8-(3,3-Dimethylallyl)-Substituted Flavonoid Glycosides from the Aerial Parts of *Epimedium koreanum*

by **Hai-Yu Zhao**, **Li Fan**, **Lei Zhou**, **Jian Han**, **Bao-Rong Wang**, and **De-An Guo***

The State Key Laboratory of Natural and Biomimetic Drug, School of Pharmaceutical Sciences, Peking University, Beijing 100083, P. R. China
(phone/fax: +86-10-82802700; e-mail: gda5958@163.com)

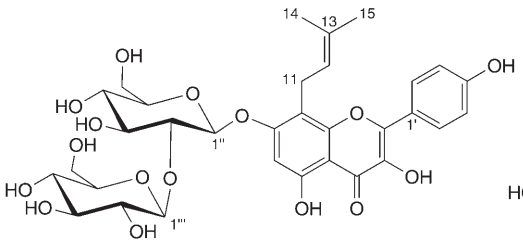
Ten 8-(3,3-dimethylallyl)-substituted flavonoid glycosides, including the four new flavonol glycosides **1** and **3**–**5** and the new flavanonol glycoside **2**, besides five known flavonol glycosides, were isolated from the aerial parts of *Epimedium koreanum* NAKAI. Their structures were determined by spectroscopic methods, including UV, IR, 1D- and 2D-NMR, ESI-MS^n, HR-ESI-MS, and circular dichroism (CD) experiments.

Introduction. – The aerial parts of *Epimedium koreanum* NAKAI have been used in China for over 2000 years for various medicinal treatments, in particular as tonic, antirheumatic, and aphrodisiac [1]. Earlier chemical investigation on *E. koreanum* afforded a series of characteristic 8-(3,3-dimethylallyl)-substituted flavonoids, which have been reported to possess multiple biological activities such as androgenic, antioxidant and antidepressant-like actions [2–5]. Our investigation aimed at finding new active chemical constituents from this plant led to the isolation of ten compounds, including the four new flavonol glycosides **1** and **3**–**5**, the new flavanonol glycoside **2**, and five known flavonol glycosides[1]) (flavonol = 3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one, flavanonol = 2,3-dihydro-3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one).

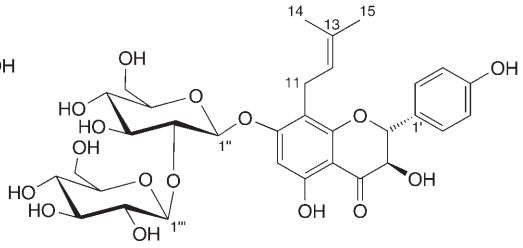
Results and Discussion. – Compound **1** was obtained as a yellow amorphous solid. The molecular formula of **1** was determined as $C_{32}H_{38}O_{16}$ based on the HR-ESI-MS ($[M+H]^+$ at *m/z* 679.2225). The UV absorption maxima (250, 267, and 373 nm) suggested its flavonol skeleton. Detailed analysis of the ^{1}H-NMR chemical shifts and coupling constants of the protons of the sugar moieties indicated them to be β-glucopyranose residues [6], which was also supported by acidic hydrolysis and subsequent TLC analysis of the sugars. The structure of **1** was established as 8-(3,3-dimethylallyl)-4′,5,7-trihydroxyflavonol 7-[*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside][1]).

The ^{1}H-NMR spectrum of **1** (*Table 1*) showed an aromatic OH group corresponding to OH–C(5) at δ(H) 12.46 (*s*). The presence of four aromatic protons forming an *AA′BB′* system at δ(H) 8.03 (*d*, J = 8.5 Hz) and 6.93 (*d*, J = 8.5 Hz) along with a *s* at δ(H) 6.57 (*s*) resembled those of a 5,7,4′-trioxygenated flavonol. Additionally, signals at δ(H) 5.17 (*t*, J = 6.0), 3.36–3.40 (*m*), and 3.48–3.55 (*m*) as well as

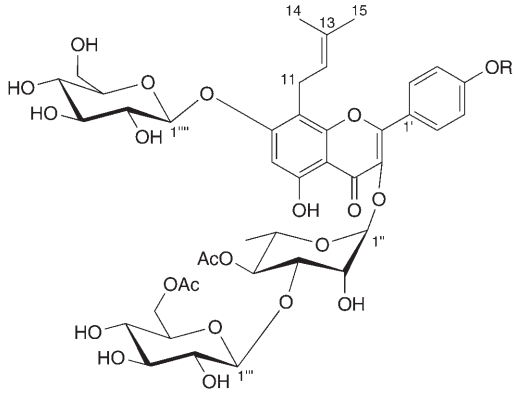
[1]) Arbitrary atom numbering of **1**–**5**; for systematic names, see *Exper. Part.*



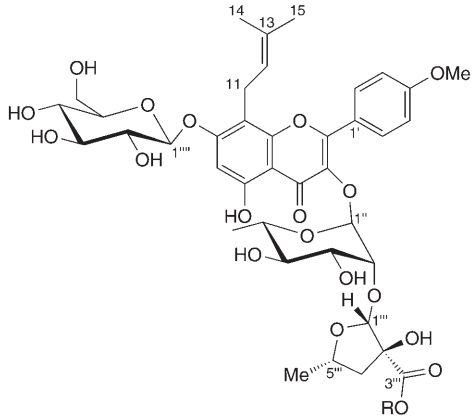
1[1])



2[1])



3 R = H[1])
6 R = Me[1])



4 R = Et[1])
5 R = H[1])

those of two Me groups at δ(H) 1.61 and 1.75 (2*s*) revealed the presence of a 3,3-dimethylallyl (= 3-methylbut-2-en-1-yl) group. Two sugar moieties were observed with anomeric protons appearing at δ(H) 5.20 (*d*, J = 6.5 Hz, H–C(1″)) and δ(H) 4.56 (*d*, J = 7.5 Hz, H–C(1‴)). The resonances of the protons were assigned with the help of the HMQC experiment. The ^{13}C-NMR spectrum of **1** (*Table 1*) confirmed the presence of a 3,3-dimethylallyl group (δ(C) 21.3, 122.8, 130.6, 25.4, and 17.9). The signal of C(8) at δ(C) 108.1 was shifted downfield by 14.6 ppm compared to kaempferol (= 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) which suggested the 3,3-dimethylallyl group to be attached at C(8) [7]. This was further supported by the HMBC correlation of C(8) with H–C(12) (δ(H) 5.17 (*t*, J = 6.0)). In the sugar parts, the downfield shift of C(2″) at δ(C) 81.1 together with the upfield shift of C(1‴) at δ(C) 103.9 established the attachment of the terminal glucose unit to C(2″). Finally, the HMBC plot showed a cross-peak H–C(1″) (δ(H) 5.20)/C(7) (δ(C) 159.5) which indicated that the [*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]oxy residue was attached to C(7) (*Fig.*).

Compound **2** was isolated as a light yellow amorphous solid. The molecular formula $C_{32}H_{40}O_{16}$ was established by HR-ESI-MS ([M + Na]$^+$ at m/z 703.2207). The UV spectrum of **2** showed absorptions at 289 and 344 (sh) nm, suggesting the presence of a flavanonol derivative. Some changes in the NMR spectra (*Table 1*) revealed that **2** was the corresponding flavanonol of **1**. This was also supported by a comparison of the molecular formula of **2** with that of **1**. Compound **2** was shown to have the (2*R*,3*R*)-

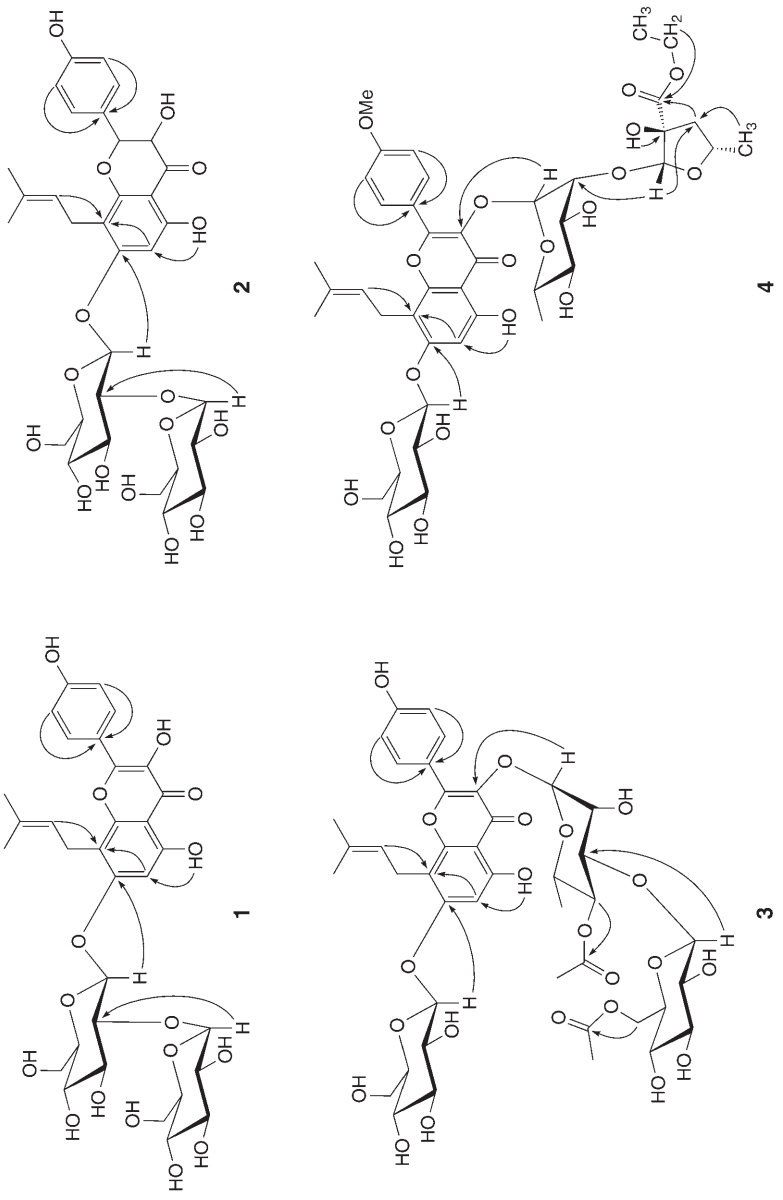


Figure. *Key HMBC correlations* (H → C) *for* **1**–**4**

Table 1. *^{13}C- and ^{1}H-NMR Data* (500 (^{1}H) and 125 MHz (^{13}C), (D_6)DMSO) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1[1])		**2**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2) or H–C(2)		147.5	5.05 (d, J = 11.5)	82.8
C(3) or H–C(3)		135.7	4.56 (d, J = 11.5)	71.9
C(4)		176.3		199.1
C(5)	12.46 (s, OH)	159.2	11.83 (s, OH)	160.9
H–C(6)	6.57 (s)	97.0	6.29 (s)	95.1
C(7)		159.5		162.6
C(8)		108.1		109.1
C(9)		152.7		158.7
C(10)		104.4		101.8
CH_2(11)	3.36–3.40, 3.48–3.55 (2m)	21.3	3.03–3.09, 3.28–3.33 (2m)	21.3
H–C(12)	5.17 (t, J = 6.0)	122.8	5.10 (t, J = 6.0)	122.7
C(13)		130.6	1.50 (s)	130.0
Me(14)	1.61 (s)	25.4	1.58 (s)	25.6
Me(15)	1.75 (s)	17.9		17.6
C(1′)		121.8		127.7
H–C(2′)	8.03 (d, J = 8.5)	129.4	7.32 (d, J = 8.5)	129.2
H–C(3′)	6.93 (d, J = 8.5)	115.4	6.79 (d, J = 8.5)	114.8
C(4′)		158.4		157.6
H–C(5′)	6.93 (d, J = 8.5)	115.4	6.79 (d, J = 8.5)	114.8
H–C(6′)	8.03 (d, J = 8.5)	129.4	7.32 (d, J = 8.5)	129.2
Glc (inner)				
H–C(1″)	5.20 (d, J = 6.5)	98.2	5.11 (d, J = 6.5)	98.0
H–C(2″)	3.63–3.66 (m)	81.1	3.59–3.62 (m)	81.3
H–C(3″)	3.12–3.14 (m)	76.2	3.09–3.15 (m)	76.2
H–C(4″)	3.23–3.40 (m)	69.3	3.22–3.25 (m)	69.2
H–C(5″)	3.47–3.55 (m)	76.8	3.49–3.56 (m)	76.9
CH_2(6″)	3.48–3.55, 3.66–3.70 (2m)	60.6	3.43–3.47, 3.66–3.70 (2m)	60.4
Glc (terminal)				
H–C(1‴)	4.56 (d, J = 7.5)	103.9	4.53 (d, J = 8.0)	104.1
H–C(2‴)	3.23–3.40 (m)	74.6	3.22–3.25 (m)	74.7
H–C(3‴)	3.59–3.66 (m)	76.2	3.49–3.56 (m)	76.3
H–C(4‴)	3.23–3.40 (m)	69.6	3.29–3.35 (m)	69.4
H–C(5‴)	3.07–3.10 (m)	76.9	2.97–3.05 (m)	77.0
CH_2(6‴)	3.55–3.60, 3.66–3.70 (2m)	60.5	3.43–3.47, 3.66–3.70 (2m)	60.3

configuration, based on the positive *Cotton* effects at 241 and 338 nm in the CD spectrum of **2** [8]. Compound **2** was identified as (2*R*,3*R*)-8-(3,3-dimethylallyl)-2,3-dihydro-4′,5,7-trihydroxyflavonol 7-[*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside][1]).

The ^{1}H-NMR spectrum of **2** was very similar to that of **1**, except for two additional signals at δ(H) 5.05 (d, J = 11.5 Hz) and 4.56 (d, J = 11.5 Hz). In the ^{13}C-NMR spectrum, compared to **1**, the signals at δ(C) 176.3, 147.5, and 135.7 were replaced by signals at δ(C) 199.1, 82.8, and 71.9. The location of the sugar chain and the inter-glycoside linkage were established from the HMBC cross-peaks H–C(1″) (δ(H) 5.11)/C(7) (δ(C) 162.6) and H–C(1‴) (δ(H) 4.53)/C(2″) (δ(C) 81.3) (*Fig*).

Compound **3**, a yellow amorphous solid, had the molecular formula $C_{42}H_{52}O_{22}$, as deduced from the HR-ESI-MS ($[M + Na]^+$ at m/z 931.2857). The IR spectra showed absorption bands for OH groups (3386 cm^{-1}), a flavonol carbonyl group (1739 cm^{-1}), and ester groups (1650 and 1597 cm^{-1}). A comparison of the ^{1}H- and ^{13}C-NMR data of **3** (*Table 2*) with those of the known epimedokoreanoside I (**6**) suggested that the structures of both compounds were similar but that a MeO group was missing in **3**. The structure of compound **3** was elucidated as 8-(3,3-dimethylallyl)-4′,5,7-trihydroxyflavonol 3-[*O*-6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-4-*O*-acetyl-α-L-rhamnopyranoside] 7-(β-D-glucopyranoside)[1]).

The ^{1}H-NMR spectrum of **3** confirmed the presence of a 3,3-dimethylallyl and two acetyl groups, and of one rhamnose and two glucose moieties. The ^{13}C-NMR spectral data of **3** clearly showed the characteristic signals of a 3,3-dimethylallyl, one rhamnose, and two glucose moieties, and of two acetyl groups at δ(C) 170.3, 169.7, 20.6, and 20.7. The positions of the three sugar units and two acetyl groups were confirmed by the HMBC experiment. Thus, the correlation H–C(1‴) (δ(H) 5.00)/C(7) (δ(C) 160.5) indicated that a glucose unit was attached to C(7) (*Fig.*). The correlation C(3) (δ(C) 133.2)/H–C(1″) (δ(H) 5.42) and the cross-peak H–C(1‴) (δ(H) 4.28)/C(3″) (δ(C) 77.2) suggested the attachment of the rhamnose unit at C(3) and of the other glucose unit at C(3″) of the rhamnose unit. The HMBC plot also revealed that the sugar proton H–C(4″) (δ(H) 4.83 (*t*, J = 10.0)) was correlated with δ(C) 170.3, and CH_2(6‴) (δ(H) 4.11–4.16 and 4.25–4.32 (2*m*)) with δ(C) 169.7, indicating the linkage of

Table 2. *^{13}C- and ^{1}H-NMR Data* (500 MHz (^{1}H) and 125 MHz (^{13}C), (D_6)DMSO) *of* **3**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
C(2)		157.7	3-*O*-Rha		
C(3)		133.2	H–C(1″)	5.42 (br. *s*)	100.8
C(4)		178.0	H–C(2″)	3.15–3.20 (*m*)	69.7
C(5)	12.55 (*s*, OH)	159.0	H–C(3″)	3.40–3.50 (*m*)	77.2
H–C(6)	6.62 (*s*)	98.2	H–C(4″)	4.83 (*t*, J = 10.0)	71.2
C(7)		160.5	H–C(5″)	3.29–3.33 (*m*)	68.4
C(8)		108.3	Me(6″)	0.72 (*d*, J = 6.0)	17.0
C(9)		153.0	*Me*COO–C(4″)	1.95 (*s*)	170.3, 20.6
C(10)		105.4	Glc at Rha		
CH_2(11)	3.35–3.40,	21.4	H–C(1‴)	4.28 (*d*, J = 6.5)	104.9
	3.52–3.58 (2*m*)		H–C(2‴)	2.97–3.03 (*m*)	72.9
H–C(12)	5.17 (*t*, J = 6.0)	122.2	H–C(3‴)	3.74–3.87 (*m*)	76.6
C(13)		131.1	H–C(4‴)	3.15–3.20 (*m*)	69.7
Me(14)	1.68 (*s*)	25.4	H–C(5‴)	3.48–3.57 (*m*)	73.6
Me(15)	1.59 (*s*)	17.8	CH_2(6‴)	4.11–4.16,	63.9
C(1′)		120.0		4.25–4.32 (2*m*)	
H–C(2′)	7.79 (*d*, J = 8.0)	130.6	*Me*COO–C(6‴)	1.97 (*s*)	169.7, 20.7
H–C(3′)	6.93 (*d*, J = 8.0)	115.7	7-*O*-Glc		
C(4′)		161.3	H–C(1⁗)	5.00 (*d*, J = 6.5)	100.6
H–C(5′)	7.79 (*d*, J = 8.0)	115.7	H–C(2⁗)	3.35–3.49 (*m*)	73.3
H–C(6′)	6.93 (*d*, J = 8.0)	130.6	H–C(3⁗)	3.29–3.33 (*m*)	76.6
			H–C(4⁗)	3.15–3.20 (*m*)	70.3
			H–C(5⁗)	3.42–3.48 (*m*)	76.6
			CH_2(6⁗)	3.40–3.46,	60.6
				3.68–3.75 (2*m*)	

the two acetyl groups with OC(4″) and OC(6‴) [9] (*Fig.*). Additionally, this was also supported by the ESI-MS^3 experiment. The ESI-MS of **3** gave an $[M-H]^-$ ion at *m/z* 907. Its fragmentation was triggered by initial loss of the glucose moiety to yield a prominent ion at *m/z* 745. In the MS^3 of *m/z* 745, losses of an acetyl group, Glc(OAc), and both Glc(OAc) and Rha(OAc) were observed with the formation of ions at *m/z* 703 (*i.e.*, 745 – 42), 541 (*i.e.*, 745 – 204), 353 (*i.e.*, 745 – 204 – 188), respectively.

Compound **4** was isolated as yellow amorphous solid. The molecular formula $C_{41}H_{52}O_{19}$ was deduced from HR-ESI-MS ($[M+Na]^+$ at *m/z* 871.2967). The UV absorption maxima (269, 315, and 354 nm) indicated the presence of a flavonol skeleton. The ^{1}H- and ^{13}C-NMR data of **4** (*Table 3*) supported the identification of the aglycone as anhydroicaritin (= 8-(3,3-dimethylallyl)kaempferol 4′-methyl ether). Additionally, the presence of a rhamnose, a glucose, and a dideoxyfuranose subunit was detected. The spectral data of the dideoxyfuranose subunit were in agreement with those observed in calactinic acid methyl ester and further cardenolides obtained recently from *Asclepias curassavica* and *Calotropis gigantean* [10][11]. Compound **4** was assigned as 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-3,5-dideoxy-2-*C*-(ethoxycarbonyl)-β-D-*erythro*-pentofuranosyl-(1 → 2)-α-L-rhamnopyranoside] 7-(β-D-glucopyranoside)[1]).

The glucose and rhamnose units of **4** were placed at C(7) and C(3), respectively, on the basis of the HMBC cross-peaks δ(H) 4.99/δ(C) 160.5 and δ(H) 5.36/δ(C) 134.4. The dideoxyfuranosyl subunit was revealed by the ^{1}H,^{1}H-COSY cross-peaks H–C(5‴) (δ(H) 4.22–4.28)/CH_2(4‴) (δ(H) 1.93–1.97) and Me(6‴) (δ(H) 1.12). It was also supported by the H,C long-range correlations H–C(1‴) (δ(H) 5.13)/C(2‴) (δ(C) 83.6) and C(4‴) (δ(C) 40.3). Connectivity of the Et group to C(3‴) and of the OH group to C(2‴) were established from the long-range HMBC correlations MeCH_2O–C(3‴) (δ(H) 4.04–4.09)/C(3‴) (δ(C) 170.4) and OH–C(2‴) (δ(H) 5.90)/C(2‴) (δ(C) 83.6). The HMBC cross-peak H–C(1‴) (δ(H) 5.13)/δ(C) 74.8 which was ascribed to C(2″) of the rhamnose unit based on ^{1}H,^{1}H-COSY and HMBC experiments, indicated that the dideoxyfuranose unit was attached to C(2″) of the rhamnose residue.

Compound **5** was also obtained as a yellow amorphous solid. It was assigned the molecular formula $C_{39}H_{48}O_{19}$ deduced from HR-ESI-MS ($[M-H]^-$ at *m/z* 819.2711). Its flavonol skeleton was detected from the UV absorption maxima (270, 314, and 359 nm). The ^{1}H- and ^{13}C-NMR data of **5** (*Table 3*) were very similar to **4**, except for the absence of the Et group. The similarity of the NMR data and the molecular formula suggested that **5** was a deethylated derivative of **4**. This was further supported by ^{1}H,^{1}H-COSY and HMBC experiments, as well as by the ESI-MS^3 fragmentation behavior of **5**. The quasimolecular $[M-H]^-$ ion at *m/z* 819 yielded a $[(M-Glc)-H]^-$ ion at *m/z* 657. The MS^3 fragmentation from this ion gave daughter ions at *m/z* 513 $[(M-Glc-144)-H]^-$ and 367 $[(M-Glc-144-Rha)-H]^-$. Thus, **5** was elucidated as 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-2-*C*-carboxy-3,5-dideoxy-β-D-*erythro*-pentofuranosyl-(1 → 2)-α-L-rhamnopyranoside] 7-(β-D-glucopyranoside)[1]).

The remaining five flavonol glycosides were identified as 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3,7-di(β-D-glucopyranoside) [12], korepimedoside A (= 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-4-*O*-acetyl-α-L-rhamnoside]) [13], korepimedoside C (= 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-β-D-glucopyranosyl-(1 → 3)-4-

Table 3. *^{13}C- and ^{1}H-NMR Data* (500 (^{1}H) and 125 MHz (^{13}C), (D_6)DMSO) *of* **4** *and* **5**. δ in ppm, J in Hz.

Position	**4**[1])		**5**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)		157.2		157.3
C(3)		134.4		134.4
C(4)		178.1		178.3
C(5)	12.60 (*s*, OH)	159.0	12.61 (*s*, OH)	159.2
H–C(6)	6.62 (*s*)	98.1	6.63 (*s*)	98.1
C(7)		160.5		160.6
C(8)		108.3		108.4
C(9)		152.9		153.0
C(10)		105.5		105.5
CH_2(11)	3.38–3.41, 3.54–3.58 (2*m*)	21.3	3.39–3.42, 3.52–3.56 (2*m*)	21.5
H–C(12)	5.15 (*t*, J = 6.0)	122.0	5.17 (*t*, J = 6.0)	122.2
C(13)		131.0		131.2
Me(14)	1.59 (*s*)	25.3	1.59 (*s*)	25.5
Me(15)	1.67 (*s*)	17.7	1.68 (*s*)	17.9
MeO	3.84 (*s*)	55.4	3.84 (*s*)	55.5
C(1′)		122.0		122.2
H–C(2′)	7.88 (*d*, J = 8.5)	130.4	7.89 (*d*, J = 8.5)	130.5
H–C(3′)	7.12 (*d*, J = 8.5)	114.0	7.13 (*d*, J = 8.5)	114.2
C(4′)		161.4		161.5
H–C(5′)	7.12 (*d*, J = 8.5)	114.0	7.13 (*d*, J = 8.5)	114.2
H–C(6′)	7.88 (*d*, J = 8.5)	130.4	7.89 (*d*, J = 8.5)	130.5
3-*O*-Rha				
H–C(1″)	5.36 (br. *s*)	100.6	5.45 (*s*)	100.6
H–C(2″)	4.16 (br. *s*)	74.8	4.16 (br. *s*)	75.1
H–C(3″)	3.08–3.12 (*m*)	70.5	3.08–3.12 (*m*)	70.6
H–C(4″)	2.95–2.99 (*m*)	71.3	2.95–2.99 (*m*)	71.6
H–C(5″)	3.69–3.72 (*m*)	70.1	3.69–3.72 (*m*)	70.4
Me(6″)	0.78 (*d*, J = 6.0)	17.4	0.78 (*d*, J = 6.0)	17.5
Furanose				
H–C(1‴)	5.13 (*s*)	107.2	5.07 (*s*)	107.5
C(2‴)	5.90 (*s*, OH)	83.6		83.6
C(3‴)=O		170.4		172.4
CH_2(4‴)	1.93–1.97 (*m*)	40.3	1.92–1.96 (*m*)	40.3
H–C(5‴)	4.22–4.28 (*m*)	74.8	4.15–4.24 (*m*)	75.1
Me(6‴)	1.12 (*d*, J = 6.0)	22.4	1.11 (*d*, J = 6.0)	22.6
Me*CH_2*O–C(3‴)	4.04–4.09 (*m*)	60.3		
MeCH_2O–C(3‴)	1.19 (*d*, J = 7.5)	14.0		
7-*O*-Glc				
H–C(1⁗)	4.99 (*d*, J = 7.0)	100.5	5.01 (*d*, J = 7.0)	100.5
H–C(2⁗)	3.40–3.49(*m*)	73.3	3.40–3.49 (*m*)	73.4
H–C(3⁗)	3.32–3.33 (*m*)	76.5	3.32–3.33 (*m*)	76.6
H–C(4⁗)	3.14–3.16 (*m*)	69.6	3.10–3.19 (*m*)	69.7
H–C(5⁗)	3.40–3.49 (*m*)	77.1	3.40–3.49 (*m*)	77.2
CH_2(6⁗)	3.41–3.44, 3.69–3.72 (2*m*)	60.6	3.40–3.44, 3.70–3.72 (2*m*)	60.7

O-acetyl-α-L-rhamnoside] 7-(β-D-glucopyranoside)) [14], epimedokoreanoside I (= 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-4-*O*-acetyl-α-L-rhamnoside] 7-(β-D-glucopyranoside)) (**6**) [15], and caohuoside B (= 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-5,6-*O*-diacetyl-β-D-glucopyranosyl-(1 → 3)-4-*O*-acetyl-α-L-rhamnoside] 7-(β-D-glucopyranoside)) [16], respectively. Their properties and spectral data were almost identical to those reported earlier. 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3,7-di(β-D-glucopyranoside) was isolated from a naturally growing plant for the first time; it had been previously reported in the cell suspension cultures of *Vancouveria hexandra* [12]. The last four of these known flavonol glucosides have been already found in *E. koreanum* before [13–16].

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Experimental Part

General. Column chromatography (CC): macroporous resin (*AB-8*; *Tianjin Nankai Chemical Factory*, P. R. China), polyamide (60–90 mesh; *Zhejiang Taizhou Chemical Factory*, P. R. China), *Sephadex LH-20* (*Pharmacia*), *C-18* reversed-phase silica gel (50 μm; *Merck*). HP-TLC: silica gel plate (*Merck*). Prep. HPLC: *Spectra-P-100* pump (*Thermo Separation Products*), connected to a *Spectra-UV-100* detector (*Thermo Separation Products*), *Zorbax-SB-ODS* column (20 mm i.d. × 150 mm, 5 μm; *Agilent*), flow rate 2.0 ml/min; wavelength detection at 270 nm. M.p.: *X-6* melting-point apparatus (*Beijing TECH Instrument Co., Ltd.*); uncorrected. Optical rotation: *AA10R* digital polarimeter; in MeOH at 25°. UV: *TU-1901-UV-VIS* spectrophotometer. CD Spectra: *Jasco J-810* spectropolarimeter. IR: *Nicolet Avatar-FT-IR* spectrometer; KBr pellets. NMR: *Bruker ARX-400* and *DRX-500* spectrometers; in (D_6)DMSO. ESI-MS^n: *Finnigan LCQ-Advantage* ion-trap mass spectrometer (*Thermo Finnigan*, San Jose, CA, USA). HR-ESI-MS: *Bruker APEX IV FT-MS.*

Plant Material. The aerial parts of *Epimedium koreanum* NAKAI (Berberidaceae) were bought from *Sinopharm Company*, Beijing, China, and identified by professor *De-An Guo*, School of Pharmaceutical Sciences, Peking University. The voucher specimen *yyh101* was deposited in the School of Pharmaceutical Sciences, Peking University, Beijing, P. R. China.

Extraction and Isolation. The powdered aerial parts (5 kg) of *E. koreanum* NAKAI (Berberidaceae) was refluxed with 70% EtOH (100 l) 3 times for 2 h. The extract was concentrated and the residue suspended in H_2O (5 l) and partitioned successively with petroleum ether (5 × 5 l), AcOEt (5 × 5 l), and BuOH (5 × 5 l). The AcOEt fraction (70 g) was then subjected to CC (polyamide, $CHCl_3$/MeOH 60:1 → 1:1): *Fractions 1–6*. *Fr. 4* was applied to CC (polyamide, $CHCl_3$/MeOH 25:1) and open CC (*ODS*, 40–80% aq. MeOH): pure korepimedoside A (20 mg), and a crude mixture which was further purified by prep. HPLC (MeCN/H_2O 40:60) to yield **1** (15 mg), **2** (8 mg), and **3** (16 mg). *Fr. 5* was applied to CC (polyamide, $CHCl_3$/MeOH 15:1), open CC (*ODS*, 20–50% aq. MeOH), and finally purified by prep. HPLC (MeCN/H_2O 25:75): **4** (5 mg), **5** (8 mg), 8-(3,3-dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3,7-di(β-D-glucopyranoside) (15 mg), and caohuoside B (20 mg). The BuOH fraction (80 g) was fractioned by CC (macroporous resin (bead diameters 0.3–1.2 mm), H_2O, 30% EtOH, 70% EtOH, 100% EtOH): 4 fractions. The fraction of 70% EtOH (35 g) yielded korepimedoside C (10 mg) and epimedokoreanoside I (**6**; 20 mg) by CC (polyamide, $CHCl_3$/MeOH 40:1 → 1:1) and repeated open CC (*ODS*, 60–80% aq. MeOH).

Acid Hydrolysis of Glycosides. Solns. of **1**–**5** in MeOH were applied onto an HP-TLC silica gel plate (10 × 10 cm) and hydrolyzed with HCl vapor for 40 min at 50–60°. Sugars were identified by comparison with authentic samples. After elution with $CHCl_3$/MeOH/H_2O 15:6:2, glucose was detected from **1** and **2** and glucose and rhamnose from **3**–**5** (spraying with 2% naphthalen-1-ol/sulfuric acid/EtOH, then heating at 105°) [17].

*8-(3,3-Dimethylallyl)-4′,5,7-trihydroxyflavonol 7-[*O*-β-*D*-Glucopyranosyl-(1→2)-β-*D*-glucopyranoside]* (= *7-[(2-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranosyl)oxy]-3,5-dihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-en-1-yl)-4*H*-1-benzopyran-4-one*; **1**): Yellow amorphous solid. M.p. 103–105°. $[\alpha]_D^{25} = -41.2$ ($c = 0.017$, MeOH). UV (MeOH): 250 (4.23), 267 (4.22), 373 (3.83). IR (KBr): 3405, 2921, 2854, 1650, 1601, 1510, 1451, 1352, 1177, 1076, 894. ^{1}H- and ^{13}C-NMR: *Table 1.* ESI-MS: 677 ($[M-H]^-$), 557, 515, 395, 353. HR-ESI-MS: 679.2225 ($[M+H]^+$, $C_{32}H_{39}O_{16}^+$; calc. 679.2233).

*(2*R*,3*R*)-8-(3,3-Dimethylallyl)-2,3-dihydro-4′,5,7-trihydroxyflavonol 7-[*O*-β-*D*-Glucopyranosyl-(1→2)-β-*D*-glucopyranoside]* (= *(2*R*,3*R*)-7-[(2-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranosyl)oxy]-2,3-dihydro-3,5-dihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-en-1-yl)-4*H*-1-benzopyran-4-one*; **2**): Light yellow amorphous solid. M.p. 123–125°. $[\alpha]_D^{25} = -21.1$ ($c = 0.019$, MeOH). UV (MeOH): 289 (4.37), 344 (3.71). CD (MeOH, $c = 0.009$; λ ($[\theta]$): 338 (+6266), 323 (0), 291 (23249), 268 (0), 241 (+4220). IR (KBr): 3415, 2925, 1641, 1586, 1451, 1253, 1176, 1075, 988, 837. ^{1}H- and ^{13}C-NMR: *Table 1.* ESI-MS: 703 ($[M+Na]^+$), 684, 589, 476, 458, 363. HR-ESI-MS: 703.2207 ($[M+Na]^+$, $C_{32}H_{40}O_{16}Na^+$; calc. 703.2209).

*8-(3,3-Dimethylallyl)-4′,5,7-trihydroxyflavonol 3-[*O*-6-*O*-Acetyl-β-*D*-glucopyranosyl-(1→3)-4-*O*-acetyl-α-*L*-rhamnopyranoside] 7-(β-*D*-Glucopyranoside)* (= *3-{[4-*O*-Acetyl-3-*O*-(6-*O*-acetyl-β-*D*-glucopyranosyl)-6-deoxy-α-*L*-mannopyranosyl]oxy}-7-(β-*D*-glucopyranosyloxy)-5-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-en-1-yl)-4*H*-1-benzopyran-4-one*; **3**): Yellow amorphous solid. M.p. 131–133°. $[\alpha]_D^{25} = -75$ ($c = 0.012$, MeOH). UV (MeOH): 270 (4.70), 320 (4.40), 353 (4.36). IR (KBr): 3386, 2922, 1739, 1650, 1597, 1441, 1375, 1223, 1177, 1076, 1047, 823. ^{1}H- and ^{13}C- NMR: *Table 2*. ESI-MS: 907 ($[M-H]^-$). HR-ESI-MS: 931.2857 ($[M+Na]^+$, $C_{42}H_{52}O_{22}Na^+$; calc. 931.2843).

*8-(3,3-Dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-3,5-Dideoxy-2-*C*-(ethoxycarbonyl)-β-*D*-erythro-pentofuranosyl-(1→2)-α-*L*-rhamnopyranoside] 7-(β-*D*-glucopyranoside)* (= *3-{[6-Deoxy-2-*O*-[3,5-dideoxy-2-*C*-(ethoxycarbonyl)-β-*D*-*erythro*-pentofuranosyl]-α-*L*-mannopyranosyl}oxy}-7-(β-*D*-glucopyranosyloxy)-5-hydroxy-2-(4-methoxyphenyl)-8-(3-methylbut-2-en-1-yl)-4*H*-1-benzopyran-4-one*; **4**): Yellow amorphous solid. M.p. 90–92°. $[\alpha]_D^{25} = -57.9$ ($c = 0.019$, MeOH). UV (MeOH): 269 (4.25), 315 (3.98), 354 (3.81). IR (KBr): 3305, 2929, 2859, 1645, 1556, 1439, 1374, 1179, 1079. ^{1}H- and ^{13}C-NMR: *Table 3.* ESI-MS: 871 ($[M+Na]^+$), 677, 531, 369. HR-ESI-MS: 871.2967 ($[M+Na]^+$, $C_{41}H_{52}O_{19}Na^+$; calc. 871.2995).

*8-(3,3-Dimethylallyl)-5,7-dihydroxy-4′-methoxyflavonol 3-[*O*-2-*C*-Carboxy-3,5-dideoxy-β-*D*-erythro-pentofuranosyl-(1→2)-α-*L*-rhamnopyranoside] 7-[β-*D*-glucopyranoside)* (= *3-{[2-*O*-(2-*C*-Carboxy-3,5-dideoxy-β-*D*-*erythro*-pentofuranosyl)-6-deoxy-α-*L*-mannopyranosyl]oxy}-7-(β-*D*-glucopyranosyloxy)-5-hydroxy-2-(4-methoxyphenyl)-8-(3-methylbut-2-en-1-yl)-4*H*-1-benzopyran-4-one*; **5**): Yellow amorphous solid. M.p. 94–96°. $[\alpha]_D^{25} = -55.6$ ($c = 0.018$, MeOH). UV (MeOH): 270 (4.27), 314 (4.02), 359 (3.89). IR (KBr): 3422, 2925, 2850, 1650, 1599, 1510, 1440, 1378, 1180, 1076. ^{1}H- and ^{13}C-NMR: *Table 3.* ESI-MS: 819 ($[M-H]^-$). HR-ESI-MS: 819.2711 ($[M-H]^-$, $C_{39}H_{47}O_{19}^-$; calc. 819.2723).

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