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Fatty Acid Composition of Polar Lipids in *Ceratodon purpureus* and *Pleurozium schreberi*

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Abstract

The total amount of fatty acids in the mono- (MGDG) and diglycosyl diglyceride (DGDG) and more polar lipid fractions of frozen *Ceratodon purpureus* shoots was 4.6, 3.4 and 4.0 mg/g dry weight, respectively. The respective values for the tops of frozen *Pleurozium schreberi* were 2.6, 3.3 and 3.8 mg/g dry weight. The molar ratios MGDG/DGDG and MGDG + DGDG/chlorophyll were 1.3 and 3.7, respectively, for *C. purpureus* and 0.8 and 3.5 for *P. schreberi.*

In *C. purpureus* the main fatty acids in the MGDG fraction were C 18:3ω3 (44% of the total fatty acids) and C 16:3ω3 (26%); in the DGDG fraction C 18:3ω3 (70%); and in the more polar lipid fraction C 18:3ω3 (26%) and C 16:0 (25%). The proportion of C 20 polyunsaturated fatty acids was 15, 12 and 19% of the total fatty acids found in the MGDG, DGDG and more polar lipid fractions, respectively.

In *P. schreberi* the proportion of C 20 polyunsaturated fatty acids was high in all polar lipid fractions (47, 42 and 25% in MGDG, DGDG and more polar lipid fractions, respectively). In addition, MGDG and DGDG fractions contained abundantly C 18:3ω3 (32 and 45%, respectively), and the more polar lipid fraction both C 18:3ω3 (24%) and C 16:0 (27%).

Introduction

Recent investigations indicate a great variation in the fatty acid composition of glycolipids of various moss species. Among the major components are C 16:3ω3, C 18:3ω3, C 20:4ω6 and C 20:5ω3 acids, contrasting with higher plants where C 18:3ω3 is the only main component (Hitchcock and Nichols 1971) plus C 16:3ω3 accumulated in the MGDG fraction of some species (Jamieson and Reid 1971).

In the glycolipids of moss species such as *Polytrichum commune* (protonemata), *Ceratodon purpureus* (Karunen 1977b, 1978) and *Fontinalis antipyretica* (Jamieson and Reid 1976) and in the liverwort *Barbilophozia barbata* (Karunen 1977b), C 18:3ω3 + C 16:3ω3 seems to predominate, while in *Hypnum cupressiforme* (Nichols 1965), *Mnium medium*, *M. cuspidatum*, *Hylocomium splendens*, *Pleurozium schreberi* (Gellerman *et al.*1975) and *Plagiothecium laetum* (Karunen 1977b) there are also considerable amounts of C 20 polyunsaturated fatty acids.

How do the component fatty acids of glycolipids within these two groups of mosses change in response to different environments? To answer this question we chose the moss material within the two groups so that the physiological condition would be different from that in the previous investigations. Thus, *Ceratodon purpureus*, which was actively growing when utilized in the previous experiment (Karunen 1977b), was now collected in frozen condition. *Pleurozium schreberi*, previously collected from a dry, unshaded roadsite cut in Alaska (Gellerman *et al.*1975), was now collected in frozen condition from moist shaded pine forest in southwestern Finland.

Abbreviations: BDS, butane-1,4-diol succinate; FFAP, poly(ethylene glycol 2-nitroterephthalate); GLC, gas liquid chromatography; ECL, equivalent chain length; MGDG, monoglycosyl diglyceride; DGDG, diglycosyl diglyceride; tr, trace amount = <0.1%; n.d., not detected.

Material and Methods

The mosses *Ceratodon purpureus* (Hedw.) Brid. and *Pleurozium schreberi* (Brid.) Mitt. were collected from natural habitats in southwestern Finland. At the time of collection (Nov. 1977) the moss material was frozen and covered with snow. Ecologically the species represent two different habitats. C. purpureus grows on rocks exposed to sunshine, whereas P. schreberi grows in shaded, moist pine forests. The upper green part of C. purpureus was utilized for lipid analysis and the topmost green part of $1\frac{1}{2}$ cm of P. schreberi, thus avoiding the interference of senescing tissue.

Lipids were extracted immediately and separated into fractions by methods described previously (Karunen 1977a). Quantitative and qualitative GLC analyses of methyl esters

of the lipid fractions were carried out before and after hydrogenation on a Perkin Elmer Model F 30 gas chromatograph using FFAP- and BDS-coated glass capillary columns (Karunen 1977a, b, 1978). Authentic fatty acid methyl esters, lin log plots and the ECL tables published by Jamieson (1975) were used for identification purposes.

To confirm the identification the fatty acid methyl esters of the more polar lipid fraction of both species were also analysed on a LKB 9000 gas chromatograph–mass spectrometer equipped with a FFAP coated glass capillary column. The samples were run isothermally at 220°C for 31 min and then programmed to 250°C at the rate of 4°C/min. Operating conditions for the mass spectrometer were: molcular separator temperature 280°C, ion source temperature 290°C, and ionizing potential 20 eV. The more polar lipid fractions were chosen for mass spectrometric analysis, since they contained all the peaks found in the other two fractions (MGDG and DGDG) as well as several additional peaks.

The quantification of the MGDG and DGDG fractions was carried out using the correction factors calculated according to the proportions of Christie *et al.* (1970). In this investigation each value represented is based on six unindependent replicates and at least twelve chromatograms.

Results and Discussion

The total amount of fatty acids in the MGDG, DGDG and more polar lipid fractions obtained from C. purpureus shoots was 4.6, 3.4 and 4.0 mg/g dry weight, respectively. The respective values for the green tops of P. schreberi were 2.6, 3.3 and 3.8 mg/g dry weight. The molar ratio MGDG/DGDG was 1.3 for C. purpureus and 0.8 for P. schreberi. Thus the ratio was higher in the frozen C. purpureus shoots than in the actively growing C. purpureus, where it was exceptionally low, *i.e.* 0.4 (Karunen 1977b). The molar ratio MGDG + DGDG/chlorophyll $a + b$ was 3.7 in C. purpureus and 3.5 in P. Schreberi. The values fall

Table 1. *Fatty acid composition of different lipid fractions of Ceratodon purpureus.* The symbol ω indicates the distance of the double bond from the methyl terminus. Data are given ±SE. Data for collection of Oct. 10, 1976, from Karunen (1977b).

	Collected Nov. 1977			Collected Oct. 10, 1976	
Fatty acid	MGDG %	DGDG %	More polar %	MGDG %	DGDG %
14:0	0.2 ± 0.03	0.3 ± 0.03	0.3 ± 0.02	0.4	0.2
15:0	0.1 ± 0.01	0.2 ± 0.01	1.0 ± 0.02	0.2	0.1
16:0	2.1 ± 0.12	2.9 ± 0.20	25.4 ± 0.84	2.9	2.8
16:1	0.5 ± 0.02	0.2 ± 0.01	0.7 ± 0.01	0.3	0.2
16:1ω13 trans	n.d.	n.d.	1.8 ± 0.12	n.d.	n.d.
16:2ω6	2.4 ± 0.02	0.5 ± 0.01	0.1 ± 0.01	1.4	0.4
16:3ω3	25.5 ± 0.06	7.6 ± 0.09	0.5 ± 0.07	38.1	13.3
17:0	0.2 ± 0.01	0.3 ± 0.02	0.9 ± 0.05	tr	tr
18:0	0.8 ± 0.05	0.7 ± 0.04	2.7 ± 0.20	0.7	0.3
18:1	0.7 ± 0.01	0.7 ± 0.01	1.6 ± 0.05	0.3	0.5
18:2ω6	4.6 ± 0.03	2.8 ± 0.07	12.2 ± 0.70	3.1	1.8
18:3ω6	1.1 ± 0.01	0.4 ± 0.01	1.5 ± 0.09	0.7	0.4
18:3ω3	44.3 ± 0.20	69.5 ± 0.31	26.3 ± 0.31	45.3	70.2
18:4ω3	0.7 ± 0.01	1.3 ± 0.07	0.9 ± 0.08	0.9	2.3
20:0	0.3 ± 0.03	0.2 ± 0.03	1.6 ± 0.22	0.2	tr
20:2ω6	tr	tr	0.2 ± 0.03	tr	tr
20:3ω6	0.1 ± 0.01	0.2 ± 0.02	1.2 ± 0.07	tr	tr
20:3ω3	0.4 ± 0.02	0.5 ± 0.02	0.4 ± 0.06	tr	0.3
20:4ω6	10.8 ± 0.11	2.1 ± 0.05	9.0 ± 0.41	2.3	1.6
20:4ω3	tr	0.4 ± 0.03	0.6 ± 0.04	tr	0.2
20:5ω3	4.0 ± 0.04	8.2 ± 0.12	7.8 ± 0.13	1.0	4.7
22:0	0.4 ± 0.01	0.1 ± 0.01	1.4 ± 0.02	tr	tr
24:0	n.d.	n.d.	0.8 ± 0.13	n.d.	n.d.
Other	tr–0.3	tr–0.4	tr–0.3	tr–1.1	tr–0.4
Saturated	4.1	4.7	34.1	6.3	3.5
Unsaturated	95.1	94.4	64.8	93.7	96.5
Total ω6	19.0	6.0	24.2	7.5	4.2
Total ω3	74.9	87.5	36.5	85.3	91.0
C12–C16	30.8	11.7	29.8	45.2	17.0
C18	52.2	75.4	45.2	51.0	75.5
C20	15.6	11.6	20.8	3.5	6.8

within the ranges found in Bryophyta and in higher plants (cf. Karunen 1977b). It seems, however, that the frozen C. purpureus shoots contain more glycolipids relative to chlorophyll than do the actively growing shoots.

The identification of the fatty acid methyl esters of various lipid fractions isolated from C. purpureus and P. schreberi was based on their analysis on capillary columns of different polarity, as originally suggested by Jamieson and Reid (1969) and Jamieson (1975) and earlier used in this laboratory (Karunen 1977a, b, 1978, Karunen and Liljenberg 1978). The ECL values of the polyunsaturated fatty acid methyl esters derived from the various polar lipid fractions of C. purpureus and P. schreberi were equal or very close to the values found by Jamieson (1975).

In the present investigation we confirmed the identification by analysing the fatty acid methyl esters of the more polar lipid fractions of C. purpureus and P. schreberi by mass spectrometry. Thus, all of the fatty acids of the more polar lipid fractions listed in Tables 1 and 2 have been characterized by both their molecular ions and fragmentation pattern.

The saturated fatty acid methyl esters exhibited the typical fragmentation pattern for such esters: *m/e* 74 or *m/e* 75 as the base peak, ions of the series $CH_3OCO(CH_2)_n^+$, hydrocarbon ions from the saturated series C_nH_{2n+1} and the formation of M-29, M-31 and M-43 ions (cf. McCloskey 1970).

For the monoenoic fatty acid methyl esters the same prominent peaks were found as reported for methyl oleate by Hallgren *et al.* (1959).

The mass spectra of methyl linoleate showed the same fragmentation pattern as reported by Hallgren *et al.* (1959). The prominent peaks of the other dienoic fatty acid methyl esters (C 16:2, C 20:2) were the same as for methyl linoleate, but of different intensities.

The trienoic, tetraenoic and pentaenoic fatty acid methyl esters exhibited two different types of spectra, characteristic of $\omega 3$ and $\omega 6$ isomers (cf. Holman and Rahm 1966). The double bonds in other isomers are not easily located from the mass spectra data and the isomers are left unidentified in this investigation.

The fatty acid composition of the MGDG and DGDG fractions of C. purpureus (Table 1) was rather similar to the composition reported earlier (Karunen 1977b). In the present investigation, however, the proportions of C 20 polyunsaturated fatty acids were higher in both glycolipid fractions: 15.7% and 11.6% in MGDG and DGDG fractions, respectively, compared with the earlier values 3.3% and 6.8%, respectively (Karunen 1977b). Further, the present MGDG and DGDG fractions contained less shorter chain (C 12–C 16) fatty acids and more longer chain (C 20) fatty acids. The Ceratodon material collected in 1977 was from the same type of habitat (unshaded rocks) in SW-Finland as that collected in 1976, though from a different locality. Therefore, we consider that though some differences

Table 2. *Fatty acid composition of different lipid fractions of Pleurozium schreberi.* Otherwise as in Table 1.

Fatty acid	MGDG %	DGDG %	More polar %
14:0	0.2 ± 0.02	0.1 ± 0.01	0.2 ± 0.03
15:0	tr	tr	0.3 ± 0.01
16:0	2.7 ± 0.30	4.0 ± 0.18	27.4 ± 1.03
16:1	0.4 ± 0.04	0.2 ± 0.02	0.7 ± 0.03
16:1 trans	n.d.	n.d.	1.6 ± 0.06
16:2ω6	1.7 ± 0.05	0.8 ± 0.05	0.2 ± 0.01
16:3ω3	8.2 ± 0.24	1.8 ± 0.05	0.3 ± 0.02
17:0	tr	tr	0.2 ± 0.01
18:0	0.4 ± 0.08	0.3 ± 0.01	0.6 ± 0.03
18:1	1.8 ± 0.08	2.4 ± 0.06	2.7 ± 0.05
18:2ω6	3.3 ± 0.12	1.5 ± 0.09	9.5 ± 0.24
18:3ω6	0.7 ± 0.02	0.4 ± 0.01	0.9 ± 0.05
18:3ω3	31.5 ± 0.66	44.8 ± 0.38	23.9 ± 0.40
18:4ω3	0.3 ± 0.01	0.1 ± 0.01	0.2 ± 0.02
20:0	tr	tr	0.3 ± 0.05
20:2ω6	tr	tr	0.3 ± 0.02
20:3ω6	0.5 ± 0.04	1.2 ± 0.04	0.4 ± 0.02
20:3ω3	1.4 ± 0.09	2.3 ± 0.05	0.8 ± 0.05
20:4ω6	31.7 ± 0.46	20.8 ± 0.22	18.3 ± 0.56
20:4ω3	0.2 ± 0.03	0.4 ± 0.02	tr
20:5ω3	13.7 ± 0.40	17.7 ± 0.49	5.2 ± 0.28
22:0	0.2 ± 0.01	0.1 ± 0.01	1.1 ± 0.06
24:0	n.d.	n.d.	3.9 ± 0.90
Other	tr–0.9	tr–0.6	tr–0.2
Saturated	3.6	4.5	34.0
Unsaturated	95.4	94.4	65.0
Total ω6	37.9	24.7	29.6
Total ω3	55.1	67.1	30.4
C12–C16	13.3	6.9	30.7
C18	38.0	49.5	37.8
C20	47.4	42.4	25.3

may be due, for example to edaphic differences between the habitats, the major differences probably are due to other ecophysiological factors. In 1976 the samples were collected in early October at a time when the moss was actively growing. In 1977 the material was collected in November and the moss had been exposed to temperatures below 0°C for a longer period. Thus, the differences might be an indication of the low temperature acclimatization of C. purpureus moss. In higher plants low temperatures change the fatty acid pattern towards more unsaturated fatty acids (cf. *e.g.* Gerloff *et al.* 1966, De la Roche *et al.* 1972, Grenier *et al.* 1972, Kuiper and Stuiver 1972, Willemot *et al.* 1977, Smoleńska and Kuiper 1977). Thus, the response of mosses to low temperatures appears similar to that of higher plants.

The changes in the fatty acid composition of glycolipids — the main lipids of chloroplast membranes — increase the membrane fluidity (cf. Cherry 1976), so enabling the physiological activity of membranes at low temperatures. In general, the degree of unsaturation of chloroplast lipids in mosses (Nichols 1965, Gellerman *et al.* 1975, Jamieson and Reid 1976, Karunen 1977b, 1978) is much higher than in higher plants (Hitchcock and Nichols 1971) and possibly

contributes to the fact that in many mosses the optimum temperature for photosynthesis is rather low, so that some mosses are capable of photosynthesis even below 0°C (Rastorfer 1970, Kallio and Heinonen 1973, 1975, Oechel and Collins 1976).

The seasonal difference in the fatty acid pattern of the MGDG and DGDG fractions of C. purpureus may be influenced by light conditions as well as by temperature. In 1977 the material was covered with snow, so receiving much less light than in the snowless October of 1976. Controlled experiments in laboratory conditions are required to distinguish the effects of temperature and light conditions on the seasonal differences in the fatty acid composition of mosses.

The MGDG and DGDG fractions of P. schreberi contained a higher proportion of polyunsaturated fatty acids than C. prupureus (Table 2). Since P. schreberi grows in moister and much more shaded habitats than C. purpureus, these findings lend further support to earlier indications that mosses adapted to moist habitats (Anderson *et al.* 1974) and low light intensity (Karunen 1977b) contain a high degree of unsaturated C 20 (even C 22) fatty acids. However, *Fontinalis antipyretica*, which grows in water but unshaded, contains C 20 polyunsaturated fatty acids as minor components in MGDG and DGDG (Jamieson and Reid 1976). Interestingly, the proportions of various fatty acids in the MGDG and DGDG fractions of P. schreberi are rather similar to those found by Gellerman *et al.* (1975) in the corresponding fractions of P. schreberi collected from Kantishna, Alaska, from a quite different habitat, *i.e.* a dry and unshaded roadsite cut.

The more polar lipid fractions of C. purpureus and P. schreberi (Tables 1 and 2) contained a higher proportion of saturated fatty acids than the MGDG and DGDG fractions. The proportions of C 20 polyunsaturated and C 18:3ω3 acids were lower and the proportion of C 16:0 considerably higher. The same pattern of fatty acid distribution was found in *Plagiothecium laetum* (Karunen 1977b). A high proportion of C 16:0 acid was likewise found in the more polar lipid fractions of *Fontinalis antipyretica* (Jamieson and Reid 1976) and *Barbilophozia barbata* (Karunen 1977b), though in the latter species the proportion of C 20 polyunsaturated fatty acids was higher in the more polar lipid fraction than in the glycolipid fractions (Karunen 1977b). As in higher plants (*e.g.* Mazliak 1977), palmitic acid appears to be enriched in the phospholipid classes of mosses (Gellerman *et al.* 1975, Nichols 1965).

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