



A DIFURANOFLAVONE FROM *EPIMEDIUM KOREANUM*

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(Received in revised form 1 August 1994)

Key Word Index—*Epimedium koreanum*; flavone; epimedokoreanin A; flavonol glycoside; sagittatoside B; ikarisoside F; epimedin B and epimedin C.

Abstract—A new difuranoflavone, epimedokoreanin A, was isolated from the aerial parts of *Epimedium koreanum*, in addition to four known flavonol glycosides, sagittatoside B, ikarisoside F, epimedin B and epimedin C.

INTRODUCTION

In our previous paper the isolation and structural elucidation of a new flavonol glycoside, caohuosome-B, was reported from *Epimedium koreanum* Nakai [1]. In the course of our search for bioactive principles in *Epimedium* species, as well as a chemotaxonomic investigation of the genus *Epimedium*, further constituents of the aerial parts of *E. koreanum* Nakai (Berberidaceae) were investigated. The present paper deals with the structure of a new flavone together with four known flavonol glycosides.

RESULTS AND DISCUSSION

After repeated polyamide and silica gel chromatography of the ethyl acetate soluble portion of a 95% ethanol extract and final purification by Sephadex LH-20 chromatography, **1–5** were isolated pure, and deduced to be flavonoids from the Shinoda test.

Epimedokoreanin A (**1**), mp 235–237°, was obtained as a yellow amorphous powder. The IR spectrum showed absorption bands at 3400 (OH), 2980, 2920 (saturated CH), 1650 (α, β -unsaturated C=O), 1600, 1580, 1500, 1450 (C=C) cm^{-1} . The UV spectrum showed absorptions at 258, 274 (sh) and 346 nm. Bathochromic shifts on addition of shift reagents (NaOMe, AlCl₃, AlCl₃–HCl, NaOAc, NaOAc–H₃BO₃) indicated that **1** is a flavone with a free hydroxyl group at C-5 and the absence of a free hydroxyl group at C-7 [2].

The HREIMS of **1** displayed the molecular ion peak at *m/z* 452.1483 (calcd 452.1470), consistent with the molecular formula C₂₅H₂₄O₈. In the ¹H NMR spectrum of **1**, the singlet at δ 6.62 was the characteristic signal of the H-3 in flavones [3]. The singlet at δ 12.94, due to a chelated C₅-OH group, and one singlet at δ 6.26 assignable to H-6 [3], suggested that **1** is 5,7,8-trisubstituted in ring A. Furthermore, the presence of aromatic signals at δ 7.37 (brs) and 7.54 (brs) assignable, respectively, to H-2'

and H-6' [4], suggested that **1** is 3',4',5'-trisubstituted in ring B. The signals at δ 2.93 (2H, *d*, *J* = 6.6 Hz), 4.21 (1H, *t*, *J* = 6.6 Hz), 1.71 (3H, *s*), 4.69 (1H, *brs*) and 4.61 (1H, *brs*) suggested the presence of 5'-isopropenylidihydrofuran (2",3":7,8) [5], which was confirmed by ¹H–¹³C COSY and ¹H–¹³C long range coupling obtained by HMBC (heteronuclear multiple bond correlation). Figure 1 showed the cross-peaks of the protons at δ 1.71 (H-8") with the carbons at δ 110.0 (C-7") and 73.4 (C-5"), the protons at δ 4.69 and 4.61 (H-7") with the carbons at δ 73.4 (C-5") and 17.5 (C-8"), the protons at δ 2.93 (H-4") with the carbons at δ 162.2 (C-7) and 155.0 (C-9), and the proton at δ 6.26 (H-6) with the carbons at δ 104.2 (C-8) and 103.4 (C-10). On the other hand, the signals at δ 5.23 (1H, *d*, *J* = 4.2 Hz), 4.22 (1H, *d*, *J* = 4.2 Hz), 1.19 (3H, *s*), 1.10 (3H, *s*), 5.78 (1H, *brs*) and 4.65 (1H, *brs*) suggested the presence of a 4"-hydroxyl-5"--(1-hydroxyl-1-methyl)ethyl-dihydrofuran(2",3":4',5') moiety, which was also confirmed by ¹H–¹³C COSY and ¹H–¹³C long range coupling obtained by HMBC. As shown in Fig. 1, the protons at δ 1.19 (H-7") and 1.10 (H-8") showed cross-peaks with the carbon at δ 97.6 (C-5"), the proton at δ 4.22 (H-5") showed a cross-peak with the carbon at δ 151.3 (C-4'), the protons at δ 7.39 (H-2') and 7.54 (H-6') showed cross-peaks with the carbons at δ 163.6 (C-2) and 151.3 (C-4'). The presence of C₃-OH (δ 9.80 in the ¹H NMR spectrum) was also indicated by the presence of a cross-peak of the proton at δ 7.39 (H-2') with the carbon at δ 141.6 (C-3') in the ¹H–¹³C long range coupling obtained by HMBC. Thus, the structure of **1** is 5"-isopropenyl-dihydrofuran(2",3":7,8)-4"-hydroxy-5"--(1-hydroxyl-1-methyl)ethyl-dihydrofuran(2",3":4',5')-5,3'-dihydroxyflavone.

Compounds **2** (mp 161–162°), **3** (mp 180–181°), **4** (mp 178–180°) and **5** (mp 170–171°) were obtained as yellow amorphous powders. The aglycone was determined as anhydroicaritin for **2**, **4** and **5**, and desmethylanhydroicaritin for **3**. The IR, UV, mass, ¹H and ¹³C NMR

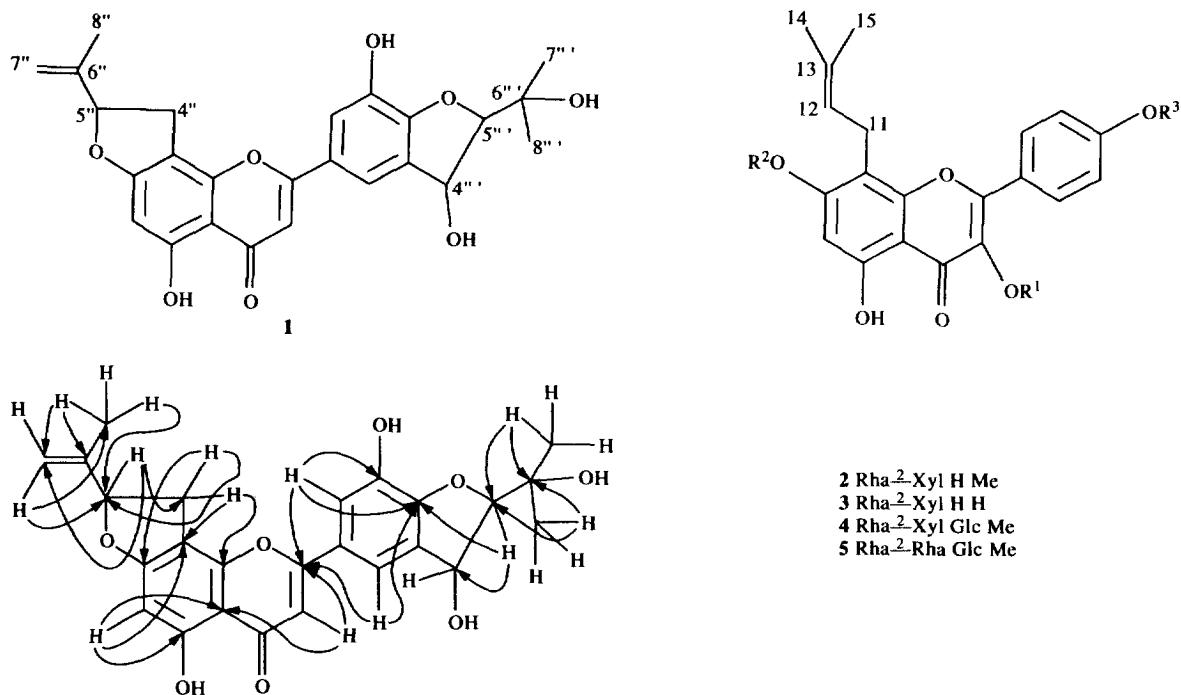


Fig. 1. The structure of **1–5** and the ^1H – ^{13}C long range coupling obtained by HMBC for **1**.

spectral data suggested that **2–5** were the known flavonol glycosides, sagittatoside B [6], ikarisoside F [7], epimedin B and epimedin C [8].

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR spectra were measured with Varian VXR-300 for **1** and FX-100 for **2–5** in DMSO-d_6 ; ^1H – ^{13}C COSY and ^1H – ^{13}C long range coupling for **1** were measured with Bruker AM-500; the chemical shifts were given in δ values (ppm) with TMS as the int. standard. HREI mass spectrum was obtained with AEI MS-50.

Extraction and isolation of flavonoids. Fr. 11 of the EtOAc-soluble portion described in detail in the previous paper [1] was chromatographed on a silica gel column with CHCl_3 –MeOH (in gradient) as the eluent to yield 15 subfrs. Subfr. 5 was rechromatographed over a polyamide column eluted with CHCl_3 –MeOH (10:1) to give subfrs A–F. Subfr. C was subjected to a Sephadex LH-20 column eluted with MeOH, yielding **1** (90 mg). Compound **2** (75 mg) from fr. 7, **3** (42 mg) from fr. 13, **4** (110 mg) and **5** (45 mg) from fr. 6 were obtained with silica gel and polyamide chromatography, and final purification with Sephadex LH-20 chromatography.

Compound 1 (epimedokoreanin A). Yellow amorphous powder (MeOH). mp 235–237°, $\text{C}_{25}\text{H}_{24}\text{O}_8$. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 258, 274 (sh), 346, + NaOMe: 274, 310 (sh), 382, + AlCl_3 : 270, 302, 358, 390 (sh), + AlCl_3 –HCl: 270, 302, 350, 390 (sh), + NaOAc: 258, 274, 330, 350, + NaOAc– H_3BO_3 : 258, 274, 330 (sh), 350. EIMS (m/z rel. int.): 452 ([M] $^+$, 35), 411 (18), 314 (12), 252 (40), 228 (15), 187 (65), 150 (10), 69 (100), 59 (10). ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.6 (C-2), 102.7 (C-3), 181.9 (C-

4), 159.1 (C-5), 98.3 (C-6), 162.2 (C-7), 104.2 (C-8), 155.0 (C-9), 103.4 (C-10), 123.5 (C-1'), 114.4 (C-2'), 141.6 (C-3'), 151.3 (C-4'), 132.4 (C-5'), 115.1 (C-6'), 29.4 (C-4''), 73.4 (C-5''), 147.9 (C-6''), 110.0 (C-7''), 17.5 (C-8''), 71.6 (C-4'''), 97.6 (C-5'''), 69.7 (C-6'''), 26.0 (C-7'''), 25.2 (C-8''').

Compounds 2–5. IR, UV, mass, ^1H and ^{13}C NMR spectral data agree with published results.

Acknowledgements—We thank the Analytical Laboratory of IMPLAD; the NMR service of Beijing Institute of Microchemistry, Beijing Medical University and Beijing Agricultural University; the MS service of Institute of Chemistry, Chinese Academy of Sciences for provision of spectroscopic facilities.

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