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THREE FLAVONOL GLYCOSIDES FROM *EPIMEDIUM KOREANUM*

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Key Word Index—*Epimedium koreanum*; Berberidaceae; flavonol glycosides; icariin; 2D NMR.

Abstract—Three new flavonol glycosides: 4′-methoxy-5-hydroxy-8-3,3-dimethylallylflavone 3-glycosyl(1→2)rhamnoside-7-glucoside, 3-xylosyl(1→2)rhamnoside-7-glucoside and 3-rhamnosyl(1→2)rhamnoside-7-glucoside and icariin were characterized from the aerial parts of *Epimedium koreanum*.

INTRODUCTION

Plants of the genus *Epimedium* (Berberidaceae) are well known as a tonic in Japan and China. It has been reported that *E. grandiflorum* Morr. contains several flavonol glycosides such as icariin [1–4] in the aerial parts and epimedoside A-E [5–8] in the underground parts, whereas *E. koreanum* Nakai contains icariin and epimedoside A [9] in the underground parts. Recently, we have investigated a number of *Epimedium* species [10, 11] and isolated three new flavonol glycosides together with icariin from the aerial parts of *E. koreanum*. This paper describes the structural investigation of these glycosides.

RESULTS AND DISCUSSION

Three new flavonol glycosides (**1**–**3**) and icariin were isolated from the aerial parts of *E. koreanum* by repeated preparative HPLC of the *n*-butanol fraction.

Compound **1** was obtained as an amorphous solid, $C_{39}H_{50}O_{20}$, FABMS *m/z*: 839 $[M+1]^+$. Upon acid hydrolysis, **1** gave the same aglycone as that of icariin, i.e. 4′-methoxy-3,5,7-trihydroxy-8-3,3-dimethylallylflavone (**4**), glucose and rhamnose. The FABMS of **1** showed peaks at *m/z* 839, 677, 531 and 369, which were ascribed respectively to $[M+1]^+$, $[M+1-162]^+$, $[M+1-162-146]^+$ and $[M+1-162\times2-146]^+$ ions, suggesting the presence of one rhamnose and two glucose moieties in the molecule. The UV spectrum of **1** (see Experimental) was similar to and gave the same shifts as that of icariin, indicating that the sugars were attached to the aglycone at C-3 and C-7. The FABMS and UV data showed that one glucose and one rhamnose were attached at C-3 and C-7, and another glucose was attached to one of these two sugars.

The unambiguous assignments of ^{1}H NMR (400 MHz in CD_3OD) and ^{13}C NMR (100 MHz in CD_3OD) signals of **1** were achieved on the basis of combinations of one-dimensional (1D) and two-dimensional (2D) NMR techniques, such as INEPT, ^{1}H–^{1}H COSY or ^{1}H–^{13}C COSY. They were especially useful in deciding the position of attachment of the terminal sugar. The results are presented in Table 1 (^{1}H NMR) and Table 2 (^{13}C NMR). Three signals derived from anomeric protons

Table 1. ^{1}H NMR spectral data for the flavonol glycosides, **1**–**3**

H	1	2	3
6	6.66 *s*	6.62 *s*	6.65 *s*
12	5.19 *t* (6.0)	5.17 *t* (6.0)	5.19 *t* (5.9)
14	1.64 *s*	1.64 *s*	1.64 *s*
15	1.72 *s*	1.72 *s*	1.72 *s*
2′,6′	7.86 *d* (9.0)	7.81 *d* (9.0)	7.82 *d* (9.0)
3′,5′	7.09 *d* (9.0)	7.05 *d* (9.0)	7.04 *d* (9.0)
OMe	3.90 *s*	3.88 *s*	3.88 *s*
Glc-1	5.07 *d* (7.3)	5.06 *d* (6.8)	5.08 *d* (7.3)
Rha-1	5.72 *d* (1.5)	5.43 *d* (2.0)	5.52 *d* (1.5)
Rha-2	4.31 *dd* (1.5, 3.4)	4.22 *dd* (2.0, 3.5)	4.30 *dd* (1.5, 3.2)
Rha-6	0.93 *d* (5.6)	0.99 *d* (5.6)	0.94 *d* (5.9)
Terminal	Glc-1	Xyl-1	Rha-1
	4.44 *d* (7.8)	4.31 *d* (7.6)	5.03 *d* (1.5)
			Rha-6
			1.22 *d* (6.1)

400 MHz, CD_3OD, TMS as int. standard, *J* (Hz) in parentheses. Glc = glucose, Rha = rhamnose, Xyl = xylose.

Table 2. ^{13}C NMR spectral data for the flavonol glycosides, 1–3

C	1	2	3
2	155.89	155.72	155.71
3	137.56	137.74	137.33
4	180.87	180.83	180.68
5	162.95	162.75	162.75
6	100.40	100.33	100.30
7	161.74	161.66	161.63
8	108.49	108.39	108.37
9	160.22	159.81	159.82
10	111.59	114.45	112.76
11	23.57	23.56	23.54
12	124.35	124.43	124.35
13	133.50	133.40	133.41
14	26.59	26.58	26.58
15	19.08	19.10	19.10
1′	124.72	124.60	124.55
2′,6′	132.73	132.56	132.55
3′,5′	116.08	115.97	115.97
4′	164.39	164.22	164.19
OMe	56.90	56.89	56.87
Glucose			
1	102.89	102.83	102.82
2	75.78	75.72	75.69
3	79.15	79.03	78.90
4	72.11	72.05	72.02
5	79.15	79.03	78.90
6	63.32	63.28	63.26
Rhamnose			
1	103.45	103.86	103.12
2	83.22	83.13	79.62
3	72.78	72.75	72.79
4	74.34	74.43	74.28
5	72.69	72.60	72.72
6	18.41	18.43	18.53
Terminal	Glucose	Xylose	Rhamnose
1	107.77	108.29	104.35
2	76.21	75.98	72.72
3	78.77	78.56	73.08
4	71.96	71.73	74.74
5	78.77	67.80	71.05
6	63.32		18.61

100 MHz, CD_3OD, TMS as int. standard.

The chemical shifts were assigned on the basis of ^1H–^{13}C COSY spectra.

were observed in the ^1H NMR spectrum of **1** (Table 1). The signal at 5.07 ppm was assigned to the H-1 glucose attached to the aglycone and the diaxial coupling (J = 7.3 Hz) between the H-1 glucose and H-2 glucose indicated the β-configuration. Similarly, the signals at 4.44 ppm (J = 7.8 Hz) and 5.72 ppm (J = 1.5 Hz) were assigned to the H-1 terminal glucose (β-configuration) and H-1 rhamnose (α-configuration), respectively. Furthermore, the 2D NOESY spectrum of **1** showed the NOE between the H-1 of the glucose attached to the aglycone (5.07 ppm) and the H-6 of the aglycone (6.66 ppm), which indicated that one glucose and one rhamnose were attached to the aglycone at C-7 and C-3, respectively. The ^{13}C NMR spectrum of **1** (Table 2) gave a downfield shift (83.22 ppm) of the C-2 rhamnose from the corresponding chemical shift value [12], suggesting that the terminal glucose was attached at C-2 of the rhamnose. On the basis of these data, **1** was identified as 4′-methoxy-5-hydroxy-8-3,3-dimethylallylflavone 3-*O*-β-D-glucopyranosyl (1→2)-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside.

Compound **2** was obtained as an amorphous solid, $C_{38}H_{48}O_{19}$, FABMS *m/z*: 809 $[M+1]^+$. Acid hydrolysis of **2** gave the same aglycone (**4**) as in **1**, glucose, rhamnose and xylose. The FABMS and UV data of **2** showed that one glucose and one rhamnose were attached at C-3 and C-7, respectively, and one xylose was attached to either of these two sugars. The ^1H NMR spectrum of **2** (Table 1) gave three signals from anomeric protons at 4.31 ppm (J = 7.6 Hz), 5.06 ppm (J = 6.8 Hz) and 5.43 ppm (J = 2.0 Hz), which were assigned to H-1 xylose (β-configuration), H-1 glucose (β-configuration) and H-1 rhamnose (α-configuration), respectively. The NOE between H-1 of the glucose and H-6 of the aglycone indicated that the glucose was attached to the aglycone at C-7. The ^{13}C NMR spectrum of **2** (Table 2) showed a downfield shift (83.13 ppm) of the C-2 rhamnose, suggesting that xylose was attached at C-2 of the rhamnose. Compound **2** is therefore identified as 4′-methoxy-5-hydroxy-8-3,3-dimethylallylflavone 3-*O*-β-D-xylopyranosyl (1→2)-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside.

Compound **3** was obtained as an amorphous solid, $C_{39}H_{50}O_{19}$, FABMS *m/z*: 823 $[M+1]^+$. Acid hydrolysis of **3** gave the aglycone **4**, glucose and rhamnose. The FABMS and UV data of **3** showed that one glucose and one rhamnose were attached at C-3 and C-7, respectively and another rhamnose was attached to one of these two sugars. The ^1H NMR spectrum of **3** (Table 1) gave three signals from anomeric protons at 5.03 ppm (J = 1.5 Hz), 5.08 ppm (J = 7.1 Hz) and 5.52 ppm (J = 1.5 Hz), which were assigned to the H-1 terminal rhamnose (α-configuration), H-1 glucose (β-configuration) and H-1 rhamnose (α-configuration), respectively. The NOE between H-1 of the glucose and H-6 of the aglycone indicated that the glucose was attached to the aglycone at C-7. The ^{13}C NMR spectrum of **3** (Table 2) showed a downfield shift (79.62 ppm) of the C-2 rhamnose attached to the aglycone, suggesting that the terminal rhamnose was attached at C-2 of the first rhamnose.

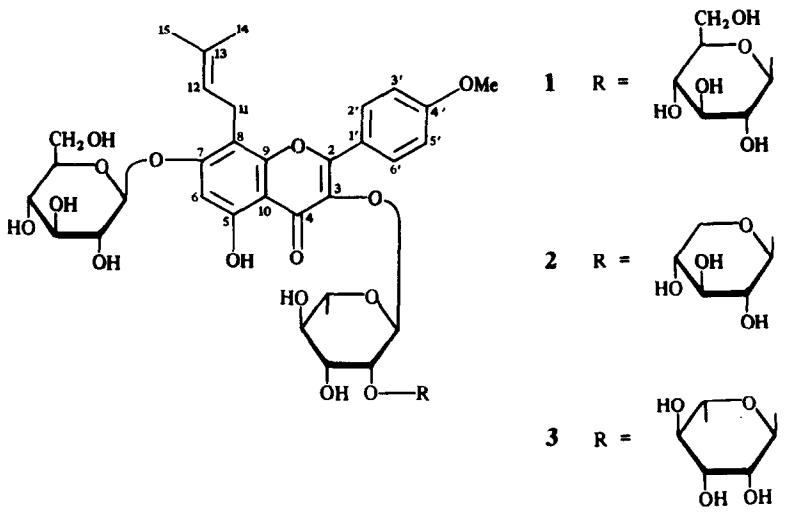
From these data, **3** is characterized as 4′-methoxy-5-hydroxy-8-3,3-dimethylallylflavone 3-*O*-α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside.

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR spectra were measured with JEOL JNM-GX400 in CD_3OD and the chemical shifts given in δ values (ppm) with TMS as the int. standard. Prep. HPLC was carried out on a CIG column system (Kusano Scientific Co., Tokyo) with ODS-silica (30 μm) as the stationary phase.

Isolation. The dried aerial parts of *E. koreanum* (320 g), purchased from Nippon Funmatsu Yakuhin Co., Ltd, were extracted with H_2O–EtOH (7:3, 3 l) × 2. The concd extract (1 l) was extracted successively with *n*-hexane, $CHCl_3$ and *n*-BuOH. The *n*-BuOH fraction (24 g) was subjected to prep. HPLC on ODS-silica with H_2O–MeCN (7:3). Repeated HPLC afforded **1** (30 mg), **2** (50 mg), **3** (100 mg) and icariin (200 mg).

Compound **1**. An amorphous solid, mp 180–182° (dec.). $[\alpha]_D^{20}$ −106.3° (EtOH; c = 0.25). FABMS *m/z* (%): 839 (M + 1, 1), 677 (1), 531 (35), 369 (100), 313 (55). Calc. for $C_{39}H_{50}O_{20} \cdot H_2O$: C, 54.67; H, 6.12. Found: C, 54.62; H, 6.30%. UV λ_{max}^{EtOH} nm (log ε):



Icariin R = H

270 (4.41), 314 (4.16); +$AlCl_3$:276 (4.33), 304 (4.15), 336 (4.13); +NaOAc:270 (4.39), 316 (4.14).

Compound **2**. An amorphous solid, mp 172–174° (dec.). $[\alpha]_D^{20}$ −108.5° (EtOH; c 0.47). FABMS *m/z* (%): 809 (M + 1, 5), 677 (2), 531 (45), 369 (100), 313 (57). Calc. for $C_{38}H_{48}O_{19} \cdot 3/2H_2O$: C, 54.61; H, 6.15. Found: C, 54.75; H, 6.12%. UV λ_{max}^{EtOH} nm (log ε): 270 (4.38), 314 (4.13); +$AlCl_3$:280 (4.33), 305 (4.15), 339 (4.17); +NaOAc:269 (4.36), 315 (4.11).

Compound **3**. An amorphous solid, mp 162–164° (dec.). $[\alpha]_D^{20}$ −104.7° (EtOH; c 0.69). FABMS *m/z* (%):823 (M + 1, 8), 677 (7), 531 (50), 369 (100), 313 (45). Calc. for $C_{39}H_{50}O_{19} \cdot H_2O$: C, 55.71; H, 6.23. Found: C, 55.70; H, 6.45%. UV λ_{max}^{EtOH} nm (log ε): 269 (4.36), 313 (4.10); +$AlCl_3$: 280 (4.32), 305 (4.01), 340 (4.17); +NaOAc: 269 (4.33), 315 (4.08).

Icariin. Yellow needles, mp 255–257°. FABMS *m/z* (%): 677 (M + 1, 15), 531 (45), 369 (100), 313 (45). UV λ_{max}^{EtOH} nm (log ε): 269 (4.30), 313 (4.04); +$AlCl_3$: 280 (4.27), 304 (4.06), 339 (4.13); +NaOAc: 269 (4.28), 315 (4.01). Icariin was identified by direct comparison with an authentic specimen (HPLC, UV, IR).

Hydrolysis of flavonoids. The glycosides in 25% H_2SO_4–MeOH were refluxed for 4 hr. The concd solns were diluted with H_2O, extracted with $CHCl_3$, neutralized with $Ba(OH)_2$ and evapd.

Identification of sugars and aglycones. The sugar mixture in the H_2O fractions of **1**–**3** were silylated in the usual way with TMCS and HMDS in C_5H_5N and subjected to GLC (2% OV-1; column temp 150–250°, 10°/min.; detection temp. 300°; N_2, 50 ml/min.) along with silyl derivatives of standard sugars (R_t: 3.3, 3.8 min for rhamnose; 3.9, 4.4 min for xylose; 5.5, 6.3 min for glucose). Aglycones in the $CHCl_3$ fractions were subjected to TLC (Merck, silica gel 60 F_{254}, Art. 5715; *n*-hexane–EtOAc, 1:1) all having the same R_f of 0.50 and identified as **4**, 4′-metoxy-3,5,7-trihydroxy-8-3,3-dimethylallylflavone, by comparison with an authentic sample.

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