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DISTRIBUTION OF GLYCOLIPIDS AND PHOSPHOLIPIDS IN *PTERIDIUM AQUILINUM*

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Abstract—The glycolipids and phospholipids in fronds and rhizomes of *Pteridium aquilinum* were determined. The total quantity of polar lipid decreased towards the base of the frond, but increased in the storage rhizome. The monogalactosyl diglyceride/digalactosyl diglyceride ratio was 1·8 in the pinnae; 1·0 in the lower petiole and 0·3 in the storage rhizome.

INTRODUCTION

THE GLYCOLIPID and phospholipid composition of those few bulky storage tissues which have been examined[1-4] is similar to that of leaf material, where these lipids are most abundant and for which much quantitative information is available.[5-8] The role of polar lipids as structural components of chloroplast membranes is well established,[9] while it has been suggested that in storage tissues they are located predominantly in the amyloplasts.[5] Polar lipids have also been implicated in fatty acid biosynthesis[10] and in the metabolism and transport of sugar within the chloroplast[11] and the amyloplast.[12]

To provide further information on this subject, we have surveyed the lipid composition of the leaf and storage tissues of bracken (*Pteridium aquilinum*) examining also a series of intermediate sections of the stem (rachis and petiole), etiolated to varying degrees, as a check upon the quantities of "background" lipids not associated with amyloplasts or chloroplasts. The pinnae contain most of the chlorophyll and lipid material in the frond. During the early part of the growing season, the storage rhizomes contain 15–25% starch. Intact tissues were analysed rather than isolated plastids, because mechanical and enzymic degradation[13,14] is possible during the isolation process.

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RESULTS AND DISCUSSION

Bracken plants were divided into six sections:[15] (1) Pinnae; (2) Upper 15 cm of rachis; (3) Upper 15 cm of petiole; (4) Lower 10 cm of petiole; (5) Upper, frond-bearing rhizome; (6) Lower, storage rhizome. The analytical methods were basically those of Roughan and Batt.[5,16] However, it was found that commercial silica gel for TLC gave unacceptably high blanks in the direct determination of glycolipids by the phenol–sulphuric acid method. Typical figures were: Kieselgel G (Merck A. G., Darmstadt), 30 μg/g, expressed as glucose; Kieselgel H, 55 μg/g; Kieselgel HR, 9 μg/g. Kieselgel HR was therefore purified further and reinforced with analytical grade $CaSO_4$, giving thin layers which contained negligible amounts of carbohydrate and phosphate, had a high capacity and were robust enough to withstand spraying.

TABLE 1. QUANTITIES OF GLYCOLIPIDS AND PHOSPHOLIPIDS (μmol/g fr. wt), AND OF CHLOROPHYLL (mg/g fr. wt) IN DIFFERENT PARTS OF THE BRACKEN PLANT

	DGD	MGD	SL	GC	SG	PI	PC	PG	DPG	Chlorophyll
Pinnae	2·54	4·8	0·23	0·25	0·16	0·22	1·14	0·15	0·07	1·33
Upper rachis	0·32	0·48	0·05	0·15	0·09	0·09	0·37	0·09	0·06	0·13
Upper petiole	0·13	0·17	0·07	0·09	0·08	0·03	0·14	0·05	0·03	0·07
Lower petiole	0·10	0·10	0·04	0·06	0·06	0·03	0·18	Trace	Trace	Trace
Frond-bearing rhizome	0·04	0·03	—	0·03	Trace	Trace	0·01	Trace	0·01	—
Storage rhizome	0·21	0·07	Trace	0·20	0·09	0·01	0·07	0·02	0·02	—

Abbreviations: DGD—digalactosyl diglyceride; MGD—monogalactosyl diglyceride; SL—sulpholipid; GC—glucocerebroside; PI—phosphatidyl inositol; PC—phosphatidyl choline; PG—phosphatidyl glycerol; DPG—diphosphatidyl glycerol.

The distribution of glycolipids and phospholipids in bracken fronds and rhizomes is shown in Table 1. The figures are the mean values of from between two and five determinations; typical relative errors vary between ±4% (e.g. MGD in pinnae, 4·8 μmol/g) and ± 30% (e.g. MGD in frond-bearing rhizome, 0·03 μmol/g). In addition, the rhizomes contained two unidentified glycolipids which released glucose on acid hydrolysis. They ran just behind and just ahead of digalactosyl diglyceride on TLC. Neither glycolipid contained any phosphate. Only traces of phosphatidyl ethanolamine were detected and phosphatidic acid, a common product of enzymic degradation,[1] was absent.

The overall lipid composition of the pinnae is qualitatively similar to that of *Dryopteris* fronds,[17,18] and quantitatively similar to that of *Blechnum* fronds,[5] though with lower proportions of PG, DPG and PE. The molar ratio MGD : DGD increases from 1·0 in the etiolated lower petiole to 1·9 in the pinnae, the same trend as has been noted when etiolated tissues of higher plants turn green.[7,19]

The total amount of polar lipid material is greatest in the pinnae and in the storage rhizomes, which contain the largest amounts of chlorophyll and starch respectively. However, the lipid proportions in the rhizomes are quite different from those in the

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fronds. In particular, the MGD : DGD ratio is well below unity, as in parsnip roots,[5] potato tubers[3] and apples.[4] It seems likely that this is a typical feature of amyloplast galactolipids.

EXPERIMENTAL

Plant material. Fronds and rhizomes of *Pteridium aquilinum* (L.) Kuhn were collected from woodland at Auchentorlie, Dunbartonshire, on 29 May at 11:30 hr. (Some lipids are known to show diurnal variability[5,20].) The fronds had one pair of pinnae unfolded.

Extraction and isolation of lipids. Portions (10–20g) of fresh tissue were extracted as described by Roughan and Batt,[5] within 1·5 hr of collection. Neutral and zwitterionic lipids were separated from acidic lipids on 2 × 5 cm columns of DEAE-cellulose prior to TLC in $CHCl_3$–MeOH–HOAc–H_2O (85 : 15 : 10 : 3).[21] Kieselgel HR (Merck A. G., Darmstadt) was washed successively with MeOH, $CHCl_3$, MeOH, and H_2O on a Büchner funnel and then passed through a 100 mesh stainless steel sieve. 10% (w/w) of analytical grade $CaSO_4$ was added immediately before spreading. Layers (0·25 or 0·5 mm thick) were activated at 120° for 30 min. Initial location was with I_2 vapour.

Identification. All lipid components were checked for homogeneity by 2-D TLC in $CHCl_3$–MeOH–HOAc–H_2O (85 : 15 : 10 : 3) followed by $CHCl_3$–MeOH–7M NH_3 (65 : 30 : 4)[7] and were identified by their chromatographic behavior on DEAE-cellulose and silica gel, by their reaction with 50% H_2SO_4 α-naphthol–H_2SO_4, Liebermann–Burchard reagent, and ninhydrin, and by their phosphate and carbohydrate contents. In addition, glycolipid bands were scraped from the plate and hydrolysed with 0·5 M H_2SO_4 for 1 hr at 100°,[16] without prior removal from the adsorbent. The supernatant was neutralised with ion-exchange resin (AGl–X4 200–400 mesh, carbonate form, and a little AG50–X4, 200–400 mesh, hydrogen form), and evaporated to dryness *in vacuo*. The sugars released were dissolved in satd benzoic acid and identified by PC in EtOAc-pyridine–H_2O (12:5:4).

Quantitative determination. The carbohydrate and phosphate contents of the lipid bands were determined according to Roughan and Batt[16] and Rouser *et al.*,[22] respectively. Chlorophyll was determined by Arnon's[23] method, checked with Bruinsma's coefficients.[24]

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