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SHORT COMMUNICATION

THE LIPIDS OF A MOSS (*HYPNUM CUPRESSIFORME*) AND OF THE LEAVES OF GREEN HOLLY (*ILEX AQUIFOLIUM*)

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Abstract—Fatty acid distribution in the lipids of a moss (*Hypnum cupressiforme*) and the green leaves of holly (*Ilex aquifolium*) is consistent with that in the green leaves of deciduous higher plants. In moss lipids, the polyunsaturated C_{20} acids are mainly concentrated in the galactosyl diglycerides, and in both moss and holly lipids, *trans*-3-hexadecenoic acid occurs only in the phosphatidyl glycerol fraction.

INTRODUCTION

The leaf lipids of several higher plants, namely spinach,[1] red clover,[2,3] runner bean,[4] alfalfa,[5] castor[6] and lettuce[6] have now been partially or wholly characterized, and the close similarity in lipid composition of all such deciduous tissues has been established. There is, however, a lack of comparable information for the evergreens, and lower plants such as the mosses. Mosses are of particular interest since Gellerman and Schlenk[7] have shown that they contain arachidonic acid, an acid previously undetected in plant tissues.

RESULTS AND DISCUSSION

The major fatty acid-containing lipids of green holly leaves (*Ilex aquifolium*) and moss (*Hypnum cupressiforme*) are those glycolipids and phospholipids commonly found in the photosynthetic tissue of higher plants, namely, the galactosyl diglycerides, sulpholipid, cardiolipin, phosphatidyl-choline, -ethanolamine, -inositol and -glycerol. Only very minor quantities of triglycerides are present. Holly leaf extracts also contain a large quantity of an acid- and alkali-stable lipid which gives a positive Lieberman–Burchard reaction, and migrates more slowly than β-sitosterol on thin layers of silica gel using hexane: diethyl ether (85:5 v/v) as mobile phase (R_f values: β-sitosterol 0·11; unknown lipid, 0·05).

The fatty acid composition of the lipids of moss and holly leaves is given in Tables 1 and 2 respectively. Previous studies on the fatty acid composition of various plant tissues[1,2–6,8] have indicated a preferential concentration of the more highly unsaturated fatty acids in the galactosyl diglycerides. Tables 1 and 2 show that this rule may also be extended to the lipids

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[1] C. F. Allen, P. Good, H. F. Davis and S. D. Fowler, *Biochem. Biophys. Res. Comm.* **15**, 424 (1964).
[2] R. O. Weenink, *J. Sci. Food Agric.* **12**, 34 (1961).
[3] R. O. Weenink and F. B. Shorland, *Biochim. Biophys. Acta* **84**, 613 (1964).
[4] P. S. Sastry and M. Kates, *Biochemistry* **3**, 1271 (1964).
[5] J. S. O'Brien and A. A. Benson, *J. Lipid Res.* **5**, 432 (1964).
[6] B. W. Nichols and A. T. James, Unpublished observations.
[7] J. L. Gellerman and H. Schlenk, *Experientia* **20**, 426 (1964).
[8] B. W. Nichols and A. T. James, *Fette, Seifen, Anstrichmittel* **66**, 1003 (1964).

TABLE 1. THE FATTY ACID COMPOSITION OF THE LIPIDS OF *Hypnum cupressiforme*

Lipids	Fatty acid																
	14:0	14:2	14:3	16:0	16:1*	16:1†	16:2	16:3	18:0	18:1	18:2	18:3	20:4	20:4‡	20:4‡	20:5	22:0
Total lipids	1·2	t	1·9	13·5	5·1	t	t	1·9	1·7	7·3	19·7	23·5	t	9·2	2·5	7·2	4·6
Monogalactosyl diglyceride	t	—	t	2·3	0·8	—	2·0	10·7	t	1·2	4·1	48·3	2·3	22·5	3·7	11·2	3·1
Digalactosyl diglyceride	t	—	—	6·2	1·8	—	1·2	1·7	1·6	2·5	4·6	62·2	2·3	11·5	—	4·4	2·6
Phosphatidyl glycerol	1·7	t	t	31·8	t	17·7	t	—	2·9	11·8	10·3	19·3	t	2·9	—	t	t
Sulphoquinovosyl diglyceride	2·2	1·1	t	35·0	12·8	—	t	—	3·0	7·5	6·3	25·7	1·2	2·0	—	—	t
Phosphatidyl choline	—	—	—	17·2	6·9	—	—	—	2·7	11·4	26·9	27·4	—	7·5	—	—	4·9
Phosphatidyl ethanolamine	1·0	t	t	12·5	12·4	—	1·4	—	2·5	11·6	20·9	21·0	t	16·8	—	—	3·3
Cardiolipin	t	—	—	27·7	13·3	—	2·2	—	7·0	25·9	14·4	7·6	—	3·6	—	—	—

* A mixture of the 7-, 9- and 11-isomers.

‡ Retention volumes were 5·4, 6·0 and 6·5 relative to 16:0 on polyester columns.
t—trace amounts.

TABLE 2. THE FATTY ACID COMPOSITION OF THE LIPIDS OF *Ilex aquifolium*

Lipid	Fatty acid								
	12:0	14:0	16:0	16:1*	16:1†	18:0	18:1	18:2	18:3
Total lipid	t	t	22·0	t	t	t	2·5	13.8	60.2
Monogalactosyl diglyceride	—	—	1·0	—	—	t	t	2.3	96·7
Digalactosyl diglyceride	—	—	13·4	—	—	t	t	6.9	79.7
Phosphatidyl glycerol	5·0	5·6	38·9	—	12.2	2·3	17·9	9·0	15·3
Sulphoquinovosyl diglyceride	2·1	2·2	33·7	3·1	—	3·7	12·4	4.3	38·5
Phosphatidyl choline	t	1.5	23.8	t	—	1·6	3·9	24·8	41·9
Phosphatidyl ethanolamine	—	—	18·3	3·7	—	—	—	33·4	44·6

* Δ^9; † Δ^3.

of moss and holly. The galactosyl diglycerides of the moss possess not only a high proportion of α-linolenic acid but they also contain most of the C_{20} polyunsaturated acids, while the corresponding lipids of holly leaves possess a high linolenic acid content comparable to that in deciduous leaves.

In most of the species of green leaf so far studied the monogalactosyl diglyceride (MGDG) fraction contains a higher proportion of linolenic acid than any other lipid from the same tissue, including digalactosyl diglyceride (DGDG). There are a few green tissues in which the MGDG has a lower linolenic acid content than the DGDG, for example spinach[1] and moss, but the former lipid contains an increased quantity of 7,10,13-hexadecatrienoic acid[7] so that the total proportion of trienoic acids in the lipid is still higher than that in any other. Superficially, it might seem permissible to argue that this hexadecatrienoic acid may be the immediate precursor of α-linolenic acid (9,12,15-octadecatrienoic acid) by a process of chain elongation, but James[9] and Harris and James[10] have shown that in higher plants the C_{18} dienoic and trienoic acids are formed by desaturation of oleic acid. This 7,10,13-hexadecatrienoic acid may result from the desaturation of 7-hexadecenoic acid which Gellerman and Schlenk[7] have also shown to be present in the hexadecenoic acids of moss. It seems likely that the same mechanism accounts for the presence of the same hexadecatrienoic acid in spinach lipids, although it has never been demonstrated that these contain 7-hexadecenoic acid. There is no evidence that 9-hexadecenoic acid can be desaturated in a similar manner, a reaction which would result in the formation of 9,12,15-hexadecatrienoic acid containing a terminal methylene group. An analogous situation also exists in the lipids of *Chlorella vulgaris* in which the dienoic acid content of the MGDG fraction is comprised of both C_{16} and C_{18} dienoic acids; whereas the DGDG fraction contains only a very small quantity of hexadecadienoic acid.[11]

In the holly leaves and moss studied, *trans*-3-hexadecenoic acid occurs only in the phosphatidyl glycerol fraction a specificity already observed in the leaf lipids of spinach,[1] red clover,[3] castor,[6] and also in light grown *Chlorella vulgaris*.[9] Gellerman and Schlenk[7] did not detect this acid in their moss extracts, probably because of its small overall concentration in the total lipids.

[9] A. T. JAMES, *Biochim. Biophys. Acta* **57**, 167 (1962).
[10] R. V. HARRIS, B. J. B. WOOD and A. T. JAMES, *Biochem. J.* **94**, 22 (1965).
[11] B. W. NICHOLS, *Biochim. Biophys. Acta* In press.

Thus the specificities already observed regarding the fatty acid distribution in the lipids of *Chlorella vulgaris* and in the leaves of deciduous plants may also be generally applicable to the lipids of the photosynthetic tissues of evergreens and mosses.

EXPERIMENTAL

Tissues were macerated with isopropyl alcohol and the residue re-extracted with chloroform:methanol (2:1 v/v) until devoid of pigment. No attempt was made to separate the photosynthetic tissue of the moss from the woody tissue. Extracts were dried *in vacuo*, redissolved in chloroform and the lipids fractionated by a combination of column chromatography on DEAE cellulose[8] and preparative thin-layer chromatography on silica gel.[8]

Fatty acid methyl esters were prepared by refluxing lipid fractions with methanol: benzene: sulphuric acid (150:75:10 v/v) and the esters analysed by gas–liquid chromatography in a Pye Argon chromatograph employing ethylene glycol adipate and Apiezon L as stationary phases. Confirmatory evidence for the identity of each acid was obtained by fractionating the methyl esters according to degree of unsaturation on thin layers of silica gel impregnated with silver nitrate, followed by further fractionation according to chain length by gas–liquid chromatography on Apiezon L.

The location of double bonds in the C_{16} monoenoic acids was determined by permanganate-periodate oxidation of their methyl esters[12] (as isolated by preparative gas–liquid chromatography), followed by GLC of the methyl esters of the reaction products. The double bond location of the hexadecatrienoic acid in the moss lipids is assumed on the basis of the evidence obtained by Gellerman and Schlenk[7] on a variety of mosses and on the observations of other workers (see Shorland[13]) that this is the only hexadecatrienoic acid found in plant leaves. The major C_{20} tetraenoic acid presumably possesses the 5,8,11,14-configuration by analogy with the findings of Gellerman and Schlenk[7] for other mosses; the minor C_{20} acids of the same degree of unsaturation but of slightly different retention volumes on polyester columns are probably positional isomers of arachidonic acid.

SUMMARY

The fatty acid composition of the lipids of a moss (*Hypnum cupressiforme*) and the green leaves of a holly (*Ilex aquifolium*) is similar to that of the green leaves of deciduous higher plants. In moss lipids, the polyunsaturated C_{20} acids are mainly concentrated in the galactosyl diglycerides, and in both moss and holly lipids *trans*-3-hexadecenoic acid occurs only in the phosphatidyl glycerol fraction.

[12] C. Hitchcock and A. T. James, *Kerntechnik* 7, 5 (1965).

[13] F. B. Shorland, In *Chemical Plant Taxonomy* (Edited by T. Swain), Academic Press, London (1963).