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QUERCETIN-3-*O*-α-[2-*O*-*p*-HYDROXYBENZOYL-4-*O*-*p*-COUMAROYLRHAMNOPYRANOSIDE], AN AGLYCONE-LIKE FLAVONOL GLYCOSIDE FROM *LIBOCEDRUS BIDWILLII*

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Key Word Index—*Libocedrus bidwillii*; Cupressaceae; leaf; quercetin 3-*O*-rhamnoside, mixed acylation; *p*-coumaroyl; *p*-hydroxybenzoyl; aglycone-like.

Abstract—Quercetin-3-*O*-α-[2-*O*-*p*-hydroxybenzoyl-4-*O*-*p*-coumaroylrhamnopyranoside], a new natural product with unusual mixed acylation, has been found accompanying the biflavonoids in *L. bidwillii*. Aglycone-like chromatographic properties resulted in this compound being missed in the initial chemotaxonomic screening of flavonoid glycosides in *Libocedrus*.

INTRODUCTION

As part of a continuing chemotaxonomic study of New Zealand conifers, the flavonoid glycosides in New Zealand and Pacific island representatives of the genus *Libocedrus* are currently under investigation. Previous work in this series has covered *Podocarpus* s.l. [1, 2], *Dacrydium* s.l. [3] and *Phyllocladus* [4]. Biflavonoids have been encountered in all genera and indeed have been the subject of special studies by others [5, 6]. The present paper details the structure elucidation of an unusual glycosidic component, unexpectedly encountered amongst the biflavonoids of *L. bidwillii*.

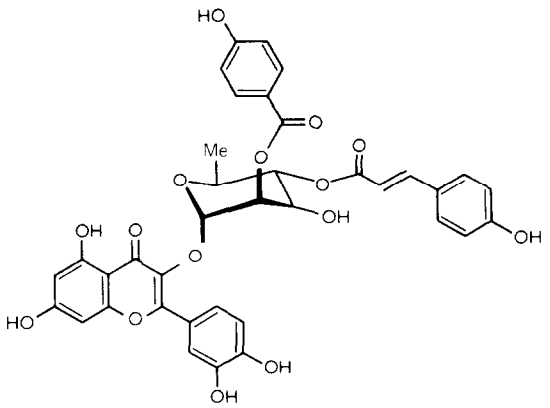
RESULTS AND DISCUSSION

The flavonoid aglycones in *L. bidwillii* were isolated by polyamide column chromatography of a crude aqueous methanol extract. The final fraction from this column was eluted with methanol and contained a range of biflavonoids which on TLC appeared as UV-absorbing spots which turned yellow-green when sprayed with diphenylboric acid 2-aminoethyl ester (NA). In addition, this fraction contained another UV-absorbing component (**1**) which, because it turned bright orange with NA, was thought to be a representative of the rare [7] luteolin-containing biflavonoid group. Preparative TLC on silica gel followed by RP-HPLC, separated **1** from the accompanying biflavonoids. The absorption spectra of **1** indicated the presence of free 5,7,3′ and 4′-hydroxyl groups, but the spectrum in methanol is dominated by intense absorption at 302–317 nm which is commonly associated with cinnamic acid acylation [8]. Indeed acid hydrolysis of **1** produced *p*-coumaric acid along with *p*-hydroxybenzoic acid, quercetin and rhamnose so confirming acylation and discounting the biflavone formulation. Alkaline treatment yielded quercetin-3-*O*-rhamnoside and thus defined **1** as a poly-acylated quercetin-3-*O*-rhamnoside.

The ^{1}H NMR spectrum of **1** confirmed many of the above features. In addition, it revealed the rhamnosyl moiety to be α-linked ($J_{H\text{-}1/H\text{-}2}=2.0$ Hz) and in the pyranose form [9], features which are confirmed by the ^{13}C NMR spectrum. The presence in **1** of a single *trans*-*p*-coumaroyl residue was indicated by the two, one proton doublets ($J=16$ Hz) at δ 6.33 and 7.52 representing the α- and β-protons respectively. Doubled *ortho*-coupled signals for the 2,6- and 3,5-proton pairs of the two acyl groups were also evident. These integrated for a total of eight protons thus defining **1** as a quercetin-3-*O*-rhamnoside diacylated with one *p*-coumaroyl and one *p*-hydroxybenzoyl function.

The sites of acylation were established by assigning and distinguishing signals representing each rhamnose proton, by selective homonuclear decoupling. It is clear from these data that the H-2 and H-4 signals are both some 1.3 ppm downfield from their normal positions (see Table 1). Accordingly, acylation at the rhamnose 2- and 4-hydroxyls is indicated. This is confirmed by the ^{13}C NMR spectrum which exhibits the α- and β-acylation shifts (relative to quercetin-3-*O*-rhamnoside) expected for 2,4-diacylation (see Table 1). All other structural features referred to above were also confirmed by the ^{13}C NMR spectrum.

FDCI mass spectrometry of **1** with ammonia as reactant gas failed to yield a molecular ion. This is not unusual for flavonol glycosides di-acylated with aromatic acids which are known to produce molecular ions of low stability [10]. The base peak at *m/z* 303 (quercetin + H^+) was accompanied by fragment ions representing rhamnose (*m/z* 164), both acids with their acyl ions (*m/z* 137, 121 and 165, 147) and *p*-coumaroyl-dehydrorhamnose (*m/z* 292). The absence of a diacylated dehydrorhamnose fragment is consistent with a previous observation on a similar compound [10]. Both coumaroyl and *p*-hydroxybenzoyl moieties are represented in the monoacylated di-dehydrorhamnose fragments (*m/z* 248 and 274) and in the monoacylated tri-dehydrorhamnose fragments (*m/z* 230 and 256). These data provide confirmation that **1** is a quercetin rhamnoside diacylated on the rhamnose, but in the absence of more detailed information on the fragmentation routes of *O*-rhamnosides, are insufficient to permit assignment of either acyl group to C-2 or C-4.



The relative positions of the two acyl functions were finally revealed in an ^1H–^1H homonuclear NOESY experiment. Strong NOE interactions were observed as expected, between the *ortho*-related protons in the quercetin B-ring and in both of the acyl functions, the H-2 and H-3 of rhamnose, and the H-α, H-β and H-2/6 of the *p*-coumaroyl moiety. In addition, NOE interactions were seen between the quercetin H-2′ and the α- and β-protons of the *p*-coumaroyl group. These effects were confirmed by selective decoupling of the olefinic protons which produced an 18% enhancement of the H-2′ signal on

Table 1. ^1H NMR spectra of **1** and reference compounds*

	1 (DMSO-d_6)	**1** (Me_2CO-d_6)	Q-3-*O*-rha 2″-gallate (TMS ether in $CDCl_3$) [15]	Q-3-*O*-rha 4″-acetate (TMS ether in $CDCl_3$) [14]
Quercetin				
H-6	6.23 (*br s*)	6.27 (*br s*)	6.26 (*d*, 2.0)	6.07 (*d*, 2.0)
H-8	6.43 (*d*, 2.5)	6.48 (*br s*)	6.37 (*d*, 2.0)	6.31 (*d*, 2.0)
H-2′	7.35 (*d*, 1.5)	7.41 (*br s*)	7.37 (*d*, 2.0)	7.12 (*m*)
H-5′	6.97 (*d*, 8.3)	7.09 (*d*, 9.0)	6.97 (*d*, 8.0)	6.73 (*d*, 8.0)
H-6′	7.28 (*dd*, 8.3, 2)	7.42 (*br d*, 9.0)	7.51 (*dd*, 8, 2)	7.12 (*m*)
5-OH	12.49	12.62		
Rhamnose				
H-1	5.56 (*d*, 2.0)	5.83 (*br s*)	5.66 (*d*, 2.0)	5.11 (*d*, 2.0)
H-2	5.47 (*t*, 2.0)†	5.60 (*m*)	5.56 (*t*, 2.0)	4.17 (*t*, 2.0)
H-3	4.06 (*dd*, 9.5, 2)†	4.20 (*m*)	4.02 (*dd*, 8, 2)	3.83 (*dd*, 10, 2)
H-4	4.84 (*t*, 9.7)†	4.96 (*t*, 9.5)	3.57 (*t*, 8.0)	4.80 (*t*, 10.0)
H-5	—	—	3.4 (*m*)	3.17 (*qd*, 6, 10)
H-6	0.91 (*d*, 6.0)	0.94 (*d*, 6.0)	0.99 (*d*, 6.0)	0.72 (*d*, 6.0)
p-OH Benzoyl				
H-2′6′	7.61 (*d*, 8.4)†	7.61 (*m*)		
H-3′5′	6.81 (*d*, 8.3)†	6.89 (*d*, 8.3)		
				p-coumaroyl [16] (DMSO-d_6)
p-Coumaroyl				
H-α	6.33 (*d*, 16.0)†	6.44 (*d*, 16.0)		6.39 (*d*, 17.0)
H-β	7.52 (*d*, 16.0)†	7.61 (*m*)		7.52 (*d*, 17.0)
H-2′6′	7.69 (*d*, 7.7)†	7.61 (*m*)		7.54 (*d*, 9.0)
H-3′5′	6.80 (*d*, 7.9)†	6.89 (*d*, 8.3)		6.83 (*d*, 9.0)

*Coupling constants for *d*, *dd*, *t* and *q* in parentheses are in Hz.
—Obscured by solvent.
†Assignment confirmed by ^1H–^1H decoupling expts.

irradiation of H-β but only a 2% enhancement on irradiation of H-α. Molecular models reveal that H-2′ can only approach closely to H-β if the *p*-coumaroyl group is sited at C-4 of the rhamnose. This is because the diaxial relationship between the C-1 and C-2 hydroxyls of α-rhamnopyranose necessitates a wide separation of the C-1 linked quercetin and any acyl function at C-2, with the rhamnose ring effectively separating the two (see structure **1**). In contrast, an equatorially linked *p*-coumaroyl function at C-4 can readily assume conformations in which either the α- or β-protons approach to within one bond length of H-2′. On this basis, the structure of **1** is defined as quercetin-3-*O*-α-[2-*O*-*p*-hydroxybenzoyl-4-*O*-*p*-coumaroylrhamnopyranoside](1).

Two unusual features of this compound are its low polarity and its mixed acylation. Thus its chromatographic behaviour is more akin to that of a flavonoid aglycone than of a glycoside. On TLC and HPLC it is exceedingly difficult to separate from biflavonoids such as amentoflavone, and on 2D-PC it was missed completely in our initial survey of the *Libocedrus* flavonoid glycosides, because it ran in the region where normally only flavone and biflavone aglycones are found [8]. These unusual properties clearly result from the acylation of two of the three available hydroxyls on the rhamnose. Mixed acylation as in **1**, although it has been reported previously (e.g. [11, 12]), is most unusual for flavonoid glycosides which are commonly mono-, and occasionally di- and tri-acylated with the same acyl functions.

EXPERIMENTAL

Plant material. Libocedrus bidwillii was collected at Christchurch botanical gardens, New Zealand and a voucher specimen (CHR 418729) deposited in the Botany Division, DSIR herbarium at Lincoln.

Techniques and apparatus. 2D-PC: Whatman 3MM paper, TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and 15% HOAc. Spray: 1% diphenylboric acid 2-aminoethyl ester (NA) in MeOH. HPLC: Versapack C-18 column, MeOH–H_2O–HCO_2H, 270:230:1, detection at 350 nm. Absorption spectroscopy and shift reagents: as detailed in ref. [8]. MS: reactant gas, NH_3.

Isolation of **1**. Dried, ground leaf material (100 g) was extracted twice with MeOH–H_2O (9:1) and the combined extracts column chromatographed on polyamide. H_2O with increasing amounts of MeOH eluted polar components first. The final (100% MeOH) fraction was subjected to preparative 1D-PC (in TBA) and the high mobility 'biflavonoid' fraction isolated. Further column chromatography of this fraction on silica gel using $CHCl_3$–MeOH–HCO_2H (1000:10:1, 190:10:1, 90:10:1 and 40:10:1) and on Merck RP-8 using MeOH–H_2O–HCO_2H (20:20:1 to 90:10:1) yielded a semi-pure fraction containing **1**. This was further purified by TLC (silica gel $CHCl_3$–MeOH–HCO_2H, 90:10:1) and the final traces of amentoflavone were removed by HPLC.

Compound **1**. R_fs: PC, 0.93 (TBA), 0.11 (15% HOAc); TLC (silica gel), 0.11 ($CHCl_3$–MeOH–HCO_2H, 180:20:1), 0.37 (toluene–HCO_2Et–HCO_2H, 5:4:1), 0.06 and 0.11 (C_6H_6–pyridine–HCO_2H, 36:9:5 and 90:20:7). Comparable R_fs for amentoflavone (PC, TLC solvents as above): 0.91, 0.13, 0.17, 0.41, 0.11, 0.28. λ_{max}^{MeOH}:nm 220, 258sh, 268, 300sh, 317, 360sh; (NaOMe) 274, 312sh, 367(inc); (NaOAc) 225sh, 278, 302sh, 317, 370sh; (NaOAc/H_3BO_3) 225sh, 265, 302sh, 317, 370; ($AlCl_3$) 220sh, 279, 302sh, 318, 427; ($AlCl_3$/HCl) 220sh, 277, 302sh, 318, 394. ^{13}C NMR (ppm in DMSO-d_6 ref. 39.50): *quercetin*; 160.9 (C-2), 133.9 (C-3), 182.4 (C-4), 163.5 (C-5), 101.0 (C-6), 165.8 (C-7), 95.8 (C-8), 161.6 (C-9), 107.0 (C-10), 123.3 (C-1′), 118.3 (C-2′), 148.9 (C-3′), 149.7 (C-4′), 118.6 (C-5′), 124.0[d] C-6′), *rhamnose*; 99.9 (C-1), 72.4 (C-2), 67.0 (C-3), 74.0 (C-4), 69.1 (C-5), 16.2 (C-6), cf. rhamnose in quercetrin [13] 101.9, 70.4, 70.6, 71.5, 70.1, 17.3, *coumaroyl*; 170.6[a] (CO), 118.3 (C-α), 149.4 (C-β), 128.2 (C-1), 133.9[c](C-2), 117.8[e] (C-3), 164.3[b](C-4). 177.7[e] (C-5), 133.8[c](C-6), *p-hydroxybenzoyl*; 169.0[a] (CO), 124.2[d] (C-1), 136.4[c] (C-2), 116.8[e] (C-3), 164.5[b] (C-4), 116.5[e] (C-5), 136.3[c] (C-6). FDCI-MS (rel. intensities) *m/z*: 303 (100), 293 (13), 292 (10), 287 (30), 275 (19), 274 (8), 257 (10), 256 (8), 248 (9), 231 (28), 230 (11), 195 (34), 177 (8), 168 (40), 165 (23), 164 (24), 153 (10), 152 (12), 151 (53), 149 (12), 147 (40), 137 (64), 136 (10), 129 (42), 124 (30), 121 (100), 119 (15), 111 (34), 110 (22), 109 (24), 97 (13), 94 (46). Alkaline hydrolysis: was carried out in a syringe with 2 M NaOH at room temp. (see ref. [8]). TLC analysis of products (cellulose, TBA, BEW and 15% HOAc) identified quercetin-3-rhamnoside. Acid hydrolysis (2 M HCl, reflux, 5 min) gave products identified by TLC and PC co-chromatography with authentic samples: quercetin (cellulose, BEW, TBA and 50% HOAc); rhamnose silica gel, *n*-BuOH–Me_2CO: 1.6%aq. NaH_2PO_4, 5:4:1 and cellulose, *n*-BuOH–pyridine–HOAc–H_2O, 5:1:3:3); acids cellulose, 5% NH_4OH in EtOH and 15% HOAc and silica gel, $CHCl_3$–MeOH–HCO_2H, 180:20:1), R_f values; *p*-coumaric (0.62, 0.53, 0.34) and *p*-OH benzoic (0.48, 0.71, 0.32) respectively. Spray reagent for acids: 1% $K_3Fe(CN)_6$–15% $FeCl_3$ (1:1).

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