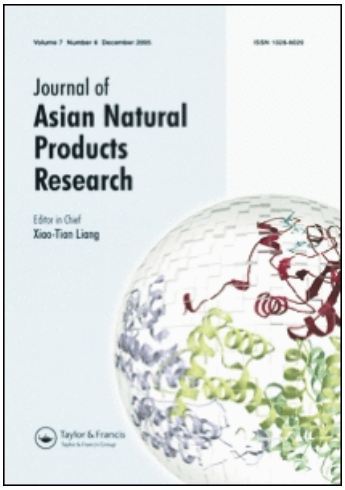

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Flavone c-glycosides from *Trollius ledebouri* reichb

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A new acylated C-glycosylflavone (**1**) was isolated from *Trollius ledebouri* Reichb together with two known C-glycosyflavones (**2**, **3**). The structures were elucidated by spectroscopic methods, including HRMS, IR, ^{1}H and ^{13}C NMR and 2D experiments (COSY, HMQC and HMBC). The anti-inflammatory activities of **1**–**3** were tested on TPA-induced mice ear edema (*in vivo*).

Keywords: *Trollius ledebouri* Reichb; C-glycosylflavone; Anti-inflammation

1. Introduction

The genus *Trollius* comprises about 16 species; three of them grow widely in China [1]. The flowers of *T. chinensis* Bunge and *T. ledebouri* Reich are often used for treating upper respiratory infection, pharyngitis, tonsillitis and bronchitis in Chinese folk medicine [2], and have been reported to possess antibacterial and antiviral activities [3–4]. Clinical studies using *T. chinensis* in the treatment of upper respiratory infections showed a combined efficacy of 92.7% [2]. Organic acids and flavonoids [5–10], especially flavone C-glycosides [11], have been isolated from this genus previously. Here, we report the isolation and structural elucidation of a new acylated C-glycosylflavone and two known compounds from *T. ledebouri*, along with their *in vivo* anti-inflammatory activities.

2. Results and discussion

The individual ethanol extract of the flower of *T. ledebouri* was successively treated with petroleum ether, chloroform, ethyl acetate and n-butanol. The ethyl acetate soluble portion was subjected to repeated chromatography alternating between polyamide and Sephadex LH-20 using chloroform–methanol gradients. A new acylated C-glycosylflavone (**1**) and two known C-glycosylflavones (**2**, **3**) were isolated and purified from this portion.

Compound **1**, obtained as a yellow powder, has the molecular formula $C_{27}H_{30}O_{12}$ determined by HREIMS (*m*/*z* 546.1733 [M^{+}]). It showed positive reactions with HCl/Mg and

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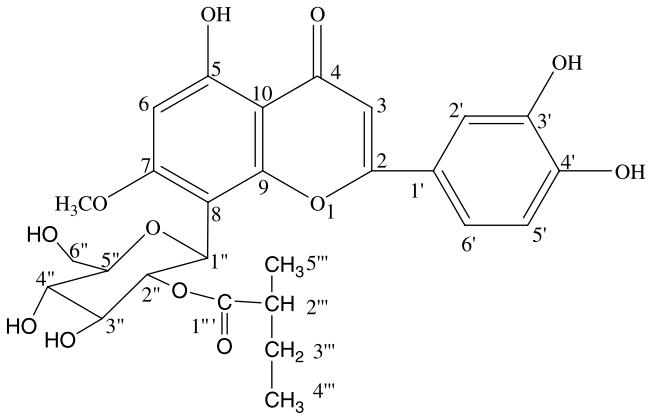
$AlCl_3$/EtOH. The UV spectrum of **1** displayed maximum absorptions at 331 nm and 290 nm, indicating that **1** is a flavone derivative. A free 5-OH was confirmed by the bathochromic shift in the presence of $AlCl_3$. The IR spectrum showed the presence of hydroxyl (3409 cm^{-1}), conjugated and 5-OH chelated ketone (1623 cm^{-1}) and aromatic rings (1504 cm^{-1}). The ^{1}H NMR spectrum of **1** (table 1) indicated the presence of an ABX system, due to the protons H-2′ (δ 7.54, d, $J = 2.2$ Hz), H-6′ (δ 7.63, dd, $J = 2.2$, 8.4 Hz), H-5′ (δ 6.91, d, $J = 8.4$ Hz) of a 3′, 4′-dihydroxyphenyl moiety, and two singlets at δ 6.71, 6.49, which were considered to be the H-3 signal in C-ring and the H-6 signal in 5, 7, 8 tri-substituted A-ring of a flavone, respectively. The ^{13}C NMR (table 1) showed, in addition to the 15 aglycone carbon signals, six sugar carbon signals, a carbon signal at 56.4 ascribable to a methoxyl, and another five signals (δ 174.3, 40.1, 25.6, 11.0, 16.3) apparently due to an acyl group. The sugar moiety was determined to be β-glucose from ^{1}H and ^{13}C NMR data. The resonances of the glucosyl residue were assigned from H-HCOSY, HMQC and HMBC data using the anomeric proton at δ 4.86 (d, $J = 9.8$ Hz) as a starting point. The carbon signals of the glucosyl at δ 82.1, 75.6, 71.4, 70.6, 70.4 and 61.1 suggested that **1** is a flavone C-glycoside. The HMBC spectral analysis (figure 1) revealed the correlation peaks between the glucosyl anomeric proton H-1″ (δ 4.86, d, $J = 9.8$ Hz) with the carbon signals at δ 103.1 (C-8), 162.6 (C-7), and 155.5 (C-9), therefore, the connection of the sugar moiety with C-8 of the aglycon was confirmed. The proton signal (3H, δ 3.86, s) of the methoxyl group exhibited HMBC correlation with the carbon signal (δ 162.6) of C-7, indicating that the methoxyl group is attached to the C-7 position. The ^{13}C NMR signals ascribable to an acyl group, namely, the carbonyl signal at δ 174.3 (C-1‴) and four aliphatic carbon signals at δ 16.3 (C-5‴), δ 11.0 (C-4‴), δ 40.1 (C-2‴), δ 25.6 (C-3‴), together with the aliphatic ^{1}H NMR signals of a doublet at δ 0.68 (3H, dd, $J = 7.0$ Hz), a triplet at δ 0.57 (3H, t, $J = 7.5$Hz) and two multiplets at δ 2.0 (1H, m) and δ 1.2 (2H, m), indicated the presence of a 2-methylbutyryl group. The EIMS of **1** gave a major fragment ion at *m/z* 461, which was in accordance with cleavage of the ester bond of a 2-methylbutyryl moiety. Finally, the position of the acyl group was determined at C-2″ of the sugar moiety by the long-range correlation between the proton at 5.35 (H-2″, $J = 9.7$ Hz) and the carbonyl signal at δ 174.3 (C-1‴) from the HMBC spectrum. Based upon the above observations, the structure of compound **1** was established

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (300 MHz) spectral data of **1** (DMSO-d_6).

Position	δ_H *(J in Hz)*	δ_C	*Position*	δ_H *(J in Hz)*	δ_C
2		164.4	6′	7.63 dd (2.2, 8.4)	119.5
3	6.71 s	102.3	1″	4.86 d (9.8)	70.6
4		182.0	2″	5.35 t (9.8)	71.4
5		161.6	3″	3.3–4.0 m	75.6
6	6.49 s	94.4	4″	3.3–4.0 m	70.4
7		162.6	5″	3.3–4.0 m	82.1
8		103.1	6″	3.3–4.0 m	61.8
9		155.5	1‴		174.3
10		104.1	2‴	2.0 m	40.1
1′		121.7	3‴	1.2 m	25.6
2′	7.54 d (2.2)	114.0	4‴	0.58 t (7.5)	11.0
3′		145.7	5‴	0.68 d (7.0)	16.3
4′		149.7	OCH_3-7	3.86 s	56.4
5′	6.91 d (8.4)	115.6			

Note: assignments based on HMQC and HMBC experiments.



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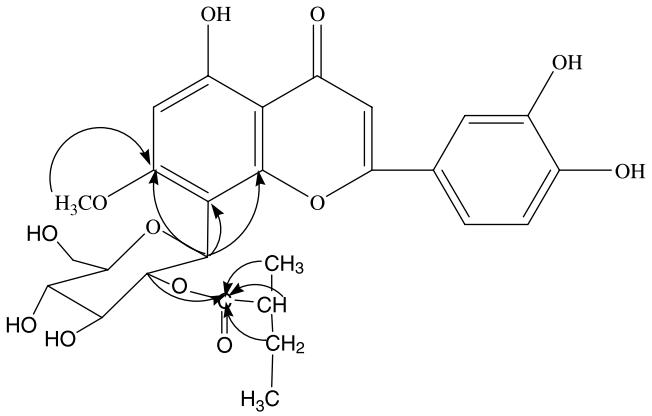

Figure 1. Selected HMBC correlations of **1**.

as 7-methoxyl, 2″-*O*-(2‴-methylbutyryl) orientin, which is a novel acylated flavone C-glycoside.

The known compounds **2** and **3** were identified to be 2″-*O*-(2‴-methylbutyryl) isoswertisin and 2″-*O*-(3‴, 4‴-dimethoxybenzoyl) vitexin by comparing the spectroscopic results with published data [11].

Compounds **1**, **2** and **3** (10 mg/kg) showed significant anti-inflammatory effects on TPA-induced ear edema with inhibitory rates of 58.6%, 35.5%, and 27.6%, respectively. Leigongtengduotai (10 mg/kg), an anti-inflammatory herbal medicine used in China, was used as positive control in the assays and exhibited 47.8% reduction of the edematous response.

3. Experimental

3.1 General experimental procedures

Melting points were determined with X4 melting point apparatus (uncorrected, Beijing science and technology company). Optical rotations were measured with a PE-243

spectrometer. UV spectra were measured with a CINTRO-20 spectrometer (Australia). IR spectra were measured with Nicolet Manga spectrometer (American Micronicolet company), NMR spectra were obtained with Varian^{unity} INOVA 600 MHz spectrometer, ESI-MS spectra were obtained with API3000 spectrometer (American ABI), HREI-MS was obtained with Micromass ZabSpec spectrometer (70 eV), polyamide and Sephadex LH-20 were used for column chromatography. TLC analysis was performed on polyamide film (Zhejiang Taizhou Chemical Industry Company; $CHCl_3$:MeOH:HCOOH, 60:20:1, MeOH:HOAc:H_2O, 95:5:5).

3.2 Plant material

The flowers of *Trollius ledebouri* Reichb (Ranunculaceae) were collected in Daxing'anling district in Heilongjiang Province of China, in May 2002, and authenticated by North Medical Corporation of Daxin'anling. A voucher specimen has been deposited at the library of Beijing Institute of pharmacology and toxicology.

3.3 Extraction and isolation

The plant material (2 kg) was extracted with ethanol to afford an EtOH extract (200 g), which was suspended in water, and partitioned successively with petroleum, chloroform, ethyl acetate and n-butanol, respectively. Compounds **1**, **2**, **3** were obtained from the ethyl actetate portion (40 g) by chromatographic isolation on polyamide with a $CHCl_3$–MeOH gradient system (96:4–0:1) and purification on Sephadex LH-20 (60 × 2 cm) with MeOH.

Compound **1**: yellow powder, mp 155–157°C, $[\alpha]_D^{20}$-60 (MeOH, C = 0.06), UV λ_{max}^{MeOH} 331, 290, 233 nm; HREIMS (*m*/*z*): 546.1733 $[M]^+$ (calcd. for $C_{27}H_{30}O_{12}$, 546.1737), 461, 426,366, 329, 247, 195, 85; IR ν_{max}^{KBr} cm^{-1}: 3409, 1623, 1504; ^1H NMR and ^{13}C NMR (see table 1).

3.4 TPA-induced mice ear edema

Anti-inflammatory activity was evaluated by the method described by Xu SY *et al.* [12]. Edema was induced on the right ear of a mouse by topical application of 15 ug/ear of TPA. Groups of 10 female mice were treated *p.o.* with the compounds at doses of 10 mg/kg, dissolved in H_2O. Mice were administrated twice 2 h before the application of TPA and 12 h after the application of TPA. The edema induced by TPA was determined as the increase in weight of the punch biopsies of the right ear compared with those of the left ear and the inhibition rate of the edema was calculated thereout.

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