Notes

Flavonoid Glycosides from *Viscum alniformosanae*

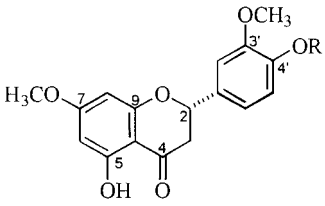
Cheng-Jen Chou,* Han-Chieh Ko, and Lie-Chwen Lin

National Research Institute of Chinese Medicine, 155-1, Sec. 2, Li-Nung St., Taipei (11221), Taiwan, Republic of China

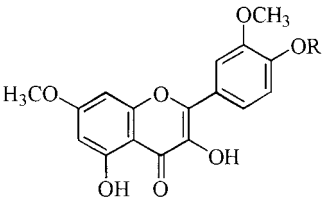
Received February 10, 1999

Two new flavonoid glycosides, (2S)-5-hydroxy-7,3′-dimethoxyflavanone−4′-*O*-*β*-[apiosyl (1 → 2)]glucoside (**1**) and rhamnazin-4′-*O*-*β*-[apiosyl (1 → 2)]glucoside (**2**) were isolated from *Viscum alniformosanae*. Structures were elucidated by NMR and mass spectroscopic analysis.

Viscum alniformosanae Hay. (Loranthaceae) is a perennial parasitic plant occurring in the central mountains of Taiwan at about 2000 m.[1] Its leaves and stems have been used in the treatments of arthritis and hypertension,[2] but *V. alniformosanae* has not been investigated previously. Preliminary TLC investigations revealed flavonoid components. The leaves and stems of *V. alniformosanae* were extracted with EtOH and partitioned successively with EtOAc, *n*-BuOH, and H_2O. Chromatographic separation of the *n*-BuOH layer resulted in the isolation of two new flavonoid glycosides. In this paper we report the isolation and structure elucidation of **1** and **2**.



1 R= apiosyl-(1→2)-β-glucoside



2 R= apiosyl-(1→2)-β-glucoside

Compound **1** was obtained as colorless needles. The molecular formula of **1** was established as $C_{28}H_{33}O_{15}$ by HRFABMS, and confirmed by ^{13}C NMR and DEPT analysis, which was in accord with a flavanone having one hydroxyl, two methoxyl, pentosyl and hexosyl substitutions. Negative ion FABMS of **1** showed a quasi-molecular ion $[M - H]^-$ at *m*/*z* 609, and a fragment peak at *m*/*z* 315 $[M - H - 132 - 162]^-$ indicated the loss of a pentosylhexoxyl moiety from the quasi-molecular ion. The absorption maxima at 332 (sh) and 285 nm in the UV spectrum, and the signals at *δ* 5.52, 2.77, and 3.35 in the ^1H NMR spectrum suggested **1** to be a flavanone.[3] The presence of three spin-coupling systems in the H−H COSY spectrum of **1** indicated substitutions at 5, 7, 3′, and 4′. In the ^1H NMR, a D_2O exchangeable singlet at *δ* 12.07 (DMSO-d_6) indicated that C-5 was hydroxylated. The ^1H NMR signals at *δ* 3.78 and 3.81 were correlated to the ^{13}C NMR signals at *δ* 148.8 and 167.4, respectively, in its COLOC NMR spectrum, suggesting that one methoxyl group was located on C-7, and one methoxyl group was located on C-3′ or C-4′. After hydrolysis of **1**, glucose and apiose were detected by TLC,[4] and the aglycon was identified (EIMS, ^1H and ^{13}C NMR) as 5,4′-dihydroxy-7, 3′-dimethoxyflavanone.[5] The sugar moiety of **1** was identified as apiosyl (1 → 2) glucose by the ^{13}C NMR data, which were in agreement with published data for the sugar moiety of (2*S*)-homoeriodictyol-7-*O*-[apiosyl (1 → 2)] glucoside.[6] Assignment of the *β*-configuration to glucose at the anomeric carbon was based on the C-1″ chemical shift and the chemical shift and large coupling constant of the anomeric proton (*δ* 4.96, d, *J* = 7.2 Hz). Hydrolysis of **1** indicated that the sugar linkage to the aglycon was at C-4′. The circular dichroism (CD) spectrum of **1** exhibited a positive Cotton effect at 331 nm ([*θ*] +13 677) and a negative Cotton effect at 289 nm ([*θ*] −44 151). Therefore, C-2 was assigned the *S* configuration[7] and **1** was elucidated to be (2S)-5-hydroxy-7,3′-dimethoxyflavanone-4′-*O*-*β*-[apiosyl (1 → 2)] glucoside.

The molecular formula of **2** was established as $C_{28}H_{31}O_{16}$ by HRFABMS, and confirmed by ^{13}C NMR and DEPT analysis. This was consistent with a flavonol having two hydroxyl, two methoxyl, and a pentosylhexosyl substitutions. Negative ion FABMS of **2** showed a quasi-molecular ion $[M - H]^-$ at *m*/*z* 623, and two fragment peaks at *m*/*z* 491 and 329 indicated losses of pentosyl and hexosyl moieties from the quasi-molecular ion with hexose as the inner monosaccharide moiety. Absorption maxima at 365 and 253 nm in the UV spectrum, and the signals of C-2 at *δ* 146.3, C-3 at *δ* 136.6, and C-4 at *δ* 176.1 in the ^{13}C NMR spectrum suggested, that **2** was a flavonol.[3,8] Two spin-coupling systems in the H−H COSY spectrum of **2** indicated that the flavonol had 5, 7, 3′, and 4′ substituents. The ^1H NMR signals at *δ* 3.84 and 3.87, which were correlated to the ^{13}C NMR signals at *δ* 148.5 and 165.0, respectively, in its COLOC spectrum, suggested that one methoxyl group was at C-7, and the other methoxyl group was at C-3′ or C-4′. The sugar moiety of **2** was identified as apiosyl-(1 → 2)-*β*-glucoside, which was evident by comparison of ^1H and ^{13}C NMR spectra with those of

* To whom correspondence should be addressed. Tel.: +886-2-8201999, ext. 7101. Fax: +886-2-28264276. E-mail: choucj@cma23.nricm.edu.tw.

10.1021/np990049m CCC: $18.00
Published on Web 09/09/1999

compound **1**. The NOESY spectrum of **2**, which displayed correlations between H-5′ and δ 5.08 and between H-2′ and 3′-OCH_3, indicated that the sugar linkage to the aglycon in **2** was at C-4′. Thus, compound **2** was rhamnazin-4′-*O*-β-[apiosyl (1 → 2)] glucoside.

Experimental Section

General Experimental Procedures. Mps were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were obtained as KBr pellets on a Perkin-Elmer 781 IR spectrometer. UV spectra were obtained on a Hitachi U-3200 spectrophotometer in MeOH. CD spectra were recorded on a JASCO J-715 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter in MeOH. ^{1}H and ^{13}C NMR spectra were measured with a Bruker ACP-300 spectrometer with deuterated DMSO as internal standard. The NOESY experiment was recorded on a Bruker Avance 500 MHz. FABMS were recorded in the negative ion mode on a JEOL JMX-HX 110 spectrometer.

Plant Material. The leaves and stems of *V. alniformosanae* Hayata were collected at Hsinchiayan, Taichung Hsien, Taiwan, in February 1997. A voucher specimen has been deposited in the herbarium of the Department of Botany of the National Taiwan University.

Extraction and Isolation. The air-dried leaves and stems of *V. alniformosanae* (9.8 kg) were extracted with 95% EtOH (×3). A solvent was evaporated in vacuo at ca. 50 °C to give 2.9 kg of residue. The crude extract was partitioned in succession between H_2O and EtOAc, followed by *n*-BuOH, yielding 456, 560, and 1500 g, respectively. The *n*-BuOH extract was subjected to silica gel CC with a gradient of MeOH in $CHCl_3$, and 10 fractions were collected. Fraction 5 was rechromatographed over Sephadex LH-20, eluting with MeOH to give twelve fractions (I−XII). Fractions were collected in 300-mL portions and pooled according to their TLC profile in EtOAc−MeOH−H_2O (5:1:0.6 v/v). Of these fractions IV, IX, and XII were individually further purified by Sephadex LH-20 column (MeOH) and silica gel MPLC (MeOH gradient in EtOAc) to give syringin[4,9] (135 mg) from fraction IV, homoflavoyadorinin-B[6] (16 mg) and (2*S*)-5-hydroxy-7,3′-dimethoxyflavanone-4′-*O* -β-[apiosyl (1 → 2)] glucoside (**1**, 51 mg) from fraction IX, and rhamnazin-4′-*O*-β-[apiosyl (1 → 2)] glucoside (**2**, 21 mg) from fraction XII.

Compound 1: colorless needles; mp 249−251 °C; $[\alpha]_D$ −97°- (*c* 0.1, MeOH), and gave a positive MgHCl test; UV (MeOH) λ_{max} (log ϵ) 332 (sh, 3.63), 285 (4.37) nm; IR(KBr) ν_{max} 3450 (OH), 1630 (C=O), 1580 (aromatic), 1510, 1460, 1380, 1300, 1260, 1150, 1070, 1020, 820 cm^{-1} ; CD (C 2.6 × 10 $^{-4}$ M, MeOH) 331 ([θ] +13677), 289 ([θ] − 44151) nm; FABMS (neg. mode) *m*/*z* 609 [M − H] $^-$ (100), 315 [M − H − 132 − 162] $^-$ (25); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 5.52 (1H, dd, *J* = 2.7, 12.9 Hz, H-2), 2.77 (1H, dd, *J* = 2.7, 17.0 Hz, H*cis*-3), 3.35 (1H, m, H*trans*-3), 6.08 (1H, d, *J* = 2.0 Hz, H-6), 6.13 (1H, d, *J* = 2.0 Hz, H-8), 7.15 (1H, br s, H-2′), 7.08 (1H, d, *J* = 8.3 Hz, H-5′), 6.99 (1H, dd, *J* = 1.1, 8.3 Hz, H-6′), 3.81 (3H, s, 7-OMe), 3.78 (3H, s, 3′-OMe), 12.07 (1H, s, 5-OH), 4.96 (1H, d, *J* = 7.2 Hz, H-1″), 5.41 (1H, br s, H-1‴), 3.58 (1H, d, *J* = 9.0 Hz, H-4‴a), 4.04 (1H, d, *J* = 9.0 Hz, H-4‴b), 3.30 (2H, d, *J* = 5.4 Hz, H-5‴); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 78.5 (d, C-2), 42.0 (t, C-3), 55.8 (q, OMe), 55.7 (q, OMe), 196.6 (s, C-4), 163.1 (s, C-5), 94.6 (d, C-6), 167.4 (s, C-7), 93.7 (d, C-8), 162.7 (s, C-9), 102.5 (s, C-10), 131.9 (s, C-1′), 111.2 (d, C-2′), 148.8 (s, C-3′), 146.5 (s, C-4′), 114.9 (d, C-5′), 119.0 (d, C-6′), 98.4 (d, C-1″), 77.1 (d, C-2″), 75.0 (d, C-3″), 69.9 (d, C-4″), 76.8 (d, C-5"), 60.6 (t, C-6"), 108.2 (d, C-1‴), 76.0 (d, C-2‴), 79.9 (s, C-3‴), 73.8 (t, C-4‴), 64.4 (t, C-5‴); HRFABMS *m*/*z* 609.1837 [M−H] $^-$ (calcd 609.1819 for $C_{28}H_{33}O_{15}$).

Hydrolysis of 1. Compound **1** (15 mg) was dissolved in EtOH−5% HCl (1:1) (20 mL) and refluxed for 3.5 h. After removal of the EtOH under reduced pressure, the precipitate was filtered to give the aglycon (3 mg), it was identified as 5,4′-dihydroxy-7, 3′-dimethoxyflavanone by comparison of its EIMS, ^{1}H and ^{13}C NMR spectra with literature data.[5] The filtrate was neutralized with $BaCO_3$. The sugar components were identified by TLC as apiose and glucose.[4]

Compound 2: yellow powder; mp 215−218 °C, and gave a positive MgHCl test; UV (MeOH) λ_{max} (log ϵ) 365 (4.26), 268 (sh,4.10), 253 (4.26) nm; IR (KBr) νmax 3350 (OH), 1650 (C=O), 1580 (aromatic), 1500, 1460, 1360, 1240, 1210, 1150, 1070, 1030, 820, 790 cm^{-1} ; FABMS (neg. mode) *m*/*z* 623 [M − H] $^-$ (100), 491 [M − H − 132]$^-$ (6), 329 [M − H − 132 − 162]$^-$ (69); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.35 (1H, d, *J* = 1.9 Hz, H-6), 6.79 (1H, d, *J* = 1.9 Hz, H-8), 7.80 (1H, br s, H-2′), 7.22 (1H, d, *J* = 9.0 Hz, H-5′), 7.78 (1H, dd, *J* = 2.1, 9.0 Hz, H-6′), 3.87 (3H, s, 7-OMe), 3.84 (3H, s, 3′-OMe), 9.57 (3-OH), 12.37 (5-OH), 5.08 (1H, d, *J* = 7.5 Hz, H-1″), 5.43 (1H, br s, H-1‴), 3.61 (1H, d, *J* = 9.2 Hz, H-4‴a), 4.07 (1H, d, *J* = 9.2 Hz, H-4‴b), 3.31 (2H, d, *J* = 5.4 Hz, H-5‴); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 146.3 (s, C-2), 136.6 (s, C-3), 56.1 (q, OMe), 55.8 (q, OMe), 176.1 (s, C-4), 160.3 (s, C-5), 97.4 (d, C-6), 165.0 (s, C-7), 92.1 (d, C-8), 156.1 (s, C-9), 104.0 (s, C-10), 124.3 (s, C-1′), 111.7 (d, C-2′), 148.5 (s, C-3′), 147.9 (s, C-4′), 114.7 (d, C-5′), 121.1 (d, C-6′), 98.1 (d, C-1"), 77.1 (d, C-2″), 75.0 (d, C-3″), 69.9 (d, C-4″), 76.9 (d, C-5″), 60.5 (t, C-6″), 108.3 (d, C-1‴), 76.0 (d, C-2‴), 79.9 (s, C-3‴), 73.9 (t, C-4‴), 64.4 (t, C-5‴); HRFABMS *m*/*z* [M − H] $^-$ 623.1615 (calcd 623.1612 for $C_{28}H_{31}O_{16}$).

Acknowledgment. We are grateful to the National Science Council, the Republic of China, for support of this research under Grant NSC 87-2314-B007-004.

References and Notes

(1) Chiu, S. T. In *Flora of Taiwan: Loranthaceae*, 2nd ed.; Editorial Committee of the Flora of Taiwan; Taipei, 1996; Vol. II, pp 282−284.
(2) Chiu, N. Y.; Chang, K. H. *The Illustrated Medicinal Plants of Taiwan*; Southern Materials Center: Taipei, 1991; Vol. II, p 33.
(3) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; Parts II and III.
(4) Wagner, H.; Feil, B.; Seligmann, O.; Petricic, J.; Kalogjera, Z. *Planta Med*. **1986**, 102−104.
(5) Rauter, A. P.; Branco. I.; Maria, Z. T.; Pais, M. S.; Gonzaiez, A. G.; Bermjo, J. B. *Phytochemistry* **1989**, *28*, 2173−2175.
(6) Fukunaga, T.; Kajkawa, I.; Nishiya, K.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull.* **1989**, *37*, 1300−1303.
(7) Gaffield, W. *Tetrahedron* **1970**, *26*, 4093−4108.
(8) Markham, K. R.; Mohan Chari, V. In *The Flavonoids: Advances in Research*; Harborne, J. B., Mabry, T. J., Eds.; Chapman and Hall: New York, 1982; Chapter 2, p 24.
(9) Fukunaga, T.; Kajikawa, I.; Nishiya, K.; Watanabe, Y.; Takeya, K. *Chem. Pharm. Bull.* **1987**, *35*, 3292−3297.

NP990049M