6.48 (1H, *d*, $J = 10.4$ Hz), 6.04 (1H, *s*), 5.47 (1H, *d*, $J = 10.4$ Hz), 5.02 (1H, *d*, $J = 11.6$ Hz), 4.53 (1H, *d*, $J = 11.6$ Hz), 3.61 (1H, *br*), 1.45 (3H, *s*), 1.42 (3H, *s*); ^{13}C NMR (Me_2CO-d_6) δ: 199.15 (*s*), 164.81 (*s*), 163.61 (*s*), 159.40 (*s*), 158.33 (*s*), 151.66 (*s*), 130.73 (*d*), 129.40 (*s*), 128.09 (*s*), 117.08 (*d*), 116.47 (*d*), 116.32 (*d*), 103.16 (*s*), 102.44 (*s*), 98.31 (*d*), 84.90 (*d*), 79.53 (*s*), 73.58 (*d*), 29.12 (*q*), 28.87 (*q*); IR ν_{max} cm^{-1}: 3400, 1710, 1640, 1620, 1580; UV λ_{max} nm: 227, 272, 296, 310, 359; EIMS *m/z* 354 (M^+, 27%), 339 (43), 321 (18), 219 (59), 218 (8), 203 (100), 192 (10), 177 (30), 136 (8); HRMS *m/z* 354.1120 (M^+, calcd for $C_{20}H_{18}O_6$: 354.1103).

Synthesis of hiravanone (**1**) *from* **3**. A solution of **3** (0.10 g) in EtOAc was added to a basic Al_2O_3 (2.41 g). The solvent was evapd to dryness, and 3-methylbut-2-enyl bromide (0.28 g) in *n*-hexane–ether (1:1) (12 ml) added and left for 68 hr. Al_2O_3 was filtered off and washed with CH_2Cl_2. The combined organic layer was evapd and the residue subjected to prep. TLC to give **1** as a colourless oil (24 mg) (17% yield), which was found to be identical with natural hiravanone by IR, ^{1}H NMR and co-TLC comparisons.

Synthesis of **2** *from* **4**. A soln of **4** (0.10 g) in EtOAc was added to basic Al_2O_3 (2.41 g). The solvent was evapd to dryness, and 3-methylbut-2-enyl bromide (0.28 g) in *n*-hexane–Et_2O (1:1) (12 ml) was added and left overnight. Al_2O_3 was filtered off and washed with CH_2Cl_2. The combined organic layer was evapd and the residue subjected to prep. TLC to give **2** (7 mg) as a colourless oil; ^{1}H NMR δ: 12.33 (1H, *s*, OH), 7.04 (1H, *d*, $J = 1.5$ Hz), 6.91 (1H, *dd*, $J = 1.5$ and 8.8 Hz), 6.87 (1H, *d*, $J = 8.8$ Hz), 6.36 (1H, *s*, OH), 5.69 (1H, *br*, OH), 5.29 (1H, *dd*, $J = 12.5$ and 3.0 Hz), 5.23 (1H, *t*, $J = 7.1$ Hz), 5.19 (1H, *t*, $J = 7.1$ Hz), 3.92 (3H, *s*), 3.34 (2H, *d*, $J = 7.1$ Hz), 3.30 (2H, *d*, $J = 7.1$ Hz), 3.02 (1H, *dd*, $J = 12.5$ and 16.9 Hz), 2.79 (1H, *dd*, $J = 3.0$ and 16.9 Hz), 1.81 (3H, *s*), 1.74 (3H, *s*), 1.72 (6H, *s*); IR ν_{max} cm^{-1}: 3550, 3400 (*br*), 1640, 1520; UV λ_{max} nm: 231, 290, 340; EIMS *m/z* 438 (M^+, 100%), 423 (21), 395 (10), 383 (45), 370 (14), 367 (41), 339 (17), 327 (27), 288 (7), 273 (31), 260 (21), 246 (24), 233 (55), 232 (27), 231 (27), 217 (48), 189 (56), 177 (66), 150 (4).

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TWO FLAVANONES FROM THE ROOT BARK OF *LESPEDEZA DAVIDII*

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Key Word Index—*Lespedeza davidii*; Leguminosae; root bark; lespedezaflavanone C; lespedezaflavanone D.

Abstract—Two new flavanones have been isolated from the root bark of *Lespedeza davidii* and their structures established as 8,3′-di-γ,γ-dimethylallyl-5,7,4′-trihydroxy-(2*R*,3*R*)-flavanonol and 8,5′-di-γ,γ-dimethylallyl-5,7,2′4′-tetrahydroxy-(2*S*)-flavanone on the basis of spectroscopic evidence.

INTRODUCTION

The roots and leaves of *Lespedeza davidii* Franch., which grows in Zhejiang province, have been used as a Chinese drug, he-xue-dan, for the treatment of dysentery and fever. In a previous communication, we reported that two new flavanones, lespedezaflavanone A and lespedezaflavanone B, had been obtained from this plant [1]. During further studies on the same species, we have now isolated two additional new flavanones.

RESULTS AND DISCUSSION

Lespedezaflavanone C (**1**). The IR spectrum showed strong absorptions at 1634 cm^{-1} (chelated C=O group) and 3430 cm^{-1} (OH). The UV spectrum (λ_{max}^{MeOH} 296 nm) suggested a flavanone structure [2]. Its ^{1}H NMR spectrum showed four hydroxy groups (C-5, C-7, C-4′ and C-3), four aromatic protons (C-6, C-2′, C-5′ and C-6′) [3]. It also indicated the presence of two γ,γ-dimethylallyl groups [4].

In the mass spectrum, the ion peak at *m/z* 220 and 204 were derived from a retro-Diels–Alder fragmentation. In view of the ^{1}H NMR spectral data, the fragment at *m/z* 220 must contain the A-ring. It loses C_4H_7 to yield the ion peak at *m/z* 165 and, therefore, the A-ring contains one γ,γ-dimethylallyl group. On the other hand, the ion peak at *m/z* 204 arises from the B-ring. It loses C_4H_7 to yield an ion peak at *m/z* 149, therefore the B-ring must also contain one γ,γ-dimethylallyl group. Thus one γ,γ-dimethylallyl group is attached to the A-ring and the other to the B-ring.

Positive UV shifts on the addition of sodium acetate, and aluminium chloride indicated that the three hydroxyl groups at C-5, C-7 and C-4′ were free. If the γ,γ-dimethylallyl group was at C-6, there would not have been a positive shift with aluminium chloride, so one γ,γ-dimethylallyl group must be located at C-8 [5].

Because the ^{1}H NMR spectrum (B-ring) of **1** showed ABX-type proton signals, and there are two protons at lower fields the γ,γ-dimethylallyl group in the B-ring must at C-3. Thus **1** is 8,3′-di-γ,γ-dimethylallyl-5,7,4′-trihydroxy-flavanol. As the ^{1}H NMR spectrum showed *trans* diaxial coupling ($J = 13.0$ Hz) between H-2 and H-3, **1** must have (*R,R*)-configuration at C-2, 3 [6].

Lespedezaflavanone D (**2**). The IR spectrum showed strong absorptions at 1630 cm^{-1} (chelated C=O group) and 3300 cm^{-1} (OH). The UV spectrum (λ_{max}^{MeOH} 295 nm) suggested a flavanone structure. Its ^{1}H NMR spectrum showed four hydroxy groups, the three aromatic protons. It also indicated the presence of two γ,γ-dimethylallyl groups. The MS spectrum of **2** is similar to **1** with ion peaks at *m/z* 220, 204, 165 and 149, so that one of the γ,γ-dimethylallyl groups in **2** is attached to the A-ring and the other to the B-ring. The base peak at *m/z* 406 was derived from loss of water at the C-2 position [7]. As with **1**, **2** shows positive UV shifts on addition of sodium acetate, and aluminium chloride, indicating the presence of three hydroxyl groups at C-5, C-7 and C-4′, and the γ,γ-dimethylallyl group at C-8. Because the ^{1}H NMR spectrum (B-ring) of **2** showed two aromatic proton singlets, the γ,γ-dimethylallyl group in the B-ring must be located at C-5′. Thus **2** is 8,5′-di-γ,γ-dimethylallyl-5,7,2′4′-tetrahydroxyflavanone. As the specific optical rotation of **2** has a minus sign, and the ^{1}H NMR spectrum showed *trans*-diaxial coupling ($J = 13.0$ Hz) between H-2 and H-3, as in other natural flavanones, **2** must have the (*S*)-configuration at C-2.

EXPERIMENTAL

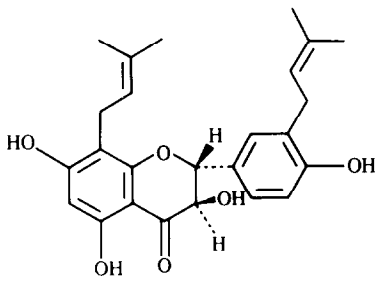
Mps: uncorr. ^{1}H NMR spectra were measured at 90 MHz; chemical shifts are given on the ppm scale with TMS as int. standard. CC was carried out on silica gel (120–160 mesh) and TLC on silica gel G_{F254}. Spots on TLC were visualized by spraying with phosphomolybdic acid and heating. The following solvent systems were employed: solvent A: C_6H_6–Me_2CO (4:1); solvent B: C_6H_6–HCO_2Et (9:1).

Plant material. The root bark of *Lespedeza davidii* was collected at Tianmu mountain in Zhejiang province, China, and authenticated by Prof. Y. K. Yang. A voucher specimen has been deposited in the Herbarium, Department of Botany, China Pharmaceutical University.

Extraction and isolation. Dried root bark of *Lespedeza davidii* was extracted with EtOH and the EtOAc soluble portion separated on a silica gel column, eluted with cyclohexane–EtOAc. The (7:1) fraction was recrystallized from a petrol–EtOAc to give **1**. The mother liquor yielded **2**.

Lespedezaflavanone C (**1**). Colourless needles, mp 161–163°. Green-brown with $FeCl_3$, Gibbs test (−). Mg–HCl (+). $[\alpha]_D$ +17.24°, (MeOH; *c* 0.58). MS *m/z*: 424 $[M]^+$ $C_{25}H_{28}O_6$ 21.74%), 395 (16.13%), 221 (48.71%), 220 (2.89%), 204 (15.18%), 202 (91.36%), 177 (19.21%), 165 base peak (100%), 149 (16.10%). UV λ_{max}^{MeOH} nm (log ε): 296 (4.20), 341 (3.61) (sh); +NaOMe 334 (4.42); +$AlCl_3$ 320 (4.35); +$AlCl_3$ + HCl 316 (4.24); +NaOAc 335 (4.33); +NaOAc + H_3BO_3 296 (4.18). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1640 (C=O), 1620, 1500 (arom, C=C), 1380, 1460 (CH_3). ^{1}H NMR ($CDCl_3$): 11.17, 6.45 and 6.10 (each 1H, *s*, shifted in DMSO-d_6 to 12.00, 10.95 and 9.60 OH × 3), 4.95 (1H, *d*, $J = 13.0$ Hz, H-2), 4.50 (1H, *d*, $J = 13.0$ Hz, H-3), 7.39 (2H, *dd*, $J = 8.5, 2.5$ Hz, H-2′, H-6′), 6.97 (1H, *d*, $J = 8.5$ Hz, H-5), 6.05 (1H, *s*, H-6) 3.37, 3.29 (each 2H, *d*, $J = 7.0$ Hz, Ar–C$\underline{H}_2$–CH = × 2), 5.31, 5.19 (each 1H, *m*, CH_2–C$\underline{H}$ = × 2), 1.77, 1.71 [each 6H, *s*, $(Me)_2$ × 2].

Lespedezaflavanone D (**2**). Yellow needles, mp 162–163°. Green-brown with $FeCl_3$. Gibbs reaction (−). Mg–HCl (+). $[\alpha]_D$ −23.1° (MeOH; *c* 0.131). MS *m/z*: 424 ($[M]^+$ $C_{25}H_{28}O_6$, 21.41%), 406 (100%), 363 (62.65%), 220 (6.61%), 204 (4.94%), 177 (40.42%), 165 (90.89%), 149 (38.35%), UV λ_{max}^{MeOH} nm (log ε): 295 (4.18); +NaOMe 334 (4.39); +$AlCl_3$ 320 (4.32); +$AlCl_3$ + HCl 316 (4.24); +NaOAc 335 (4.32); +NaOAc + H_3BO_3 296 (4.15). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1640 (C=O), 1620, 1520 (arom. C=C), 1390, 1370 (CH_3). ^{1}H NMR ($CDCl_3$): 12.01, 6.45, 6.40 and 6.03 (each 1H, *s*, OH × 4), 5.50 (1H, *dd*, $J = 2.5$, 13.0 Hz, H-2), 2.75 (1H, *dd*, $J = 2.5$, 17.0 Hz, H-3β), 3.15 (1H, *dd*, $J = 13.0$, 17.0 Hz, H-3α), 5.95 (1H, *s*, H-6), 7.35 (1H, *s*, H-6′), 6.94 (1H, *s*, H-3′), 1.80, 1.70 (each 3H, *s*, Me × 2), 1.60 (6H, *s*, Me × 2), 3.22, 3.31 (each 2H, *d*, $J = 7.0$ Hz Ar–C$\underline{H}_2$–CH = × 2), 5.22 (2H, *m*, CH_2–C$\underline{H}$ = × 2).



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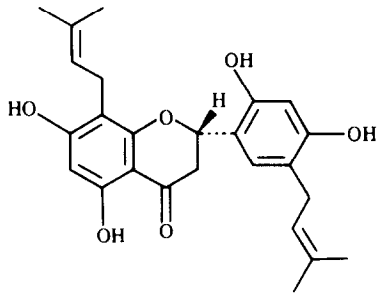

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QUERCETIN-3-*O*-α-[2-*O*-*p*-HYDROXYBENZOYL-4-*O*-*p*-COUMAROYLRHAMNOPYRANOSIDE], AN AGLYCONE-LIKE FLAVONOL GLYCOSIDE FROM *LIBOCEDRUS BIDWILLII*

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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.