Two C-Methylated Flavonoid Glycosides from the Roots of Sophora leachiana

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J. Nat. Prod., **1991**, 54 (4), 1144-1146• DOI: 10.1021/np50076a042 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 4, pp. 1144-1146, Jul-Aug 1991

TWO *C*-METHYLATED FLAVONOID GLYCOSIDES FROM THE ROOTS OF *SOPHORA LEACHIANA*

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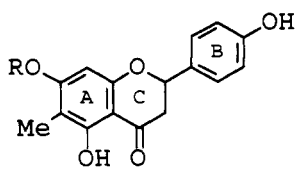
ABSTRACT.—5,7,4′-Trihydroxy-6-methylflavanone (poriol), its 7-*O*-β-D-glucopyranoside (poriolin), and a new flavonol glycoside were isolated from the roots of *Sophora leachiana*. Spectroscopic analysis established the structure of the new flavonol to be 8-methylkaempferol-7-*O*-β-D-glucopyranoside.

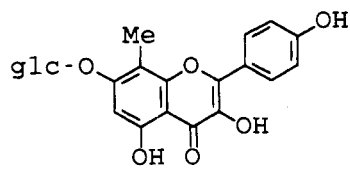
In a previous paper (1), we reported the isolation and structural determination of sophoraflavanone G and a new flavanone, named leachinone A, as major constituents of the roots of *Sophora leachiana* M.E. Peck (Leguminosae). [*Sophora leachiano* used in our previous paper (1) must be changed to *S. leachiana*.] In our continuing study of chemical constituents with medicinal properties, two flavonoid glycosides and an aglycone, one of which is a new compound, were obtained from the polar fraction of the rhizomes and roots.

After repeated purification of an Me_2CO extract of the rhizomes and roots with Si gel chromatography and recrystallization, two glycosides **1** and **3** were obtained. Compound **1** was a colorless powder and showed a positive $FeCl_3$ test. In the ^{1}H-nmr spectrum, three typical one-proton double doublets at δ 2.76 (J = 17, 3 Hz), 3.22 (J = 17, 17 Hz) and 5.46 (J = 17, 3 Hz) assigned to the protons at C-3 and C-2 indicated that **1** had a flavanone skeleton. One set of two-proton doublets at δ 6.90 and 7.38 (each J = 8 Hz) showed that the B ring is oxygenated at C-4′. Furthermore, a methyl group (δ 2.02), an aromatic proton (δ 6.30) assignable to the A ring, and two hydroxyl groups (δ 8.60 and 12.28) were observed. The latter hydroxyl group (δ 12.28) was assigned to a chelated group at C-5. The eims gave a fragment peak at *m/z* 286, corresponding to the aglycone moiety, and other prominent fragments at *m/z* 193, 180, 167, 166, 138, and 120. Among these, *m/z* 167 $[A]^+$ and 166 $[A_1 + H]^+$ showed that the A ring bore two oxygen functions and a methyl group that was substituted to its nucleus. The peak at *m/z* 120 $[B]^+$ showed that the B ring also bore a hydroxyl group.

From the above data, the aglycone of **1** was identified as a naringenin derivative with a *C*-substituted methyl group on the A ring. With the addition of $AlCl_3$/HCl, no significant shift was observed in the uv spectrum, which suggested that the *C*-methyl group was substituted at C-6 (2). In the ^{1}H-^{13}C long range COSY, the chelated hydroxyl group caused three cross peaks at δ



1 R=glc
2 R=H



3

104.6, 107.4, and 161.9. The signal at δ 161.9 was assigned to C-5; the signal at δ 107.4, correlated with the *C*-methyl protons, was assigned to C-6, and the other carbon signal (δ 104.6) was attributed to C-10. From the data just described, the aglycone of **1** was characterized at 6-methylnaringenin. The position of a glucose moiety on **1** was located at C-7 as follows. An anomeric proton (δ 5.06, $J=7$ Hz) caused a cross peak with a singlet signal of H-8, and the anomeric proton also correlated with H-8 in the ^{1}H-^{13}C long range COSY. Accordingly, the structure of **1** was established as 6-methyl-5,7,4′-trihydroxyflavanone-7-*O*-β-D-glucopyranoside (poriol-7-*O*-β-D-glucopyranoside) (poriolin), which had previously been isolated from the species *Pseudotsuga menziesii* (3) and *Leucothoe keiskei* (4). The aglycone of **1**, poriol [**2**], was also isolated in the present study from a CH_2Cl_2 fraction of the roots.

Compound **3** was obtained as a yellow powder. It showed a positive $FeCl_3$ test and a negative Gibbs test. The uv spectrum indicated that **3** had a flavonol skeleton. In the ^{1}H-nmr spectrum, a set of two-proton doublets at δ 6.97 and 8.12 (each $J=9$ Hz), a singlet aromatic proton at δ 6.64, and three hydroxyl groups at δ 9.55, 10.10, and 12.40 (chelated) in addition to a singlet at δ 2.25 based on a *C*-methyl group were observed. A fragment peak corresponding to the aglycone appeared at *m/z* 300 in the eims, as well as other peaks at *m/z* 272 and 121. The negative ion fabms showed $[M-H]^-$ at *m/z* 461, indicating that **3** is a *C*-methylkaempferol glucoside. Location of the *C*-methyl on the A ring was indicated as follows. In the ^{1}H-^{13}C long-range COSY, a chelated hydroxyl group located at C-5 caused three cross peaks between carbon signals at δ 97.3, 104.4, and 160.2, and the signal at δ 97.3 caused also a cross peak between an aromatic proton on the A ring. These findings showed that no substituent existed at the ortho position of the chelated hydroxyl group at C-5. Consequently, the structure of the aglycone moiety was concluded to be 8-methylkaempferol. In a kaempferol derivative substituted with an allyl group at C-8, such as hexandrasides A and B (5) from the leaves of *Vancouveria hexandra* (Hook.) Morr. & Decne. (Berberidaceae), a proton signal based on H-6 appeared at δ 6.60 in a 7-*O*-glycoside form and δ 6.30 in an aglycone form. These results were applied to **3** (H-6 δ 6.64) concerning the position of the glucose moiety. Therefore, the new flavonol glycoside of **3** was determined to be 8-methylkaempferol-7-*O*-β-D-glucopyranoside.

C-Methylflavonoids occur abundantly in ferns, gymnosperms, and the Ericaceae but are rarely found in leguminous plants (6).

EXPERIMENTAL

PLANT MATERIAL.—The roots of *S. leachiana* were collected near Galice, Oregon in July 1988. Voucher specimens are deposited at the Herbarium of Gifu Pharmaceutical University.

EXTRACTION AND ISOLATION OF COMPOUNDS **1–3**.—An Me_2CO extract of the rhizomes and roots was subjected to Si gel cc eluted with solvent systems of $CHCl_3$/MeOH and $CHCl_3$/MeOH/H_2O to give **1** (60 mg) and **3** (35 mg). A CH_2Cl_2 extract gave **2** (5 mg) after Si gel chromatography eluted with *n*-hexane–Me_2CO (5:1).

Poriolin [**1**].—A colorless amorphous powder (MeOH): $[\alpha]_{26}$ 8.0 ($c=0.1$); eims *m/z* (%) 286 (71), 193 (16), 180 (22), 167 (100), 166 (62), 138 (58), 120 (40); uv λ max (MeOH) (log ε) 286 (3.7), 343 (3.4), (+NaOMe) 286, 320 sh, 420, (+$AlCl_3$) 285, 343, 420 inf., (+$AlCl_3$/HCl) 285, 344, 419 inf.; ^{1}H nmr (270 MHz, DMSO-d_6) δ 2.02 (3H, s, C-Me), 2.76 (1H, dd, $J=17$, 3 Hz, H-3eq), 3.22 (1H, dd, $J=17$, 17 Hz, H-3ax), 3.50–3.92 (m, sugar protons), 5.06 (1H, d, $J=7$ Hz, H-1″), 5.46 (1H, dd, $J=17$, 3 Hz, H-2), 6.30 (1H, s, H-8), 6.90 (2H, d, $J=8$ Hz, H-3′, -5′), 7.38 (2H, d, $J=8$ Hz, H-2′, -6′), 12.28 (1H, s, 5-OH); ^{13}C nmr see Table 1.

Poriol [**2**].—Colorless needles: mp 217° (MeOH); eims was completely identical to that of compound **1**; ^{1}H nmr (270 MHz, Me_2CO-d_6) δ 1.97 (3H, s, C-Me), 2.71 (1H, dd, $J=17$, 3 Hz, H-3ax), 3.16 (1H, dd, $J=17$, 17 Hz, H-3eq), 5.41 (1H, dd, $J=17$, 3 Hz, H-2), 6.03 (1H, s, H-8), 6.91 (2H, d, $J=8$ Hz, H-3′, -5′), 7.36

TABLE 1. ^{13}C-nmr Spectral Data of Compounds **1** and **3**. All carbons were assigned by ^{1}H-^{13}C, ^{1}H-^{13}C long-range COSY and INEPT methods.

Carbon	**1**[a]	**3**[b]
C-2	80.5	147.3
C-3	44.2	135.8
C-4	198.6	176.4
C-5	161.9	158.2
C-6	107.4	97.3
C-7	164.9	160.2
C-8	95.3	104.1
C-9	162.5	152.8
C-10	104.6	104.4
C-1′	131.2	121.9
C-2′	129.6	129.5
C-3′	116.7	115.6
C-4′	159.2	159.3
C-5′	116.7	115.6
C-6′	129.6	129.5
C-1″	101.6	100.3
C-2″	75.0	73.2
C-3″	78.2	76.4
C-4″	71.6	69.6
C-5″	78.4	77.1
C-6″	62.0	60.6
Me	7.8	7.7

[a]Measured in Me_2CO-d_6.
[b]Measured in DMSO-d_6.

(2H, d, J=8 Hz, H-2′, -6′), 8.54, 9.55 (1H, each s, 7-OH and 4′-OH), 12.45 (1H, s, 5-OH).

8-Methylkaempferol-7-O-β-D-glucopyranoside [**3**].—A yellow powder: $FeCl_3$ (+), Gibbs test (−); negative ion fabms *m/z* [M − H]$^-$ 461, [aglycone − H]$^-$ 299; eims *m/z* (rel. int.) 300 (100), 272 (9), 150 (8), 121 (14); uv λ max (MeOH) (log ε) 273 (4.2), 330 (4.1), 380 (3.9), (+NaOMe) 261, 416, (+$AlCl_3$) 266, 360, 434, (+$AlCl_3$/HCl) 269, 360, 436, (+NaOAc) 271, 328, 384, (+NaOAc/H_3BO_3) 272, 325, 380; ^{1}H nmr (270 MHz, DMSO-d_6) δ 2.25 (3H, s, C-Me), 3.30–3.40 (m, H-2″, -3″), 3.15 (1H, m, H-4″), 3.48 (1H, m, H-5″), 3.50 and 3.75 (1H, each m, H-6″), 5.00 (1H, d, J=7 Hz, H-1″), 6.64 (1H, s, H-6), 6.97 (2H, d, J=9 Hz, H-3′, -5′), 8.12 (2H, d, J=9 Hz, H-2′, -6′), 9.55, 10.10 (1H, s, 3- and 4′-OH), 12.40 (1H, s, 5-OH); ^{13}C nmr see Table 1.

LITERATURE CITED

1. M. Iinuma, T. Tanaka, M. Mizuno, Y. Shirataki, I. Yokoe, M. Komatsu, and F.A. Lang, *Phytochemistry*, **29**, 2667 (1990).
2. E.A. Sherif, R.K. Gupta, and M. Krishnamurti, *Tetrahedron Lett.*, **21**, 641 (1980).
3. W.E. Hillis and N. Ishikura, *Aust. J. Chem.*, **22**, 483 (1969).
4. A. Ogiso and I. Kashida, *Phytochemistry*, **11**, 3545 (1972).
5. M. Mizuno, M. Iinuma, T. Tanaka, N. Sakakibara, J. Murata, H. Murata, and F.A. Log, *Phytochemistry*, **29**, 1277 (1990).
6. B.A. Bohm, in: "The Flavonoids, Advances in Research Since 1980." Ed. by J.B. Harborne, Chapman and Hall, New York, 1988, p. 379.

Received 17 December 1990