Phytochemistry, 1973, Vol. 12, pp. 1377 to 1381 Pergamon Press Printed in England.

LIPID METABOLISM IN THE FERN *POLYPODIUM VULGARE*

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(*Received* 20 *November* 1972. *Accepted* 8 *December* 1972)

Key Word Index—*Polypodium vulgare*; Pteridophyta; lipids; triglycerides; fatty acids; spore germination.

Abstract—The early stages of spore germination in *Polypodium vulgare* involve the catabolism of endogenous triglyceride which is accompanied by the *de novo* synthesis of several classes of neutral and polar lipid. These newly synthesized lipids include triglycerides which possess different fatty acid compositions from those in dormant spores and resemble the triglyceride fraction found in the sporophyte frond tissue. The C_{20} acids which are present in the non-chloroplast lipids of the sporophyte frond tissue do not occur in the spore to an appreciable extent.

INTRODUCTION

ALTHOUGH the major changes in lipid composition and metabolism which accompany seed germination are now well understood, the biochemical processes involving lipid which take place during the germination of fern spores remain undetermined. In this paper we describe comparative compositional and metabolic studies on spores, and on gametophyte and sporophyte frond tissues of the fern *Polypodium vulgare* L.

RESULTS

Dormant spores of *Polypodium vulgare* contained 50% lipophilic material of which 73% was triglyceride and only 10% phospholipid and glycolipid (Table 1). The major fatty acids of the spore triglyceride were oleic and linoleic acids; the more polar lipids contained higher proportions of palmitic and C_{20} acids (Table 2).

In contrast to the spores, sporophyte frond tissue contained 1·9% lipid of which only 12% was triglyceride, the remainder comprising phospholipids, glycolipids, sterol esters and pigment (Table 1). Other differences occurred in the fatty acid composition of the component lipids which in the sporophyte frond tissue contained a far higher proportion of linolenic acid and acids of chain length above C_{18} than was found in the dormant spores (Table 2).

Spores which had imbibed water and germinated partially to develop rhizoids possessed similar lipid and fatty acid compositions to the dry spores. On the other hand, fully-germinated spores which had produced both rhizoids and short chains of vegetative cells (comprising the gametophyte) contained far less fat than the ungerminated and partially-germinated spores, the relatively greater fall in concentration of triglyceride compared with that of phospholipids and glycolipids being particularly noticeable. An increase in the levels of linolenic and C_{20} acids also occurred (Tables 1, 2 and 4).

TABLE 1. FAT CONTENT OF TISSUES FROM *Polypodium vulgare*

Tissue	Total lipid % of fr. wt	Lipid classes (% of total lipid) Sterols	Diglycerides	Triglycerides	Sterol esters	Free fatty acids	Phospholipids and glycolipids	Chlorophyll % of fr. wt
Spore								
Dormant	50·0	5 5	5·2	73 0	4·7	1·6	10 0	0·032
Partially-germinated (6 day stage)	50·2	2·6	7·2	72·4	3·9	3·9	10 0	0·042
Fully-germinated in presence of [^{14}C]-acetate (10 day stage)	42·3	n.d	n d	n.d.	n d.	n.d.	n.d.	0 103
Fully-germinated (15 day stage)	25·3	5·3	8·5	63·8	4·6	1·5	16 3	0·250
Sporophyte frond	1·9	5 4	3 4	12·1	15 0	3·9	60·0	n d.

The distribution of ^{14}C-activity between the individual lipid classes, and between their component fatty acids, of spores germinated in the presence of Na[2-^{14}C]acetate are given in Table 5. During germination radioactivity was primarily incorporated into neutral lipid classes, particularly triglyceride, but the labelled fatty acids in this fraction were not those which predominate in the dormant spore triglyceride.

TABLE 2. FATTY ACID COMPOSITION OF TOTAL LIPID EXTRACTS FROM TISSUES OF *Polypodium vulgare*

Tissue	Fatty acid (% of total) 14:3	16 0	16 1 Δ^9	16 1 Δ^3	16.2	16.3	18.0	18.1	18 2	18·3 $\Delta^{6,9,12}$	18·3 $\Delta^{9,12,15}$	20·0	20·3	20.4	22.0	20.5	24:0
Spore																	
Dormant	—	5	t	—	—	—	1	66	24	1	1	—	1	1	—	—	—
Partially-germinated (6 day stage)	—	5	t	—	—	—	t	66	24	2	1	—	1	1	—	—	—
Fully-germinated in presence of [^{14}C]acetate (10 day stage)	—	5	t	—	—	—	t	67	24	2	1	—	1	1	—	—	—
Fully-germinated (15 day stage)	—	4	t	—	—	—	t	62	24	2	1	—	1	2	1	1	1
Sporophyte frond	2	17	1	1	1	3	2	6	13	1	30	2	2	13	3	2	4

DISCUSSION

From the data presented here it is evident that fern spores resemble the seeds of many higher plants in containing relatively high proportions of lipid, most of which is triglyceride. Similarly the acyl lipid composition of the sporophyte frond tissue resembles that of the corresponding tissues of higher plants, although the fatty acid composition differs in con-

taining substantial quantities of the C_{20} polyenoic acids which are more characteristic of the photosynthetic tissues of mosses and ferns.[1,2]

Thus *Polypodium vulgare* clearly resembles the many families of higher plant in which the lipids and fatty acids of the seed tissue differ both qualitatively and quantitatively from those of the vegetative tissue. No comparable data are available for other fern species although Karunen[3] has reported that moss spores contain triglycerides and sterol esters as the major lipid classes; the same author also showed, however, that the spore triglycerides contained substantial quantities of linolenic and C_{20} polyenoic acids, which contrasts with our results with the fern. Karunen did not report the fatty acid composition of the corresponding vegetative tissue so that it is not possible to compare the difference in fatty acid composition in the two types of moss tissue.

TABLE 3. FATTY ACID COMPOSITION OF LIPID FRACTIONS FROM THE SPOROPHYTE FROND TISSUE OF *Polypodium vulgare*

	Fatty acid (% of total)																	
			16·1							18·3								
Class of lipid*	14·3	16·0	Δ^9	Δ^3	16 2	16.3	18:0	18·1	18·2	$\Delta^{6,9,12}$	$\Delta^{9,12,15}$	20·0	20·3	20·4	22:0	20:5	24:0
Total lipid	2	17	1	1	1	3	1	6	13	1	30	2	2	13	3	2	4
TG	1	8	1	—	t	1	2	10	14	1	24	1	5	24	2	3	3
PC	—	16	1	—	1	—	6	11	22	1	12	1	9	19	2	—	—
PI	2	34	3	—	1	1	3	5	10	—	13	1	4	18	1	2	3
PG	3	25	2	14	1	t	2	27	6	1	6	1	3	4	1	2	6
MGDG	3	5	9	—	2	6	1	3	5	—	49	—	3	8	—	4	—
DGDG	—	11	1	—	1	3	1	4	7	1	65	—	1	5	—	2	—
SQDG	1	41	2	—	t	t	1	35	4	1	10	—	2	4	—	—	—
U_1	2	10	2	—	1	4	2	4	5	—	54	—	4	5	—	2	5
U_2	4	5	1	—	t	1	4	5	4	t	35	—	7	6	1	3	23

* Abbreviations: TG, triglyceride; PC, PI, PG, phophatidyl-choline, -inositol, and -glycerol; MGDG monogalactosyl diglyceride; DGDG, digalactosyl diglyceride; SQDG, sulphoquinovosyl diglyceride (sulpholipid); U_1, U_2, unidentified lipids, possibly tri- and tetra-galactosyl diglycerides respectively.

In one respect our data for fern leaves (Table 3) contrast with those previously obtained for the photosynthetic tissue of ferns and mosses, namely that in *P. vulgare* the C_{20} polyenoic acids accumulate preferentially in those lipids not normally associated with the photosynthetic apparatus (triglyceride, phosphatidyl–choline, –ethanolamine and –inositol) rather than the chloroplast lipids (galactosyl diglycerides, sulpholipid and phosphatidyl glycerol) whereas former studies with other species showed that arachidonic acid and related acids were mainly located in the chloroplast.[2]

TABLE 4. FATTY ACID COMPOSITIONS OF LIPID FRACTIONS FROM DORMANT SPORES OF *Polypodium vulgare*

	Fatty acid (% of total)														
		16·1						18:3							
Class of lipid	16:0	Δ^9	Δ^3	16·2	18:0	18·1	18:2	$\Delta^{6,9,12}$	$\Delta^{9,12,15}$	20.0	20:3	20:4	22:0	24:0
Total lipid	5	t	—	t	1	66	24	1	1	t	1	1	t	t
TG	5	t	—	—	t	70	23	1	1	—	t	t	—	—
PC	12	2	—	1	3	29	40	2	1	t	4	2	1	3
PI	18	1	—	1	5	18	41	1	1	1	8	2	2	2
PG	33	1	1	1	8	17	16	—	—	2	11	2	4	5
MGDG	22	6	—	1	7	32	19	1	2	1	4	t	1	2
DGDG	17	2	—	3	11	12	38	1	7	1	11	—	—	—
SQDG	15	1	—	1	7	49	9	1	1	2	9	—	3	3

[1] SCHLENK, H. and GELLERMAN, J. L. (1965) *J. Am. Oil Chemists' Soc.* **42**, 504.
[2] NICHOLS, B. W. (1965) *Phytochemistry* **4**, 769.
[3] KARUNEN, P. (1971) *Phytochemistry* **10**, 2811.

From our studies certain aspects of spore germination in *P. vulgare* have become evident. Firstly, the fact that the germinated spores, which had produced gametophyte tissues, contained only half the quantity of lipid present in ungerminated spores indicates that a large proportion of the original lipid (mainly triglyceride) was catabolized during the germination process. Because such analyses alone did not indicate whether the lipids in the gametophyte were mainly those of the original spore which had not been catabolized, or whether they had been synthesized *de novo* during germination, we carried out experiments employing Na[^{14}C]acetate to follow lipid synthesis during this process. The location of label in the lipids and fatty acids (Table 5) indicated some surprising aspects of the lipid metabolism operative at this stage of spore germination. In particular the major part of the total ^{14}C activity incorporated into lipid appeared in neutral lipid fractions (tri- and di-glycerides, sterol esters) with only a small proportion accumulating in the more polar lipids typical of sporophyte frond tissue, despite the appreciable level of chlorophyll at this stage.

TABLE 5. ACTIVITY IN LIPIDS AND COMPONENT FATTY ACIDS OF SPORES GERMINATED IN PRESENCE OF Na-[2-^{14}C]ACETATE

Lipid	Total activity in lipid (%)	Activity in fatty acid (% of total activity in component fatty acids) 16 0	16 1 Δ^9	16 1 Δ^3	16 2	16 3	18 0	18 1	18 2	18 3 $\Delta^{6,9,12}$	18 3 $\Delta^{9,12,15}$	20 3	20 4
Total	100 0	7	4	t	1	2	5	13	15	4	3	21	28
TG	23 9	6	2	—	—	—	3	8	9	8	12	23	31
DG	21 4	5	1	—	—	—	4	12	16	14	7	18	23
FFA	1 9	13	4	—	1	3	8	15	17	—	11	14	14
PC	1 2	9	2	—	—	—	1	16	26	6	4	9	28
PG	4 4	27	13	3	1	9	2	11	5	3	3	8	16
PI	0 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MGDG	2 1	6	2	—	4	7	3	13	17	—	49	—	—
DGDG	1 3	16	2	—	—	—	3	13	28	—	38	—	—
SQDG	1 2	55	4	—	—	—	4	30	7	—	—	—	—
Sterols	19 9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sterol esters	19 4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sterol glycosides	2 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

In higher plants, by contrast, it has been established that germination of seeds initially results in the catabolism of storage lipid, usually triglyceride, and the subsequent development of root and vegetative tissue is accompanied by the synthesis of the classes of lipid which typify these tissues, namely phospholipids and glycolipids.[4, 5]

To summarize, the results from the present study clearly show that catabolism of triglyceride fatty acids certainly occurs at the early stages of fern spore germination and in this respect this process resembles that in seed germination of higher plants. On the other hand, the labelling experiments show equally clearly that at this stage the major end-products of lipid synthesis are primarily neutral lipids rather than the phospholipids and glycolipids found in plant membranes and which are most heavily labelled at comparable stages of seed germination. Nevertheless the radioactive fatty acids present in the triglycerides and other lipids of the germinated spores were those more typical of these fractions in the sporophyte frond tissue of *P. vulgare* rather than those in the dormant spore. We therefore conclude that at this early stage of gametophyte development the fatty acid synthesizing systems involved in the synthesis of lipids like those of sporophyte frond tissue are operative, but

[4] ZIMMERMAN, D. C. and KLOSTERMAN, H. J. (1965) *J. Am. Oil Chemists' Soc.* **42**, 58.
[5] KATAYAMA, M. and FUNAHASHI, S. (1969) *J. Biochem. (Tokyo)* **66**, 479.

that the phospholipids and glycolipids typical of such tissues have still not started to accumulate to an appreciable extent. The significance of the synthesis of these neutral lipids at this early stage of gametophyte development is not clear.

EXPERIMENTAL

Plant materials. Fronds of *Polypodium vulgare* bearing ripe sporangia were dried at room temp. between sheets of paper. The released spores were collected from the paper and purified by sieving through lens tissue. 150 mg of surface-sterilized spores were sown aseptically in 100 ml of Knop's solution in a conical flask and the cultures maintained at 23° under continuous fluorescent light at an intensity of 600 lx. Partially-germinated spores, which had produced rhizoids, were harvested after 6 days, fully-germinatesd spores, with photosynthetic gametophyte tissue, were harvested after 15 days. The sporophyte frond tissue used comprised the sterile pinnae of fronds from which spores had been collected.

Radio-labelling studies. 1 g of fern spores were added to 30 ml Knops solution containing 100 μCi of Na[2-^{14}C]acetate. After 2 days, when imbibition was complete, the spore suspension was diluted to 1000 ml to permit germination and the tissues harvested after a further 8 days.

Extraction of lipids. Ungerminated, partially-germinated and fully-germinated spores were recovered from the cultures by centrifugation and were then disintegrated and extracted by resuspending them in a small vol. of $CHCl_3$–MeOH (2:1), adding an equal vol. of glass 'Ballotini' balls of mixed sizes and shaking the mixture vigorously for one hour on a wrist action shaker in a stoppered glass tube. After filtering, the residue of broken cells and glass balls was re-extracted with more solvent, filtered, and the two $CHCl_3$–MeOH extracts combined. Sporophyte frond tissue was macerated with *iso*-PrOH and the filtered residue then re-extracted with $CHCl_3$–MeOH.

Lipid analyses. Individual lipids were quantitated gravimetrically after separation by a combination of column chromatography on DEAE-cellulose and preparative TLC on silica gel.[6] Fatty acid analyses were obtained by GLC of their methyl esters, prepared by refluxing lipid fractions with MeOH–C_6H_6–H_2SO_4 (20:10:1).[7] ^{14}C-Activity in lipids was determined by standard scintillation techniques and activity in individual fatty acids was obtained by radiochemical–GLC.[7]

Chlorophyll. Chlorophyll was determined by the method of Arnon.[8]

[6] NICHOLS, B. W. and JAMES, A. T. (1964) *Fette Seifen Anstrichmittel* **66**, 1003.
[7] NICHOLS, B. W. and WOOD, B. J. B. (1968) *Lipids* **3**, 46.
[8] ARNON, D. I. (1949) *Plant Physiol.* **24**, 1.