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NEW FLAVONOL GLYCOSIDE FROM *EPIMEDIUM ACUMINATUM*

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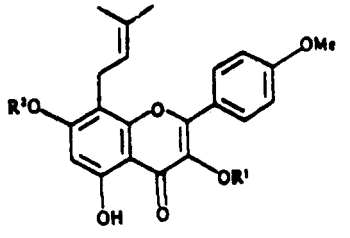
ABSTRACT.—A novel glycoside, cuhuoside [**1**], was isolated from the aerial parts of *Epimedium acuminatum* along with six known flavonols (tricin, icaritin, icariside I, baohuoside I, icariin, and baohuoside VI) which were isolated from this species for the first time. Structures were identified by means of chemical and spectroscopic methods. The structure of cuhuoside [**1**] was elucidated as 3,5,7-trihydroxy-4′-methoxy-8-prenylflavone-7-*O*-[β-D-glucopyranosyl-1→4)-β-D-glucopyranoside].

Epimedium acuminatum Franch (Berberidaceae) has been used to invigorate the kidneys and strengthen "yang" in Chinese herbal medicine. The species is native to Si Chuan, Yun Nan, Gui Zhou, Shan Xi, and Hu Bei Provinces in China. In this paper, we report the isolation and structural elucidation of a novel flavonol glycoside, cuhuoside [**1**], together with tricin, icaritin, icariside I, baohuoside I, icariin, and baohuoside VI, from the aerial parts (1–4).

RESULTS AND DISCUSSION

Cuhuoside was isolated as an amorphous powder that responded to the Molish and Shinoda (Mg-HCl) tests. Its ir spectrum showed a strong absorption band at 1650^{-1} cm for a chelated carbonyl group. Its fabms showed peaks at *m/z* 693 $[M+1]^+$, 531 $[M-162+H]^+$, 369 $[M-2\times162+H]^+$, and 313 $[369-C_4H_8+H]^+$, clearly suggesting the presence of two hexose moieties in the molecule. In its ^{1}H-nmr spectrum, the presence of peaks at δ 8.13 (2H, d, J=8.5 Hz), 7.13 (2H, d, J=8.5 Hz), and 6.59 (1H, s) suggested that the structure must be based on that of kaempferol with a substituent at C-8 which gave the characteristic signals of an isopentenyl group observed at δ 1.62 (3H, s), 1.81 (3H, s), 3.13 (2H, d), 5.15 (1H, br t, J=5.0 Hz). The spectrum further showed the presence of an MeO group. The signals at 5.34 (1H, br, J=6.5 Hz), 5.36 (1H, br, J=6.5 Hz) were assigned to H-1 of the two glucoses. Its uv spectrum (λ max, nm) in MeOH showed absorption at 374 (band I), indicating the presence of a free OH at C-3. The bathochromic shifts of band I with NaOMe and $AlCl_3$/HCl, 72 and 58 nm, respectively, are characteristic features of 3-OH and 5-OH substituents. The lack of a shift of band II with NaOAc indicated that the OH at C-7 was substituted. This was supported by the enzymatic hydrolysis of cuhuoside [**1**] with β-amylase, which gave D-glucose and an aglycone whose uv spectrum in MeOH/NaOAc gave a consistent bathochromic shift (270–279 nm), indicating a free OH at C-7. Acid hydrolysis gave D-glucose and the same aglycone as that of enzymatic hydrolysis. The aglycone was identified as a 4′-methoxy-8-prenylkaempferol (icaritin), the same aglycone as that of icariin.



1 R^1=H, R^2=Glc (1→4) Glc

^{13}C-nmr chemical shifts showed the presence of signals of icariside I and one glucose. The signals of sugar moieties were similar to those of cellubiose. Sig-

nals at δ 100.59 and 105.3 showed the linkage between sugars and the aglycone and glucose to be of the β configuration. The C-4 signal (δ 76.78) of the glucose of the 7-*O*-glucosyl moiety showed a downfield shift of 6.9 ppm upon comparison with the corresponding C-4 signal (δ 69.84 ppm) of a flavonol 7-*O*-glucoside, revealing a 1→4 linkage between the 7-*O*-glucosyl and the terminal glucosyl moiety (5,6). On the basis of these data, cuhuoside [**1**] was elucidated as 3,5,7-trihydroxy-4′-methoxy-8-prenyl-flavone-7-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside].

The identification of the other six known flavonol glycosides was made by chemical and spectroscopic methods (uv, ir, ^{1}H nmr, ^{13}C nmr, fabms, enzymatic and acid hydrolysis and co-tlc with authentic samples).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Boetius micro-melting point apparatus and are uncorrected. The ir spectra were obtained on a Nicolet-20 SXB FT-IR. Uv spectra were obtained on a Specord UV VIS. Nmr spectra were recorded on a VXR-300 nmr spectrometer. Ms spectra were obtained on an MAT 711 (fd) and a KYKY znp-5 A (fab).

PLANT MATERIAL.—*E. acuminatum* was collected in August 1988 at Gui Yang, Gui Zhou Province, China. A voucher specimen is deposited in the Herbarium of Beijing College of Traditional Chinese Medicine, Beijing, China.

EXTRACTION AND ISOLATION.—The dried and powdered aerial parts (2 kg) were throughly extracted with 95% EtOH. The concentrated extract (250 g) was partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc (8 g) and *n*-BuOH fractions (10 g) were chromatographed on Si gel columns eluted with $CHCl_3$-MeOH-HCOOH (150:1:0.5) and $CHCl_3$-MeOH (8:2→7:2), respectively, to afford compound **1** (38 mg), tricin (50 mg), icaritin (30 mg), icariside I (28 mg), baohuoside I (40 mg), icariin (200 mg), and baohuoside VI (6 mg), after recrystallization.

Cuhuoside [**1**].—Yellow amorphous solid, mp 258–260°; uv max nm (MeOH) 228, 269, 327, 374; (NaOMe) 261, 281, 353, 446, strength of band I not changed after 5 min; ($AlCl_3$) 280, 356, 432; ($AlCl_3$/HCl) 238, 260, 280, 354, 432; ^{1}H nmr (DMSO-d_6) δ 1.61, 1.75 (each 3H, s, Me-4″, Me-5″), 3.13 (2H, d, H-1″), 3.84 (3H, s, OMe), 5.15 (1H, br t, H-2″), 5.36 (1H, br s, glc H-1), 6.59 (1H, s, H-6), 7.13 (2H, d, J=8.5 Hz, H-3′, -5′), 8.13 (2H, d, J=8.5 Hz, H-2′, -6′), 9.64 (1H, s, 3-OH), 12.43 (1H, s, 5-OH); fab/fd ms *m/z* $[M+H]^+$ 693, $[M-162+H]^+$ 531, $[M-2\times 162+H]^+$ 369, $[369-C_4H_7+H]^+$ 313; ^{13}C nmr see Table 1.

ENZYMATIC HYDROLYSIS OF CUHUOSIDE [**1**].—NaOAc/HOAc buffer solution (pH 5.0) containing **1** (5 mg) and β-amylase (2 mg) was incubated at 37° for 24 h. After filtration, a product was obtained by extraction of the filtrate with EtOAc: uv λ max nm (MeOH) 270, 352, 375; (NaOAc) 279, 320, 393. The product did not respond to the

TABLE 1. ^{13}C-nmr Spectral Data of Cuhuoside [**1**] in DMSO-d_6.

Carbon	δ	Carbon	δ
C-2	147.00	C-3″	129.45
C-3	136.26	C-4″	25.76
C-4	176.50	C-5″	18.20
C-5	160.50	OMe	55.64
C-6	97.59	glc-1	100.59
C-7	160.68	glc-2	73.55
C-8	108.17	glc-3	76.70
C-9	152.77	glc-4	76.78
C-10	105.20	glc-5	77.33
C-1′	122.43	glc-6	60.83
C-2′	129.45	glc′-1	105.30
C-3′	114.26	glc′-2	73.45
C-4′	158.60	glc′-3	76.78
C-5′	114.26	glc′-4	69.84
C-6′	129.45	glc′-5	77.33
C-1″	21.71	glc′-6	60.87
C-2″	123.51		

Molish test. Its R_f value was equal to that of icaritin on tlc analysis.

ACID HYDROLYSIS OF CUHUOSIDE [**1**].—A 3% H_2SO_4 solution (5 ml) of **1** (2 mg) was heated under reflux for 2 h. The solution was neutralized with $BaCO_3$, and the filtrate was subjected to tlc [eluent $CHCl_3$-MeOH-H_2O (30:12:4), lower phase]. D-Glucose was detected by spraying with 0.2% naphthoresorcinol-EtOH (1:1) (heating 105°).

A solution of MeOH (5 ml) containing 2 mg of **1** was applied at a point about 1 cm from the bottom edge of the hptlc Si gel plate (10×30 cm) and was hydrolyzed with HCl vapor for 48 h. The lower layer of a mixture of a $CHCl_3$-MeOH-H_2O (30:12:4) was used as the developing solvent. The identification of D-glucose was carried out by comparison with authentic sugars on the same plate.

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