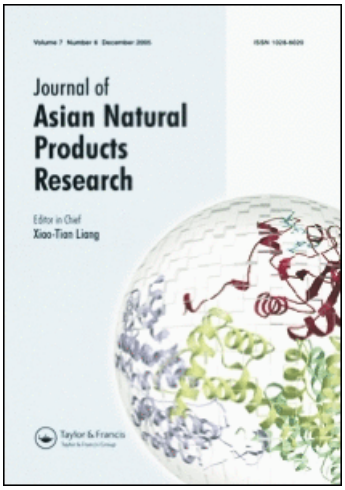

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Wang, Hong-Bing , Zhang, Hua-Ping , Yao, Guang-Min , Wang, Yu-Bo and Qin, Guo-Wei(2007) 'New sesquiterpene and phenolic glucosides from *Saussurea involucrata*', Journal of Asian Natural Products Research, 9: 7, 603 — 607
To link to this Article: DOI: 10.1080/10286020600882528
URL: http://dx.doi.org/10.1080/10286020600882528

Journal of Asian Natural Products Research, Vol. 9, No. 7, October–November 2007, 603–607

New sesquiterpene and phenolic glucosides from *Saussurea involucrata*

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(Received 22 February 2006; revised 11 May 2006; in final form 18 May 2006)

One new guaiane-type sesquiterpene glucoside (**1**) and one new phenolic glucoside (**2**) were isolated from the whole herb of *Saussurea involucrata.* Their structures were established by spectroscopic methods, mainly 1D and 2D NMR, and mass spectral analysis.

Keywords: Sesquiterpene glucoside; Phenolic glucoside; *Saussurea involucrata*

1. Introduction

The genus *Saussurea* (Compositae) contains more than 300 species, distributed in north-temperate zone of the world. China is a main producing area having more than 200 species of the genus. A literature survey showed that *Saussurea* plants contain rich flavonoids, coumarins and sesquiterpene lactones [1]. The species *S. involucrata* is one of the plants used as "snow-lotus flower", a famous Tibetan medicine for treatment of rheumatic arthritis and gynopathy [2]. Several sesquiterpene compounds have been reported from this plant [3]. In our continuing chemical study on the plant we have isolated one new sesquiterpene glucoside (**1**), one new phenolic glucoside (**2**) (figure 1) and three known sesquiterpene compounds which are first found in the plant. This paper describes the isolation and structural elucidation of the two new compounds.

2. Results and discussion

Compound **1**, a colourless gum, exhibited an ion peak at *m/z* 428.1613 in HRESIMS, consistent with a molecular formula of $C_{21}H_{32}O_9$. The IR spectrum exhibited the presence of hydroxyl (3446 cm^{-1}), γ-lactone (1774 cm^{-1}), and double bond (1639 cm^{-1}). The ^1H NMR spectrum (table 1), being characteristic as a guaiane-type sesquiterpenoid [4,5], showed the signals for one methyl at δ 1.15 (3H, d, $J = 6.6$ Hz), one exomethylene at δ 4.91 and 5.08

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020600882528

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of **1**-**2** (ppm, *J* in Hz).

Position	***1***	***1***	*position*	***2***	***2***
1	2.47	50.4	1		111.3
2	1.83, m, 1.70, m	27.1	2		158.7
3	2.35, m, 2.52, m	31.3	3	6.73, d (8.3)	108.3
4		152.9	4	7.25, br t (8.3)	134.5
5	2.67, br t (10.0)	54.1	5	6.58, d (8.3)	112.1
6	4.13, t (10.1)	83.4	6		160.3
7	2.35, m	49.4	7		170.5
8	1.43, m, 2.08, m	26.1	8	3.88, s	56.3
9	1.58, m, 1.91, m	29.6	1′		137.8
10		77.1	2′	7.48, br d (7.4)	129.7
11	2.34, m	45.6	3′	7.36, br t (7.2, 7.7)	130.0
12		182.1	4′	7.30, m	129.7
13	1.15, d (6.6)	13.9	5′	7.36, br t (7.2, 7.7)	130.0
14	3.28, m, 3.80, d (10.3)	78.3	6′	7.48, br d (7.4)	129.7
15	4.91, 5.08 br s	110.5	7′	5.38, s	68.5
1′	4.28, d (7.7)	105.5	1″	4.94, d (7.5)	103.3
2′	3.47 *m*	75.7	2″	3.45 *m*	75.4
3′	3.40 *m*	78.5	3″	3.41 *m*	78.4
4′	3.41 *m*	72.2	4″	3.38 *m*	71.7
5′	3.19 *m*	78.6	5″	3.16 *m*	78.7
6′	3.66 *dd* (12.0,4.8)	63.2	6″	3.65 *dd* (12.0,4.8)	63.0
	3.82 *dd* (12.0,2.4)			3.80 *dd* (12.0,2.4)	

1 and **2** were measured in CD_3OD.

(each 1H, br s), one oxygenated methine at δ 4.13 (1H, t, $J = 10.1$ Hz), and one oxygenated methylene at δ 3.28 (1H, m) and 3.80 (1H, d, $J = 10.3$ Hz). Besides it showed additional signals for a glucose unit (δ 4.28, d, $J = 7.7$ Hz, H-1'; 3.47, 3.40, 3.41, 3.19, each 1H, m; 3.66, dd, $J = 12.0$, 4.8 Hz; 3.82, dd, $J = 12.0$, 2.4 Hz, H_2-6'). Hydrolysis of **1** with β-glucosidase afforded glucose as detected by co-TLC with authentic sample. The ^{13}C NMR spectrum (table 1) exhibited signals for 21 carbons including one CH_3, six CH_2 (one olefinic, one oxygenated), five CH (one oxygenated), three quaternary carbons (one olefinic, one carbonyl, and one oxygenated) and one glucose unit (δ105.5, 75.7, 78.5, 72.2, 78.6, 63.2), confirming that compound **1** was a guaiane-type sesquiterpene glucoside. Further comparative studies revealed that ^{1}H and ^{13}C-NMR data of the aglycone moiety of **1** were very similar with those of 10β, 14-dihydroxy-11βH-guai-4(15)-ene-12, 6α-olide [6], a known sesquiterpene glucoside. In the HMBC spectrum of **1**, the correlation of H-1′ of glucose (δ 4.28) with C-14 (δ 78.3) was observed, suggesting glucosylation was at C-14.

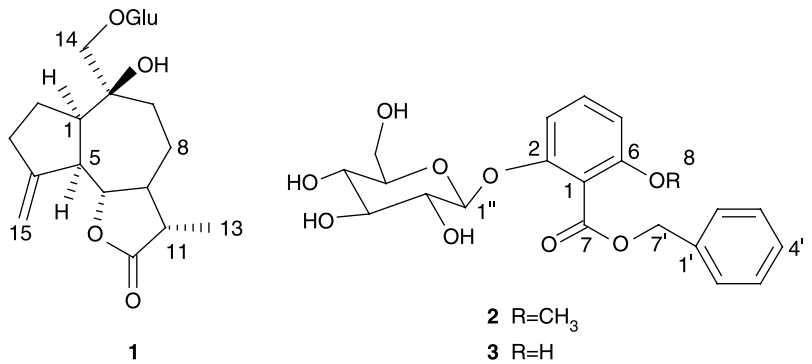
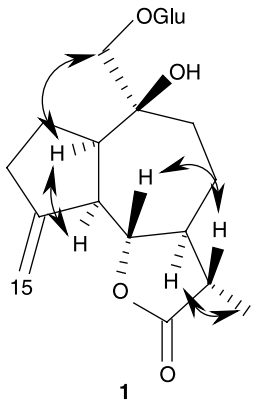


Figure 1. Structure of compounds **1** and **2**.

The β-glucosyl linkage was deduced from coupling constant of the anomeric proton at δ 4.28 ($J = 7.7$ Hz, H-1'). In the ROESY spectrum, the correlation between H-5 and H-1; H-14 and H-1; H-7 and H-13, and H-6 and H-11 were observed, indicating that H-1, 5, 7, 14 were in α-orientation, while H-6, 11 were β-oriented (figure 2). The unambiguous assignments of all protons and carbons were made by ^{1}H-^{1}H COSY, HMBC and HMQC analyses. On the basis of above evidence, **1** was established as 10β, 14 – dihydroxy -11βH – guai - 4 (15) - ene-12, 6α - olide 14 - O - β – D - glucoside.

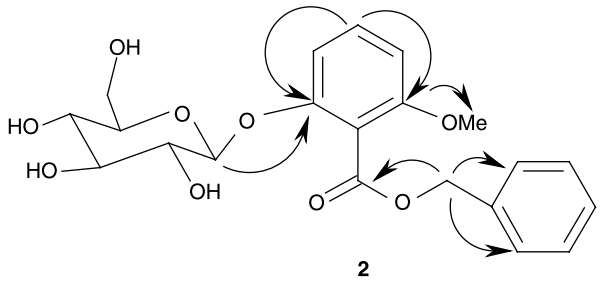
Compound **2**, a white powder, was assigned the molecular formula $C_{21}H_{24}O_9$ by its HRESIMS (*m/z* 443.1162 $[M + Na]^+$). It exhibited an UV absorption maximum at 294 nm, which implied the presence of a highly conjugated system. The IR spectrum showed the presence of carbonyl group (1722 cm^{-1}) and aromatic ring (1602, 1495 cm^{-1}). The ^{1}H NMR spectrum (table 1) showed the presence of one 1, 2, 6-trisubstituted benzene (δ 6.73, d, $J = 8.3$ Hz, H-3; 7.25, br t, $J = 8.3$ Hz, H-4; 6.58, d, $J = 8.3$ Hz, H-5), one ester benzyl (δ 7.48, 2H, br d, $J = 7.4$ Hz, H-2′, 6′; 7.36, 2H, br t, $J = 7.4$, 7.7 Hz, H-3′, 5′; 7.30, 1H, m, H-4′; 5.38, 2H, s, H_2-7′), one methoxyl group (δ 3.88, s) and an additional glucose unit (δ 4.94, 1H, d, $J = 7.5$ Hz, H-1"; 3.45, 3.41, 3.38, 3.16, each 1H, m; 3.65, dd, $J = 12.0$, 4.8 Hz, 3.80, dd, $J = 12.0$, 2.4 Hz, each 1H, H_2-6"). The ^{13}C NMR spectrum (table 1) exhibited signals for 21 carbons arisen from one carbonyl, two aromatic rings, one benzylic, one methoxyl and one glucose unit (δ103.3, 75.4, 78.4, 71.7, 78.7, 63.0). Hydrolysis of **2** with β-glucosidase afforded glucose as detected by co-TLC with authentic sample, which confirmed compound **2** was a phenolic glucoside. The β-glucosyl linkage was deduced from a coupling constant of the anomeric proton at δ 4.28 ($J = 7.5$ Hz). Further studies revealed that ^{1}H- and ^{13}C-NMR data of **2** were very similar with those of a known compound **3** [7], except for one more O-methyl group in **2**. The HMBC correlation (figure 3) indicated that **2** was a C_6-methyl ether of **3** (H_2-7′/C-7; H-1″/C-2; H_3-8/C-6). On all above evidence compound **2** was established as benzyl 2-hydroxy-6-methoxybenzoate 2- O - β – D – glucoside.

Besides compounds **1** and **2**, we have obtained three known sesquiterpenoid and its glucosides, which were first reported from this plant. Their structures were determined by comparison of spectral data with literature values as 3β – hydroxyl – 11βH - 11, 13-dihydrodehydrocostuslactone [8], 3β – hydroxyl − 11βH – 11, 13 – dihydrodehydrocostuslactone 3 – O- β – D – glucoside [8] and 3α, 8α–dihydroxy-11βH – 11, 13 – dihydrodehydrocostuslactone 3 – O- β – D – glucoside [9]. All isolated compounds were



1

Figure 2. Key ROESY correlations of **1**.



2

Figure 3. Key HMBC correlations of **2**.

tested for murine lymphocyte proliferation induced by concanavalin A (Con A) (5 μg/mL) or lipopolysaccharide (LPS)(10μg/mL) at concentration of 10^{-5} to 10^{-7} M, and P-388 cell lines *in vitro* at concentration of 10^{-4} to 10^{-8} M but did not show significant immunomodulatory activities or toxicities.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba Sepa-300 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. NMR spectra were run in CD_3OD on a Bruker AM-400 spectrometer with TMS as internal standard. ESIMS were measured using a Finnigan LCQ-DECA instrument, and HRESIMS data were obtained on Bruker FTMS Apex IIIspectrometer. Column chromatographic separations were carried out on silica gel H-60 (Qingdao Marine Chemical Group Corporation, Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) were used for analytical TLC.

3.2 Plant material

The dried whole plant materials were purchased from Xinjiang Autonomous Region, China. It was identified by Prof. Shao-Qing Cai of College of Pharmacy, Beijing University, Beijing, China. A voucher specimen (No. 02-02-19) is deposited at the Herbarium of College of Pharmacy, Beijing University, Beijing, China.

3.3 Extraction and isolation

The dried whole plants of *Saussurea involucrata* (3.0 kg) were ground and percolated with 95% EtOH (5L × 3) at room temperature. The filtrate was concentrated in vacuum. The residue was added with water and then extracted with petroleum ether, EtOAc, and *n*-BuOH (500ml × 3), successively. The EtOAc extract (50 g) was subjected to column chromatography over silica gel H-60 eluted with $CHCl_3$-MeOH (50:1-5:1) to afford five fractions (A-E). Fraction B was subjected to repeated column chromatography over silica gel H-60 with $CHCl_3$-MeOH (30:1-5:1) and Sephadex LH-20 with MeOH to yield compound **1**

(7 mg). By similar separation procedures mentioned above compound **2** (9 mg) was obtained from fraction C and three known compounds were obtained from fractions D and E, respectively.

10β, 14 – Dihydroxy -11βH – guai - 4 (15) - ene-12, 6α - olide 14 - O - β – D - glucoside **(1)**, $[\alpha]_D^{20}$ + 38.0 (c 0.1, MeOH); IR (KBr) $\nu_{max}(cm^{-1})$ 3446 (OH), 1774 (γ-lactone) and 1639 (double bands). ^1H and ^{13}C NMR data see table 1; ESIMS: *m/z* 429 $[M + H]^+$, HRESIMS: *m/z* 429.1613 $[M + H]^+$ (calcd for $C_{21}H_{33}O_9$, 429.1624).

Benzyl 2-hydroxy-6-methoxybenzoate 2- O - β – D – glucoside **(2)**, $[\alpha]_D^{20}$ -35.0 (c 0.1, MeOH); IR (KBr) $\nu_{max}(cm^{-1})$ 1722 (carbonyl group) and 1602, 1495 (aromatic ring); UV (MeOH) λ_{max}: 294 nm. ^1H and ^{13}C NMR data see table 1; ESIMS: *m/z* 443 $[M + Na]^+$, HRESIMS: *m/z* 443.1162 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_9Na$, 443.1124).

3.4 Hydrolysis of compounds 1 and 2

Compound **1** (3 mg) was suspended in water (8 ml) and incubated with almond β-glucosidase (20 mg) for 24 h at 37 °C. The solution was extracted with $CHCl_3$ to remove aglycone. Produced sugar was remained in water solution, and was detected by HPTLC (Merck) developed with Me_2CO–2 mM NaOAc (17:3, v/v) and sprayed with 0.2% naphthoresorcinol in Me_2CO–3N H_3PO_4 (5:1, v/v) followed by heating at 105 °C for 5 min. D-Glucose (Chemprosa Holding AG) was used as standard. The same method was used to identify the sugar moiety of compound **2**.

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