



## A DIFURANOFLAVONE FROM *EPIMEDIUM KOREANUM*

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**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, caohuoside-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 ( $\alpha,\beta$ -unsaturated C=O), 1600, 1580, 1500, 1450 (C=C)  $\text{cm}^{-1}$ . The UV spectrum showed absorptions at 258, 274 (sh) and 346 nm. Bathochromic shifts on addition of shift reagents (NaOMe,  $\text{AlCl}_3$ ,  $\text{AlCl}_3\text{-HCl}$ , NaOAc, NaOAc- $\text{H}_3\text{BO}_3$ ) 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  $\text{C}_{25}\text{H}_{24}\text{O}_8$ . In the  $^1\text{H}$  NMR spectrum of **1**, the singlet at  $\delta$ 6.62 was the characteristic signal of the H-3 in flavones [3]. The singlet at  $\delta$ 12.94, due to a chelated  $\text{C}_5\text{-OH}$  group, and one singlet at  $\delta$ 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  $\delta$ 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  $\delta$ 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''-isopropenyldihydrofuran (2'',3'':7,8) [5], which was confirmed by  $^1\text{H}\text{-}^{13}\text{C}$  COSY and  $^1\text{H}\text{-}^{13}\text{C}$  long range coupling obtained by HMBC (heteronuclear multiple bond correlation). Figure 1 showed the cross-peaks of the protons at  $\delta$ 1.71 (H-8'') with the carbons at  $\delta$ 110.0 (C-7'') and 73.4 (C-5''), the protons at  $\delta$ 4.69 and 4.61 (H-7'') with the carbons at  $\delta$ 73.4 (C-5'') and 17.5 (C-8''), the protons at  $\delta$ 2.93 (H-4'') with the carbons at  $\delta$ 162.2 (C-7) and 155.0 (C-9), and the proton at  $\delta$ 6.26 (H-6) with the carbons at  $\delta$ 104.2 (C-8) and 103.4 (C-10). On the other hand, the signals at  $\delta$ 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  $^1\text{H}\text{-}^{13}\text{C}$  COSY and  $^1\text{H}\text{-}^{13}\text{C}$  long range coupling obtained by HMBC. As shown in Fig. 1, the protons at  $\delta$ 1.19 (H-7''') and 1.10 (H-8''') showed cross-peaks with the carbon at  $\delta$ 97.6 (C-5'''), the proton at  $\delta$ 4.22 (H-5''') showed a cross-peak with the carbon at  $\delta$ 151.3 (C-4'), the protons at  $\delta$ 7.39 (H-2') and 7.54 (H-6') showed cross-peaks with the carbons at  $\delta$ 163.6 (C-2) and 151.3 (C-4'). The presence of  $\text{C}_3\text{-OH}$  ( $\delta$ 9.80 in the  $^1\text{H}$  NMR spectrum) was also indicated by the presence of a cross-peak of the proton at  $\delta$ 7.39 (H-2') with the carbon at  $\delta$ 141.6 (C-3') in the  $^1\text{H}\text{-}^{13}\text{C}$  long range coupling obtained by HMBC. Thus, the structure of **1** is 5''-isopropenyl-dihydrofuran (2'',3'':7,8)-4'''-hydroxy-5'''-(1-hydroxy-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 anhydrocarotin for **2**, **4** and **5**, and desmethylanhydrocaritin for **3**. The IR, UV, mass,  $^1\text{H}$  and  $^{13}\text{C}$  NMR

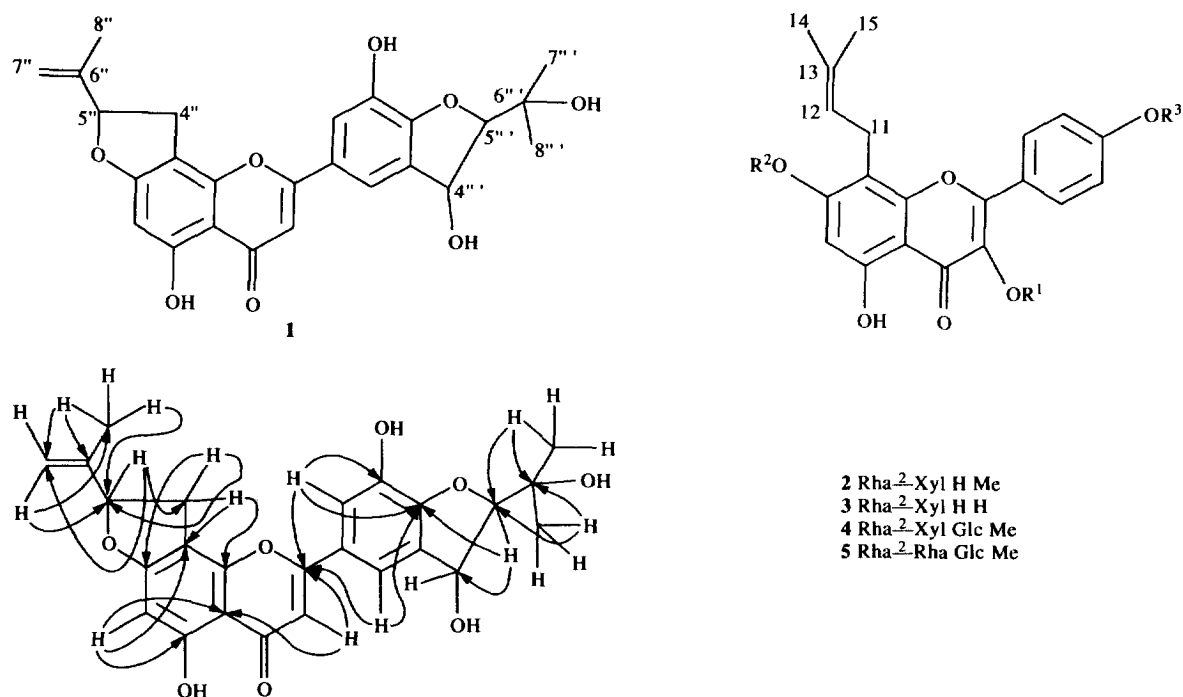


Fig. 1. The structure of 1-5 and the <sup>1</sup>H-<sup>13</sup>C long range coupling obtained by HMBC for 1.

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

#### EXPERIMENTAL

Mps: uncorr. <sup>1</sup>H and <sup>13</sup>C NMR spectra were measured with Varian VXR-300 for 1 and FX-100 for 2-5 in DMSO-d<sub>6</sub>; <sup>1</sup>H-<sup>13</sup>C COSY and <sup>1</sup>H-<sup>13</sup>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<sub>3</sub>-MeOH (in gradient) as the eluent to yield 15 subfrs. Subfr. 5 was rechromatographed over a polyamide column eluted with CHCl<sub>3</sub>-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°. C<sub>25</sub>H<sub>24</sub>O<sub>8</sub>. UV λ<sub>max</sub><sup>MeOH</sup> nm: 258, 274 (sh), 346, + NaOMe: 274, 310 (sh), 382, + AlCl<sub>3</sub>: 270, 302, 358, 390 (sh), + AlCl<sub>3</sub>-HCl: 270, 302, 350, 390 (sh), + NaOAc: 258, 274, 330, 350, + NaOAc-H<sub>3</sub>BO<sub>3</sub>: 258, 274, 330 (sh), 350. EIMS (m/z (rel. int.): 452 ([M]<sup>+</sup>, 35), 411 (18), 314 (12), 252 (40), 228 (15), 187 (65), 150 (10), 69 (100), 59 (10). <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): 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, <sup>1</sup>H and <sup>13</sup>C NMR spectral data agree with published results.

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