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Flavone *C*-glycosides from flowers of *Trollius ledebouri*

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Abstract

The ethanol extract of the flowers of *Trollius ledebouri* yielded four flavone *C*-glycosides, 2″-*O*-vanilloylvitexin, 2″-*O*-feruloylorientin, 2″-*O*-β-L-galactopyranosylvitexin, and 2″-*O*-β-L-galactopyranosylorientin, along with known compounds, 6″-*O*-acetylorientin, 2″-*O*-(4‴-hydroxybenzoyl)vitexin, vitexin, and orientin. Their structures were elucidated by means of UV, IR, MS and NMR spectroscopic analyses.
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Keywords: *Trollius ledebouri* Reichb; Ranunculaceae; Flavone *C*-glycosides; Acylated flavone *C*-glycosides

1. Introduction

In a previous paper (Zou et al., 2004), we have described the isolation and structure elucidation of six new acylated flavone *C*-glycosides from the flowers of *Trollius ledebouri* Reichb. (Ranunculaceae). In this paper, we report the isolation and structure elucidation of four new compounds, 2″-*O*-vanilloylvitexin (**1**), 2″-*O*-feruloylorientin (**2**), 2″-*O*-β-L-galactopyranosylvitexin (**3**), and 2″-*O*-β-L-galactopyranosylorientin (**4**), along with known compounds, 6″-*O*-acetylorientin (**5**), 2″-*O*-(4‴-hydroxybenzoyl)vitexin (**6**), vitexin (**7**), and orientin (**8**).

2. Results and discussion

An EtOH extract of the flowers of *T. ledebouri* was concentrated and partitioned further into petroleum ether, ethyl acetate, *n*-butanol and H_2O soluble fractions. The ethyl acetate solubles were subjected to successive medium pressure liquid chromatography (MPLC) and repeated Sephadex LH-20 column chromotography, to afford the four acylated flavone *C*-glycosides (**1**, **2**, **5**, **6**) and the flavone *C*-glycosides (**7**, **8**). Two of these were isolated from the genus *Trollius* for the first time, and identified as 6″-*O*-acetylorientin (**5**) and 2″-*O*-(4‴-hydroxybenzoyl)vitexin (**6**), although they had been isolated previously from *Odontosoria gymnogrammoides* (Hori et al., 1987) and puriri wood (Horowitz and Gentili, 1966). Another two compounds were previously reported from flowers of *T. ledebouri* (Huang et al., 2000), and identified as vitexin (**7**) (Zhang and Xu, 2003) and orientin (**8**) (Kato and Morita, 1990). The *n*-butanol fraction was subjected to macro resin D101 column, further purified by MPLC and Sephadex LH-20 to give two unusual flavone *C*-diglycosides (**3**), (**4**) which were linked in position C-2″ to L-galactose.

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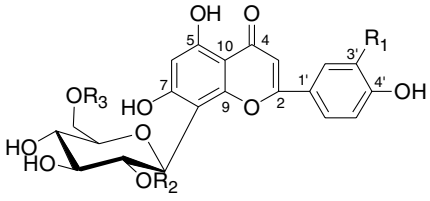
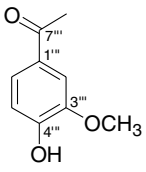
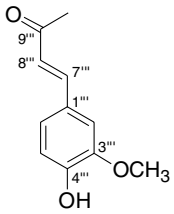




vanilloyl



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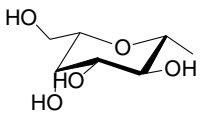
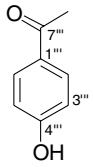


β-L-galactopyranosyl

	R_1	R_2	R_3
1	H	vanilloyl	H
2	OH	feruloyl	H
3	H	β-L-galactopyranosyl	H
4	OH	β-L-galactopyranosyl	H
5	OH	H	acetyl
6	H	4‴-hydroxybenzoyl	H
7	H	H	H
8	OH	H	H



4‴-hydroxybenzoyl

The molecular formula of **1** was deduced as $C_{29}H_{26}O_{13}$ from positive ESI-MS and HRFAB-MS spectroscopic analyses. The IR spectrum showed the presence of two carbonyl groups at 1690 and 1660 cm^{-1}. The ^1H NMR spectrum of **1** (Table 1) exhibited signals at δ 13.12, 10.99, 10.37, and 9.84, indication of four aromatic hydroxyl group substitutions, whereas a singlet at δ 13.12 was assigned to the hydroxyl group linked to the C-5 of the flavone aglycone. Resonances at δ 8.12 (2H, *dd*, $J = 9.0$, 3.0 Hz) and δ 6.94 (2H, *dd*, $J = 9.0$, 3.0 Hz) suggested the presence of AA′BB′-system on the B-ring. An ABX-system [δ 7.25 (1H, *dd*, $J = 8.0$, 3.0 Hz), 7.20 (1H, *d*, $J = 3.0$ Hz), and 6.76 (1H, *d*, $J = 8.0$ Hz)] was assigned to the protons signals in another 1,3,4-trisubstituted aryl ring. A singlet for three protons was also observed at δ 3.75, which indicated the presence of a methoxyl group substituted on an aromatic ring. For the sugar moiety, a resonance for an anomeric proton was observed at δ 5.02 (1H, *d*, $J = 10$ Hz). The ^{13}C NMR spectrum (Table 1) revealed 29 carbon signals, which suggested the presence of a flavonoid moiety, a saccharide moiety, and an acyl group in **1**. The six carbon signals of the sugar moiety were at δ 71.0, 72.5, 75.9, 70.6, 82.1 and 61.0, suggesting that **1** is a flavone *C*-glycoside. The sugar moiety was determined to be β-glucose from analyses of ^1H and ^{13}C NMR spectroscopic data. Compared with the resonances of the known compound **7**, the data suggested the presence of the vitexin skeleton moiety in **1**. The signals of C-1″ (δ 71.0) and C-3″ (δ 75.9) of the sugar moiety showed upfield shifts of 2.4 and 2.7 ppm, compared with the corresponding data (δ 73.4, 78.6) of **7**. These data revealed that the acyl group was attached to the C-2″ hydroxyl of the sugar moiety. The acyl group was determined by HMBC experiment. From the HMBC spectrum, a correlation between the proton H-6‴ (δ 7.25, 1H, *dd*, $J = 8.0$, 3.0 Hz) and the C-4‴ (δ 151.2) signals was observed, which showed the presence of vanilloyl moiety in **1**. The correlation between the proton H-2″ (δ 5.52, 1H, *t*, $J = 10$ Hz) and a carbon signal at δ 164.7 further confirmed that the vanilloyl group was attached to the C-2″ hydroxyl of the sugar moiety. From these findings, compound **1** was identified as 2″-*O*-vanilloylvitexin.

Compound **2** was obtained as a yellow powder. A quasi-molecular ion peak was observed at *m/z*: 625.4 $[M + H]^+$ in the positive ESI-MS, and the formula was assigned as $C_{31}H_{28}O_{14}$ by HRFAB-MS, *m/z*: 647.1382 $[M + Na]^+$ (calcd for $C_{31}H_{28}O_{14}Na$, 647.1377). The IR spectrum also showed the presence of two carbonyl groups at 1700 and 1650 cm^{-1}, whereas the strong absorption at 970 cm^{-1} revealed the presence of *trans*-double bond in **2**. The ^1H NMR spectrum (Table 1) showed two ABX-systems, [δ 7.63 (1H, *dd*, $J = 8.0$, 2.0 Hz), 7.56 (1H, *br s*), and 6.91 (1H, *d*, $J = 8.0$ Hz)] and [δ 7.02 (1H, *br d*, $J = 8$ Hz), 7.22 (1H, *br s*), and 6.75 (1H, *d*, $J = 8$ Hz)], which revealed the presence of two trisubstituted phenyl rings. The resonances for δ 7.29 (1H, *d*, $J = 16$ Hz) and δ 6.21 (1H, *d*, $J = 16$ Hz) were due to *trans*-olefinic hydrons. The ^{13}C NMR spectrum of **2** revealed 31 carbon signals, 21 of which were identical to those reported for orientin (**8**). The remaining 10 signals were indicative of the presence of a feruloyl group. The ^1H and ^{13}C NMR spectroscopic analyses suggested that **2** major differed from **1** only in the acyl group. HMQC and HMBC correlations allowed for the complete assignments of the proton and carbon signals of **2**. Thus, the structure of **2** was established as 2″-*O*-feruloylorientin.

Compound **3** was also obtained as a yellow powder. The molecular formula was deduces as $C_{27}H_{30}O_{15}$ from ^{13}C NMR and positive ESI-MS (*m/z*: 595 $[M + H]^+$)

Table 1
^{1}H NMR (500 MHz, J in Hz) and ^{13}C NMR (125 MHz) spectroscopic data for compounds **1**–**4** in DMSO-d_6

Position	1[a]		2[a]		3[a]		4[a]	
	H	C	H	C	H	C	H	C
2		164.1		164.2		163.6		163.8
3	6.82 *s*	102.5	6.68 *s*	102.4	6.74 *s*	102.5	6.61 *s*	102.5
4		182.0		181.9		181.9		181.9
5		160.6		160.6		160.5		160.5
6	6.19 *s*	97.7	6.16s	97.7	6.20s	98.3	6.20 *s*	98.2
7		162.1		162.1		161.2		162.6
8		102.4		102.4		103.8		103.8
9		156.3		156.5		156.2		156.2
10		103.9		103.9		103.8		103.7
1′		121.6		122.0		121.6		122.0
2′	8.12 *dd* (9.0, 3.0)	129.1	7.56 *br s*	114.1	8.01 *d* (8.5)	128.8	7.45 *d* (2.0)	114.0
3′	6.94 *dd* (9.0, 3.0)	115.9		145.8	6.90 *d* (8.5)	115.9		145.8
4′		161.2		149.7		161.2		149.7
5′	6.94 *dd* (9.0, 3.0)	115.9	6.91 *d* (8.0)	115.7	6.90 *d* (8.5)	115.9	6.86 *d* (8)	115.7
6′	8.12 *dd* (9.0,3.0)	129.1	7.63 *dd* (8.0, 2.0)	119.4	8.01 *d* (8.5)	128.8	7.50 *dd* (8, 2.0)	119.2
1″	5.02 *d* (10)	71.0	4.92 *d* (10)	71.1	4.81 *d* (10)	71.4	4.78 *d* (10)	71.4
2″	5.52 *t* (10)	72.5	5.45 *t* (10)	72.1	4.04 *t* (9)	81.9	4.03 *t* (9)	81.9
3″	3.63 *m*	75.9	3.59 *t* (10)	75.9	3.51 *t* (9)	78.7	3.49 *t* (9)	78.8
4″	3.55 *m*	70.6	3.51 *m*	70.7	3.45 *t* (9)	69.9	3.42 *t* (9)	70.1
5″	3.41 *m*	82.1	3.38 *m*	82.2	3.26 *m*	81.7	3.27 *m*	82.0
6″	3.84 *m*, 3.62 *m*	61.0	3.86 *m*, 3.64 *m*	61.3	3.76 *br d* (11), 3.54 *m*	60.9	3.78 *br d* (11), 3.57 *m*	61.3
1‴		120.7		125.5	3.91 *d* (7.5)	106.1	3.90 *d* (7.5)	106.1
2‴	7.20 *d* (3.0)	112.4	7.22 *br s*	111.0	3.15m	71.8	3.13 *m*	71.8
3‴		147.1		147.9	3.07 *m*	73.1	3.06 *m*	73.1
4‴		151.2		149.2	3.47 *m*	66.7	3.45 *m*	66.7
5‴	6.76 *d* (8.0)	114.9	6.75 *d* (8)	115.4	2.78 *m*	74.0	2.77 *m*	74.1
6‴	7.25 *dd* (8.0, 3.0)	123.3	7.02 *br d* (8)	123.0	3.12 *m*, 2.50 *m*	58.1	3.09 *m*, 2.70 *m*	58.1
7‴		164.7	7.29 *d* (16)	144.6				
8‴			6.21 *d* (16)	114.3				
9‴				165.5				
3‴-OCH_3	3.75 *s*	55.6	3.79 *s*	55.6				
5-OH	13.12 *s*		13.16 *s*		13.15 *s*			13.14 *s*
7-OH	10.99 *s*							
4′-OH	10.37 *s*							
4‴-OH	9.84 *s*							

[a] Assignments based on HMQC and HMBC.

analyses. In the ^{1}H NMR spectrum of **3** (Table 1), the doublet at δ4.81 (J = 10 Hz) due to an anomeric proton and the AB-type signals (δ8.01 and 6.90, J = 8.5 Hz) assignable to four aryl protons on B-ring suggested the presence of a vitexin moiety. The doublet at δ 3.91 (J = 7.5 Hz), however, was due to another anomeric proton. Taking into account the ^{13}C NMR spectroscopic data, and the 13° of unsaturation calculated from the empirical formula of **3**, it was suggested that **3** has vitexin moiety in addition to a sugar moiety. The substitution position and assignment of ^{13}C NMR spectral data was confirmed by analysis of HMQC and HMBC spectra. The correlations of 2″-H/C-1‴ and 1‴-H/C-2″ in the HMBC spectrum confirmed that the terminal sugar was linked to C-2″ of glucose. In the ^{1}H NMR spectrum, the doublet at δ 3.91 (J = 7.5 Hz) suggested that the configuration of the terminal sugar was β. In order to confirm the pattern and configuration of the terminal sugar, a NOESY analysis was carried out. The correlation between the proton at δ 3.91 and the proton at δ 4.04 indicated that the anomeric proton of the terminal sugar was on the same side as the proton of C-2″. Together with the correlations of 1‴-H/3-H‴, 5‴-H, 4‴-H, the terminal sugar moiety was confirmed as β-L-galactose. Thus, the structure of compound **3** is proposed to be 2″-*O*-β-L-galactopyranosylvitexin.

The positive ESI-MS spectrum of flavonoid **4** gave a quasi molecular ion at m/z 611 $[M + H]^+$, which differed from that of flavonoid **3** by 16 mass units. The ^{1}H NMR spectrum showed a ABX-type system of the protons at δ 7.50, 7.45, and 6.86, which suggested the B-ring had two hydroxyl groups. Compound **4** according to ^{13}C NMR spectroscopic analyses was also a flavone *C*-diglycoside bearing two hexoses. As an analogy to **3**, the interpretation of comprehensive NMR experiments led to the structure of 2″-*O*-β-L-galactopyranosylorientin. Signals

of the two β-glycosyl moieties were completely assigned from the HMQC and HMBC spectra. The analysis of the NOESY spectrum confirmed the pattern of the terminal glycosyl as β-L-galactose. Finally, the structure of compound **4** was defined as 2″-*O*-β-L-galactopyranosylorientin.

In conclusion, we have isolated four new flavone *C*-glycosides, and four known compounds. It is very interesting that the isolated flavonoids were flavone *C*-8 glycosides. And the flavone C-diglycosides (**3** and **4**) have a unique structure as natural products, in which the terminal sugar is β-L-galactose. As for its possible biosynthesis of all isolated flavone *C*-glycosides from *T. ledebouri*, the pathway is not clear and needs further study.

3. Experimental

3.1. General

NMR spectra were recorded in DMSO-d_6 with an INOVA 500 NMR spectrometer, using visual DMSO-d_6 resonances (^{1}H δ 2.49, ^{13}C δ 39.4) for internal reference. Melting points were determined with an X4 micro-melting point apparatus and are uncorrected. The $[\alpha]_D$ values were obtained in MeOH at 20 °C on a Perkin–Elmer 341 digital polarimeter. UV spectra were recorded on a Hitachi UV-2201 spectrophotometer and IR spectra with an Impact 400 FTIR spectrometer. Mass spectra were recorded on an Auto-Spec Ultima-TOF spectrometer.

Silica gel 60H and silica gel 100–200 mesh (both from Qingdao Haiyang Chemical Co., Qingdao, P.R. China) were used for column chromatography. Sephadex LH-20 (25–100 μm, Sigma–Aldrich) was used for chromatography.

3.2. Plant material

The flowers of *T. ledebouri* Reichb. were collected from Chengde (P.R. China) in August 2002, and identified by Prof. Wen-Yan Lian. A voucher specimen is deposited at the Institute of Medicinal Plant Development (IMPLAD), under the number HB-02-0128.

3.3. Extraction and isolation

Dried flowers (15 kg) were extracted with 95% ethanol two times under reflux conditions. The concentrated extract was suspended in water and successively extracted with petroleum ether, EtOAc, and *n*-BuOH. The EtOAC-solubles (150 g) were subjected to silica gel (100–200 mesh) CC, eluting with a $CHCl_3$–MeOH gradient to afford 15 fractions. Fractions 10 and 11 were subjected to MPLC ($CHCl_3$:MeOH:H_2O = 90:10:1), then further purified by repeated Sephadex LH-20 CC (MeOH:H_2O = 10:1) to give **1** (80 mg), **2** (181 mg), **5** (110 mg), **6** (130 mg), **7** (50 mg), and **8** (257 mg). The *n*-BuOH-solubles (998 g) were dissolved in water, then subjected to D-101 macro resin CC and eluted with an H_2O–EtOH gradient (100:0; 90:10; 30:70; 50:50; 5:95) to give five fractions. The H_2O–EtOH (1:9) fraction (62 g) was subjected to MPLC (EtOAc:MeOH:H_2O, gradient), and repeatedly purified with Sephadex LH-20 (MeOH:H_2O = 10:1) to give **3** (140 mg), and **4** (500 mg).

3.3.1. 2″-*O*-Vanilloylvitexin (*1*)

Yellow powder; m.p. 266–267 °C; $[\alpha]_D^{20}$ −221.1° (MeOH, *c* 0.038); UV λ_{max}^{MeOH} nm (log ε): 258 (4.65), 294 (4.50), 332 (4.48); IR ν_{max}^{KBr} cm^{-1}: 3440, 3400, 1690, 1660, 1600, 1580, 1500, 1440, 1360, 1280, 1240, 1210, 1110, 1080, 1000, 840, 760; ESI-MS (positive) *m/z*: 583.5 $[M + H]^+$; HRFAB-MS (positive) *m/z*: 583.1517 $[M + H]^+$ (calcd for $C_{29}H_{27}O_{13}$, 583.1452); for ^{1}H NMR and ^{13}C NMR spectra, see Table 1.

3.3.2. 2″-*O*-Feruloylorientin (*2*)

Yellow powder; m.p. 234–236 °C; $[\alpha]_D^{20}$ −73.7° (MeOH, *c* 0.048); UV λ_{max}^{MeOH} nm (log ε): 275 (4.54), 335 (4.66); IR ν_{max}^{KBr} cm^{-1}: 3400–3200 (br), 1700, 1650, 1610, 1580, 1560, 1510, 1340, 1250, 1160, 1120, 970, 840, 820; ESI-MS (positive) *m/z*: 625.4 $[M + H]^+$; HRFAB-MS (positive) *m/z*: 647.1382 $[M + Na]^+$ (calcd for $C_{31}H_{28}O_{14}Na$, 647.1377); for ^{1}H NMR and ^{13}C NMR spectra, see Table 1.

3.3.3. 2″-*O*-β-L-Galactopyranosylvitexin (*3*)

Yellow powder; m.p. 260–262 °C; $[\alpha]_D^{20}$ −37.2° (MeOH, *c* 0.022); UV λ_{max}^{MeOH} nm (log ε): 274 (4.51), 335 (4.45); IR ν_{max}^{KBr} cm^{-1}: 3400–3200 (br), 1650, 1600, 1570, 1500, 1360, 1240, 1180, 1050, 830; ESI-MS (positive) *m/z*: 595.4 $[M + H]^+$; for ^{1}H NMR and ^{13}C NMR spectra, see Table 1.

3.3.4. 2″-*O*-β-L-Galactopyranosylorientin (*4*)

Yellow powder; m.p. 218–220 °C; $[\alpha]_D^{20}$ 28.9° (MeOH, *c* 0.045); UV λ_{max}^{MeOH} nm (log ε): 258 (4.48), 270 (4.48), 348 (4.42); IR ν_{max}^{KBr} cm^{-1}: 3400–3200, 1650, 1600, 1500, 1430, 1360, 1250, 1080, 1040, 840; ESI-MS (positive) *m/z*: 611.3 $[M + H]^+$; for ^{1}H NMR and ^{13}C NMR spectra, see Table 1.

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