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Acylated flavone 8-*C*-glucosides from the flowers of *Trollius chinensis*

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ABSTRACT

Ten new flavone 8-*C*-glucosides, trollichinensides A–J, with the substitution of various acyls at C-2″, C-3″, or C-6″ of the glucose moiety, together with thirteen known ones were isolated from the flowers of *Trollius chinensis*. Spectroscopic analyses including NMR and HRESIMS resulted in the establishment of the structures of these constituents and electronic circular dichroism (ECD) experiment was employed for the determination of the absolute configuration of the glucose residue in these molecules.

1. Introduction

The plants of *Trollius* genus (Ranunculaceae), which are mainly distributed in north China and widely used as folk medicines for the treatment of upper respiratory infections, are rich in organic acids, flavone *C*-glycosides, and the assembly of these two structural moieties, acylated flavone 8-*C*-glycosides, which have been isolated from *T. ledebouri* (Wu et al., 2006, 2009, 2011a; Zou et al., 2004, 2005) and *T. chinensis* (Cai et al., 2006; Li et al., 2009), as well as some phenylethanols (Liu and Luo, 2010; Wu et al., 2011a, 2011b), alkaloids (Wang et al., 2004a), and diterpenoids (Zou et al., 2006). A preliminary phytochemical investigation by us into the flavone 8-*C*-glycosides from *T. chinensis* led to the isolation of six ones (Li et al., 2009). While, considering of the existence of four flavone 8-*C*-glucosides including vitexin, orientin, isoswertisin, and isoswertiajaponin in *T. chinensis* (Wang et al., 2004b), together with various organic acids like veratric acid, benzoic acid, vanillic acid, and 4-hydroxybenzoic acid, the structural diversity of the acylated flavone 8-*C*-glucosides in *T. chinensis* was subjected to reexamination, resulting in ten new acylated flavone 8-*C*-glucosides and thirteen known ones whose isolation and structural elucidation are reported as follows.

2. Results and discussion

Detailed separation and purification by chromatographic methods on the ethyl acetate extract of the flowers of *T. chinensis* yielded ten new flavone 8-*C*-glucosides (**1**–**10**, Fig. 1) and thirteen known ones including 2″-*O*-(2‴-methylbutyryl)vitexin (Zou et al., 2004), 2″-*O*-(4‴-hyroxybenzoyl)vitexin (Brum-Bousquet et al., 1977), 2″-*O*-vanilloylvitexin (Zou et al., 2005), 2″-*O*-veratroylvitexin (Zou et al., 2004), 3″-*O*-(2‴-methylbutyryl)vitexin (Li et al., 2009), 2″-*O*-acetylorientin (Kato and Morita, 1990), 2″-*O*-(2‴-methylbutyryl)orientin (Zou et al., 2004), 2″-*O*-veratroylorientin (Zou et al., 2004), 6″-*O*-acetylorientin (Hori et al., 1987), 2″-*O*-(2‴-methylbutyryl)isoswertisin (Zou et al., 2004), 3″-*O*-(2‴-methylbutyryl)isoswertisin (Zou et al., 2004), 2″-*O*-(2‴-methylbutyryl)isoswertiajaponin (trollisin I) (Cai et al., 2006; Wu et al., 2006), and 2″-*O*-veratroylisoswertiajaponin (trollisin II) (Cai et al., 2006) which were identified by comparison of their NMR data with those reported.

The issue needs to be addressed in the structural elucidation of acylated flavone 8-*C*-glucosides from *Trollius* genus by NMR technique is the substituting position of acyl moieties. Till now, acylation has occurred at C-2″, 3″, and 6″ of glucose. Although HMBC is the most accurate spectroscopic method to assign the acyl, proton signal on the acylated carbon of glucose measured in deuterated dimethyl sulfoxide can be employed as another indicator. The chemical shifts of H-2″ of C-2″-acylated flavone 8-*C*-glucosides were observed at the range from δ_H 5.30–5.50 as a triplet with the coupling constant of 9–10 Hz, and the resonances for H-3″ of C-3″-acylated ones were at δ_H 4.85–5.10 as a triplet. The 6″-acylated flavone 8-*C*-glucosides could be readily identified from the resonances for H-6″ at δ_H 4.00–4.50 or for C-6″ at about δ_C 64.5. What's more interesting, the veratroyl (Li et al., 2009; Zou et al., 2004), vanilloyl (Zou et al., 2005), or 4-hydroxylbenzoyl connected to C-2″, or feruloyl linked to C-3″of the glucose moiety of flavone-8-*C*-glucoside exhibited long-range shielding effect leading to the up-field shift of H-6 by about 0.1–0.2 chemical shift. For instance, H-6 of vitexin at δ_H 6.26 (Wang et al., 2004b) up-field shifted to δ_H 6.09 (Zou et al., 2004) and H-6 for isoswertisin at δ_H 6.52 (Wang et al., 2004b) shifted to

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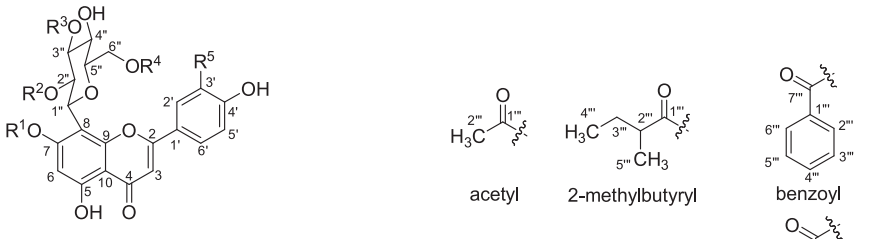

1 R^1 = H, R^2 = H, R^3 = veratroyl, R^4 = H, R^5 = H
2 R^1 = H, R^2 = H, R^3 = feruloyl, R^4 = H, R^5 = H
3 R^1 = H, R^2 = H, R^3 = H, R^4 = veratroyl, R^5 = H
4 R^1 = H, R^2 = vanilloyl, R^3 = H, R^4 = H, R^5 = OH
5 R^1 = H, R^2 = H, R^3 = acetyl, R^4 = H, R^5 = OH
6 R^1 = H, R^2 = H, R^3 = 2-methylbutyryl, R^4 = H, R^5 = OH
7 R^1 = H, R^2 = H, R^3 = veratroyl, R^4 = H, R^5 = OH
8 R^1 = CH_3, R^2 = feruloyl, R^3 = H, R^4 = H, R^5 = H
9 R^1 = CH_3, R^2 = benzoyl, R^3 = H, R^4 = H, R^5 = OH
10 R^1 = CH_3, R^2 = feruloyl, R^3 = H, R^4 = H, R^5 = OH

Fig. 1. Structures of flavone-8-*C*-glucosides from *Trollius chinensis*.

δ_H 6.33 (Li et al., 2009) when a veratroyl was connected to C-2″.

Compound **1**, an amorphous yellow powder, exhibited the molecular formula of $C_{30}H_{28}O_{13}$ determined from HRESIMS (*m/z* 597.1601 $[M+H]^+$, 619.1423 $[M+Na]^+$). ^1H NMR of **1** displayed the chelated phenolic hydroxyl at C-5 at δ_H 13.16 (1H, brs) and H-3 proton signal at δ_H 6.79 (1H, s), which were characteristic markers for flavone skeleton instead of flavonol, and an AA′BB′ coupling system at ring-B composed of two sets of doublet signals at δ_H 8.06 (2H, d, J = 8.5 Hz, H-2′, 6′) and 6.94 (2H, d, J = 8.5 Hz, H-3′, 5′). Additionally, the chemical shifts for H-6 at δ_H 6.25 and C-6 at δ 98.4, as well as the anomeric proton at δ_H 4.86 (1H, d, J = 9.8 Hz) and the six oxygenated aliphatic carbons at δ_C 73.6, 68.8, 80.4, 68.6, 81.6, and 60.8 assigned **1** a vitexin scaffold. The acyl moiety in **1** was determined to be veratroyl from the proton signals of an ABX system at δ_H 7.50 (1H, brd, J = 8.3 Hz), 7.37 (1H, brs), and 7.03 (1H, d, J = 8.3 Hz) and two methoxyl group signals at δ_H 3.79 and 3.71. Furthermore, the characteristic proton at δ_H 5.10 (1H, t, J = 9.6 Hz) was assigned to H-3″ and its long-range correlation with the carbonyl of veratroyl moiety at δ_C 165.1 established the structure of **1** as 3″-*O*-veratroylvitexin, given the trivial name of trollichinenside A.

Compound **2**, with the molecular formula of $C_{31}H_{28}O_{13}$ determined from the HRESIMS (*m/z* 609.1601 $[M+H]^+$), was obtained as an amorphous yellow powder. ^1H and ^{13}C NMR spectra of **2** displayed the 3″-*O*-acylated vitexin structure, as well as the proton and carbon signals for *E*-feruloyl moiety including the *trans*-coupling olefinic protons at δ_H 7.48 and 6.46 (J = 15.9 Hz). NOESY experiment was used to assign the methoxy group on C-3‴ of the feruloyl group. Finally, **2** was determined to be 3″-*O*-feruloylvitexin, named trollichinenside B.

Based on the quasi-molecular ion peak in HRESIMS at *m/z* 597.1600 $[M+H]^+$, compound **3**, a yellow amorphous powder, was given a molecular formula of $C_{30}H_{28}O_{13}$ and assigned a acylated vitexin derivative from the ^1H and ^{13}C NMR data. The acyl moiety of **3** could be unambiguously deduced to be veratroyl from the ABX coupling aromatic protons at δ_H 7.40 (1H, dd, J = 8.4, 1.9 Hz), 7.32 (1H, d, J = 1.9 Hz), and 6.82 (1H, d, J = 8.4 Hz), as well as two methoxyl groups at δ_H 3.77 and 3.69. Additionally, the resonances for H-6″ at δ_H 4.52 (1H, d, J = 11.5 Hz) and 4.33 (1H, m), and C-6″ at δ_C 64.7 showed the substitution of veratroyl at C-6″ of glucose. Consequently, the structure of compound **3** could be elucidated to be 6″-*O*-veratroylvitexin, with the trivial name of trollichinenside C.

The ^1H and ^{13}C NMR spectra of compound **4** with the molecular formula of $C_{29}H_{26}O_{14}$ determined from HRESIMS (*m/z* 599.1390 $[M+H]^+$), displayed signals for orientin and vanilloyl. The characteristic proton triplet for H-2″ at δ_H 5.49 (1H, t, J = 9.7 Hz), together with its HMBC correlation with the carbonyl carbon of vanilloyl group at δ_C 164.7, established **4** as 2″-*O*-vanilloylorientin, named trollichinenside D.

The following three compounds including **5**, **6**, and **7** had the molecular formulae of $C_{23}H_{22}O_{12}$ (HRESIMS *m/z* 491.1188 $[M+H]^+$), $C_{26}H_{28}O_{12}$ (*m/z* 533.1653 $(M+H)^+$), and $C_{30}H_{28}O_{14}$ (*m/z* 613.1545 $[M+H)^+$), respectively. All of these three compounds were assigned to be 3″-*O*-acylated orientin derivatives for their ^1H NMR data, especially the H-3″ for compounds **5** at δ_H 4.84 (1H, t, J = 9.2 Hz), **6** at δ_H 4.88 (1H, t, J = 9.2 Hz), and **7** at δ_H 5.09 (1H, t, J = 8.6 Hz), respectively. Furthermore, the acyls in these three compounds were identified as acetyl for compound **5** (δ_H 1.97 in ^1H and δ_C 169.9 and 21.2 in ^{13}C NMR), 2-methylbutyryl for **6** (δ_C 175.4, 40.4, 26.4, 16.6, and 11.3 in ^{13}C NMR), and veratroyl for **7** (δ_C 165.1, 152.6, 148.2, 123.2, 122.7, 111.8, 111.0, 55.7, and 55.4 in ^{13}C NMR). Finally, HMBC experiments unambiguously determined the structures for compounds **5** as 3″-*O*-acetylorientin (trollichinenside E), **6** as 3″-*O*-(2‴-methylbutyryl)orientin (trollichinenside F), and **7** as 3″-*O*-veratroylorientin (trollichinenside G).

The molecular formula of $C_{32}H_{30}O_{13}$ was give to compound **8**, on the basis of its HRESIMS data (*m/z* 623.1762 $[M+H]^+$). **8** was determined to be an isoswertisin derivative (Wang et al., 2004b) by analyses on ^1H and ^{13}C NMR data. Furthermore, the proton resonance for H-2″ at δ_H 5.43 (1H, t, J = 9.6 Hz) and the NMR data for a feruloyl moiety, as well as detailed NOESY and HMBC analyses, established the structure of compound **8** as 2″-*O*-feruloylisoswertisin, given the trivial name of trollichinenside H.

The molecular formulae of compounds **9** and **10** were established as $C_{29}H_{26}O_{12}$ (HRESIMS *m/z* 567.1485 $[M+H]^+$) and $C_{32}H_{30}O_{14}$ (*m/z* 639.1709 $[M+H]^+$), respectively. These two compounds could be determined to be 2″-*O*-acylated isoswertiajaponin derivatives since the protons of an ABX coupling system on ring-B of flavone skeleton were observed in the ^1H NMR spectra and one methoxyl group signal was observed to display NOESY correlation with the proton singlet of H-6. Furthermore, **9** was determined to be 2″-*O*-benzoylisoswertiajaponin (trollichinenside I) for the proton signals of a benzoyl moiety at δ_H 7.68 (2H, d, J = 7.3 Hz), 7.57 (1H, m), and 7.43 (2H, t, J = 7.7 Hz), and compound **10** to be 2″-*O*-feruloylisoswertiajaponin (trollichinenside J) by NMR analyses.

Although the sugar moiety of all these flavone-8-*C*-glycosides could be readily determined as glucose with a *β*-configuration for the glycosidic bond judged by the ^{13}C NMR data and coupling constant of the anomeric proton, the issue of the absolute configuration of the glucose moiety in these molecules remained unsolved.

There has been one report on the establishment of the glucose in C-glucosides as d-form by oxidative hydrolysis with ferric chloride followed by GC detection (Shimamura et al., 2006). However, a non-consumable approach would be preferred by the chemists focusing on natural products. Around 1980s, Dr. Gaffield reported the application

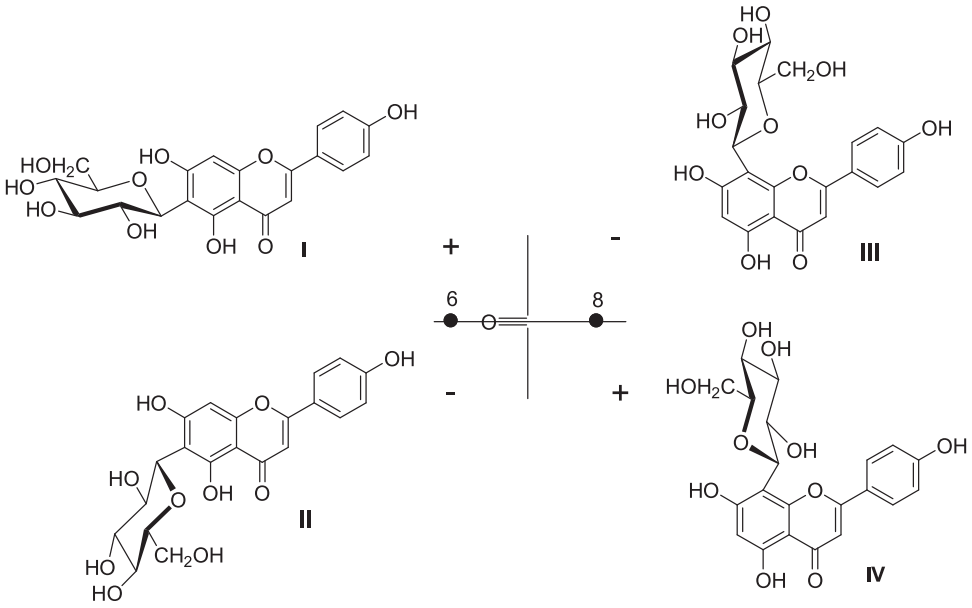

Fig. 2. Rotational conformers of flavone 8-*C*-*β*-D-glucoside and 6-*C*-*β*-D-glucoside and their projections in quadrant sector diagram.

of electronic circular dichroism (ECD) experiment to determine the glycosidation position of flavone 6 or 8-*C*-glycosides according to their Cotton effect at 250–275 nm (Gaffield et al., 1978), and further the absolute configuration of the anomeric carbon of arabinose residues (Gaffield et al., 1984), providing an alternative way to determine the absolute configuration of the glucose moiety in flavone-8-*C*-glucosides.

First of all, the sign of the ECD band (250–275 nm) related to the charge-transfer transition of ring-A and 4-carbonyl (Gaffield et al., 1978) was determined by whichever sector was predominantly occupied by the asymmetric carbon nearest to the glycosidic bond (C-2″) in the glucose moiety attached to C-6 or C-8 of the flavone, in the postulated quadrant viewing from the 4-carbonyl to C-10 (Fig. 2). The second issue to be addressed was the existence of two rotational conformers for flavone 6-*C*-glucosides (conformers I and II in Fig. 2) or 8-*C*-glucosides (III and IV in Fig. 2) with the plane of the sugar moiety nearly perpendicular to ring-A, which should be differentiated by NOESY experiment before the analysis on the ECD data. Finally, the conformer established was put into the quadrant to analyze the sector contribution and correlated with the ECD data.

Supposing that the glucose moiety in compounds **1–10** had the D-configuration resulting in a $^4C_1$ conformation, the observation of the major nuclear Overhauser effects (NOEs) between H-2″, H-4″, and H-6″ of the glucose moiety and H-2′ and H-6′ on ring-B, and minor NOE cross peaks between H-2′ and H-6′ and H-1″, H-3″, and H-5″ in the NOESY spectra of compounds **2**, **3**, **4**, **8**, **9**, and **10**, disclosed that these flavone 8-*C*-*β*-glucosides were mixtures of two rotational conformers with one (III) in predominance. This established conformer (III) with C-2″ in the upper right sector would lead to a negative ECD absorption at 250–275 nm, resembling the ECD data of compounds **8** (Fig. S47), **7** (Fig. S40), and **3** (Fig. S18) with the substitution of acyls at C-2″, C-3″, or C-6″ of the glucose moiety, as well as that of isoswertisin without the substation of acyl (Fig. S61). Therefore, the absolute configuration of the glucose moiety in **1–10** was confirmed to be D-form, for that those with the substitution of L-glucose ($^1C_4$ conformation) and the same NOESY properties would set C-2″ to the lower right sector, resulting in the positive Cotton effect.

In order to verify this proposal, the flavone-6-*C*-glucoside, isoorientin was prepared from orientin isolated from *T. chinensis*, by the Wesley-Moser rearrangement reaction followed by HPLC separation. The glycosidic bond was unambiguously determined to be *β*-form by the coupling constant value of the anomeric proton. Although there was no NOE correlation observed between the protons in the sugar moiety and those on ring-A in the NOESY spectrum, isoorientin was judged to adopt predominantly conformation I (Fig. 2) with reference to the crystal structure reported for swertisin (Ohba et al., 2004). Therefore, D-configuration was assigned to the glucose in isoorientin by the positive Cotton effect at 260–275 nm (Fig. S63).

The results above enriched the structural diversity of flavone-8-*C*-glucosides from *Trollius* genus by reporting firstly the substitution of phenolic acyls at C-3″ of the glucose moiety and that of benzoyl on the sugar residue. Furthermore, it intrigues us to explore that if all of the organic acids in the plant share the possibility to be attached to C-2″, C-3″, and C-6″ of glucose and there is the existence of the substitution of acyls at C-4″. Additionally, ECD experiment was applied for the determination of the absolute configuration of sugar moiety in flavone-8-*C*-glucosides.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. IR spectra were obtained on a Bruker IFS-55 spectrometer with KBr pellets. ECD spectra were obtained on a Bio-Logic MOS-450 spectrometer. Mass spectra were performed on an Agilent 1100 mass spectrometer (ESIMS) and a Bruker micrOTOF-Q spectrometer (HRESIMS). The 1D and 2D NMR spectra were recorded in DMSO-d_6 on a Bruker ARX-300 and AV-600 NMR spectrometers with TMS as an internal standard. Semi-preparative HPLC was carried out using a RP-C_{18} column (YMC ODS-A, 20 × 250 mm, 5 μm), a Shimadzu LC-6AD pump, and a Shimadzu SPD-20A UV detector. The detector wavelength was set at 210 nm. The mobile phase was MeOH-H_2O system with or without 0.1% acetic acid. Silica gel (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (GE Healthcare), and RP-C_{18} (50 μm, YMC Co., Ltd, Kyoto, Japan) were used for column chromatography.

3.2. Plant material

The air-dried flowers of *Trollius Chinensis* Bunge were purchased from Tongrentang drugstore, Shenyang, Liaoning Province, China, in November 2005, and were authenticated by Professor Qi-Shi Sun (Shenyang Pharmaceutical University). A voucher specimen (JLH-

200530) has been deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China.

3.3. Extraction and isolation

The air-dried flowers (5.0 kg) were decocted with water for three times. The extract was concentrated and added to EtOH until the concentration of EtOH was 80% (v/v). After being laid aside overnight at room temperature, the supernatant was evaporated in vacuo to give a brown gum (920 g). Half of the gum was suspended in water (1.5 L), and then partitioned successively with CH_2Cl_2 (3 × 1.5 L), EtOAc (3 × 1.5 L) and *n*-BuOH (3 × 1.5 L). The EtOAc extract (30 g) was subjected to silica gel column chromatography eluted with CH_2Cl_2-MeOH in a gradient (100:5–0:100) to afford four fractions (Fr. E1-Fr. E4). The fraction Fr. E1 (15 g) was chromatographed over silica gel using a gradient system of CH_2Cl_2-MeOH (100:0–100:5). The collected fractions were combined on the basis of their TLC characteristics to yield ten fractions (Fr. E1-A to Fr. E1-J). The fraction Fr. E1-E was put on a Sephadex LH-20 column chromatography eluted with MeOH and combined into three fractions (Fr. E1-E1 to Fr. E1–E3), monitored by TLC. Subfraction Fr. E1–E2 was separated by semi-preparative HPLC using MeOH-H_2O (61:39, 6.0 mL/min) as the mobile phase to afford compounds of 3″-*O*-(2‴-methylbutyryl)isoswertisin (41.1 mg, 37 min) and 2″-*O*-(2‴-methylbutyryl)isoswertisin (176.9 mg, 45 min). The fraction Fr. E1-F was isolated by semi-preparative HPLC using MeOH-H_2O (47:53, 6.0 mL/min) to give Fr. E1-F1 fraction, which was further purified by a preparative TLC with solvent system of CH_2Cl_2-MeOH (8:1) to provide trollisin I (379.7 mg). The fraction E2 (11 g) was subjected to passage over a silica gel column eluted with CH_2Cl_2-MeOH in a gradient (100:0–100:10) to give ten fractions (Fr. E2-A to Fr. E2-J). Subfraction Fr. E2-C was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH-H_2O (0:1–9:1) to give three subfractions (Fr. E2-C1 to Fr. E2-C3). Subfraction Fr. E2-C2 was separated over Sephadex LH-20 eluted with MeOH, to give 2″-*O*-(2‴-methylbutyryl)vitexin (468.2 mg). Subfraction Fr. E2-C3 was separated by semi-preparative HPLC, using MeOH-H_2O (59:41, containing 0.1% acetic acid, 3.0 mL/min) as the mobile phase, to give **7** (7.4 mg, 27.72 min), **1** (17.0 mg, 33.47 min), **6** (13.7 mg, 37.25 min), trollisin II (53.5 mg, 45.55 min), 3″-*O*-(2‴-methylbutyryl)vitexin (10.8 mg, 49.96 min), **8** (46.8 mg, 53.18 min), **9** (22.4 mg, 65.33 min), **3** (8.2 mg, 88.57 min). Subfraction Fr. E2-D was separated over ODS, using MeOH-H_2O (0:1–9:1) to yield three subfractions (Fr. E2-D1 to Fr. E2-D3). Subfraction Fr. E1-D1 was separated by semi-preparative HPLC on an ODS column eluted with MeOH-H_2O (52:48, containing 0.1% acetic acid, 3.0 mL/min) as the mobile phase, and then every fraction was put on Sephadex LH-20 eluted with MeOH for further purification, to give **5** (5 mg, 27.96 min), 2″-*O*-acetylorientin (20.9 mg, 31.44 min), **4** (9.3 mg, 39.82 min), 6″-*O*-acetylorientin (29.8 mg, 43.72 min), 2″-*O*-(4‴-hyroxybenzoyl)vitexin (9.6 mg, 46.85 min), 2″-*O*-vanilloylvitexin (27.2 mg, 49.13 min), 2″-*O*-(2‴-methylbutyryl)orientin (64.9 mg, 67.25 min). Subfraction Fr. E2-D2 was put on a Sephadex LH-20 and then separated by a preparative TLC with a solvent system of CH_2Cl_2-MeOH-HAc (5:1:0.1) to provide compounds 2″-*O*-veratroylorientin (61.9 mg) and 2″-*O*-veratroylvitexin (30.7 mg). Subfraction Fr. E2-D3 was separated by semi-preparative HPLC using MeOH-H_2O (57:43, containing 0.1% acetic acid, 3.0 mL/min) as the mobile phase to give **2** (6.5 mg,

Table 1
^{1}H NMR data of compounds **1–5** in DMSO-d_6.

	1[a] δ_H (*J* in Hz)	**2**[b] δ_H (*J* in Hz)	**3**[a] δ_H (*J* in Hz)	**4**[a] δ_H (*J* in Hz)	**5**[a] δ_H (*J* in Hz)
2					
3	6.79 s	6.63 s	6.74 s	6.65 s	6.64 s
4					
5					
6	6.25 s	6.09 s	6.17 s	6.07 s	6.26 s
7					
8					
9					
10					
1′					
2′	8.06 d (8.5)	8.00 d (8.7)	7.94 d (8.7)	7.55 d (2.2)	7.47 brs
3′	6.94 d (8.5)	6.91 d (8.7)	6.89 d (8.7)		
4′					
5′	6.94 d (8.5)	6.91 d (8.7)	6.89 d (8.7)	6.89 d (8.4)	6.87 d (8.0)
6′	8.06 d (8.5)	8.00 d (8.7)	7.94 d (8.7)	7.62 dd (8.4, 2.2)	7.53 brd (8.0)
5-OH	13.16 brs	13.12 brs	13.17 brs	13.10 brs	13.16 brs
7-OCH_3					
1″	4.86 d (9.8)	4.85 d (9.4)	4.81 d (9.8)	5.00 d (10.1)	4.77 d (9.8)
2″	4.24 m	4.10 m	4.04 m	5.49 t (9.7)	3.98 t (9.5)
3″	5.10 t (9.6)	5.01 t (9.2)	3.63 m	3.61 m	4.84 t (9.2)
4″	3.74 m	3.62 m	3.45 m	3.51 t (9.4)	3.55 t (9.3)
5″	3.40 m	3.38 m	3.34 m	3.40 m	3.36 m
6″	3.76 m	3.76 m	4.52 d (11.5)	3.85 brd (10.7)	3.78 brd (11.0)
	3.59 m	3.59 m	4.33 m	3.64 m	3.61 dd (11.0, 5.7)
1‴					
2‴	7.37 brs	7.28 brs	7.32 d (1.9)	7.19 d (1.9)	1.97 s
3‴					
4‴					
5‴	7.03 d (8.3)	6.75 d (8.1)	6.82 d (8.4)	6.74 d (8.3)	
6‴	7.50 brd (8.3)	7.06 brd (8.1)	7.40 dd (8.4, 1.9)	7.23 dd (8.3, 1.9)	
7‴		7.48 d (15.9)			
8‴		6.46 d (15.9)			
9‴					
3‴-OCH_3	3.71 s	3.77 s	3.69 s	3.74 s	
4‴-OCH_3	3.79 s		3.77 s		

[a] Data collected at 600 MHz.
[b] Data collected at 300 MHz.

35.00 min) and **10** (21.8 mg, 48.17 min).

3.3.1. Trollichinenside A (1)

Yellow powder; $[\alpha]_D^{20}$ −98 (*c* 0.02, MeOH); IR (KBr) ν_{max} 3414, 2978, 2841, 1651, 1452, 1407 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 597.1601 $[M+H]^+$ (calcd. for $C_{30}H_{29}O_{13}$, 597.1603), 619.1423 $[M+Na]^+$ (calcd. for $C_{30}H_{28}O_{13}Na$, 619.1422).

3.3.2. Trollichinenside B (2)

Yellow powder; $[\alpha]_D^{20}$ −101 (*c* 0.03, MeOH); ^1H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 609.1601 $[M+H]^+$ (calcd. for $C_{31}H_{29}O_{13}$, 609.1603).

3.3.3. Trollichinenside C (3)

Yellow powder; $[\alpha]_D^{20}$ −47 (*c* 0.02, MeOH); ECD (MeOH): 265 (−2.5); IR (KBr) ν_{max} 3438, 2974, 1755, 1654, 1604, 1513, 1452, 1383, 1271, 1180 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 597.1600 $[M+H]^+$ (calcd. for $C_{30}H_{29}O_{13}$, 597.1603).

3.3.4. Trollichinenside D (4)

Yellow powder; $[\alpha]_D^{20}$ −81 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3378, 2970, 1656, 1606, 1515, 1372, 1282 cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 1 and 3; (+)-ESIMS *m/z* 599.2 $[M+H]^+$, 621.1 $[M+Na]^+$, 431.1 $[M+H-$ vanillic acid$]^+$; (–)-ESIMS *m/z* 597.0 $[M-H]^-$, 428.9 $[M-H-$ vanillic acid$]^-$; HRESIMS *m/z* 599.1390 $[M+H]^+$ (calcd. for $C_{29}H_{27}O_{14}$, 599.1395).

3.3.5. Trollichinenside E (5)

Yellow powder; $[\alpha]_D^{20}$ −22 (*c* 0.13, MeOH); IR (KBr) ν_{max} 3353, 1725, 1662, 1611, 1579, 1516, 1434, 1366, 1253 cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 1 and 3; (−)-ESIMS *m/z* 489.0 $[M-H]^-$, 428.9 $[M-H-$ acetic acid$]^-$; HRESIMS *m/z* 491.1188 $[M+H]^+$ (calcd. for $C_{23}H_{23}O_{12}$, 491.1184).

3.3.6. Trollichinenside F (6)

Yellow powder; $[\alpha]_D^{20}$ −48 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3438, 2975, 1653, 1514, 1364, 1262, 1191 cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 533.1653 $[M+H]^+$ (calcd. for $C_{26}H_{29}O_{12}$, 533.1654).

3.3.7. Trollichinenside G (7)

Yellow powder; $[\alpha]_D^{20}$ −173 (*c* 0.01, MeOH); ECD (MeOH): 271 (−3.0). IR (KBr) ν_{max} 3438, 2978, 2841, 1750, 1644, 1516, 1451, 1382, 1273, 1180 cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 613.1545 $[M+H]^+$ (calcd. for $C_{30}H_{29}O_{14}$, 613.1552).

3.3.8. Trollichinenside H (8)

Yellow powder; $[\alpha]_D^{20}$ −212 (*c* 0.10, MeOH); ECD (MeOH): 261 (−5.2), 279 (−4.8). IR (KBr) ν_{max} 3414, 2977, 1753, 1654, 1602,

Table 2
^1H NMR data of compounds **6–10** in DMSO-d_6.

	6[a] δ_H (*J* in Hz)	**7**[a] δ_H (*J* in Hz)	**8**[a] δ_H (*J* in Hz)	**9**[a] δ_H (*J* in Hz)	**10**[b] δ_H (*J* in Hz)
2					
3	6.51 s	6.66 s	6.86 s	6.73 s	6.72 s
4					
5					
6	6.12 s	6.26 s	6.42 s	6.30 s	6.40 s
7					
8					
9					
10					
1′					
2′	7.41 brs	7.49 brs	8.13 d (8.8)	7.58 brs	7.57 brs
3′			6.94 d (8.8)		
4′					
5′	6.81 d (7.9)	6.92 d (8.4)	6.94 d (8.8)	6.92 d (8.4)	6.90 d (8.4)
6′	7.48 brd (7.9)	7.58 brd (8.4)	8.13 d (8.8)	7.66 dd (8.4, 2.0)	7.63 brd (8.4)
5-OH	13.19 brs	13.19 brs	13.32 brs	13.28 brs	13.32 brs
7-OCH_3			3.83 s	3.76 s	3.83 s
1″	4.81 d (9.3)	4.85 d (9.8)	4.96 d (10.1)	5.05 d (10.1)	4.95 d (10.1)
2″	4.01 m	4.27 t (9.3)	5.43 t (9.6)	5.51 t (9.5)	5.42 t (9.4)
3″	4.88 t (9.2)	5.09 t (8.6)	3.59 m	3.65 m	3.57 m
4″	3.52 t (9.4)	3.73 m	3.54 t (9.2)	3.55 t (9.4)	3.51 m
5″	3.36 m	3.40 m	3.38 m	3.42 m	3.37 m
6″	3.76 m	3.76 m	3.82 m	3.87 dd (13.0, 10.9)	3.85 m
	3.58 dd (11.8, 5.9)	3.57 m	3.61 m	3.66 m	3.64 m
1‴					
2‴	2.32 m	7.39 brs	7.21 d (1.7)	7.68 d (7.3)	7.20 brs
3‴	1.55 m			7.43 t (7.7)	
	1.35 m				
4‴	0.80 t (6.7)			7.57 m	
5‴	1.05 d, (6.7)	7.03 d (8.4)	6.75 d (8.2)	7.43 t (7.7)	6.75 d (8.2)
6‴		7.53 brd (8.4)	7.02 dd (8.2, 1.7)	7.68 d (7.3)	7.01 brd (8.2)
7‴			7.27 d (15.9)		7.26 d (15.9)
8‴			6.17 d (15.9)		6.16 d (15.9)
9‴					
3‴-OCH_3		3.71 s	3.77 s		3.77 s
4‴-OCH_3		3.79 s			

[a] Data collected at 600 MHz.
[b] Data collected at 300 MHz.

Table 3
^{13}C NMR data of compounds **1–10** in DMSO-d_6.

	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]	**10**[b]
2	164.0	164.0	163.3	164.2	164.1	163.8	164.6	164.6	164.7	164.7
3	102.6	102.3	102.4	102.4	102.6	102.1	102.5	102.5	102.5	102.4
4	182.1	182.0	181.7	181.9	182.0	181.6	182.0	182.3	182.2	182.2
5	160.7	160.6	160.6	160.6	160.6	160.7	160.7	161.7	161.7	161.7
6	98.4	98.8	98.5	97.8	98.2	98.8	98.3	94.9	94.7	94.8
7	163.2	163.2	163.3	162.5	162.8	163.8	163.1	162.9	162.7	162.8
8	103.8	104.0	104.2	102.4	103.7	103.8	103.7	103.6	103.2	103.5
9	156.2	156.4	156.2	156.3	156.0	156.3	156.2	155.7	155.6	155.7
10	104.0	104.0	104.2	103.7	104.0	103.8	103.9	104.4	104.3	104.4
1′	121.7	121.8	121.5	122.0	122.0	121.4	121.9	121.5	121.8	121.8
2′	129.1	128.8	128.4	114.1	114.0	113.6	114.0	129.3	114.2	114.2
3′	115.9	116.0	116.0	145.9	145.9	146.2	146.0	116.0	146.0	146.0
4′	161.4	161.3	161.3	149.8	149.7	150.7	149.9	161.5	150.1	150.2
5′	115.9	116.0	116.0	115.7	115.8	115.8	115.6	116.0	115.8	115.9
6′	129.1	128.8	128.4	119.5	119.4	119.3	119.4	129.3	119.7	119.7
7-OCH_3								56.8	56.6	56.8
1″	73.6	74.0	73.8	71.1	73.5	73.8	73.5	71.0	70.9	71.0
2″	68.8	68.9	70.9	72.5	68.5	68.6	68.7	72.3	73.2	72.2
3″	80.4	79.7	78.6	76.1	79.9	79.4	80.4	75.8	75.9	75.9
4″	68.6	68.5	70.7	70.8	68.5	68.6	68.8	70.6	70.7	70.8
5″	81.6	81.6	78.6	82.3	81.8	81.9	81.8	82.2	82.5	82.4
6″	60.8	60.8	64.7	61.4	61.1	61.3	61.2	61.0	61.3	61.4
1‴	122.7	125.7	121.8	120.8	169.9	175.4	122.7	125.5	129.7	125.5
2‴	111.8	110.9	111.7	112.5	21.2	40.4	111.8	111.1	128.9	111.2
3‴	148.2	148.0	148.3	147.1		26.4	148.2	148.0	128.6	148.0
4‴	152.7	149.3	152.8	151.2		11.3	152.6	149.4	133.2	149.4
5‴	111.0	115.4	110.8	114.9		16.6	111.0	115.6	128.6	115.6
6‴	123.2	123.2	123.2	123.3			123.2	123.1	128.9	123.0
7‴	165.1	144.6	165.6	164.7			165.1	144.8	164.8	144.7
8‴		115.4						114.2		114.2
9‴		166.5						165.6		165.5
3‴-OCH_3	55.4	55.7	55.4	55.6			55.4	55.7		55.7
4‴-OCH_3	55.7		55.6				55.7			

[a] Data collected at 150 MHz.
[b] Data collected at 75 MHz.

1514, 1448, 1368, 1336, 1275, 1248 cm^{-1}. ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 623.1762 $[M+H]^+$ (calcd. for $C_{32}H_{31}O_{13}$, 623.1759).

3.3.9. Trollichinenside I (9)

Yellow powder; $[\alpha]_D^{20}$ −125 (*c* 0.02, MeOH); IR (KBr) ν_{max} 3438, 2981, 1752, 1645, 1450, 1366, 1268, 1206, 1126 cm^{-1}. ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectroscopic data, see Tables 2 and 3; HRESIMS *m/z* 567.1485 $[M+H]^+$ (calcd. for $C_{29}H_{27}O_{12}$, 567.1497).

3.3.10. Trollichinenside J (10)

Yellow powder; $[\alpha]_D^{20}$ −99 (*c* 0.53, MeOH); IR (KBr) ν_{max} 3406, 2923, 1737, 1703, 1653, 1638, 1598, 1455, 1352, 1253 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (DMSO-d_6, 75 MHz) spectroscopic data, see Tables 2 and 3; (+)-ESIMS *m/z* 639.2 $[M+H]^+$, 661.1 $[M+Na]^+$; (–)-ESIMS *m/z* 637.0 $[M-H]^-$; HRESIMS *m/z* 639.1709 $[M+H]^+$ (calcd. for $C_{32}H_{31}O_{14}$, 639.1708).

3.3.11. Isoswertisin

^{1}H NMR (DMSO-d_6, 300 MHz): δ_H 13.36 (1H, brs, 5-OH), 10.32 (1H, brs, 4′-OH), 8.04 (2H, d, *J* = 8.7 Hz, H-2′, 6′), 6.90 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 6.83 (1H, s, H-3), 6.52 (1H, s, H-6), 4.72 (1H, d, *J* = 9.6 Hz, H-1″), 3.88 (3H, s, 7-OCH_3). ECD (MeOH): 266 (– 4.6).

3.4. Preparation and identification of isoorientin

Orientin of 50 mg was suspended in 50 mL 6% aqueous hydrochloride solution which was refluxed for 7 hs. The reactant solution was neutralized by sodium carbonate to pH = 7 and then partitioned with *n*-butanol to give a yellow powder, which was separated by semi-preparative HPLC on a RP-C_{18} column (YMC ODS-A, 20 × 250 mm, 5 μm) eluted with MeOH-H_2O (44:56, containing 0.1% acetic acid, 3.0 mL/min) as the mobile phase to give orientin (19.0 mg, 36.62 min) and isoorientin (14.3 mg, 39.23 min). (–)-ESIMS: 447.0 $[M-H]^-$; ^{1}H NMR (DMSO-d_6, 300 MHz): δ_H 13.60 (1H, brs, 5-OH), 7.33 (1H, brd, *J* = 8.4 Hz, H-6′), 7.29 (1H, brs, H-2′), 6.75 (1H, d, *J* = 8.4 Hz, H-5′), 6.47 (1H, s, H-3), 6.25 (1H, s, H-8), 4.55 (1H, d, *J* = 9.9 Hz, H-1″), 4.06 (1H, t, *J* = 8.9 Hz, H-2″), 3.66 (1H, d, *J* = 11.2 Hz, H-6″a), 3.41 (1H, brd, *J* = 11.2 Hz, H-6″b), 3.10–3.20 (3H, H-3″, 4″, 5″). ECD (MeOH): 270 (+1.2).

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.phytol.2018.04.019.

References

Brum-Bousquet, M., Tillequin, F., Paris, R.R., 1977. The C-flavonosides of *Sarothamnus scoparius*. Isolation of a new compound, the 6″-O-acetylscoparoside. Lloydia 40, 591–592.

Cai, S.Q., Wang, R.F., Yang, X.W., Shang, M.Y., Ma, C.M., Shoyama, Y., 2006. Antiviral flavonoid-type *C*-glycosides from the flowers of *Trollius chinensis*. Chem. Biodivers. 3, 343–348.

Gaffield, W., Horowitz, R.M., Gentili, B., Chopin, J., Bouillant, M.L., 1978. Circular dichroism of C-glycosylflavones, a chiroptical method for differentiating 6-C-, 8-C- and 6, 8-di-C-β-glycosyl isomers. Tetrahedron 34, 3089–3096.

Gaffield, W., Besson, E., Chopin, J., 1984. Effect of conformation and configuration of

arabinose residues on the circular dichroism of *C*-glycosylflavones. Phytochemistry 23, 1317–1322.
Hori, K., Satake, T., Yamaguchi, H., Saiki, Y., Murakami, T., Chen, C.M., 1987. Chemical and chemotaxonomical studies of filices. LXXII. Chemical studies on the constituents of *Odontosoria gymnogrammoides* Christ. Yakugaku Zasshi 107, 774–779.
Kato, T., Morita, Y., 1990. C-glycosylflavones with acetyl substitution from *Rumex acetosa* L. Chem. Pharm. Bull. 38, 2277–2280.
Li, Z.L., Li, D.Y., Hua, H.M., Chen, X.H., Kim, C.S., 2009. Three new acylated flavone *C*-glycosides from the flowers of *Trollius chinensis*. J. Asian Nat. Prod. Res. 11, 426–432.
Liu, Z.Y., Luo, D.Q., 2010. Chemical constituents of *Trollius chinensis*. Chin. Tradit. Herb. Drugs 41, 370–373.
Ohba, S., Yoshida, K., Kondo, T., 2004. Swertisin dihydrate. Acta Cryst. C60, 893–896.
Shimamura, N., Miyase, T., Umehara, K., Warashina, T., Fujii, S., 2006. Phytoestrogens from *Aspalathus linearis*. Biol. Pharm. Bull. 29, 1271–1274.
Wang, R.F., Yang, X.W., Ma, C.M., Cai, S.Q., Li, J.N., Shoyama, Y., 2004a. A bioactive alkaloid from the flowers of *Trollius chinensis*. Heterocycles 63, 1443–1448.
Wang, R.F., Yang, X.W., Ma, C.M., Liu, H.Y., Shang, M.Y., Zhang, Q.Y., Cai, S.Q., Park, J.H., 2004b. Trollioside, a new compound from the flowers of *Trollius chinensis*. J. Asian Nat. Prod. Res. 6, 139–144.
Wu, X.A., Zhao, Y.M., Yu, N.J., 2006. Flavone c-glycosides from *Trollius ledebouri* reichb. J. Asian Nat. Prod. Res. 8, 541–544.
Wu, X.A., Zhao, Y.M., Zhu, J., Wu, J., Zhu, S.Q., Xu, G.S., 2009. Acylated flavone *C*-glycosides from *Trollius ledebouri* Reichb. Nat. Prod. Res. Dev. 21, 1–5.
Wu, L.Z., Wu, H.F., Xu, X.D., Yang, J.S., 2011a. Two new flavone *C*-glycosides from *Trollius ledebourii*. Chem. Pharm. Bull. 59, 1393–1395.
Wu, L.Z., Xu, X.D., Yang, J.S., 2011b. Chemical constituents of water soluble fraction of *Trollius ledebouri* Reichb. Chin. Pharm. J. 46, 745–747.
Zou, J.H., Yang, J.S., Zhou, L., 2004. Acylated flavone *C*-glycosides from *Trollius ledebouri*. J. Nat. Prod. 67, 664–667.
Zou, J.H., Yang, J.S., Dong, Y.S., Zhou, L., Lin, G., 2005. Flavone *C*-glycosides from flowers of *Trollius ledebouri*. Phytochemistry 66, 1121–1125.
Zou, J.H., Yang, J.S., Zhou, L., Lin, G., 2006. A new labdane type diterpenoid from *Trollius ledebouri*. Nat. Prod. Res. 20, 1031–1035.