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SHORT REPORTS

COMPOSITION OF LEAF EPICUTICULAR WAXES OF *PTERIDIUM* SUBSPECIES

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Key Word Index—*Pteridium aquilinum caudatum; P. a. aquilinum*; Polypodiaceae; bracken; leaf wax; alkyl esters; alkanols; hydrocarbons.

Abstract—The leaf epicuticular waxes of two subspecies of *Pteridium* consisted principally of alkyl esters (92%; C_{40}–C_{50}) together with small amounts of *n*-alkanols (2%; C_{24}–C_{32}) and hydrocarbons (2%; C_{27}–C_{31}). The esters comprised C_{22}–C_{32} alkanols randomly combined with C_{20}–C_{24} fatty acids.

INTRODUCTION

The genus *Pteridium* is the most widely distributed of the vascular plants, and bracken has now assumed ecological dominance in many areas. This successful adaptation has led to economic problems arising from its progressive encroachment of agricultural and forestry land. Differences in seasonal senescence [1], herbicide sensitivity [2, 3] and response to environmental stress between subspecies of *P. aquilinum* viz: *caudatum* the New Zealand form and *aquilinum*, the UK form have resulted in speculation over differences in leaf surface characteristics and added to the controversy over taxonomic classification [4]. Since the cuticular components of *Pteridium* subspecies have not been investigated as taxonomic criteria, *P. a. caudatum* and *P. a. aquilinum* were selected for a detailed comparison of epicuticular wax chemistry.

This paper describes the composition of the major wax classes and the alkanol and fatty acid fractions of the alkyl ester hydrolysis products.

RESULTS AND DISCUSSIONS

The multiple branching of the delicate pinnae precluded an accurate assessment of surface area. Expressed on either fresh or dry weight basis, similar quantitites of wax were recovered from both subspecies, (Table 1). The composition of the individual wax fractions were also very similar. Nonacosane was the main constituent of the hydrocarbon fractions; these contained almost identical proportions of five major homologues (C_{27}–C_{31}). Although the odd carbon-numbered homologues predominated, the ratio of odd to even chain components (19:10) was much lower than that commonly reported for other plant waxes. The alkanol fractions from both subspecies consisted principally (98%) of constituents of even chain length (C_{24}–C_{30}); triacontanol was the major homologue of *P. a. aquilinum* alkanols whereas octocosanol was the dominant component of the corresponding fraction of *P. a. caudatum* wax.

An homologous series (C_{40}–C_{52}) of even carbon-numbered constituents present in almost identical proportions comprised the dominant components (99%) of the alkyl ester fractions of the waxes of both species. Gas–liquid chramatography analysis of the hydrolysis products revealed that these esters were formed principally from C_{22}, C_{24} and C_{26} alkanols esterified with C_{20}, C_{22} and C_{24} fatty acids. The difference between the homologue composition of the free and esterified alkanols was especially marked, the C_{28}–C_{32} constituents comprising 68–73% and 16–18% of the respective fractions.

Although primary alcohols, secondary alcohools, β-diketones and to a lesser extent hydrocarbons and triterpenoid acids comprise the dominant class of many plant leaf waxes [5], this is the first report of alkyl esters occurring as major cuticular constituents in plants. The close similarity between the waxes of the two *Pteridium* subspecies suggests that leaf wax components will be of limited use as chemotaxonomic indicators for varieties within this species. It would also appear to eliminate surface wax chemistry as a cause of differences in herbicide sensitivity.

EXPERIMENTAL

Fresh fronds of *P. aquilinum* (L.) Bonap. ssp. *caudatum* var. *esculentum* and *P. aquilinum* (L.) Kuhn ssp. *aquilinium var. aquilinum* were obtained from rhizome fragments maintained in pots for 4 months in a glasshouse, at the University of Strathclyde, Glasgow. Epicuticular waxes were removed from fresh pinnae by washing with cold chloroform. The alkyl ester fractions isolated by prep TLC were saponified using methanolic

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Table 1. Composition of *Pteridium a. aquilinum* and *P. a. caudatum* leaf waxes

(i) Total wax analysis

	Wax (% Dry wt)	% hydrocarbon	% primary alcohol	% alkyl ester	% other constituents	
aquilinum	1.3	2	2	93	3	
caudatum	1.3	4	2	92	2	

(ii) Hydrocarbons (Homologue composition %)

	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}
aquilinum	12	18	29	17	24
caudatum	15	20	26	15	23

(iii) Alkanols (homologue composition %)

	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{30}
aquilinum	12	tr	18	2	28	40
caudatum	tr	ND	25	2	48	25

(iv) Alkyl esters (homologue composition %)

	C_{40}	C_{41}	C_{42}	C_{43}	C_{44}	C_{45}	C_{46}	C_{47}	C_{48}	C_{49}	C_{50}	C_{51}	C_{52}
aquilinum	1	tr	6	tr	17	tr	24	0.5	26	0.5	17	tr	8
caudatum	0.5	tr	4	tr	16	tr	25	0.5	24	0.5	17	tr	7

(v) Ester hydrolysis products (homologue composition %)

	Fatty acid fraction			Primary alcohol fraction					
	C_{20}	C_{22}	C_{24}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}
aquilinum	47	32	27	23	42	19	8	5	tr
caudatum	40	21	12	19	39	24	12	4	2

tr = Trace, ND = Not detected.

KOH [6]. Total waxes and individual wax fractions were analysed by GLC on 1% Dexsil 300 programmed 130–350° at 6°/min using an FID with N_2 as carrier gas [7]. Alkanols and fatty acids were converted to the corresponding ethers and esters using *N,O*-bistrimethylsilylacetamide [8]. TLC was performed using Si gel G plates and toluene as solvent. All structural assignments were confirmed by GC-MS [9, 10].

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