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CONSTITUENT ACIDS OF *PINUS RADIATA* STEM CUTIN

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Key Word Index—*Pinus radiata*; Pinaceae; radiata pine; stem cutin; mono acids; di acids; hydroxy acids; capillary column GC/MS.

Abstract—Stem cutin from *P. radiata* seedlings grown under winter and summer environmental conditions comprised *n*-alkanoic, (C_{10}–C_{26}), α, ω-alkanedioic (C_{14}–C_{22}), ω-hydroxyalkanoic (C_{12}–C_{24}), hydroxy-α, ω-alkanedioic and polyhydroxyalkanoic acids. 9-Hydroxyheptadecane-1, 17-dioic, 9-hydroxyoctadecene-1, 18-dioic, 9-hydroxynonadecane-1, 19-dioic, and 10, 17-dihydroxyheptadecanoic acids are newly-identified constituents of gymnosperm cutin. Cutin grown under winter temperatures and photoperiod contained twice the amount of 9, 16-dihydroxyhexadecanoic acid than that in summer-grown cutin, suggesting that the winter-grown cutin was formed from a highly cross-linked polymer, and that summer-grown cutin contained more linear polyester portions in the polymer.

INTRODUCTION

Green shoots of *Pinus radiata* are infected by the fungal pathogen *Diplodia pinea* (Desm.) Kickx directly through the stem causing a disease known as shoot dieback [1, 2]. Infection of rapidly growing shoots occurs during late spring and summer, while the previous year's growth tissue, which has ripened over winter, is rarely colonized [1].

As part of a study concerned with the chemical and biochemical factors which may predispose *P. radiata* to infection by *D. pinea*, the effect of summer and winter temperature and light conditions on stem cutin composition has been investigated. Although cutin constitutes the major protective barrier between the plant and its environment, some pathogenic micro-organisms synthesize cutinase enzymes which enable them to breach this barrier [3]. Cutin from both angiosperms [4] and gymnosperms [5] contain C_{16}-hydroxy acids (primarily 16-hydroxy, 9, 16- or 10, 16-dihydroxy) which contain the essential structural feature (an aliphatic alcohol containing at least 16 carbon atoms) required to induce cutinase [3].

This report describes the identification, using capillary column GC/MS, of the constituent acids of *P. radiata* stem cutin from plants grown under summer and winter environmental conditions.

RESULTS AND DISCUSSION

P. radiata stem cutin from both environmental treatments comprised *n*-alkanoic (C_{10}–C_{26}), α, ω-alkanedioic (C_{14}–C_{22}), ω-hydroxalkanoic (C_{12}–C_{24}), hydroxy-α, ω-alkanedioic and polyhydroxy acids. The approximate composition of each cutin sample is summarized in Table 1, and the homologue distribution of the alkanoic, α, ω-alkanedioic, and ω-hydroxy alkanoic acids in Table 2.

The hydroxy-α, ω-alkanedioic and polyhydroxy acids identified in *P. radiata* stem cutin hydrolysate are summarized in Table 3, together with their diagnostic mass spectral fragments formed by fission α to the OTMSi function [5] and their approximate amount in each cutin sample. None of the hydroxy acid methyl ester TMSi ether mass spectra showed $[M]^+$, but all showed weak $[M-15]^+(\leq 1)$ ions, permitting the chain length to be defined. Other polyhydroxy acids are present, but their mass spectra cannot readily be interpreted.

Among the hydroxy acids identified, 9-hydroxyheptadecane-1, 17-dioic, 9-hydroxyoctadecene-1, 18-dioic, 9-hydroxynonadecane-1, 19-dioic and 10, 17-dihydroxyheptadecanoic acids have not previously been recognized as constituents of gymnosperm cutins. *P. radiata* stem cutin contains 9, 16-dihydroxyhexadecanoic acid as the principal monomer, with no evidence of the 10, 16-dihydroxy isomer being present, as was observed with *P. sylvestris* needle cutin acids [6]. *P. radiata* cutin also contains much higher proportions of hydroxyalkanedioic acids than those reported in pine needle cutin [6], and significantly higher proportions of odd-carbon chain (C_{15}, C_{17}, C_{19}) components, as well as a wide range of carbon chain lengths of the monomers instead of the usual C_{16} and C_{18} families than have been found generally in plant cutins [3–6]. The major alkanoic acid in summer cutin was C_{22} (Table 2), which was also the major alkanoic acid homologue identified in *P. radiata* bark [7].

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Table 1. Approximate composition (%) of *P. radiata* stem cutin from summer and winter environmental treatments

Monomer acid classes	Summer cutin	Winter cutin
Alkanoic	16.6	12.5
α,ω-Alkanedioic	25.4	10.9
ω-Hydroxyalkanoic	27.4	19.3
Hydroxy-α,ω-alkanedioic	7.5	9.9
Dihydroxyalkanoic	20.2	40.0
9,10,18-Trihydroxyoctadecanoic	0.1	0.2
Others (mainly polyhydroxy acids)	2.8	7.2

Table 2. Homologue content (%) of *P. radiata* stem cutin alkanoic, α,ω-alkanedioic and ω-hydroxyalkanoic acids

Acids		Summer	Winter
Alkanoic	10:0	0.6	0.2
	12:0	13.9	22.0
	14:0	4.9	7.8
	15:0	0.2	0.5
	16:1 (9)	0.3	1.2
	16:0	9.0	17.1
	17:0	0.1	0.2
	18:2	2.1	6.4
	18:1 (9)	4.7	7.5
	18:1 (11)	0.9	1.9
	18:0	3.8	4.3
	20:1	0.5	1.0
	20:0	20.3	16.8
	21:0	0.2	0.1
	22:0	25.4	10.9
	23:0	0.5	0.3
	24:0	12.0	1.2
	26:0	0.7	0.1
α,ω-Alkane-dioic	14:0	0.5	1.5
	15:0	0.7	0.7
	16:0	24.6	22.2
	17:0*	5.2	1.6
	18:2	3.6	5.1
	18:1	33.4	37.0
	18:0	7.2	10.5
	19:1	7.6	2.2
	20:0	13.3	16.6
	22:0	3.7	2.6
ω-Hydroxyal-kanoic	12:0	0.7	8.0
	14:0	4.6	15.0
	16:0	40.0	33.9
	17:0	0.4	0.3
	18:2	0.4	0.7
	18:1 (9)	34.1	27.5†
	18:0	5.5	4.4
	20:1	1.1	1.1
	20:0	9.4	6.7
	22:0	2.8	1.4
	24:0	0.4	0.1

*Probably branched.
†Calculated from mixed mass spectra.

Environmental conditions do have an effect on *P. radiata* stem cutin composition, since material grown under winter photoperiod and temperatures contained C_{12} as the major alkanoic acid (Table 2). It also contained twice the amount of 9, 16-dihydroxyhexadecanoic acid as that present in summer cutin (Table 3). This could be interpreted as indicating that the winter stem cutin polymer (which is formed more slowly than the summer cutin), is more cross-linked, whereas the summer cutin, which contains *ca* equal proportions of ω-hydroxyalkanoic, α, ω-alkanedioic, and dihydroxyalkanoic acids (Table 1), could contain more linear, estolide-like, portions in the polymer. It has been suggested that cutin of rapidly growing plant organs contains predominantly C_{16} monomers, whereas that of slower growing organs contains a mixture of C_{16} and C_{18} monomers [3]. This distinction is not well defined in the case of *P. radiata* stem cutin.

In addition to quantitative differences in the constituents of summer and winter grown cutin (Tables 1–3), the latter material also contained phenolic compounds, probably due to initial formation of bark, which contains condensed tannins of the procyanidin and prodelphinidin type [8]. Further details of *P. radiata* stem chemistry in relation to *D. pinea* resistance will be reported elsewhere.

EXPERIMENTAL

Plant material. P. radiata D. Don seedlings (1 yr old) were raised in pots from commercial seed (FRI, Rotorua, N.Z.). Seedlings were placed in growth cabinets under simulated summer (24° day, 14° night, 16 hr photoperiod), and winter (12° day, 8° night, 8 hr photoperiod) conditions for 1 month. Epidermis (*ca* 0.5 g fr. wt) was stripped in short pieces from portions of newly-grown stem. Epidermal strips were exhaustively treated with chloroform at 20° for 2 weeks with 10 changes of solvent to remove waxes, and then with $ZnCl_2$–HCl (1:2) to remove cellulose. Pure gymnosperm cuticle appears to be difficult to prepare by chemical treatment alone; material used for these studies contained various amounts of adhering epidermal cell wall.

Cutin hydrolysis. 'Cuticle' pieces were hydrolysed using 5% KOH in MeOH for 15 hr. MeOH was removed under red. pres., H_2O added to the residue, and then acidified with 2 N HCl. Organic material was extracted with Et_2O, which was washed with H_2O, dried ($MgSO_4$) and evaporated. The residue was methylated (CH_2N_2 in Et_2O–EtOH) and then analysed.

Table 3. Hydroxy-α, ω-alkanoic and polyhydroxy acids, mass spectral fragments for the methyl ester TMSi ether derivatives, and amounts in summer and winter *P. radiata* stem cutin

Acid	*m/z*(RI, %)	Composition(%)	
		Summer	Winter
7-Hydroxypentadecane-1, 15-dioic	231(50), 259(65)	0.6	0.7
8-Hydroxyhexadecane-1, 16-dioic	245(80), 259(70)	2.9	4.5
9-Hydroxyheptadecane-1, 17-dioic	259(100)	2.1	1.1
9-Hydroxyoctadecene-1, 18-dioic*	259(10), 271(100)	0.9	0.8
9-Hydroxyoctadecane-1, 18-dioic	259(45), 273(30)	0.6	1.1
9-Hydroxynonadecane-1, 19-dioic	259(85), 287(35)	0.4	1.7
9, 16-Dihydroxyhexadecanoic	259(100), 289(55)	20.0	39.0
10,17-Dihydroxyheptadecanoic	273(40), 289(25)	0.1	0.3
9, 18-Dihydroxyoctadecanoic	259(75), 317(20)	0.1	0.7
9, 18-Dihydroxy-10-methoxyoctadecanoic†			
10, 18-Dihydroxy-9-methoxyoctadecanoic†	259(100), 274(15)‡ 303(35)	0.3	0.3
9, 10, 18-Trihydoxyoctadecanoic	259(65), 361(25) 322(5)‡, 303(1)	0.1	0.2

*Position of double bond not known

†From methanolysis products of 9, 10-epoxy-18-hydroxyoctadecanoic acid.

‡Diagnostic rearrangement fragment.

Analysis. Methyl esters were separated into classes by prep. TLC on Si gel G using hexane–EtOAc (9:1). (R_f values for the esters were: alkanoic, 0.63; α, ω-alkanedioic, 0.57; hydroxy-α, ω-alkanedioic, 0.36; ω-hydroxyalkanoic, 0.20; polyhydroxyalkanoic, 0.03). Hydroxy acids were treated with BSTFA to form TMSi ethers. GC was carried out on 20 m × 0.2 mm OV1 capillary columns using He carrier gas at 1–1.5 ml/min. Purged-splitless injector and FID temps. were 300°. Column temp. was programmed from 80 to 270° at 4°/min. GC/MS was performed using a quadrupole filter instrument operating at 35 eV, 350 μA electron energy and an ion source temp. of 250°. The capillary column was coupled directly to the ion source. Chromatographic conditions were similar to those used for analytical GC.

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