Two New Flavone *C*-Glycosides from *Trollius ledebourii*

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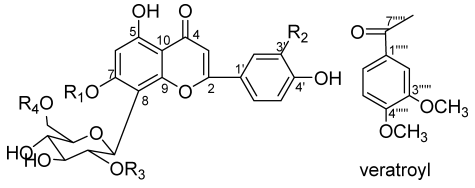
Two new flavone *C*-glycosides, trollisin A (1) and trollisin B (2), along with seven known flavonoids, isoswertisin (3), isoswertiajaponin (4), orientin (5), 2″-*O*-β-L-galactopyranosylvitexin (6), 2″-*O*-β-L-galactopyranosylorientin (7), neodiosmin (8) and acacetin-7-*O*-neohesperidoside (9) were isolated from the flowers of *Trollius ledebourii* Reichb. The structures of the new compounds were elucidated based on spectral analysis, including MS, 1D- and 2D-NMR experimentation.

Key words *Trollius ledebouri*; flavone *C*-glycoside; trollisin A; trollisin B

Trollius ledebourii Reichb (Ranunculaceae) is a herb (40—100 cm tall) found in damp grassland and widely distributed in the north-eastern regions of China. Its flowers, called "Jin Lian Hua" in Chinese, are well known for their use as a traditional folk medicine for the treatment of otitis media acuta, conjunctivitis and upper respiratory tract infection.[1] Previous studies on this species have revealed the presence of flavonoids and organic acids, some of which were reported to exhibit antiviral, antibacterial and antioxidant activities.[2] As a follow-up study on the chemical constituents of *T. ledebourii*, two new flavone *C*-glycosides (**1**, **2**) together with seven known flavonoids have been isolated.

Results and Discussion

Compound **1** was obtained as yellow powder and exhibited a positive magnesium hydrochloric acid test, indicating a flavonoid. $[\alpha]_D^{20}$ −17.1 (c=0.645, MeOH). The molecular formula was deduced to be $C_{42}H_{48}O_{23}$ by positive mode high resolution-electrospray ionization (HR-ESI)-MS data at m/z 943.2484 $[M+Na]^+$ (Calcd for $C_{42}H_{48}O_{23}Na$, 943.2484). The UV spectrum showed λ_{max} (MeOH) (log ε) at 217 (4.45), 267 (4.14), 296 (3.97), and 332 (3.96) nm characteristic of a flavone. The IR spectrum revealed the presence of hydroxy (3355 cm^{-1}), carbonyl (1653 cm^{-1}) and aromatic groups (1605, 1577, 1512, 1438 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) showed resonances at δ_H 7.92 (2H, d, J=8.4 Hz) and 6.94 (2H, d, J=8.4 Hz), suggesting the presence of an AA′BB′ system on the B-ring, corresponding to the protons H-2′, 6′ and H-3′, 5′, respectively. Two singlets at δ_H 6.68 and 6.24 were due to the protons at C-3 and C-6 in rings C and A of a flavone, respectively. In addition, signals due to two aromatic methoxy groups at δ_H 3.84 (3H, s) and 3.80 (3H, s) and an ABX system [δ_H 7.50 (1H, dd, J=8.4, 1.8 Hz), δ_H 7.38 (1H, d, J=1.8 Hz), δ_H 7.03 (1H, d, J=8.4 Hz)] corresponding to a 1, 3,4-trisubstituted aromatic ring were also revealed, indicating the presence of a dimethoxybenzoyl group. The ^{13}C-NMR spectrum (Table 1) revealed 38 carbon signals, indicating the presence of a flavonoid moiety, three saccharide moieties and a dimethoxybenzoyl moiety in **1**. From analysis of the ^{1}H- and ^{13}C-NMR data of the sugar moieties, **1** was deduced to be a flavone *C*-glycoside and three sugar moieties were determined to be β (J_H of anomeric carbons >7.0 Hz). Signals at δ_C 98.3 (C-6), 103.6 (C-8) and 80.1 (C-5″) revealed that the site of the sugar linkage in **1** should be at the C-8 position of the aglycon moiety, which was further confirmed by the correlation of the glucosyl anomeric proton H-1″ (δ 4.83, d, J=10.2 Hz) with carbon signals at δ_C 103.6 (C-8), 162.5 (C-7) and 156.2 (C-9) in the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 2). From these data, **1** had a similar pattern to the known compound 2″-*O*-(3‴,4‴-dimethoxybenzoyl) vitexin.[3] But the signal of C-2″ (δ_C 81.2) showed a downfield shift of 8.4 ppm compared with the corresponding data (δ_C 72.8) of 2″-*O*-(3‴,4‴-dimethoxybenzoyl) vitexin, which indicated the middle sugar was attached to the C-2″. This was also confirmed by the long-range correlation between C-1‴ (δ_C 105.5) and H-2″ (δ_H 4.08). Moreover, in the HMBC spectrum, observation of the cross peaks from H-6″ (δ_H 3.88, 3.55) to C-7⁗ (δ_C 164.8) of the dimethoxybenzoyl moiety suggested that the 1,3,4-trisubstituted aromatic ring was connected to the C-6″; correlations from H-1⁗ (δ_H 4.15) to C-6‴ (δ_C 69.1) and from H-6‴ (δ_H 4.21, 3.58) to C-1⁗ (δ_C 103.2) indicated that the terminal sugar was attached to C-6‴. Acid hydrolysis of **1** yielded D-glucose and L-galactose, which



	R_1	R_2	R_3	R_4
1	H	H	Glc (1→6) Gal	veratroyl
2	CH_3	H	H	Glc
3	CH_3	H	H	H
4	CH_3	OH	H	H
5	H	OH	H	H
6	H	H	Gal	H
7	H	OH	Gal	H

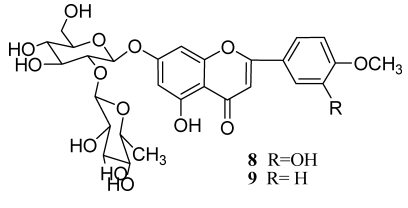


Fig. 1. Structures of Compounds **1**—**9**

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Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) Data of Compounds **1** and **2**[a,b]

No.		**1** (DMSO-d_6)		**2** (DMSO-d_6)	
		δ_H	δ_C	δ_H	δ_C
	2		163.5		164.3
	3	6.68 s	102.7	6.83 s	102.5
	4		181.8		182.3
	5		160.6		161.4
	6	6.24 s	98.3	6.52 s	95.0
	7		162.5		163.3
	8		103.6		105.5
	9		156.2		155.1
	10		103.6		104.4
	1′		121.7		121.4
	2′	7.92 (d, 8.4)	128.6	7.99 (d, 8.4)	128.8
	3′	6.94 (d, 8.4)	115.8	6.95 (d, 8.4)	115.9
	4′		161.1		161.3
	5′	6.94 (d, 8.4)	115.8	6.95 (d, 8.4)	115.9
	6′	7.92 (d, 8.4)	128.6	7.99 (d, 8.4)	128.8
	7-OCH$_3$			3.88 s	56.5
Glc	1″	4.83 (d, 10.2)	71.5	4.72 (d, 10.2)	73.2
	2″	4.08 (t, 9.0)	81.2	3.86 (t, 9.0)	70.7
	3″	3.56 m	78.4	3.27 (t, 8.4)	78.4
	4″	3.24 m	71.2	3.40 m	70.6
	5″	3.46 m	80.1	3.44 m	80.2
	6″	3.88 (t, 9.6)	61.5	4.20 (br d, 10.2)	69.3
		3.55 m		3.56 m	
Gal	1‴	4.03 (d, 7.2)	105.5	4.14 (d, 7.8)	103.2
	2‴	3.42 m	70.2	2.95 (t, 8.4)	73.3
	3‴	3.16 m	72.6	3.08 (t, 8.4)	76.8
	4‴	3.47 m	67.3	3.02 m	69.8
	5‴	3.15 m	71.5	3.03 m	76.8
	6‴	4.21(br d, 10.8)	69.1	3.62 (br d, 10.8)	60.9
		3.58 m		3.40 m	
Glc	1⁗	4.15 (d, 7.8)	103.2		
	2⁗	2.95 (t, 8.4)	73.2		
	3⁗	3.09 (t, 8.4)	76.8		
	4⁗	3.04 m	69.9		
	5⁗	3.02 m	76.8		
	6⁗	3.63 (br d, 10.2)	60.9		
		3.40 m			
Veratroyl	1⁗′		121.6		
	2⁗′	7.38 (d, 1.8)	111.8		
	3⁗′		152.9		
	4⁗′		148.3		
	5⁗′	7.03 (d, 8.4)	111.0		
	6⁗′	7.50 (dd, 8.4, 1.8)	123.2		
	7⁗′		164.8		
	3⁗′-OCH$_3$	3.84 s	55.7		
	4⁗′-OCH$_3$	3.80 s	55.6		

a) Chemical shift values were in ppm and *J* values (in Hz) were presented in parentheses. *b*) The assignments were based on HSQC, HMBC and ^{1}H–^{1}H TOCSY experiments.

were confirmed by PC and GC analysis. 1D- and 2D-nuclear Overhauser effect (NOE) analysis were also carried out. The correlation between the proton at δ_H 4.03 and the proton at δ_H 4.08 indicated that the anomeric proton of the middle sugar was on the same side as the proton of C-2″. Together with the correlations of 1‴-H/3‴-H, 5‴-H, the middle sugar moiety was confirmed to be L-galactose. Thus, the structure of **1** was established as shown in Fig. 1, named trollisin A.

Compound **2** was obtained as yellow powder and exhibited

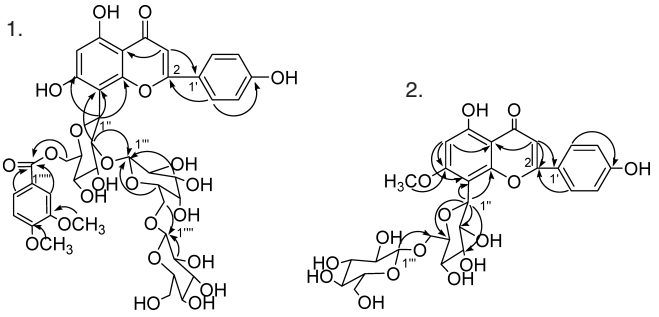

Fig. 2. Key HMBC Correlations of Compounds **1** and **2**

a positive magnesium hydrochloric acid test, indicating a flavonoid. $[\alpha]_D^{20}$ +2.18 (c=0.046, MeOH). The molecular formula was established to be $C_{28}H_{32}O_{15}$ by HR-ESI-MS at m/z 631.1639 $[M+Na]^+$ (Calcd for $C_{28}H_{32}O_{15}Na$, 631.1624). The UV spectrum showed λ_{max} (MeOH) (log ε) at 214 (4.36), 269 (4.08) and 333 (4.08) nm characteristic of a flavone. The IR spectrum showed the presence of hydroxy (3326 cm^{-1}), carbonyl (1653 cm^{-1}) and aromatic (1602, 1575, 1447 cm^{-1}) groups. The ^{1}H-NMR spectrum of **2** (Table 1) indicated the presence of an AA′BB′ system [δ_H 7.99 (2H, d, J=8.4 Hz), 6.95 (2H, d, J=8.4 Hz)] on the B-ring, the same as **1**. The ^{13}C-NMR spectrum of **2** (Table 1) revealed 28 carbon signals, of which there were two sugar moieties and a methoxyl group besides the flavone skeleton. According to above mentioned data, Compound **2** was determined to have an analogous pattern with the known compound isoswertisin.[3] By analyzing the ^{13}C-NMR data with isoswertisin, the signal of C-6″ (δ_C 69.3) of **2** was shifted downfield by 8.1 ppm, suggesting that the middle sugar was attached to C-6″, which was further confirmed by the correlation between H-1‴ (δ_H 4.14) and C-6″ (δ_C 69.3) in the HMBC spectrum (Fig. 2). Therefore, the structure of **2** was evaluated as shown in Fig. 1, named trollisin B.

By comparison with the NMR data reported in literature,[3—6] the known compounds **3**—**9** were identified as isoswertisin (**3**), isoswertiajaponin (**4**), orientin (**5**), 2″-*O*-*β*-L-galactopyranosylvitexin (**6**), 2″-*O*-*β*-L-galactopyranosylorientin (**7**), neodiosmin (**8**) and acacetin-7-*O*-neohesperidoside (**9**).

Experimental

General NMR spectra were obtained with a Bruker AV III 600 NMR spectrometer (chemical shift values are presented as δ values with tetramethylsilane (TMS) as the internal standard). UV and IR spectra were recorded on Shimadzu UV2550 and FTIR-8400S spectrometer, respectively. The optical rotations were obtained in MeOH at 20 °C on a Perkin-Elmer 341 digital polarimeter. HR-ESI-MS spectra were performed on an LTQ-Obitrap XL spectrometer. GC analysis was carried out on a GC-7890: column, DB-5 (30 m×0.32 mm×0.25 μm); detector, FID-6850 (Agilent, California, U.S.A.). C_{18} reversed-phase silica gel (40—63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), MCI gel (CHP 20P, 75—150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) and silica gel (100—200 mesh, Qingdao Marine Chemical Plant, Qingdao, People's Republic of China) were used for column chromatography. Precoated silica gel GF254 plates (Zhi Fu Huang Wu Pilot Plant of Silica Gel Development, Yantai, People's Republic of China) were used for TLC. All solvents used were of analytical grade (Beijing Chemical Plant).

Plant Material The flowers of *T. ledebourii* were collected from Chengde, Heibei (P. R. China) and identified by Prof. Wen-Yan Lian. A voucher specimen (HB-02-0128) has been deposited at the Institute of Medicinal Plant Development.

Extraction and Isolation Dried flowers (15 kg) were extracted twice

with 95% ethanol. The concentrated extract obtained under reduced pressure was suspended in water and extracted with petroleum ether, ethyl acetate and 1-butanol, successively. The water fraction (500 g) was subjected to polyamide (100—200 mesh) column chromatography, and eluted with MeOH–H_2O (0 : 1—1 : 0, v/v) to give 7 fractions. Fraction 2 (50 g) was chromatographed on a polyamide column and eluted with MeOH–$CHCl_3$ (0 : 1—3 : 4, v/v), then purified with CC over silica gel (MeOH–$CHCl_3$) and Sephadex LH-20 (MeOH) to afford **6** (33 mg) and **7** (24 mg); Fraction 4 (4 g) was isolated by reversed-phase MCI gel and MPLC elution with MeOH–H_2O (50 : 50, v/v), then purified by Sephadex LH-20 (MeOH) to give **3** (4 mg), **4** (5 mg), **5** (5 mg), **8** (3 mg) and **9** (4 mg) and by preparative HPLC elution with MeOH–H_2O (45 : 55, v/v) to afford **1** (13 mg) and **2** (2 mg).

Trollisin A (**1**): Yellow powder; $[\alpha]_D^{20}$ −17.1 (c=0.645, MeOH); UV λ_{max} (MeOH) (log ε): 217 (4.45), 267 (4.14), 296 (3.97), 332 (3.96) nm; IR (KBr) cm^{-1}: 3355, 2923, 1653, 1605, 1577, 1512, 1438, 1357, 1272, 1227, 1175, 1076, 1022, 840, 764; HR-ESI-MS m/z: 943.2484 $[M+Na]^+$ (Calcd for $C_{42}H_{48}O_{23}Na$, 943.2484). ^1H- and ^{13}C-NMR data, see Table 1.

Trollisin B (**2**): Yellow powder; $[\alpha]_D^{20}$ +2.18 (c=0.046, MeOH); UV λ_{max} (MeOH) (log ε): 214 (4.36), 269 (4.08), 333 (4.08) nm; IR (KBr) cm^{-1}: 3326, 2923, 1653, 1602, 1575, 1447, 1362, 1244, 1178, 1076, 1019, 833; HR-ESI-MS m/z: 631.1639 $[M+Na]^+$ (Calcd for $C_{28}H_{32}O_{15}Na$, 631.1624). ^1H- and ^{13}C-NMR data, see Table 1.

Determination of Sugar Components Compound **1** (2 mg) in 2% H_2SO_4 was heated at 100 °C for 4 h in a water bath. The reaction mixture was neutralized with Ba_2CO_3, filtered and then extracted with ethyl acetate. After concentration, the H_2O layer was examined by PC with 1-butanol–acetic acid–H_2O (4 : 1 : 5, choose the upper layer) and compared with authentic samples. Hydroxylamine hydrochloride (2 mg) was added to the sugar residue and then reacted in anhydrous pyridine (0.5 ml) on a magnetic stirrer at 90 °C for 0.5 h in an oil bath. After cooling, acetic anhydride (0.5 ml) was added to the reaction mixture and heated at 90 °C for 0.5 h, then the solution was dried by N_2 at 70 °C. The acetylated derivative was dissolved in $CHCl_3$ and analyzed by GC. The authentic sugars were processed using the same method as above. Column temperature 210 °C; injection temperature 240 °C; detection temperature 250 °C; carrier gas N_2 at a flow of 1.5 ml/min; D-glucose, L-galactose, 16.0 and 16.4 min, respectively.

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