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FLAVONOL GLYCOSIDES FROM *EPIMEDIUM KOREANUM*

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Abstract—A novel flavonol glycoside named caohuoside-B was isolated from the aerial parts of *Epimedium koreanum*, along with a known flavonol glycoside, epimedokoreanoside-I. Their structures were established by spectroscopic methods. The new compound was elucidated as anhydroicaritin 3-*O*-β-D-(4,6-*O*-diacetyl)glucopyranosyl-(1 → 3)-α-L-(4″-*O*-acetylrhamnopyranoside)-7-*O*-β-D-glucopyranoside.

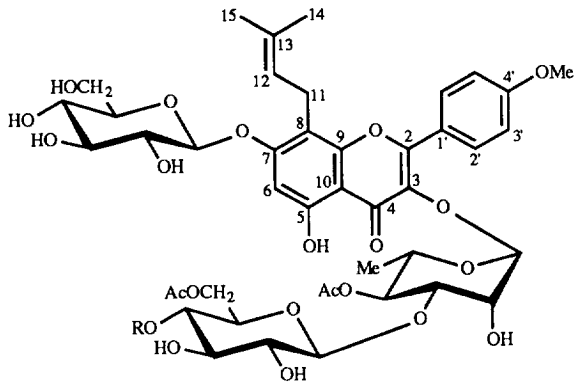
INTRODUCTION

Epimedium koreanum Nakai (*Berberidaceae*) is native to Heilongjiang province in China. This species is used as a tonic, antirheumatic and aphrodisiac in traditional Chinese medicine, together with four other Chinese Pharmacopoeia recorded species: *E. brevicornum*, *E. sagittatum*, *E. pubescens* and *E. wushanense*. Some flavonoids have been isolated previously from *E. koreanum* [1–7]. In the present paper we describe the isolation and structural elucidation of a novel flavonol glycoside together with a known flavonol glycoside, epimedokoreanoside-I from the same species.

RESULTS AND DISCUSSION

Compound **1** gave *m/z* 965 $[M + H]^+$, $C_{45}O_{56}H_{23}$ in FABMS and responded to the Molish and Shinoda (Mg–HCl) tests. Its IR spectrum showed a strong absorption band at 1650 cm^{-1} for a chelated carbonyl group. The UV spectrum of **1** in methanol showed absorptions at 266 (band II), 310 and 350 (band I) nm, indicating a C_3-*O*-substituted flavonol skeleton [8]. The bathochromic shift of band I with aluminium chloride–hydrochloride acid (60 nm) is a characteristic feature of a 5-hydroxy-3-*O*-substituted flavonol. A small bathochromic shift and decreasing intensity of band I (25 nm) with sodium methoxide suggested that there was no free 4′-hydroxyl group on the ring B [8]. The lack of a shift of band II with sodium acetate indicated that the C-7 hydroxyl group was absent or substituted. In the ^1H NMR spectrum (all protons were reasonably assigned by ^1H–^1H COSY, ^1H–^1H TOCSY and ^1H–^{13}C COSY) of **1**, a one-proton multiplet at δ3.52 and 3.38, a one-proton triplet at 5.17 in addition to two three-proton singlets at 1.59 and 1.67 showed the presence of a γ,γ-dimethylallyl group. This was also supported by signals at δ21.4 assigned to C-11, 122.0 to C-12, 131.0 to C-13, and 25.4 and 17.7 to C-14 and C-15 in the ^{13}C NMR spectrum, in which all carbons were assigned by APT, ^1H–^{13}C COSY and ^1H–^{13}C long range COSY. The B ring moiety was oxygenated only at C-4′ on account of the signals of A_2B_2 at δ7.88 and 7.16. On the basis of a three-proton singlet at δ3.87 in the ^1H NMR spectrum and the significant fragment ion at *m/z* 369 in the FABMS, the aglycone moiety of **1** was identified as anhydroicaritin (8-γ,γ-dimethylallyl-3,5,7-trihydroxy-4′-methoxyflavone). The FABMS of **1** showed peaks at *m/z* 965, 719, 531 and 369, which were ascribed respectively to $[M + H]^+$, $[M - \text{glucosyl(OAc)}_2 + H]^+$, $[M - \text{glucosyl(OAc)}_2 - \text{rhamnosyl(OAc)} + H]^+$ and $[M - \text{glucosyl(OAc)}_2 - \text{rhamnosyl(OAc)} - \text{glucosyl} + H]^+$ ions, clearly suggesting the presence of one rhamnose and two glucose moieties together with three acetyl groups in the molecule. The presence of acetyl groups was supported by three three-proton singlets at δ1.95, 1.97 and 2.03 in ^1H NMR spectrum and δ169.6, 20.4 (OAc), 169.7, 20.6 (OAc) and 170.1, 20.8 (OAc) in the ^{13}C NMR spectrum. The molar ratio, 1:2:1 of aglycone to glucose to rhamnose was confirmed by TLC-densitometry after acid hydrolysis [9].

Three anomeric protons in the ^1H NMR spectrum of **1** were observed and assigned to those of glucose δ4.38 (*d*, $J = 7.9$ Hz), glucose 4.99 (*d*, $J = 6.8$ Hz) and rhamnose 5.28 (*d*, $J = 1.5$ Hz). The glycosyl anomeric proton (δ4.99) showed a cross-peak with C-7 (δ161.6) of the aglycone in the ^1H–^{13}C long range COSY, indicating that a glucose was attached by a β-glycosidic linkage to the phenoxyl group at C-7. On the other hand, the anomeric proton of the rhamnose moiety (δ5.28) caused a cross-peak with a carbon assigned to C-3 (δ134.0), which also indicated that the rhamnose was attached to the phenoxyl group at C-3



R

1 Ac

2 H

Fig. 1. The structure of **1** and **2**.

through a glycosidic linkage. The position of the terminal glucose was determined by a carbon signal (δ77.2) assigned to C-3 of the rhamnose, which shifted downfield by *ca* 6 ppm on comparison with the corresponding signal of C-3 (δ70.9) of icariin [10]. The glycosylation shift is usually observed to be 7–10 ppm [11]. In this case, the effect of the acetyl group attached to C-4 diminished the shift value. This was also supported by the appearance of a cross-peak of the proton at C-4 (δ4.84) of the rhamnose with a carbonyl carbon of the acetyl group (δ169.6) and a cross-peak of the proton at C-1 of the terminal glucose (δ4.38) with C-3 of the rhamnose (δ77.2). This finding showed the interlinkage of the biose to be β-D-glucopyranosyl(1→3)-α-L-rhamnopyranoside, and that an acetyl group was esterified at C-4 of the rhamnose. The position of the acetyl groups on terminal glucose was determined by the carbon signals (δ71.1, 62.6) assignable, respectively, to C-4 and C-6 of the terminal glucose, which shifted downfield by *ca* 2 and 2 ppm on comparison with the corresponding signals of C-4 (δ69.1) and C-6 (60.3) of epimedin A [3], suggesting that the acetyl groups were attached, respectively, to C-4 and C-6 of the terminal glucose. These were supported by other evidence: a proton at C-4 of the terminal glucose (δ4.58) had a cross-peak with carbonyl carbon (δ169.5) of an acetyl group, two protons at C-6 of the terminal glucose (δ4.18, 3.95) had cross-peaks with carbonyl carbon (δ170.1) of another acetyl group in ^{1}H–^{13}C long range COSY. From the spectral data described above, the structure of **1** was concluded to be anhydroicaritin-3-*O*-β-D-(4,6-*O*-diacetyl) glucopyranosyl(1 → 3)-α-L-(4″-*O*-acetylrhamnopyranoside)-7-*O*-β-D-glucopyranoside and named caohuoside-B. Compound **2** was identified as the known compound epimedokoreanoside-I [5] by standard procedures (mp, IR, UV, ^{1}H NMR and ^{13}C NMR).

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 and chemical shifts given in values (ppm) with TMS as the int. standard. TLC was carried out on G-PF 254 (Merk) in EtOAc–Me_2CO–H_2O (25:5:1).

Plant material. The aerial parts of *Epimedium koreanum* were collected in September 1992 at Harbin, Heilongjiang province in China and a voucher specimen has been deposited in the Herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China.

Extraction and isolation. The dried and powdered aerial parts of *E. koreanum* (25 kg) were completely extracted with 95% EtOH. The concd extract was partitioned successively with CH_2Cl_2, EtOAc and *n*-BuOH. The EtOAc fr. (191 g) was chromatographed on a polyamide column eluted with $CHCl_3$–MeOH (gradient) to afford 15 frs. Fr. 6 was rechromatographed over a silica gel column with $CHCl_3$–MeOH (100:1, 50:1, 25:1, 10:1 and 5:1) as eluent, yielding subfrs A–D. Subfr. B was subjected to a Sephadex LH-20 column with MeOH as eluent to yield **1** (25 mg). Compound **2** (105 mg) was obtained from fr. 7 by silica gel and polyamide chromatography and final purification with Sephadex LH-20 chromatography.

Compound **1** (*caohuoside-B*). Yellow amorphous powder (MeOH, 25 mg), mp 125–127°, $C_{45}H_{56}O_{23}$, $[\alpha]_D^{20}$ −98.2 (MeOH; *c* 0.02). IR ν_{max}^{KBr} cm^{-1}: 3420, 2980, 2920, 1650, 1600, 1510, 1450, 840. UV λ_{max}^{MeOH} nm: 220 (sh), 266, 310, 350, +NaOMe: 278, 375, +$AlCl_3$: 232 (sh), 278, 302, 340, 410, +$AlCl_3$–HCl: 232 (sh), 278, 336, 410, +NaOAc: 268, 312, 350, +NaOAc–H_3BO_3: 268, 312, 350 (sh). FABMS (*m/z*): 965 $[M+H]^+$, 719 $[M-246+H]^+$, 531 $[M-246-188+H]^+$, 369 $[M-246-188-162+H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6 + D_2O): δ [aglycone moiety] 7.88 (*d*, *J* = 8.1 Hz, H-2′, 6′), 7.16 (*d*, *J* = 8.1 Hz, H-3′, 5′), 6.63 (*s*, H-6), 3.38 and 3.52 (each *m*, H_2-11), 5.17 (*t*, *J* = 7.5 Hz, H-12), 1.67 and 1.59 (each *s*, Me-14, 15), 3.87 (*s*, OMe-4′), [sugar moieties] (rha) 5.28 (*d*, *J* = 1.5 Hz, H-1), 4.16 (*d*, *d*, *J* = 1.5, 3.5 Hz, H-2), 3.62 (*d*, *d*, *J* = 3.5, 10.1 Hz, H-3), 4.84 (*d*, *d*, *J* = 10.1, 10.1 Hz, H-4), 3.19 (*d*, *q*, *J* = 10.1, 6.1 Hz, H-5), 0.71 (*d*, *J* = 6.1 Hz, H-6), 1.95 (*s*, OAc-4), (7-*O*-glc) 4.99 (*d*, *J* = 6.8 Hz, H-1), 3.26 (*d*, *d*, *J* = 6.8, 9.3 Hz, H-2), 3.29 (*d*, *d*, *J* = 9.3, 9.3 Hz, H-3), 3.17 (*d*, *d*, *J* = 9.3, 9.3 Hz, H-4), 3.37 (*d*, *d*, *d*, *J* = 9.3, 6.5, 2.5 Hz, H-5), 3.70 (*d*, *d*, *J* = 11.3, 6.5 Hz, H-6), 3.47 (*d*, *d*, *J* = 11.3, 2.5 Hz, H-6), (terminal glc) 4.38 (*d*, *J* = 7.9 Hz, H-1), 3.08 (*d*, *d*, *J* = 7.9, 9.6 Hz, H-2), 3.42 (*d*, *d*, *J* = 9.6, 9.6 Hz, H-3), 4.58 (*d*, *d*, *J* = 9.6, 9.6 Hz, H-4), 3.71 (*d*, *d*, *d*, *J* = 9.6, 5.7, 2.5 Hz, H-5), 4.18 (*d*, *d*, *J* = 10.1, 5.7 Hz, H-6), 3.95 (*d*, *d*, *J* = 10.1, 2.5 Hz, H-6), 1.97 (*s*, OAc-4), 2.03 (*s*, OAc-6). ^{13}C NMR: Table 1.

Compound **2** (*epimedokoreanoside*-I). Mp 155–157°, obtained as a yellow amorphous powder. $C_{43}H_{54}O_{22}$. IR ν_{max}^{KBr} cm^{-1}: 3400, 2980, 2920, 1650, 1600, 1510, 1440, 840. UV λ_{max}^{MeOH} nm: 220 (sh), 268, 314, 350, +NaOMe: 278, 375, +$AlCl_3$: 230 (sh), 270, 306, 334, 410, +$AlCl_3$–HCl: 230 (sh), 270, 306, 340, 410, +NaOAc: 268, 314, 350, +NaOAc–H_3BO_3: 268, 314, 350. FABMS (*m/z*): 961 $[M+K]^+$, 945 $[M+Na]^+$, 923 $[M+H]^+$, 719 [M − glucosyl(OAc) + H]$^+$, 531 [M − glucosyl(OAc) − rhamnosyl(OAc) + H]$^+$, 369 [M − glucosyl(OAc) − rhamnosyl (OAc) − glucosyl + H]$^+$. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (*d*, *J* = 9.0 Hz, H-2′, 6′), 7.16 (*d*, *J* = 9.0 Hz, H-3′,

Table 1. ^{13}C NMR spectral data of **1** and **2** (DMSO-d_6)*

	1				**2**	
Aglycone		Sugar		Aglycone	Sugar	
2	157.4	3-*O*-inner Rha		157.2	3-*O*-inner Rha	
3	134.0	1	101.3	133.8	1	100.8
4	178.1	2	69.5	178.1	2	69.3
5	160.6	3	77.2	160.5	3	77.1
6	98.3	4	71.4	98.2	4	71.1
7	161.6	5	68.3	161.6	5	68.3
8	108.5	6	17.0	108.4	6	16.9
9	153.0	4-COMe	169.6	153.0	4-COMe	169.6
10	110.8	4-COMe	20.4	110.5	4-COMe	20.6
11	21.4	terminal Glc		21.4	terminal Glc	
12	122.0	1	105.6	122.0	1	104.8
13	131.0	2	73.7	131.4	2	72.9
14	25.4	3	70.0	25.3	3	76.6
15	17.8	4	71.1	17.7	4	69.7
1′	122.0	5	71.0	122.0	5	73.6
2′	130.4	6	62.6	130.6	6	63.9
3′	114.1	4-COMe	169.7	114.2	6-COMe	170.2
4′	159.0	4-COMe	20.6	159.0	6-COMe	21.1
5′	114.1	6-COMe	170.1	114.2	7-O-Glc	
6′	130.4	6-COMe	20.8	130.6	1	100.6
OMe	55.5	7-O-Glc		55.5	2	73.3
		1	100.6		3	76.5
		2	73.3		4	70.2
		3	76.6		5	76.6
		4	69.7		6	60.6
		5	76.7			
		6	60.7			

*Chemical shifts are given in ppm values; the ^{13}C NMR spectrum of **1** was obtained in (DMSO-d_6 + D_2O).

5′), 6.64 (*s*, H-6), 5.16 (*t*, $J = 7.5$ Hz, H-12), 1.67 and 1.59 (each *s*, Me-14, 15), 3.87 (*s*, OMe-4′), 5.29 (*d*, $J = 1.5$ Hz, H-1, rha), 0.71 (*d*, $J = 6.5$ Hz, H-6, rha), 1.93 (*s*, OAc-4, rha), 4.99 (*d*, $J = 5.9$ Hz, H-1,7-*O*-glc), 4.25 (*d*, $J = 7.8$ Hz, H-1, terminal glc), 1.97 (*s*, OAc-6, terminal glc). ^{13}C NMR: Table 1.

Acid hydrolysis of glycosides [9]. Glycoside solns were applied 1 cm from the bottom edge of an HPTLC silica gel plate (10 × 10 cm) and hydrolysed with HCl vapour for 40 min at 50–60° and the plate developed in 9 ml of the lower layer of a mixt. of $CHCl_3$–MeOH–H_2O (15:6:2) to which 1 ml of HOAc was added. The identification of sugars was carried out by comparison with authentic sugar markers on the same plate. To determine the molar ratio of the sugars and aglucone of **1**, a 10% soln of **1** was heated under reflux for 10 hr. The soln was taken down to dryness and the residue prepd for quantitative determination by TLC-densitometry.

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