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FLAVONOL GLYCOSIDES FROM *EPIMEDIUM PUBESCENS*

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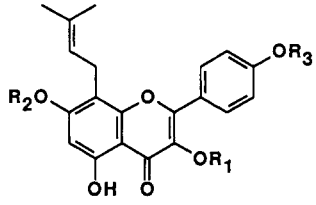
ABSTRACT.—A novel flavonol glycoside, rouhuoside [**1**], was isolated from aerial parts of *Epimedium pubescens* along with six known flavonol glycosides. Structures were established by spectroscopic and chemical methods. The structure of rouhuoside [**1**] was elucidated as 8-prenylkaempferol-3-*O*-α-L-rhamnopyranosyl(4↦1)-β-D-glucopyranosyl-7-*O*-β-D-glucopyranoside. The known compounds were identified as epimedoside C, icariside I, icariin, baohuoside VI, hyperin, and baohuoside I.

Several *Epimedium* species (Berberidaceae) have been used as tonics, aphrodisiacs, and antirheumatics in Chinese herbal medicine. The chemical constituents of *Epimedium pubescens* Maxim, recorded in the *Chinese Pharmacopoeia* (1), have not been reported, although investigations on the chemical constituents of other *Epimedium* species have been noted (2–9). In this paper, we report the isolation and structure elucidation of a novel flavonol glycoside **1** together with the identification of several known flavonol glycosides from the aerial parts of *E. pubescens*.

RESULTS AND DISCUSSION

Rouhuoside [**1**], $C_{38}H_{48}O_{20}$, was obtained as a yellow amorphous powder that responded to the Molisch and Shinoda (Mg-HCl) test. Its ir spectrum showed a strong absorption band at 1650^{-1} cm for a chelated carbonyl group. By acid hydrolysis, **1** gave an aglycone along with glucose and rhamnose. The fabms of **1** showed peaks at *m/z* 847 $[M+Na]^+$, 663 $[M-162+H]^+$, 517 $[M-162-146+H]^+$ and 355 $[M-2\times162-146+H]^+$, clearly suggesting the presence of one rhamnose and two glucose moieties in the molecule. The uv spectrum of **1** (see Experimental) was similar to that of epimedoside A (10), indicating that the sugars were attached to the C-3 and C-7 hydroxyl groups of the aglycone. Three signals derived from the anomeric protons were observed in the ^{1}H-nmr spectrum of **1**. The signal at δ 5.17 (1H, d, $J=6.3$ Hz) could be assigned to the H-1 of glucose attached to the C-7 hydroxyl; this was supported by the enzymatic hydrolysis of **1** using β-glucosidase. The hydrolysis products were D-glucose and a partial hydrolysis product, whose uv spectrum in MeOH/NaOAc gave a consistent bathochromic shift (268↦276 nm) indicating that a hydroxyl group at C-7 was released. In the case of a 3-*O*-glucoside, the relevant anomeric proton should be observed at δ 5.7–6.2 (11). Similarly, the signals at δ 5.50 (1H, br, s) and δ 5.00 (1H, d, $J=$ 6.3 Hz) were assigned to the anomeric protons of the α-rhamnose and the terminal β-glucose, respectively.

In the ^{13}C-nmr spectrum of **1**, the C-4 signal (81.4 ppm) of rhamnose showed a downfield shift of 10 ppm upon comparison with the corresponding C-4 signal (71.1 ppm) of a flavonol-3-*O*-rham-



1 R_1=Rha(4↦1)Glc, R_2=Glc, R_3=H
Glc = β-D-glucopyranosyl
Rha = α-L-rhamnopyranosyl

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noside (10), revealing a 1↦4 linkage between the 3-*O*-rhamnosyl and the terminal glucosyl moieties. On the basis of these data, **1** was elucidated as 8-prenylkaemperol-3-*O*-α-L-rhamnopyranosyl (4↦1)-β-D-glucopyranosyl-7-*O*-β-D-glucopyranoside and was named as rouhuoside [**1**].

The identification of the other six known flavonol glycosides was made by uv, ^{1}H nmr, ^{13}C nmr, fabms, and co-tlc with authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Mp's were determined on a Kofler hot stage instrument and are uncorrected. The ir spectra were obtained on a Perkin-Elmer 983G spectrometer, and uv spectra were determined on a Philips PYE Unicam PU8800 spectrometer. Nmr spectra were recorded in DMSO-d_6 on a Varian VXR-300 spectrometer, and ms spectra were obtained on a KYKY ZhP-5A spectrometer.

PLANT MATERIAL.—The aerial parts of *E. pubescens* were collected in August 1988 at Leshan county, Sichuan Province, in China. A voucher specimen is deposited in the Herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China.

EXTRACTION AND ISOLATION.—The dried and powdered aerial parts (4 kg) were thoroughly extracted with 95% EtOH. The concentrated extract (286 g) was absorbed on Amberlite D-101, and the resin was eluted with MeOH after being washed with H_2O. The MeOH eluate was chromatographed on a Sephadex LH-20 column with MeOH to afford fractions I and II. After being chromatographed on a polyamide column, eluting with $CHCl_3$-MeOH-EtCOMe-Me_2CO (10:5:2:1), fraction I gave rouhuoside [**1**] (28 mg), icariin (150 mg), baohuoside VI (122 mg), and hyperin (25 mg), and fraction II afforded epimedoside C (40 mg), icariside I (30 mg), and baohuoside I (116 mg).

ROUHUOSIDE [**1**].—Yellow amorphous solid: mp 214–217°; ir ν max (KBr) 3400, 1650, 1600, 1510, 840 cm^{-1}; uv λ max nm 220 sh, 268, 316, 348 (MeOH); 268, 388 (NaOMe); 236, 276, 304, 344, 404 ($AlCl_3$); 236, 276, 300, 336, 404 ($AlCl_3$/HCl); 270, 364 (NaOAc); 268, 316, 348 (NaOAc/H_3BO_3); ^{1}H-nmr (DMSO-d_6) δ 0.83 (3H, d, J = 6.0 Hz, rha, Me), 5.50 (1H, br s, rha, H-1), 5.01 (1H, d, J = 6.0 Hz, glu, H-1), 5.17 (1H, d, J = 6.0 Hz, glu, H-1), 1.61 (3H, s, Me-5″), 1.69 (3H, s, Me-4″), 5.19 (1H, t, J = 6.0 Hz, H-2″), 6.82 (1H, s, H-6), 6.97 (2H, d, J = 8.0 Hz, H-3′, -5′), 7.84 (2H, d, J = 8.0 Hz, H-2′, -6′); fabms *m/z* [M + Na]$^+$ 847, [M − glucosyl + H]$^+$ 663, [M − glucosyl − rhamnosyl + H]$^+$ 517, [M − 2 × glucosyl − rhamnosyl + H]$^+$ 355; ^{13}C nmr see Table 1.

TABLE 1. ^{13}C-nmr Spectral Data of Rouhuoside [**1**] (in DMSO-d_6).[a]

Carbon		Carbon[b]	
C-2	157.5	rha-1	101.1
C-3	134.7	rha-2	71.9
C-4	178.2	rha-3	70.2
C-5	160.6	rha-4	81.4
C-6	98.2	rha-5	69.5
C-7	160.5	rha-6	17.6
C-8	108.5	glc-1	100.7
C-9	153.0	glc-2	73.5
C-10	106.2	glc-3	76.8
C-1′	120.6	glc-4	70.3
C-2′	130.7	glc-5	77.4
C-3′	115.5	glc-6	60.8
C-4′	159.0	glc′-1	105.6
C-5′	115.5	glc′-2	74.0
C-6′	130.7	glc′-3	76.3
C-1″	21.7	glc′-4	70.6
C-2″	122.3	glc′-5	76.6
C-3″	131.1	glc′-6	60.5
C-4″	25.7		
C-5″	18.1		

[a]Chemical shifts are given in ppm values.
[b]rha = rhamnosyl; glc = glucosyl.

ENZYMATIC HYDROLYSIS OF **1**.—NaOAc/HOAc buffer solution (pH 5.0) (2 ml) containing **1** (1 mg) and β-glucosidase (1 mg) was incubated at 37° for 48 h. After filtration, a product was obtained by extraction with EtOAc from the filtrate: uv λ max nm 268, 320, 370 (MeOH); 276, 318 sh, 390 (NaOAc).

ACID HYDROLYSIS OF ALL GLYCOSIDES.—Each glucoside solution was applied at a point about 1 cm from the bottom edge of the hptlc Si gel plate (10 × 10 cm) and was hydrolyzed with HCl vapor for 40 min at 50–60°. Glacial HOAc (1 ml) was added to 9 ml of the lower layer of a mixture of $CHCl_3$-MeOH-H_2O (30:12:4), which was used as the developing solvent (12). The identification of sugars was carried out by comparison with authentic sugars on the same plate.

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