Flavonol Glycosides from *Epimedium pubescens*

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Five new flavonol glycosides (1, 3, 5—7) were isolated from the aerial parts of *Epimedium pubescens* Maxim., along with two known compounds, sagittasine C (2) and 4′,5-dihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-[*β*-D-xylopyranosyl(1→3)-4-*O*-acetyl-*α*-L-rhamnopyranoside]-7-*O*-*β*-D-glucopyranoside (4). The structures were elucidated on the basis of their 1D-, 2D-NMR, MS, UV and IR spectra data.

Key words *Epimedium pubescens*; Berberidaceae; flavonol glycoside

Epimedium pubescens Maxim. (Berberidaceae), together with other four species *E. brevicornum* Maxim., *E. sagittatum* Maxim., *E. wushanenes* T. S. Ying, and *E. koreanum* Nakai, is used as the official source of Traditional Chinese Medicine (TCM) "Yinyanghuo" (Chinese Pharmacopoeia 2010 Edition). It is one of the most well known herbal medicines with tonic, anti-rheumatic and aphrodisiac effects.[1,2] Previous chemical investigations on the geneus *Epimedium* have afforded a series of flavonol glycosides,[3—7] which possess multiple biological activities such as androgenic,[8] antioxidant,[9] antiosteoporosis,[10] and antidepressant-like actions.[11] Our further chemical investigation on the aerial parts of *E. pubescens* Maxim. resulted in the isolation of five new flavonol glycosides (compounds **1**, **3**, **5**—**7**) and two known compounds, sagittasine C (**2**)[3] and 4′,5-dihydroxy-8-(3,3-dimethylallyl)-flavonol 3-*O*-[*β*-D-xylopyranosyl(1→3)-4-*O*-acetyl-*α*-L-rhamnopyranoside]-7-*O*-*β*-D-glucopyranoside (**4**).[12] Their structures were elucidated on the basis of spectroscopic evidences (Fig. 1).

Compounds **1**, **3**, **5**, **6** and **7** were obtained as yellow amorphous powder. The UV absorption bands of all the compounds were at about 205, 270, 320 and 350 nm, suggesting the presence of flavonol skeleton in their structures.

The molecular formula of compound **1** was determined as $C_{33}H_{40}O_{15}$ based on the high resolution-electrospray ionization-mass spectrum (HR-ESI-MS) (*m*/*z* 677.2448 $[C_{33}H_{41}O_{15}]^+$, Calcd for 677.2440). The ^{1}H-NMR spectrum of **1** exhibited two singlet protons at δ 12.58 (1H, s, OH-5) and δ 6.28 (1H, s), respectively. Three coupled aromatic protons at δ 7.36 (1H, dd, J=8.4, 2.1 Hz), 7.34 (1H, d, J=2.1 Hz), and 7.08 (1H, d, J=8.4 Hz) suggested the presence of a 1,2,4-trisubstituted benzene ring. A methoxy signal was obversed at δ 3.85 (3H, s). A serial of proton signals at δ 5.15 (1H, t, J=7.0 Hz, H-12), 3.33 (2H, m, H-11), 1.68 (3H, s, H-15), 1.62 (3H, s, H-16) correlated with carbon signals at δ 122.4, 25.4, 17.7, 21.2 in heteronuclear single quantum coherence (HSQC) spectrum respectively, suggesting the presence of a prenyl group. In addition, the proton signals at δ 5.34 (1H, d, J=1.3 Hz, H-1″)/0.83 (3H, d, J=6.0 Hz, H-6″), and 4.86 (1H, d, J=1.1 Hz, H-1‴)/1.08 (3H, d, J=6.2 Hz, H-6‴) indicated the existence of two rhamnose moieties. It was further confirmed by the acid hydrolysis and HPLC analysis according to the method of Tanaka *et al.*[13] The absolute configuration of the sugars was determined to be the *L* configuration. The methoxy group at δ 3.85 was attached at the C-4′ (δ 160.5) due to the characteristic heteronuclear multiple bond connectivity (HMBC) correlation. The aromatic carbon signals at δ 158.8, 98.5 and 103.7 were assigned to C-5, C-6 and C-10, due to their HMBC correlations with the hydroxy group at δ 12.58 (OH-5) (Fig. 2). Therefore, the carbon signals at δ 162.6 and 105.9 were then assigned to C-7 and C-8 according to their HMBC correlations with H-6 (δ 6.28). The prenyl group was located at C-8, which was supported by the HMBC correlation between H-11 (δ 3.33, 2H, m) and C-8 (δ 105.9). Moreover, one rhamnose moiety was located at C-3 of the aglycone according to the HMBC correlation of H-1″/C-3 (δ 134.2). And the other rhamnose moiety was located at C-2″ of the inner one due to the HMBC correlations of H-1‴/C-2″ (δ 75.6) and H-2″ (δ 4.10, 1H, m)/C-1‴ (δ 101.6). Thus, the structure of **1** was deduced as 4′-methoxyl-3′,5,7-trihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-*α*-L-rhamnopyranosyl(1→2)-*α*-L-rhamnopyranoside.

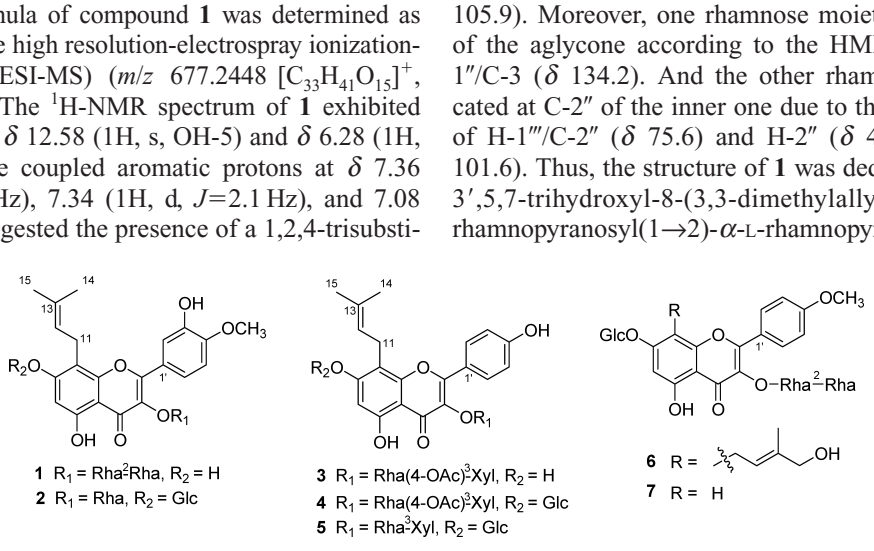

Fig. 1. Chemical Structures of **1**—**7**

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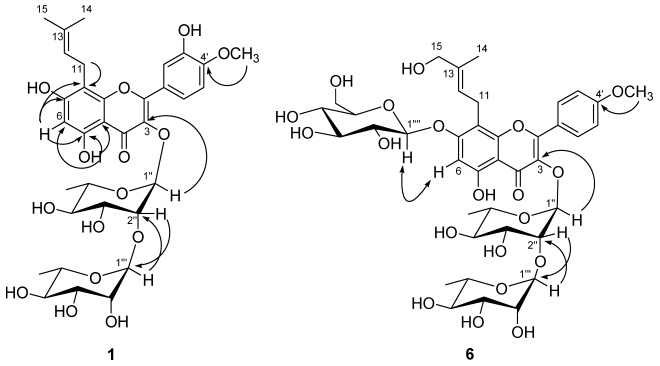

Fig. 2. Key HMBC (→) and ROESY (↔) Correlations of **1** and **6**

Compound **3** had the molecular formula $C_{33}H_{38}O_{15}$ based on the HR-ESI-MS (*m/z* 675.2296 $[C_{33}H_{29}O_{15}]^+$, Calcd for 675.2283). The ^{1}H-NMR spectrum of **3** showed a singlet signal of OH-5 at δ 12.48 (1H, s). A set of *ortho*-coupled doublet signals of four aromatic protons at δ 7.71, 6.93 (each 2H, d, J=7.8 Hz) and a one-proton singlet signal at δ 6.10 (1H, s) were also observed. The presence of an acetyl group was supported by a three-proton singlet signal at δ 1.97 in the ^{1}H-NMR spectrum and carbon signals at δ 169.7, 20.8 in the ^{13}C-NMR spectrum. Proton signals at δ 5.17 (1H, t, J=5.9 Hz, H-12), 3.34 (2H, m. H-11) as well as two methyl groups signals at δ 1.67 and 1.61 (each 3H, s, H-14, 15) revealed the presence a prenyl group. It was further confirmed by the carbon signals at δ 21.3 (C-11), 123.3 (C-12), 129.9 (C-13), 17.8 (C-14) and 25.4 (C-15) observed in the ^{13}C-NMR spectrum. Additionally, two sugar moieties were observed with protons δ 5.30 (1H, br s)/0.71 (3H, d, J=6.2 Hz) and δ 4.22 (1H, d, J=7.6 Hz) in the ^{1}H-NMR spectrum. They were identified as an L-rhamnose and a D-xylose by the acid hydrolysis experiment and HPLC analysis according to the method of Tanaka *et al.*[13] The aromatic methylene carbon signal at δ 99.5 was assigned to C-6, due to its HMBC correlation with the hydroxy group at δ 12.48 (OH-5). Therefore, the carbon signals at δ 158.9 and 106.2 were then assigned to C-7 and C-8 according to their HMBC correlations with H-6 (δ 6.10).The location of the prenyl group at the C-8 (δ 106.2) position was supported by the chemical shift value of C-6 (δ 99.5) in the ^{13}C-NMR spectrum.[3,14—16] It was further confirmed by the HMBC correlation between H-11 (δ 3.34) and C-8. In the HMBC spectrum, a correlation between the H-1″ (δ 5.30) and C-3 (δ 134.2) were observed, indicating the rhamnose moiety was attached to C-3 of the aglycone. The xylose moiety was located at C-3″ (δ 76.5) of the inner rhamnose moiety, according to the HMBC correlation of H-1‴ (δ 4.22)/C-3″. The acetyl group was located at C-4″ of the rhamnose moiety due to the correlation between H-4″ (δ 4.85) and the carbonyl carbon of the acetyl group at δ 169.7. Therefore, the structure of **3** was elucidated to be 4′,5,7-trihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-β-D-xylopyranosyl(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside.

The molecular formula of Compound **5** was established as $C_{37}H_{46}O_{19}$ by HR-ESI-MS (*m/z* 795.2726 $[C_{37}H_{47}O_{19}]^+$, Calcd for 795.2706). The NMR data of **5** were similar with those of **3**, except for the loss of the acetyl group signals and additional signals of a glucose moiety. The anomeric proton at δ 5.00 (1H, d, J=7.3 Hz, H-1⁗) indicated it to be β-glucose. The absolute configuration was further elucidated as D configuration by the acid hydrolysis experiment.[13] The glucose moiety was located at C-7 of the aglycone due to the rotating frame Overhauser effect spectroscopy (ROESY) correlation of H-6 (δ 6.63)/H-1⁗. Thus, the structure of **5** was identified as 4′,5-dihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranozside]-7-*O*-β-D-glucopyranoside.

Compound **6** was obtained with the molecular formula $C_{39}H_{50}O_{20}$ by HR-ESI-MS (*m/z* 839.2982 $[C_{39}H_{51}O_{20}]^+$, Calcd for 839.2968). The ^{1}H-NMR spectrum of **6** suggested the presence of a chelated 5-OH group (δ 12.61, 1H, s), a *penta*-substituted benzene ring (δ 6.64, 1H, s), a *para*-substituted benzene ring (δ 7.12, 7.90, each 2H, d, J=2.1 Hz), and a methoxy group (δ 3.85, 3H, s). Proton signals at δ 5.37 (1H, m, H-12), 3.73 (2H, br s, H-15), 3.60 (1H, m, H-11a), 3.47 (1H, m, H-11b), 1.66 (3H, s, H-14) in the ^{1}H-NMR spectrum, correlated with carbon signals at δ 120.9 (C-12), 66.1 (C-15), 20.8 (C-11), 13.7 (C-14) in HSQC spectrum respectively, indicating the presence of a hydroxyprenyl group. Additionally, three anomeric protons were observed at δ 5.39 (1H, d, J=1.5 Hz, H-1″), 4.88 (1H, d, J=1.1 Hz, H-1‴), and 5.01 (1H, d, J=7.5 Hz, H-1⁗) respectively, suggesting the presence of three monosaccharide moieties. They were identified as two α-L-rhamnose moieties and a β-D-glucose moiety by the acid hydrolysis experiment.[13] The methoxy group was located at C-4′ according to the HMBC correlation between the proton signal at δ 3.85 and C-4′ (δ 161.4). One rhamnose moiety was located at C-3 of the aglycone due to the HMBC correlation of H-1″/C-3 (δ 134.5). The other rhamnose moiety was located at C-2″ of the inner one due to the HMBC correlations of H-1‴/C-2″ (δ 75.5) and H-2″ (δ 4.12, 1H, m)/C-1‴ (δ 101.6). And the glucopyranose moiety was located at C-7 (δ 160.6) according to the ROESY correlation between H-1⁗ and H-6 (δ 6.64) (Fig. 2). Therefore, compound **6** was elucidated as 4′-methoxyl-5-hydroxyl-8-(3-methyl-4-hydroxyl-but-2-enyl)-flavonol 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranoside]-7-*O*-β-D-glucopyranoside.

Compound **7** was obtained with the molecular formula $C_{34}H_{42}O_{19}$ by HR-ESI-MS (*m/z* 755.2402 $[C_{34}H_{43}O_{19}]^+$, Calcd for 755.2393). The NMR spectroscopic data of **7** were similar with those of **6**, except for the loss of the signals of a hydroxyprenyl group. Thus, the structure of **7** was elucidated as 4′-methoxyl-5-hydroxyl-flavonol 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranoside]-7-*O*-β-D-glucopyranoside.

Experimental

General Procedure UV spectra were recorded on a JASCO V-550 UV/Vis spectrometer. IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. ESI-MS spectra were recorded on a Finnigan LCQ Advantage MAX mass spectrometer. HR-ESI-MS spectra were acquired using Thermo-fishier LTQ Orbitrap XL mass spectrometer. 1D- and 2D-NMR spectra were measured with a Bruker AV-400 spectrometer. Open column chromatography was performed using silica gel (200—300 mesh, Qingdao Haiyang Chemical Group Corp., Qingdao, China), ODS (50 μm, YMC, Japan), HW-40 (Tosoh, Japan) and Sephadex LH-20 (Pharmacia). Thin layer chromatography (TLC) was performed using percolated silica gel plates (silica gel GF_{254}, 1 mm, Yantai). An agilent series 1200 HPLC instrument equipped with a quaternary pump, a multiple wavelength detector, an auto sampler and a column compartment was used.

Plant Material The plant was supplied by Guizhou Tongjitang Pharmaceutical Co., Ltd., Guiyang, China, and identified by Professor Guang-Xiong

Table 1. NMR Data of Aglycone Moiety for Compounds **1**, **3**, **5**—**7** (in DMSO-d_6)

No.	**1**		**3**		**5**		**6**		**7**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	156.6		155.9		157.7		157.3		157.4	
3	134.2		133.1		134.0		134.5		134.8	
4	177.7		176.8		178.1		178.2		177.9	
5	158.8		157.9		159.0		159.1		160.9	
6	98.5	6.28 s	99.5	6.10 s	98.1	6.63 s	98.1	6.64 s	99.4	6.48 d (2.1)
7	162.6		158.9		160.4		160.6		163.1	
8	105.9		106.2		108.3		108.1		94.7	6.78 d (2.1)
9	153.7		153.9		152.9		153.0		156.1	
10	103.7		102.0		105.5		105.1		105.8	
11	21.2	3.38 m	21.3	3.34 m	21.4	3.34 m	20.8	3.47 m		
						3.55 m		3.60 m		
12	122.4	5.15 t (7.0)	123.3	5.17 t (5.9)	122.2	5.16 t (6.9)	120.9	5.37 m		
13	130.9		129.9		131.0		135.6			
14	17.7	1.68 s	17.8	1.67 s	17.8	1.69 s	13.7	1.66 s		
15	25.4	1.62 s	25.4	1.61 s	25.4	1.60 s	66.1	3.73 br s		
1′	122.5		120.6		120.3		122.1		121.9	
2′	115.4	7.34 d (2.1)	130.2	7.71 d (8.7)	130.7	7.80 d (8.7)	130.6	7.90 d (9.0)	130.6	7.88 d (8.9)
3′	146.4		115.3	6.93 d (8.7)	115.4	6.94 d (8.7)	114.0	7.12 d (9.0)	114.1	7.12 d (8.9)
4′	150.0		160.3		160.4		161.4		161.4	
5′	111.7	7.08 d (8.4)	115.3	6.93 d (8.7)	115.4	6.94 d (8.7)	114.0	7.12 d (9.0)	114.1	7.12 d (8.9)
6′	120.6	7.36 dd (8.4, 2.1)	130.2	7.71 d (8.7)	130.7	7.80 d (8.7)	130.6	7.90 d (9.0)	130.6	7.88 d (8.9)
4′-OCH_3	55.7	3.85 s					55.5	3.85 s	55.5	3.85 s
5-OH		12.58 s		12.48 s		12.57 s		12.61 s		12.65 s

Zhou, College of Pharmacy, Jinan University. A voucher specimen was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, China.

Extraction and Isolation The aerial parts of *E. pubescens* Maxim. (2 kg) were extracted twice with 60% ethanol. After removal of the ethanol *in vacuo*, the extract (245 g) was chromatographied over Diaion HP-20 resin, eluted with water, 30% and 95% ethanol in successive. The 95% ethanol eluate (80 g) was then subjected to a silica-gel column chromatography (CC) eluted with chloroform–methanol in gradient to give fourteen fractions. Fraction 7 ($CHCl_3$–MeOH 9 : 1 eluent) was then subjected to ODS CC eluted with MeOH–H_2O in gradient. And nine subfractions were obtained (7A—I). The subfraction 7H (eluted with 60% MeOH–H_2O) was further separated by Sephadex LH-20 eluted with $CHCl_3$–MeOH (1 : 1). Compound **3** (16 mg) was obtained after the purification by HW-40 CC eluted with 50% MeOH–H_2O. Fraction 11 ($CHCl_3$–MeOH 8 : 2 eluent) was chromatographied on ODS CC with MeOH–H_2O in gradient to yield 7 subfractions (11A to G). The subfraction 11B (eluted with 20% MeOH–H_2O) was applied to repeated Sephadex LH-20 CC eluted with $CHCl_3$–MeOH (1 : 1) and 35% MeOH–H_2O, respectively. Then the eluate was separated by preparative HPLC with 50% MeOH–H_2O to yield compounds **6** (21 mg) and **7** (10 mg). Fraction 10 ($CHCl_3$–MeOH 8 : 2 eluent) was also subjected to ODS CC eluted with MeOH–H_2O in gradient to yield 9 subfractions (10A—I). Fraction 10F (eluted with 50% MeOH–H_2O) was further subjected to Sephadex LH-20 eluted with 55% MeOH–H_2O and then separated by preparative HPLC (Ultimate™ XB-C18, 5 μm, 21.2×250 mm, Welch) with 50% MeOH–H_2O to yield compounds **2** (8 mg) and **5** (26 mg). Fraction 10G (eluted with 50% MeOH–H_2O) was purified by Sephadex LH-20 with 60% MeOH–H_2O to yield compound **4** (68 mg). Fraction 10I (eluted with 50% MeOH–H_2O) was subjected to Sephadex LH-20 eluted with 60% MeOH–H_2O, and then separated by preparative HPLC (Ultimate™ XB-C18, 5 μm, 21.2×250 mm, Welch) with 65% MeOH–H_2O to yield compound **1** (9 mg).

Acid Hydrolysis and HPLC Analysis The absolute configuration of the sugar moieties in the structures were determined by the method of Tanaka *et al.*[13] Compound **1** (2 mg) was hydrolyzed with 2 M HCl for 2 h at 90 °C. The mixture was evaporated to dryness under a vacuum, and then the residue was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was collected. After drying *in vacuo*, the residue was dissolved in pyridine (1 ml) containing L-cysteine methyl ester (1 mg) (Sigma, U.S.A.) and heated at 60 °C for 1 h. Then, *o*-tolyl isothiocyanate (5 μl) (Alfa Aesar, U.K.) was added to the mixture, which was heated at 60 °C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC. Analytical HPLC was performed on a Cosmosil 5C_{18}-MS-II column (250×4.6 mm i.d., 5 μm, Nacalai Tesque Inc., Japan) at 35 °C with isocratic elution of 25% CH_3CN containing 0.1% formic acid for 40 min and subsequent washing of the column with 90% CH_3CN at a flow rate 0.8 ml/min. Peaks were detected by a UV detector at 250 nm. One peak of the derivatives of **1** was obversed at t_R 29.1 (L-Rha) min. The mixture of standard monosaccharides, such as L-rhamnose, D-glucose, L-glucose, D-xylose, and L-xylose (Sigma, U.S.A.), were subjected to the same method. The peaks of the standard monosaccharide derivatives were recorded at t_R 15.9 (L-Glc), 17.2 (D-Glc), 18.7 (L-Xyl), 20.0 (D-Xyl), and 29.2 (L-Rha) min. Following the above procedure, the derivatives of **2**, **6** and **7** gave two peaks at t_R 17.2—17.3 (D-Glc) and 29.2—29.3 (L-Rha) min, respectively. The derivatives of **3** gave two peaks at t_R 20.1 (D-Xyl) and 29.3 (L-Rha) min. Those of **4** and **5** both gave three peaks at t_R 17.2—17.3 (D-Glc), 20.0—20.1 (D-Xyl), and 29.1—29.2 (L-Rha) min.

4′-Methoxyl-3′,5,7-trihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-α-L-Rhamnopyranosyl(1→2)-α-L-rhamnopyranoside (**1**): Yellow powder; UV λ_{max} (MeOH) nm (log ε): 205 (4.47), 258 (4.13, sh), 270 (4.17), 346 (3.91); IR (KBr) cm^{-1}: 3402, 2933, 1653, 1509, 1046; ^{1}H- and ^{13}C-NMR data (see Tables 1, 2); ESI-MS (positive) *m/z*: 699 $[M+Na]^+$, 1375 $[2M+Na]^+$, 677 $[M+H]^+$, ESI-MS (negative) *m/z*: 675 $[M-H]^-$, 1351 $[2M-H]^-$; HR-ESI-MS *m/z*: 677.2448 $[M+H]^+$ (Calcd for $C_{33}H_{41}O_{15}$, 677.2440).

Sagittasine C (**2**): Yellow powder; UV λ_{max} (MeOH) nm (log ε): 205 (4.50), 258 (4.14, sh), 269 (4.15), 346 (3.91); IR (KBr) cm^{-1}: 3388, 2925, 1651, 1599, 1076; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 12.59 (1H, s, 5-OH), 7.40 (1H, dd, J=8.5, 2.1 Hz, H-6′), 7.38 (1H, d, J=2.1 Hz, H-2′), 7.09 (1H, d, J=8.5 Hz, H-5′), 6.62 (1H, s, H-6), 5.25 (1H, d, J=1.3 Hz, H-1″), 5.17 (1H, t, J=7.0 Hz, H-12), 5.00 (1H, d, J=7.5 Hz, H-1‴), 4.00 (1H, br s, H-2″), 3.86 (3H, s, 4′-OCH_3), 3.72 (1H, m, H-6‴a), 3.54 (1H, m, H-11a), 3.53 (1H, m, H-3″), 3.48 (1H, m, H-6‴b), 3.43 (2H, m, H-11b, H-5‴), 3.35—3.28 (overlapped in HDO, H-2‴, 3‴), 3.26—3.12 (3H, m, H-4‴, H-4″, H-5″), 1.70 (3H, s, H-14), 1.61 (3H, s, H-15), 0.81 (3H, d, J=6.0 Hz, H-6″); ^{13}C-NMR data (DMSO-d_6, 100 MHz) δ: 178.3 (C-4), 160.5 (C-7), 159.0 (C-5), 157.4 (C-2), 152.9 (C-9), 150.2 (C-4′), 146.4 (C-3′), 134.6 (C-3), 131.1 (C-13), 122.4 (C-1′), 122.1 (C-12), 120.8 (C-6′), 115.5 (C-2′), 111.7 (C-5′), 108.3 (C-8), 105.5 (C-10), 102.0 (C-1″), 100.6 (C-1‴), 98.1 (C-6), 77.2 (C-5‴), 76.6 (C-3‴), 73.3 (C-2‴), 71.2 (C-4″), 70.6 (C-5″), 70.3 (C-3″), 70.0 (C-2″), 69.7 (C-4‴), 60.6 (C-6‴), 55.7 (4′-OCH_3), 25.4 (C-15), 21.4 (C-11), 17.7 (C-14), 17.4 (C-6″); ESI-MS (positive) *m/z*: 715 $[M+Na]^+$, 1407 $[2M+Na]^+$, 693 $[M+H]^+$, ESI-MS (negative) *m/z*: 691 $[M-H]^-$, 1383 $[2M-H]^-$; HR-ESI-MS *m/z*: 693.2404 $[M+H]^+$ (Calcd for $C_{33}H_{41}O_{16}$, 693.2389).

4′,5,7-Trihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-β-D-Xylopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranoside (**3**): Yellow powder; UV λ_{max} (MeOH) nm (log ε): 204 (4.43, sh), 270 (4.25), 316 (3.97, sh), 350 (3.91);

Table 2. NMR Data of Sugar Moieties for Compounds **1**, **3**, **5**—**7** (in DMSO-d_6)

No.	**1**		**3**		**5**		**6**		**7**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
Rhamnose-1										
1″	100.7	5.34 d (1.3)	101.2	5.30 br s	101.8	5.30 br s	100.6	5.41 d (1.3)	100.7	5.39 d (1.5)
2″	75.6	4.10 m	69.6	4.12 br s	69.5	4.13 br s	75.5	4.11 m	75.5	4.12 m
3″	70.1	3.63 m	76.5	3.80 m	80.9	3.56 m	70.1	3.59 m	70.1	3.61 m
4″	71.4	3.26—3.10 o	71.4	4.84 t (10.0)	69.9	3.40—3.27 o	71.3	3.23—3.07 o	71.3	3.22—3.08 o
5″	70.4	3.26—3.10 o	68.2	3.32 m	70.5	3.27—3.05 o	70.4	3.23—3.07 o	70.4	3.22—3.08 o
6″	17.5	0.83 d (6.0)	17.0	0.71 d (6.2)	17.4	0.84 d (6.1)	17.5	0.81 d (5.6)	17.4	0.81 d (5.6)
4″-*O*-$COCH_3$			169.7							
			20.8	1.97 s						
Rhamnose-2										
1‴	101.6	4.86 d (1.1)					101.6	4.88 br s	101.6	4.88 d (1.1)
2‴	70.2	3.68 m					70.2	3.68 m	70.2	3.68 m
3‴	70.6	3.50—3.31 o					70.7	3.36 m	70.7	3.36 m
4‴	71.9	3.26—3.10 o					71.9	3.23—3.07 o	71.9	3.22—3.08 o
5‴	68.7	3.50—3.31 o					68.8	3.39 m	68.8	3.39 m
6‴	17.5	1.08 d (6.2)					17.6	1.11 d (6.2)	17.6	1.10 d (6.1)
Xylose										
1‴			105.5	4.22 d (7.6)	105.8	4.32 d (7.3)				
2‴			72.9	2.98 m	73.8	3.08 m				
3‴			76.7	3.11 m	76.2	3.14 m				
4‴			69.5	3.30 m	69.5	3.40—3.27 o				
5‴			65.7	3.77 m	65.7	3.75 m				
				3.14 m		3.12 m				
Glucose										
1⁗					100.5	5.07 d (7.3)	99.9	5.01 d (7.5)	100.6	5.07 d (7.3)
2⁗					73.3	3.30 m	73.1	3.27 m	73.3	3.30 m
3⁗					76.6	3.30 m	76.4	3.30 m	76.6	3.31 m
4⁗					69.6	3.27—3.05 o	69.5	3.23—3.07 o	69.6	3.22—3.08 o
5⁗					77.2	3.43 m	77.2	3.44 m	77.1	3.43 m
6⁗					60.6	3.71 m	60.6	3.72 m	60.6	3.72 m
						3.48 m		3.48 m		3.48 m

"o" refers to peaks overlapped with other signals.

IR (KBr) cm^{-1}: 3419, 2926, 1651, 1612, 1044; ^{1}H- and ^{13}C-NMR data (see Tables 1, 2); ESI-MS (positive) *m/z*: 697 $[M+Na]^+$, 1371 $[2M+Na]^+$, ESI-MS (negative) *m/z*: 673 $[M-H]^-$; HR-ESI-MS *m/z*: 675.2296 $[M+H]^+$ (Calcd for $C_{33}H_{39}O_{15}$, 675.2283).

4′,5-Dihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-[*β*-D-Xylopyranosyl-(1→3)-4-*O*-acetyl-*α*-L-rhamnopyranoside]-7-*O*-*β*-D-glucopyranoside (**4**): Yellow powder; UV λ_{max} (MeOH) nm (log *ε*): 204 (4.59, sh), 270 (4.34), 318 (4.08, sh), 348 (4.03); IR (KBr) cm^{-1}: 3420, 2926, 1651, 1601, 1074; ^{1}H-NMR (DMSO-d_6, 400 MHz) *δ*: 12.53 (1H, s, 5-OH), 7.78 (2H, d, *J*=8.8 Hz, H-2′, 6′), 6.96 (2H, d, *J*=8.8 Hz, H-3′, 5′), 6.63 (1H, s, H-6), 5.32 (1H, br s, H-1″), 5.17 (1H, t, *J*=7.0 Hz, H-12), 5.00 (1H, d, *J*=7.4 Hz, H-1⁗), 4.85 (1H, t, *J*=10.0 Hz, H-4″), 4.22 (1H, d, *J*=7.6 Hz, H-1‴), 4.14 (1H, br s, H-2″), 3.80 (1H, dd, *J*=10.1, 2.3 Hz, H-3″), 3.77 (1H, m, H-5‴a), 3.73 (1H, m, H-6⁗a), 3.57 (1H, m, H-11a), 3.48 (1H, m, H-6⁗b), 3.44 (1H, m, H-11b), 3.43 (1H, m, H-5⁗), 3.40—3.25 (overlapped in HDO, H-4‴, H-5″, H-2⁗, H-3⁗), 3.17 (1H, m, H-4⁗), 3.14 (1H, m, H-5⁗b), 3.08 (1H, m, H-3‴), 2.98 (1H, m, H-2‴), 1.97 (3H, s, 4″-OAc), 1.68 (3H, s, H-14), 1.60 (3H, s, H-15), 0.72 (3H, d, *J*=6.2 Hz, H-6″); ^{13}C-NMR data (DMSO-d_6, 100 MHz) *δ*: 178.1 (C-4), 169.7 (4″-*O*-$\underline{C}OCH_3$), 160.5 (C-7), 160.5 (C-4′), 159.0 (C-5), 157.7 (C-2), 153.0 (C-9), 133.8 (C-3), 131.0 (C-13), 130.6 (C-2′, 6′), 122.1 (C-12), 120.3 (C-1′), 115.4 (C-3′, 5′), 108.3 (C-8), 105.6 (C-1‴), 105.5 (C-10), 101.3 (C-1″), 100.6 (C-1⁗), 98.1 (C-6), 77.2 (C-5⁗), 76.7 (C-3‴), 76.6 (C-3⁗), 76.5 (C-3″), 73.3 (C-2⁗), 72.9 (C-2‴), 71.3 (C-4″), 69.6 (C-4⁗), 69.6 (C-2″), 69.5 (C-4‴), 68.4 (C-5″), 65.8 (C-5‴), 60.6 (C-6⁗), 25.4 (C-15), 21.4 (C-11), 20.8 (4″-*O*-$CO\underline{C}H_3$), 17.8 (C-14), 17.0 (C-6″); ESI-MS (positive) *m/z*: 859 $[M+Na]^+$, 1695 $[2M+Na]^+$, 837 $[M+H]^+$, ESI-MS (negative) *m/z*: 835 $[M-H]^-$; HR-ESI-MS *m/z*: 837.2825 $[M+H]^+$ (Calcd for $C_{39}H_{49}O_{20}$, 837.2812).

4′,5-Dihydroxyl-8-(3,3-dimethylallyl)-flavonol 3-*O*-[*β*-D-Xylopyranosyl-(1→3)-*α*-L-rhamnopyranoside]-7-*O*-*β*-D-glucopyranoside (**5**): Yellow powder; UV λ_{max} (MeOH) nm (log *ε*): 204 (4.38, sh), 270 (4.13), 321 (3.87, sh), 350 (3.85); IR (KBr) cm^{-1}: 3409, 2924, 1651, 1600, 1075; ^{1}H- and ^{13}C-NMR data (see Tables 1, 2); ESI-MS (positive) *m/z*: 817 $[M+Na]^+$, 1611 $[2M+Na]^+$, 795 $[M+H]^+$, ESI-MS (negative) *m/z*: 793 $[M-H]^-$, 1587 $[2M-H]^-$; HR-ESI-MS *m/z*: 795.2726 $[M+H]^+$ (Calcd for $C_{37}H_{47}O_{19}$, 795.2706).

4′-Methoxyl-5-hydroxyl-8-(3-methyl-4-hydroxyl-but-2-enyl)-flavonol 3-*O*-[*α*-L-Rhamnopyranosyl(1→2)-*α*-L-rhamnopyranoside]-7-*O*-*β*-D-glucopyranoside (**6**): Yellow powder; UV λ_{max} (MeOH) nm (log *ε*): 204 (4.62, sh), 270 (4.38), 316 (4.14, sh), 348 (4.07); IR (KBr) cm^{-1}: 3408, 2930, 1651, 1597, 1062; ^{1}H- and ^{13}C-NMR data (see Tables 1, 2); ESI-MS (positive) *m/z*: 861 $[M+Na]^+$, 839 $[M+H]^+$, ESI-MS (negative) *m/z*: 873 $[M+Cl]^-$; HR-ESI-MS *m/z*: 839.2982 $[M+H]^+$ (Calcd for $C_{39}H_{51}O_{20}$, 839.2968).

4′-Methoxyl-5-hydroxyl-flavonol 3-*O*-[*α*-L-Rhamnopyranosyl(1→2)-*α*-L-rhamnopyranoside]-7-*O*-*β*-D-glucopyranoside (**7**): Yellow powder; UV λ_{max} (MeOH) nm (log *ε*): 204 (4.41, sh), 266 (4.17), 317 (3.97, sh), 342 (3.98); IR (KBr) cm^{-1}: 3420, 2931, 1654, 1603, 1066; ^{1}H- and ^{13}C-NMR data (see Tables 1, 2); ESI-MS (positive) *m/z*: 777 $[M+Na]^+$, 755 $[M+H]^+$, ESI-MS (negative) *m/z*: 789 $[M+Cl]^-$, 1507 $[2M-H]^-$; HR-ESI-MS *m/z*: 755.2402 $[M+H]^+$ (Calcd for $C_{34}H_{43}O_{19}$, 755.2393).

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References

1) Zhao H. Y., Sun J. H., Fan M. X., Fan L., Zhou L., Han J., Wang B. R., Guo D. A., *J. Chromatogr. A*, **119**, 157—181 (2008).
2) Zhao H. Y., Fan L., Zhou L., Han J., Wang B. R., Guo D. A., *Helv. Chim. Acta*, **90**, 2186—2195 (2007).
3) Wang G. J., Tsai T. H., Lin L. C., *Phytochemistry*, **68**, 2455—2464 (2007).
4) Uriburu M. L., Gil R. R., Sosa V. E., de la Fuente J. R., *Phytochemistry*,

68, 1295—1299 (2007).
5) Yu C. Y., Song L. N., Chen G., *Chin. Chem. Lett.*, **20**, 842—844 (2009).
6) Eun S. K., Mi K. K., Hyun K. K., Young I. P., Mi S. D., *Nat. Prod. Sci.*, **14**, 233—238 (2008).
7) Luo G. J., Ci X. X., Ren R., Wu Z. Y., Li H. M., Li H. Z., Li R. T., Deng X. M., *Planta Med.*, **75**, 843—847 (2009).
8) Yang J. Y., Yu Q. H., Wu J., *J. Shenyang Pharm. Univ.*, **15**, 98—101 (1998).
9) Wang T., Zhang J. C., Chen Y., Huang F., Yang M., Xiao P. G., *China J. Chin. Mat. Med.*, **32**, 715—718 (2007).
10) Peng S. L., Zhang G., He Y. X., Wang X. L., Leung P. C., Leung K., Qin L., *Bone*, **45**, 534—544 (2009).
11) Pan Y., Kong L. D., Xia X., Zhang W. Y., Xia Z. H., Jiang F. X., *Pharmacol. Biochem. Behav.*, **82**, 686—694 (2005).
12) Ueda T., Nakajima K., Chin M., Mihashi H., JP Patent 04159295 (1992).
13) Tanaka T., Nakashima T., Ueda T., Tomii K., Kouno I., *Chem. Pharm. Bull.*, **55**, 899—901 (2007).
14) Li W. K., Zhang R. Y., Xiao P. G., *Phytochemistry*, **43**, 527—530 (1996).
15) Carvalho M. G., Suzart L. R., Cavatti L. C., Kaplan M. A. C., *J. Braz. Chem. Soc.*, **19**, 1423—1428 (2008).
16) Zhang P. C., Wang S., Wu Y., Chen R. Y., Yu D. Q., *J. Nat. Prod.*, **64**, 1206—1209 (2001).