6a,9–18:2, 6a,9,12–18:3 and 6a,9,12,15–18:4 (neutral fraction); 16:0, 18:2 and 18:3 (glycolipid fraction); 16:0, 18:2 and 18:3. The main fatty acids in *P. juniperim* were 16:0, 18:0 and 18:1 (neutral fraction); 18:1, 18:2 and 18:3 (glycolipid fraction); 18:1, 18:2, 18:3 and 20:4 (phospholipid fraction).

EXPERIMENTAL

The mosses *Dicranum polysetum* Sw., *Pleurozium schreberi* (Brid.) Mitt. and *Polytrichum juniperinum* Hedw. were sampled in May 1992, in the Zhiguli nature reserve near Togliatti. Freshly collected mosses were thoroughly cleansed of extraneous matter and homogenized in a high-speed unit.

The extraction of lipids, their analyses and quantification, and the identification of individual phospholipid

classes were carried out as described elsewhere [59, 60] The total lipid extract was sepd on a silica gel column according to the method of ref. [61] into the neutral, glyco- and phospholipid frs. PLs were isolated by silica gel TLC using (i) $CHCl_3$–MeOH–benzene–NH_4OH (65:30:10:6) and (ii) $CHCl_3$–MeOH–benzene–Me_2CO–HOAc–H_2O (70:30:10:5:4:1). To identify PLs on TLC plates, the following specific reagents were used: ninhydrin 0.2% in Me_2CO for free amino groups, Dragendorff's reagent for choline-containing lipids, modified Jungnickel's reagent for P-containing lipids [62] and periodate–Schiff's reagent for PG.

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