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WAX ESTERS OF THE NEW ZEALAND SILVER FERN, *CYATHEA DEALBATA*

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Key Word Index—*Cyathea dealbata*, Cyantheaceae, New Zealand silver fern, capillary column GC/MS, epicuticular wax, wax esters

Abstract—The New Zealand silver fern epicuticular wax contained wax esters (C_{38}–C_{60}) of which the C_{40}–C_{52} homologues were shown by capillary column GC/MS to comprise C_{22}–C_{34} even and odd *n*-alkanols randomly combined with C_{16}–C_{24} even *n*-acids

INTRODUCTION

The silver fern, *Cyathea dealbata* (recently renamed *Alsophila tricolour*), is a common fern in New Zealand forests It has large (1–2 m long) fronds, the undersides of which are covered with a dense epicuticular wax, which has not previously been examined chemically It is the silvery appearance of the wax layer from which the fern derives its common name

The epicuticular waxes of ferns have been shown to contain *n*-alkanes (C_{25}–C_{33}), wax esters [giving long chain *n*-acids (C_{26}–C_{34}) and long chain *n*-alkanols (C_{23}–C_{31}) after hydrolysis], nonacosan-10-ol, nonacosan-10-one and triterpene hydrocarbons, such as fern-7-ene, fern-8-ene, fern-9(11)-diene, derived from hopane precursors, as well as serratenes [1]

Methods for the analysis of low-volatility, epicuticular wax components by GC and GC/MS have been improved through the use of fused silica capillary columns with immobilized phases allowing operation to 350°, better capillary column injection techniques, especially cool, on-column injection [2], and better GC/MS interface design [3] Wax esters can now be analysed directly by capillary column GC/MS [4–6], without the need for a hydrolysis step, in which structural information on individual homologues is lost

This paper reports the composition of silver fern epicuticular wax esters determined directly by capillary column GC and GC/MS

RESULTS AND DISCUSSION

Silver fern epicuticular wax was obtained as an off-white, very hard solid The neutral fraction (87% total wax) obtained by ion-exchange chromatography was shown by TLC to comprise one major hydrocarbon, identified by mass spectrometry, ^1H and ^{13}C NMR as fern-9(11)-ene [7] (*ca* 78% of the neutral fraction) and *ca* 20 minor components After initial separation by CC, the alkyl esters were isolated pure by prep TLC and comprised *ca* 0.2% of the neutral fraction This is a very small ester content compared with that of other plant waxes [8]

The alkyl ester fraction was shown to be composed of C_{38}–C_{60} even and odd homologues by capillary GC and the C_{40}–C_{52} esters to contain C_{14}–C_{26} even *n*-acids and C_{24}–C_{34} even and odd *n*-alkanols randomly interesterified as determined by GC/MS The *n*-acids range (C_{14}–C_{26}) is lower than that (C_{26}–C_{34}) found for other ferns [1]

The data are summarized in Table 1, which shows the percentage of each homologue in the wax ester fraction, the electron impact GC/MS $[M]^+$ observed, the collision induced probe mass spectral $[M-1]^+$ observed [6], the diagnostic electron impact mass spectral fragments [9], the acid–alkanol composition of each homologue and the approximate content of each ester in each homologue Some of the minor components did not display an $[M]^+$ in their electron impact mass spectra, but their presence and M_rs were determined independently by probe collision induced mass spectrometry Wax esters with carbon numbers greater than C_{52} failed to pass through the GC/MS transfer line and so composition data for these were not obtained

The $[R'-1]^+$ fragments of the esters were observed only for the C_{24}, C_{26} and C_{28} alkanols It is known that the abundance of the $[R'-1]^+$ ion is dependent on ester composition and chain length [9] The composition of some of the esters was, therefore, determined from the acyl fragment only In addition, the absence of $[R'-1]^+$ ions for C_{20}, C_{22} and C_{30}–C_{34} alkanols in the mass spectra of esters containing them (Table 1) did not allow accurate quantitation of the esters within each homologue by application of the formula used by Aasen *et al* [9]

The analysis of the small ester sample from New Zealand silver fern wax has demonstrated the applicability of the method for up to C_{52} esters using an unmodified GC/MS instrument (cf. ref [6]) It is unlikely, in this example, that the wax esters contribute significantly to the physical appearance of silver fern epicuticular wax, in which fern-9(11)-ene is such a dominant component

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Table 1 Composition and diagnostic mass spectra of ions of alkyl esters (RCO_2R') of the New Zealand silver fern, *Cyathea dealbata*

Carbon No	% of total	EI $[M]^+$ observed	CI $[M-1]^+$ observed*	$[RCO_2H_2]^+$ fragment	$[R'-1]^+$ fragment	Composition		Approx % within homologue
						Alkanol	Acid	
38	0 1	—	—	—	—	—	—	—
40	0 3	—	591	257	—	24	16	100
41	0 1	—	605	257	—	25	16	100
42	2 1	620	619	257	364	26	16	79
				285	336	24	18	18
				313		22	20	3
43	0 5	—	633	257	—	27	16	76
				285		25	18	24
44	5 8	648	647	257	392	28	16	32
				285	364	26	18	40
				313	336	24	20	23
				341		22	22	4
				369		20	24	1
45	1 0	—	661	257	—	29	16	34
				285		27	18	45
				313		25	20	21
46	25 1	676	675	257		30	16	23
				285	392	28	18	17
				313	364	26	20	41
				341	336	24	22	16
				369		22	24	3
47	1 1	—	689	257	—	31	16	26
				285		29	18	14
				313		27	20	51
				341		25	22	9
48	28 0	704	703	257		32	16	13
				285		30	18	8
				313		28	20	24
				341	364	26	22	34
				369	336	24	24	21
49	0 9	—	717	313	—	29	20	18
				341		27	22	65
				369		25	24	17
50	24 1	732	731	257		34	16	1
				285		32	18	2
				313		30	20	5
				341		28	22	27
				369	364	26	24	65
51	0 8	—	745	369	—	27	24	100
52	5 0	760	759	341	—	30	22	5
				369		28	24	95
53	0 3	—	—	—	—	—	—	—
54	2 6	—	787	—	—	—	—	—
55	0 1	—	—	—	—	—	—	—
56	1 3	—	—	—	—	—	—	—
58	0 6	—	—	—	—	—	—	—
60	0 3	—	—	—	—	—	—	—

*$[M+1]^+$ also observed

EXPERIMENTAL

Fronds of *Cyathea dealbata* were obtained locally near the Forest Research Institute, Rotorua, New Zealand

Wax extraction and fractionation Epicuticular wax was obtained by immersing sections of fronds in $CHCl_3$ at 20° for 1 min The crude wax was chromatographed on DEAE–Sephadex using $CHCl_3$–MeOH–H_2O (89 10 1) to recover neutral compounds and $CHCl_3$–MeOH–H_2O–HCO_2H (86 9 1 4) to elute free acids The neutral fraction was chromatographed on a column of deactivated Al_2O_3 (activity IV) using hexane–EtOAc mixtures (9 1, 4 1, 1 1) and the alkyl ester fraction obtained pure by prep TLC (R_f 0 6) on silica gel using hexane–EtOAc (9 1)

Analysis Wax esters were dissolved in CH_2Cl_2 (1 mg/ml) for injection into the chromatograph GC was carried out on both

5 m and 25 m × 0 2 mm immobilized OV-1 fused silica capillary columns, programmed from 100° to 300° at 4°/min The sample was loaded onto the column using an air-cooled, on-column syringe injector Carrier gas was He at a mean flow rate of 100 cm/sec, FID was at 300° Peak areas were determined by reporting integrator and are uncorr for relative response for esters over the C_{40}–C_{60} range GC/MS was carried out using a quadrupole filter instrument operating at 70 eV, 300 μA electron energy and an ion-source temp of 200° Other mass spectrometer parameters were chosen to maximize high mass sensitivity Spectra were taken at 2 8 sec intervals The capillary column was coupled to the mass spectrometer ion-source via an open-split interface heated maximally at 285° The approximate amount of each ester within a homologue was determined by integration of the ion chromatograms for the $[RCO_2H_2]^+$ fragments CI probe MS were obtained using CH_4 reactant gas at 0 6 Torr and an evaporation temp of 300°

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24β-ETHYLSTEROLS, *n*-ALKANES AND *n*-ALKANOLS OF *CLERODENDRUM SPLENDENS*

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Key Word Index—*Clerodendrum splendens*, Verbenaceae, 24β-ethylsterols, 24β-ethylcholesta-5,22*E*,25(27)-trien-3β-ol, 25(27)-dehydroponiferasterol, clerosterol, 25(27)-dehydrochionasterol, *n*-alkanes, *n*-alkanols, fatty alcohols, sterols

Abstract—The sterols of *Clerodendrum splendens*, an angiosperm belonging to the family Verbenaceae, were found to possess a 24β-ethyl group No other sterols were detected The major sterol was 24β-ethylcholesta-5,22*E*,25(27)-trien-3β-ol [also known as 25(27)-dehydroponiferasterol] A very small amount of what may have been its 22-dihydroderivative, clerosterol [also known as 25(27)-dehydrochionasterol] was also found The dominant *n*-alkane was C_{29} (*n*-nonacosane) and the dominant *n*-alkanol was C_{28} (*n*-octacosanol)

INTRODUCTION

In the great majority of mature angiosperms which have been investigated the dominant sterols possess a 24α-alkyl group [1] While smaller amounts of 24β-methylsterols often occur, 24β-ethylsterols have been found only rarely Examples include the Δ⁵-24β-ethylsterols of *Kalanchoe daigremontiana* [2], *Conopharyngia durissima* [3], *Enhydra fluctuans* [4, 5], *Cucurbita maxima* [6] and various species belonging to the genus *Clerodendrum* [7–13] Δ⁷-24β-Ethylsterols are well described in the family Cucurbitaceae [14–20] Interestingly, 24β-ethylsterols frequently appear to be unaccompanied by 24α-ethylsterols or by 24α- or 24β-methylsterols (in cases where the configuration at C-24 has been firmly established) However, there have been reports of the presence of sitosterol (without ^1H NMR substantiation of configuration) along with 24β-ethylsterols in the roots of *Clerodendrum paniculatum* and *Clerodendrum colebrookianum* [11] and the flowers of *Clerodendrum infortunatum* [12, 21] It has also been reported that the leaf fat (which was 4 1 % of the leaf material) of *Clerodendrum inerme* yielded two isomeric sterols with empirical formulae $C_{27}H_{46}O$, one of which was presumed to be cholesterol [22, 23]

Clerodendrum splendens, a native of Sierra Leone, became available to us through the kindness of Dr Donald G Huttleston This particular plant does not appear to have been previously investigated and it offered an