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FLAVONOIDS FROM *EPIMEDIUM WANSHANENSE*

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Key Word Index—*Epimedium wanshanense*; Berberidaceae; flavonoids; wanepimedoside A; anhydroicaritin; desmethylanhydroicaritin; icarisid I and II; quercetin; ikarisoside A and B; sagittatoside B; 2″-*O*-rhamnosylicarisid II; icariin; 2″-*O*-rhamnosylikarisoside A; epimedin B and C; diphylloside A and B.

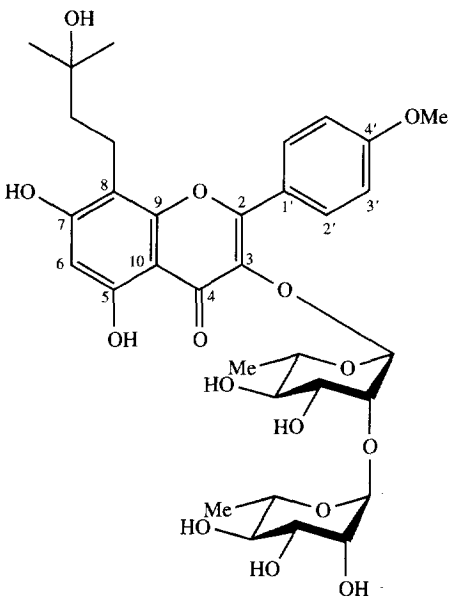
Abstract—A novel flavonol glycoside named wanepimedoside A was isolated from the whole plant of *Epimedium wanshanense*, along with fifteen known flavonoids, anhydroicaritin, desmethylanhydroicaritin, icarisid I and II, quercetin, ikarisoside A and B, sagittatoside B, 2″-*O*-rhamnosylicarisid II, icariin, 2″-*O*-rhamnosylikarisoside A, epimedin B, epimedin C, and diphylloside A and B.

INTRODUCTION

Epimedium wanshanense S. Z. He et Buo (Berberidaceae) has been used to invigorate the kidney and strengthen 'Yang' in Chinese herbal medicine. The species is native to Guizhou province in China. In previous papers [1–3], we have reported the chemical constituents of *E. koreanum*. In the course of our ethnopharmacological investigations of the genus *Epimedium*, some cardiovascular principles have been isolated from the whole plant of *E. wanshanense*. In this paper we report the isolation and structural elucidation of a novel flavonol glycoside, wanepimedoside A (**1**), together with 15 known flavonoids from the same species.

RESULTS AND DISCUSSION

Compound **1** gave *m/z* 677 $[M-H]^-$, $C_{33}H_{42}O_{15}$ in FAB mass spectrometry, 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 268 (band II), 310 and 350 (band I) nm, indicating a 3-*O*-substituted flavonol skeleton [4]. The bathochromic shift of band I with aluminum chloride–hydrochloric acid (60 nm) is a characteristic feature of 5-hydroxy-3-*O*-substituted flavonols. A small bathochromic shift and decreasing intensity of band I (30 nm) with sodium methoxide suggested that there was no free 4′-hydroxyl group in ring B [4]. The bathochromic shift of band II (4 nm) with sodium acetate revealed the presence of a free hydroxyl group at C-7. In the ^1H NMR spectrum of **1**, a two-proton multiplet at δ 2.70, a two-proton multiplet



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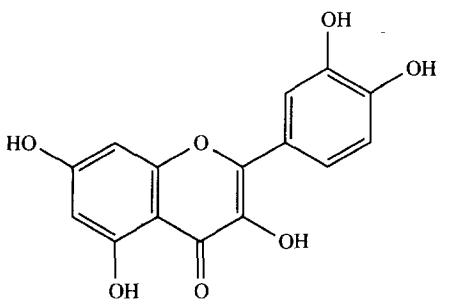

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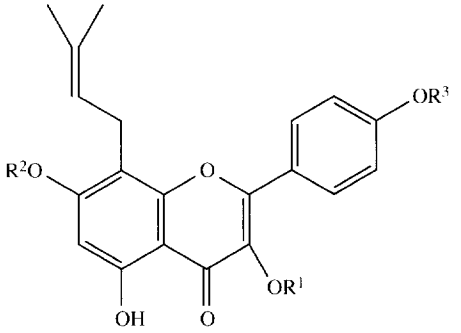
at δ 1.55 in addition to three-proton singlets at δ 1.16 and 1.15 showed the presence of a γ-hydroxy-γ,γ-dimethylpropyl group, which was supported by signals at δ 42.9 assigned to C-11, 17.3 to C-12, 68.7 to C-13, and 29.1 and 28.9 to C-14 and C-15 in the ^{13}C NMR spectrum. This was also confirmed by the presence of fragment ions at *m/z* 386 [aglycone], 368 [aglycone $-$ H_2O], 353 [aglyone $-$ H_2O $-$ Me], 313 [aglycone $-$ H_2O $-$ C_4H_7], 300 [aglycone $-$ H_2O $-$ C_5H_9 + H] and 165 (formed after retro-Diels–Alder cleavage of the fragment ion at *m/z* 368) in EI mass spectrometry [5]. The location of the γ-hydroxy-γ,γ-dimethylpropyl group at the C-8 position was supported by the ^{13}C NMR spectrum of **1** because of the chemical shift value of the carbon atom at the C-6 position (δ 98.3) [6]. The B ring moiety was oxygenated only at C-4′ because of the appearance of A_2B_2 signals at δ 7.93 and 7.09. On the basis of a three-proton singlet at δ 3.85 in the ^{1}H NMR spectrum and the presence of a fragment ion at *m/z* 135 (B_2^+) in the EI mass spectrum [7], the aglycone moiety was identified as icaritin (8-γ-hydroxy - γ,γ - dimethylpropyl - 3,5,7 - trihydroxy - 4′ - methoxyflavone). The negative ion FAB mass spectrum of **1** showed peaks at *m/z* 677, 531 and 385, which were ascribed respectively to $[M-H]^-$, $[M-$ rhamnosyl $-H]^-$ and $[M-$ rhamnosyl $-$ rhamnosyl $-H]^-$ ions, clearly suggesting the presence of two rhamnose moieties in the molecule. The nature of the sugar was confirmed by co-TLC with an authentic marker after acid hydrolysis [8].

Two anomeric protons in the ^{1}H NMR spectrum of **1** were observed and assigned to those of the inner rhamnose δ 5.38 (*d*, J = 1.5 Hz) and the terminal rhamnose δ 4.87 (*br s*), which was closely similar to those of 2″-*O*-rhamnosylicarisid II and 2″-*O*-rhamnosylikarisoside A [9], suggesting the biose had a 1 $\rightarrow$ 2 linkage, which was further supported by the fact that the ^{13}C NMR chemical shifts of the inner rhamnose C-1 and C-2 signals were displaced upfield by 1.7 ppm and downfield by 5.1 ppm, respectively, from those of



2~5,7~16

	Name	R_1	R_2	R_3
2	Anhydroicaritin	H	H	Me
3	Desmethylanhydroicaritin	H	H	H
4	Icarisid II	Rha	H	Me
5	Icarisid I	H	Glc	Me
7	Ikarisoside A	Rha	H	H
8	Sagittatoside B	Rha$\xrightarrow{2}$Xyl	H	Me
9	2″–*O*–Rhamnosylicarisid II	Rha$\xrightarrow{2}$Rha	H	Me
10	Icariin	Rha	Glc	Me
11	Ikarisoside B	Rha$\xrightarrow{2}$Glc	H	H
12	2″–*O*–Rhamnosylikarisoside A	Rha$\xrightarrow{2}$Rha	H	H
13	Epimedin B	Rha$\xrightarrow{2}$Xyl	Glc	Me
14	Epimedin C	Rha$\xrightarrow{2}$Rha	Glc	Me
15	Diphylloside B	Rha$\xrightarrow{2}$Rha	Glc	H
16	Diphylloside A	Rha$\xrightarrow{2}$Glc	Glc	H

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

Position		Position		Position	
2	156.3	13	68.7	3″	70.0
3	134.3	14	29.1	4″	71.8
4	177.9	15	28.9	5″	70.4
5	158.5	1′	122.3	6″	17.5
6	98.3	2′	130.4	1‴	101.5
7	161.7	3′	114.7	2‴	70.1
8	107.1	4′	161.2	3‴	70.6
9	153.6	5′	114.7	4‴	71.8
10	104.1	6′	130.4	5‴	69.6
11	42.9	1″	100.1	6‴	17.4
12	17.3	2″	75.4	OMe	55.4

*Chemical shifts are given in ppm values.

icarisid II [10]. Therefore, the structure of **1** is icaritin-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

Compounds **2–16** were identified by IR, UV, mass, ^1H NMR and ^{13}C NMR spectral data as the known flavonoids, anhydroicaritin [11], desmethylanhydroicaritin [12], icarisid II [10], icarisid I [13], quercetin [14], ikarisoside A [12], sagittatoside B [15], 2″-*O*-rhamnosylicarisid II [9], icariin [10], ikarisoside B [16], 2″-*O*-rhamnosylikarisoside A [9], epimedin B, epimedin C [15], diphylloside B and diphylloside A [11], respectively.

EXPERIMENTAL

All mps are uncorr. MS were obtained at 70 eV. ^1H NMR spectra were recorded at 400 MHz; chemical shifts are given in δ values (ppm) with TMS as int. standard. ^{13}C NMR spectra were obtained at 100 MHz. TLC was carried out on G-PF 254 (Merck) in $CHCl_3$–MeOH (25:1, 15:1, 10:1, 7.5:1 and 5:1).

Plant material. The whole plant of *E. wanshanense* were collected in Wanshan country, Guizhou province, China, in June 1994, and a voucher specimen has been deposited in the Herbarium of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.

Extraction and isolation. Fresh whole plants of *E. wanshanense* (6 kg) were powdered and extracted with 80% EtOH. The concd extracts (180 g) were suspended in H_2O and successively extracted with CH_2Cl_2, EtOAc and *n*-BuOH. The combined EtOAc and *n*-BuOH extracts (127 g) were chromatographed on silica gel using $CHCl_3$–MeOH as solvent to give 10 frs. Fr. 6 was rechromatographed on a silica gel column with $CHCl_3$–MeOH (5:1) as eluent, yielding subfrs A–C. Subfr. A was subjected to a Sephadex LH-20 column with MeOH as eluent to yield **1** (105 mg). Compound **2** (16 mg) and **3** (6 mg) from fr. 1; **4** (125 mg), **5** (16 mg) and **6** (5 mg) from fr. 2; **7** (25 mg) and **8** (18 mg) from fr. 4; **9** (150 mg) from fr. 5; **10** (2000 mg), **11** (20 mg) and **12** (25 mg) from fr. 6; **13** (43 mg) from fr. 7; **14** (750 mg) from fr. 8; **15** (350 mg) and **16** (15 mg) from fr. 9 were obtained by silica gel chromatography and final purification with Sephadex LH-20 chromatography.

Wanepimedoside A (**1**). Yellow amorphous powder (MeOH, 105 mg), mp 160–161°, $C_{33}H_{42}O_{15}$. IR ν_{max}^{KBr} cm^{-1}: 3400, 2980, 2920, 1650, 1610, 1510, 1430, 1260, 840. UV λ_{max}^{MeOH} nm: 268, 310, 350, +NaOMe: 280, 380, +$AlCl_3$: 270, 304, 350, 410, +$AlCl_3$ − HCl: 270, 304, 350, 410, +NaOAc: 272, 314, 350, +NaOAc − H_3BO_3: 272, 314, 350. EIMs (*m/z*): 386, 368, 353, 313, 300, 165, 135. ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.55 (1H, *s*, OH-5), 10.75 (1H, *s*, OH-7), 7.93 (2H, *d*, *J* = 8.9Hz, H-2′, 6′), 7.09 (2H, *d*, *J* = 8.9 Hz, H-3′, 5′), 6.30 (1H, *s*, H-6), 3.85 (3H, *s*, OMe-4′), 2.70 (2H, *m*, H-11), 1.55 (2H, *m*, H-12), 1.16 (3H, *s*, H-14), 1.15 (3H, *s*, H-15), 5.38 (1H, *d*, *J* = 1.5 Hz, inner Rha-H-1), 4.87 (1H, *br s*, terminal Rha-H-1), 4.10 (1H, *br s*, inner Rha-H-2), 1.09 (3H, *d*, *J* = 5.6 Hz, terminal Rha-H-6), 0.80 (3H, *d*, *J* = 5.6 Hz, inner Rha-H-6). For ^{13}C NMR, see Table 1.

Compounds **2–16**. Properties and spectra were identical to those reported earlier.

Acid hydrolysis of **1** [8]. A soln of **1** was 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 sugar was carried out by comparison with an authentic sugar marker on the same plate.

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