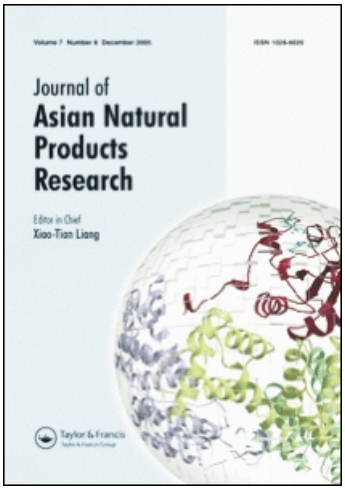

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Flavonoids from *Galium verum* L.

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Two new flavonoids, compounds **1** and **2**, together with seven known flavonoids, were isolated from *Galium verum* L. Their structures were elucidated as diosmetin 7-*O*-α-L-rhamnopyranosyl-(1-2)-[β-D-xylopyranosyl-(1–6)]-β-D-glucopyranoside (**1**) and 3,5,7, 3′,4′,3″,5″,7″,3‴,4‴-decahydroxyl-[8-CH_2-8″]-biflavone (**2**) by chemical methods and spectroscopic analyses. Compounds **3** and **4** were isolated from the genus *Galium* for the first time.

Keywords: rubiaceae; *Galium verum* L; flavonoids; methylene biflavone

1. Introduction

Galium verum L. (rubiaceae), widely distributed in China, is often used as natural dyestuff and food additive. In traditional Chinese medicine, it is often used in the treatment of phlebophlogosis and hepatitis [1]. Phytochemical investigations of *G. verum* L. have led to the isolation of several kinds of bioactive compounds such as iridoids, anthraquinones, chlorogenic acids, and flavonoids [2]. In our recent research, two new flavonoids, compounds **1** and **2**, together with isorhamnetin (**3**), isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranoside (**4**), kaempferol (**5**), quercetin (**6**), diosmetin (**7**), diosmetin 7-*O*-β-D-glucopyranoside (**8**), and diosmetin 7-*O*-β-D-xylopyranosyl-(1–6)-β-D-glucopyranoside (**9**), were obtained. In this paper, we report the isolation and structural elucidation of two new flavonoids.

2. Results and discussion

The ethanolic extract of *G. verum* L. was suspended in water and partitioned with petroleum ether, $CHCl_3$, and *n*-BuOH successively. The *n*-BuOH extract was chromatographed successively on silica gel column, Sephadex LH-20 column, and HPTLC to afford compounds **1**–**9**.

Compound **1** was obtained as a yellow amorphous powder. The molecular formula was determined as $C_{33}H_{40}O_{19}$ by ESI-MS at *m/z* 763.2 $[M + Na]^+$ and 739.0 $[M - H]^-$, and HR-ESI-MS at *m/z* 763.2068 $[M + Na]^+$. It showed a positive reaction to Molish reagent. Its structure was determined by 1D (^1H, ^{13}C) and 2D (HMQC and HMBC) NMR (Table 1; Figure 1) experiments. Comparison of the NMR spectral data of **1** with those of diosmetin (**7**) revealed that two compounds shared the same carbon skeleton except for the appearance of three sets of sugar signals in **1**,

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Table 1. NMR spectral data of compound **1** (in DMSO-d_6).

Position	δ_C (125 MHz)	δ_H (600 MHz)	HMBC	TOCSY
2	164.2			
3	104.0	6.78 (s)		
4	181.9			
5	162.6			
6	99.8	6.38 (br s)	C-5, 10	
7	162.6			
8	94.3	6.73 (br s)	C-9	
9	157.2			
10	105.8			
1′	123.0			
2′	113.3	7.45 (br s)	C-2, 4′, 6′	
3′	147.5			
4′	151.6			
5′	112.3	7.11 (d, 8.6 Hz)	C-1′, 3′	
6′	118.5	7.52 (br d, 8.6 Hz)	C-2	
Glc				
1″	98.0	5.17 (d, 7.1 Hz)	C-7	H-2″
2″	76.1	3.50	C-4″, 1‴	H″-1, 3″
3″	75.7	3.64		H-2″, 4″
4″	69.6	3.24–3.29		H-3″, 5″
5″	77.1	3.47	C-3″	H-4″, 6″
6″	68.2	3.92 (d, 10.9 Hz) 3.61 (m)	C-1⁗	H-5″
Rha				
1‴	100.5	5.13 (br s)	C-2″, 3‴, 5‴	H-2‴
2‴	70.4	3.67	C-4‴	H-1‴, 3‴
3‴	70.6	3.31		H-2‴, 4‴
4‴	72.0	3.22	C-3‴	H-3‴, 5‴
5‴	68.4	3.76		H-4‴, 6‴
6‴	18.2	1.19 (d, 6.1 Hz)	C-4‴, 5‴	H-5‴
Xyl				
1⁗	104.2	4.16 (d, 7.5 Hz)	C-6″	H-2⁗
2⁗	73.5	2.97–3.00	C-4⁗	H-1⁗, 3⁗
3⁗	76.6	3.08 (dd, 8.7, 8.3 Hz)	2-C⁗	H-2⁗, 4⁗
4⁗	69.6	3.24–2.29		H-3⁗, 5⁗
5⁗	65.7	2.97–3.00, 3.68 (dd, 5.4, 11.5 Hz)	C-1⁗, 3⁗	H-4⁗
—OCH_3	55.8	3.84 (s)	C-4′	

which was further verified by the result that, on acid hydrolysis, compound **1** gave diosmetin (**7**) as the aglycone, as well as D-glucose, L-rhamnose, and D-xylose, which were identified by GC [3]. The α-anomeric configuration for the rhamnose was judged by its C_5 chemical shift at δ 68.4 [4]. The coupling constants of the other two anomeric protons 7.1 and 7.5 Hz showed the β-configurations of the glucose and xylose linkage units, respectively. Comparison with the ^{13}C NMR spectral data of diosmetin (**7**), a downfield shift to δ 105.8 at C-10 and an upfield shift to δ 162.6 at C-7 in **1** gave a hint that the sugar moiety was attached to C-7. In the HMBC spectrum of **1**, the anomeric proton of the glucose at δ 5.17 showed a long-range correlation with C-7 confirming the above deduction. The TOCSY, HMBC, and HMQC experiments allowed identifying the spin systems of each sugar residue. In the TOCSY spectrum of **1**, the correlation between H-1″ (δ 5.17) and H-2″ (δ 3.50) was observed. A carbon signal at δ 76.1 was assigned to C-2″ by the correlation with H-2″ (δ 3.50) in the HMQC spectrum. The HMBC experiment showed correlations between H-1‴ (δ 5.13) and C-2″ (δ 76.1), and H-1⁗ (δ 4.16) and C-6″ (δ 68.2). The above evidence

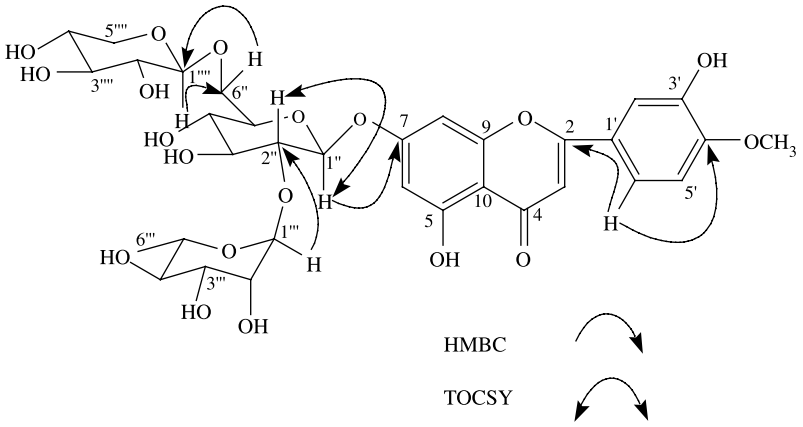

Figure 1. Key HMBC and TOCSY correlations of compound **1**.

suggested that the sugar moiety was linked to the C-7 position, and the rhamnose and the xylose were linked to the C-2″ and C-6″ positions of the glucose, respectively. Thus, the structure of compound **1** was established as diosmetin 7-*O*-α-L-rhamnopyranosyl-(1-2)-[β-D-xylopyranosyl-(1−6)]-β-D-glucopyranoside.

Compound **2** was isolated as a yellow amorphous powder. Quasi-molecular ion peaks at *m/z* 617.1 $[M + H]^+$ in ESI-MS and 639.0759 $[M + Na]^+$ in HR-ESI-MS spectra confirmed the molecular formula $C_{31}H_{20}O_{14}$. In the ^1H NMR spectrum of **2** (Table 2), an ABX spin system at δ 6.83 (d, $J = 8.5$ Hz), 7.60 (dd, $J = 8.5$, 2.0 Hz), and 7.84 (d, $J = 2.0$ Hz) was assigned to a trisubstituted B-ring of flavonoid. The ^1H NMR spectrum of **2** was almost identical to that of **6**, except for the disappearance of one proton signal at δ 6.42 and the appearance of an extra proton signal at δ 4.27 (br s, H-11), correlated with the carbon signal at δ 16.6 (C-11) in the HMQC spectrum of **2**. It was obvious that H-6 or H-8 of compound **2** was substituted. The ^{13}C NMR spectrum of **2** gave

Table 2. NMR spectral data of compound **2** (in DMSO-d_6).

Position	δ_C (125 MHz)	δ_H (600 MHz)	HMBC
2 (2″)	146.8		
3 (3″)	135.4		
4 (4″)	176.3		
5 (5″)	158.4		
6 (6″)	97.8	6.13 (s)	C-5 (5″), 7 (7″), 8 (8″), 10 (10″)
7 (7″)	162.2		
8 (8″)	104.8		
9 (9″)	153.8		
10 (10″)	102.7		
11	16.6	4.27 (br s)	C-7 (7″), 8 (8″), 9 (9″)
1′ (1‴)	122.5		
2′ (2‴)	115.3	7.84 (d, $J = 2.0$ Hz)	C-3′ (3‴), 4′ (4‴), 6′ (6‴)
3′ (3‴)	145.1		
4′ (4‴)	147.7		
5′ (5‴)	115.6	6.83 (d, $J = 8.5$ Hz)	C-1′ (1‴), 3′ (3‴), 4′ (4‴)
6′ (6‴)	120.1	7.60 (dd, $J = 8.5$, 2.0 Hz)	C-2′ (2‴), 2 (2″)
5-OH (5‴-OH)		12.53 (s)	C-5 (5″), 6 (6″), 10 (10″)

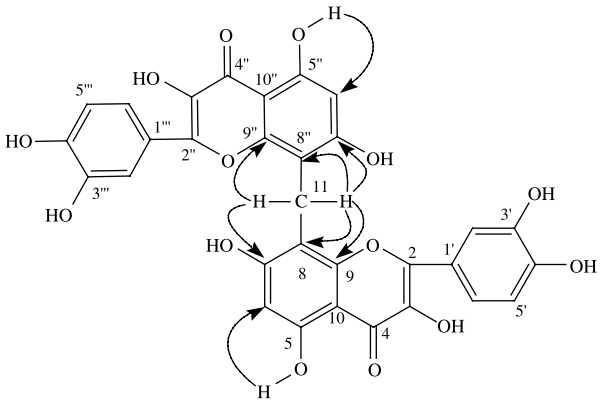

Figure 2. Key HMBC correlations of compound **2**.

16 carbon signals. Besides an unassigned carbon signal resonated at δ 16.6, the remaining 15 carbons were assigned to a flavonoid familiar to quercetin [6], except for the C-8 or C-6 resonance shifted downfield to δ 104.8. Together with the other signal at δ 97.8 (C-8 or C-6), this suggested that H-8 rather than H-6 was substituted by an aliphatic moiety [5]. The proposed partial structure was further confirmed by the HMBC experiment (Figure 2). The long-range correlations between the proton at δ 4.27 (2H, br s, H-11) and C-9 (δ 153.8), C-7 (δ 162.2) and C-8 (δ 104.8), and 5-hydroxyl proton (δ 12.53) and C-6 (δ 97.8) suggested that C-11 (δ 16.6) was attached to C-8 position.

According the MS experiments, another quercetin moiety (*m/z* 302, $C_{15}H_{10}O_7$) was needed to agree with the molecular weight. Since all the NMR spectral data have been assigned to the partial structure described above, and there is no additional signal in the ^{1}H NMR and ^{13}C NMR spectra, the only explanation of this contradiction is that the molecule is completely symmetrical and each signal in the ^{1}H NMR spectrum was doubled. Namely, two identical quercetin partial structures were bridged by a methylene at their eight positions. Thus, the structure of **2** was concluded as 3,5,7,3′,4′,3″,5″,7″,3‴,4‴-decahydroxyl-[8-CH_2-8″]-biflavone. This linkage of two flavonoid units with a methylene in compound **2** was unusual in natural products.

Compounds **3**–**9**, by comparison with the published data, were identified as isorhamnetin (**3**) [6], isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranoside (**4**) [7], kaempferol (**5**) [6], quercetin (**6**) [6], diosmetin (**7**) [8], diosmetin 7-*O*-β-D-glucopyranoside (**8**) [9], and diosmetin 7-*O*-β-D-xylopyranosyl-(1–6)-β-D-glucopyranoside (**9**) [10], respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on Yanaco MP-S3 melting point apparatus and are uncorrected. UV–Vis spectra were performed on a Shimadzu UV-260 instrument. The IR spectra were performed on Bruker IR S-55. NMR spectra were recorded on Bruker ARX-300 or Bruker ARX-600. ESI-MS was performed on Finnigan LCQ mass spectrometer. HR-ESI-MS was performed on QSTARLCQ mass spectrometer.

3.2 Plant material

The plant material of *G. verum* L. was collected at Hulin City, Heilongjiang Province, China, in September 2005, and identified by Professor Qishi Sun, Shenyang Pharmaceutical University. A voucher

specimen (No. 20050403) has been deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The air-dried herbs (20 kg) of *G. verum* L. were extracted with 95% ethanol (200 l × 3 times) for 2 h. The extracts were concentrated *in vacuo* to give a residue (1.5 kg), which was partitioned with petroleum ether, $CHCl_3$, and *n*-BuOH successively. The *n*-BuOH extract (650 g) was subjected to column chromatography (CC) over silica gel (2.5 kg) and gradiently eluted with $CHCl_3$—MeOH. Fractions 1 (100:1), 2 (100:2), 4 (100:4), 8 (100:8), 11 (100:13), and 12 (100:15) were further purified by recrystallization from MeOH to give compounds **7** (114 mg), **3** (60 mg), **5** (130 mg), **8** (2.4 g), **9** (2.7 g), and **4** (703 mg), respectively. Fraction 5 (100:5) was submitted to Sephadex LH-20 column chromatography using MeOH as eluent to afford compounds **2** (43 mg) and **6** (2.4 g). Fraction 14 (100:20) was submitted to HPTLC using $CHCl_3$—MeOH—H_2O—HOAc (65:35:10:0.15) as developing solvent to afford compound **1** (15 mg).

3.3.1 Compound *1*

Yellow amorphous solid, mp 201–203°C; IR (KBr) ν_{max} (cm^{-1}): 3408, 1657, 1612, 1498, 1382, 1261, 1177, 1046. UV λ_{max} (nm, MeOH): 344, 252, 207. ^1H and ^{13}C NMR spectral data (DMSO-d_6): see Table 1. The positively charged ESI-MS: *m/z* 779.3 $[M + K]^+$, 763.2 $[M + Na]^+$, 617.1 [M-rhamnose + $Na]^+$, 463.1 [M-xylose-rhamnose + $H]^+$; the negatively charged ESI-MS: *m/z* 739.0 $[M - H]^-$, 606.9 [M-xylose − $H]^-$, 298.8 [M-xylose-rhamnose-glucose − $H]^-$; HR-ESI-MS: *m/z* 763.2068 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_{19}Na$, 763.2062).

3.3.2 Compound *2*

Yellow amorphous solid, mp > 300°C; IR (KBr) ν_{max} (cm^{-1}): 3421, 2931, 1656, 1613, 1599, 1539, 1513, 1362, 1257, 1162, 1005. UV λ_{max} (nm, MeOH): 377, 260. ^1H and ^{13}C NMR spectral data (DMSO-d_6): (see Table 2). The positively charged ESI-MS: *m/z* 617.1 $[M + H]^+$; the negatively charged ESI-MS: *m/z* 614.9 $[M - H]^-$; HR-ESI-MS: *m/z* 639.0759 $[M + Na]^+$ (calcd for $C_{31}H_{20}O_{14}Na$, 639.0751).

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