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LUTEOLIN 7-*O*-[6-*O*-α-L-ARABINOFURANOSYL]β-D-GLUCOPYRANOSIDE AND OTHER NEW FLAVONOID GLYCOSIDES FROM NEW ZEALAND *DACRYDIUM* SPECIES

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ABSTRACT.—The New Zealand conifers, *Dacrydium laxifolium*, *Dacrydium intermedium*, and *Dacrydium colensoi* (Podocarpaceae), have yielded a number of rare or new flavonoid glycosides. These have been fully characterized by ^{1}H- and ^{13}C-nmr spectroscopy and include apigenin and luteolin 7-*O*-β-D-xylopyranosyl(1-6)β-D-glucopyranosides, 7-*O*-α-L-arabinopyranosyl(1-6)β-D-glucopyranosides, and 7-*O*-α-L-arabinofuranosyl(1-6)β-D-glucopyranosides together with quercetin 3-*O*-α-L-arabinopyranosyl(1-6)β-D-glucopyranoside. The distribution of flavonoid glycosides is of relevance to a previously proposed subdivision of this genus.

In the course of chemotaxonomic studies directed at the better classification of New Zealand conifers (1-3), a number of new flavonoid glycosides have been isolated from New Zealand *Dacrydium* species (Podocarpaceae). It has previously been proposed on morphological grounds (4) [with some prior indications from biflavonoid distribution (5)] that species of this genus should be reassigned to four separate genera, *Dacrydium*, *Halocarpus*, *Lepidothamnus*, and *Lagarostrobus*. The present paper describes the flavonoid glycoside chemistry of the New Zealand members of the proposed *Lepidothamnus* and *Lagarostrobus* genera. No previous studies of the flavonoid glycosides of these plants have been recorded.

RESULTS AND DISCUSSION

Flavonoid glycosides isolated and identified from the three New Zealand species are listed in Table 1. The common flavonoids were identified by standard techniques (6) and are not discussed further; details of the properties and structure elucidation of new or rare flavonoids are presented below.

Most of the flavonoid glycosides encountered were readily separated by a combination of cellulose column chromatography and 2D-paper chromatography. Separation of xyloglucoside and arabinoglucoside pairs, however, was exceedingly difficult. They co-chromatographed on cellulose in *t*-BuOH-HOAc-H_2O (3:1:1) (TBA), 15% HOAc, 50%HOAc and *n*-BuOH-EtOH-H_2O (20:5:11) (BEW), and on hplc but separated on polyamide using H_2O-MeOH-MEK-acetylacetone (13:3:3:1). The arabinofuranosyl-glucosides were easily separated from the equivalent arabinopyranosylglucosides by 2D-pc, being markedly more mobile in TBA. The rhamnoglucosides found in *Dacrydium intermedium* co-chromatographed with the arabinofuranosylglucosides and were detected as additional components by sugar analyses.

The structure determinations in all cases involved acid hydrolysis followed by aglycone and sugar analyses, and uv-visible absorption spectroscopy using standard procedures (6) defined the sites of glycosylation. ^{13}C-nmr spectroscopy (see spectra, Table 2) was used to confirm the sugars and to define the sugar ring size and site of interglycosidic linkage. The (1-6)-interglycosidic linkages in quercetin arabinoglucoside and in apigenin and luteolin xylo- and arabino-glucosides are clearly evidenced by the 6 to 7 ppm downfield shift in the glucose C-6 signals. The α-configuration of the arabinose and β-configurations of the xylose and glucose are also evident from the pattern of sugar carbon signals in each spectrum (7).

Of the two arabinofuranosylglucosides found, the luteolin glycoside was the major, and all nmr studies were carried out on this glycoside. On acid hydrolysis both

TABLE 1. Flavonoid Glycosides Found in Three New Zealand *Dacrydium Species*[a]

Flavonoid	*D. laxifolium*	*D. intermedium*	*D. colensoi*
	(*Lepidothamnus*)		(*Lagarostrobus*)
Kaempferol			
-3-glucoside			+
-3-galactoside			trace
Quercetin			
-3-glucoside	+	trace?	++
-3-galactoside			++
-3-rhamnoside			+
-3-arabino(1-6)glucoside			++
-3-rhamno(1-6)glucoside			trace
Apigenin			
-7-glucoside	+	+	
-7-xylo(1-6)glucoside	+	+	
-7-arabino(1-6)glucoside	+	+	
-7-arabinofuranosyl(1-6)glucoside	trace	trace	
-7-rhamno(1-6)glucoside		trace	
Luteolin			
-7-glucoside	++	++	
-7-xylo(1-6)glucoside	++	++	
-7-arabino(1-6)glucoside	++	++	
-7-arabinofuranosyl(1-6)glucoside	+	+	
-7-rhamno(1-6)glucoside		+	

[a]Unless otherwise specified, sugars are in the pyranosyl form. Number of crosses indicates relative levels within a species.

glycosides gave arabinopyranose and glucose in approximately 1:1 ratio, together with the appropriate aglycones. The arabinofuranosyl formulation of the luteolin glycoside was apparent from the distinctive ^{13}C-nmr sugar signals (Table 2) that matched well with published data (7) and, further, defined the glucose moiety as β-D-glucopyranosyl and the arabinose as α-L-arabinofuranosyl. The configurations at C-1 were confirmed by the ^{1}H-nmr spectrum in which the glucose H-1 signal appeared at 5.06 ppm with $J(1,2)=7.3$ Hz (8) and the arabinose H-1 signal at 4.72 ppm with $J(1,2)=1.3$ Hz (9). The luteolin glycoside is, thus, defined as luteolin 7-*O*-[6-*O*-α-L-arabinofuranosyl]β-D-glucopyranoside, and, by analogy, the apigenin glycoside is assigned the equivalent structure.

Of the diglycosides isolated in this study (Table 1), the rhamnoglucosides are commonly encountered in nature, but the arabino- and xylo-glucosides are all rare or previously unreported. Quercetin 3-arabino(1-6) glucoside has been reported only once [from *Nymphoides peltata* (10)], and compounds described as apigenin 7-arabinoglucoside, 7-xyloglucoside, and luteolin 7-arabinoglucoside have been reported briefly in surveys (11,12) but in no cases have they been properly characterized with respect to sugar sequence and the ring size, interglycosidic linkages, or glycoside configuration. Luteolin 7-xylo(1-6)glucoside (primeveroside) has been tentatively identified in a preliminary report on *Salix repense* (13), but the apigenin and luteolin 7-arabinofuranosylglucosides are new.

There is an apparent marked distinction between the two proposed new genera separated from *Dacrydium: Lepidothamnus* accumulating flavone monoglycosides and diglycosides and *Lagarostrobus* accumulating flavonol monoglycosides and diglycosides. Final confirmation of the revelance of these differences to the proposed reclassification of

TABLE 2. ^{13}C-nmr Data (in ppm) for the Sugar Moieties of Selected Flavonoid Glycosides[a]

Sugar Carbon Number	Quercetin 3-glucoside	Quercetin 3-galactoside	Quercetin 3-arabino(1-6)glucoside	Apigenin 7-xylo(1-6)glucoside	Apigenin 7-arabino(1-6)glucoside	Luteolin 7-xylo(1-6)glucoside	Luteolin 7-arabino(1-6)glucoside	Luteolin 7-arabinofuranosyl(1-6)-glucoside
C-1″	101.1	102.0	101.1	100.0	100.0	100.0	100.0	100.0
C-2″	74.2	71.4	74.1	73.2[c]	73.2	73.2[d]	73.2	73.2
C-3″	76.7[b]	73.4	76.5[b]	76.4[d]	76.4[b]	76.4[c]	76.4[c]	76.4[c]
C-4″	70.1	68.1	70.2	69.6	69.6	69.6	69.6	69.9
C-5″	77.7[b]	76.0	76.9[b]	76.6[d]	76.6[b]	76.7[c]	76.7[c]	75.6[c]
C-6″	61.1	60.3	67.4	68.4	67.7[c]	68.5	67.5[d]	67.0
C-1‴			102.8	104.2	102.9	104.1	103.2	108.6
C-2‴			72.6	73.4[c]	72.5	73.4[d]	72.6	82.1
C-3‴			70.6	75.8[d]	70.6	75.7[c]	70.6	77.3[c]
C-4‴			67.4	69.6	67.2[c]	69.6	67.2[d]	84.1
C-5‴			64.9	65.7	64.6	65.7	64.7	61.4

[a]Unless otherwise specified sugars are in the pyranosyl form. Assignments are based on previously published work-see Markham *et al.* (15) (flavonoid glycosides general) and Garcia-Granados and Saenz de Boruaga (16) (primeveroside).

[b,c,d]Assignments bearing the same superscript in any one spectrum may be reversed.

Dacrydium, however, must await completion of the continuing survey of New Zealand (and other) *Dacrydium* species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were measured on DMSO-d_6 solutions of components from *Dacrydium laxifolium*, the ^{1}H- spectra at 200 MHz on a Varian XL-200 and the ^{13}C- spectra at 20 MHz on a Bruker FT80A. Paper chromatography [for method etc., see Markham (6)] solvents used: *t*-BuOH-HOAc-H_2O, 3:1:1 (TBA); 15% HOAc; *n*-BuOH-EtOH-H_2O, 20:5:11 (BEW). Hplc conditions: analytical C-18 reversed phase column, detector at 350 nm, solvent=MeOH-H_2O (3:7) gave retention times (minutes) for luteolin monoglycosides and diglycosides (32 min, 26 min), apigenin monoglycosides and diglycosides (52 min, 41 min), and quercetin 3-glucoside (34 min) all from *D. laxifolium*. Voucher specimens of each plant are deposited in the Botany Division, DSIR herbarium at Christchurch, *D. laxifolium* J.D. Hook. (CHR388283), *D. intermedium* T. Kirk (CHR418734), and *Dacrydium colensoi* Hook. (CHR415906).

ISOLATION AND IDENTIFICATION TECHNIQUES.—Plant material was dried at 100°, ground to a powder, and extracted overnight with EtOH-H_2O (1:1). The extract was chromatographed on a column of cellulose (Merck, microcrystalline) in 2% HOAc. This was gradually increased to 5% HOAc, and the column was finally washed with EtOH. Individual glycosides were isolated by 1D- or 2D-paper chromatography using TBA and 15% HOAc. Low mobility components were cleaned of fluorescent contaminants in *D. intermedium* using 50% HOAc, and arabinoglucosides were separated from xyloglucosides on tlc using Bakerflex 6-F polyamide and H_2O-MeOH-methylethyl ketone-acetylacetone (aged) (13:3:3:1). Acid hydrolyses were carried out using 3N HCl-H_2O (1:1) for 7-glycosides and 3N HCl-MeOH (1:1) for 3-glycosides, and the sugars analysed by pc in *n*-BuOH-C_6H_6-pyridine-H_2O (5:1:3:3) (6). Uv-visible absorption spectra with and without the addition of diagnostic reagents were identical with those previously published for similar glycosides (14). ^{13}C-nmr spectra are detailed in Table 2, and the ^{1}H-nmr spectrum of luteolin 7-arabinofuranosyl(1-6)glucoside in DMSO-d_6 is as follows (δ values): 7.45/7.39 m (H-2′,6′); 6.83 d, J=8.2 Hz (H-5′); 6.75 d, J=2.5 Hz (H-8); 6.68 s (H-3); 6.42 d, J=2.5 Hz (H-6); 5.06 d, J=7.3 Hz (H-1 glucose); 4.72 d, J=1.3 Hz (H-1 arabinose).

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