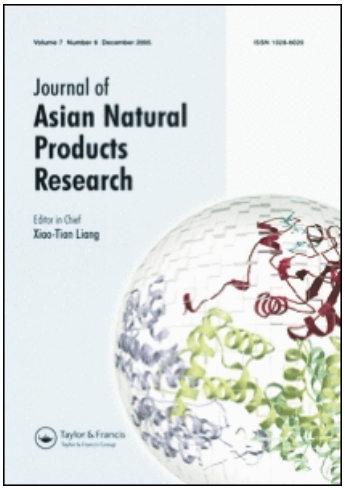

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Chemical constituents from the cell cultures of *Saussurea involucrata*

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ORIGINAL ARTICLE

Chemical constituents from the cell cultures of *Saussurea involucrata*

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The chemical constituents of the cell cultures of *Saussurea involucrata* were systematically investigated, thus a new eudesmane-type sesquiterpene together with 14 known compounds was isolated. Among them, syringin is the main compound with an isolated yield of 0.3%. The new compound was characterized as 11βH-2α-hydroxy-eudesman-4(15)-en-12,8β-olide (**1**) on the basis of extensive spectroscopic data.

Keywords: *Saussurea involucrata*; cell cultures; chemical constituents; sesquiterpene; syringin

1. Introduction

Saussurea involucrata Kar. et Kir. is one of the precious and endangered medicinal herbs in China. Therefore, the study of *in vitro* culture of this species would be necessary. There have been some reports describing the *in vitro* culture of *S. involucrata* [1,2]. Moreover, there have also been some studies of production of the principal constituents in the wild plant from cultures [3,4]. Similar to the wild plant, the extract of the cultures of *S. involucrata* has shown anti-inflammatory and analgesic activities in experimental animals [5]. However, few chemical data of the cultures are available, and there is relative scarcity of definitive evidence to prove the relationships between its main bioactive ingredients and pharmacological activity of *S. involucrata*. In this context, a systematic investigation on the chemical constituents of the cell cultures of *S. involucrata* has been carried out in our laboratory. This paper reports the isolation and structural elucidation of the chemical constituents in the cultures of *S. involucrata*.

2. Results and discussion

Various chromatographic techniques were employed to isolate and purify the 95% ethanol extract of 1.4 kg cultures of *S. involucrata*. Consequently, 15 compounds including 1 new compound and 14 known compounds were characterized by the analyses of spectroscopic data. They were determined as 11βH-2α-hydroxy-eudesman-4(15)-en-12,8β-olide (**1**), β-sitosterol, palmitic acid, hexacosanoic acid, linoleic acid, linoleic acid mono-acylglycerol, 3-*O*-β-D-glucosyl-β-sito-

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Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** (in $CDCl_3$, 600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR).[a]

Position	^{13}C NMR		^{1}H NMR
1	51.0, t	H-1α	1.17 (1H, dd, $J = 12.0, 11.4$ Hz)
		H-1β	1.90 (1H, ddd, $J = 12.0, 4.2, 1.8$ Hz)
2	67.1, d	H-2β	3.85 (1H, m)
3	46.2, t	H-3α	1.99 (1H, dd, $J = 12.0, 11.4$ Hz)
		H-3β	2.68 (1H, ddd, $J = 12.0, 4.8, 1.8$ Hz)
4	146.1, s		
5	46.1, d	H-5α	1.78 (1H, br d, $J = 12.0$ Hz)
6	26.1, t	H-6α	1.75 (1H, m)
		H-6β	1.25 (1H, br q, $J = 12.6$ Hz)
7	42.6, d	H-7α	2.10 (1H, m)
8	77.1, d	H-8α	4.71 (1H, ddd, $J = 4.8, 3.6, 1.8$ Hz)
9	41.2, t	H-9α	1.47 (1H, dd, $J = 15.0, 4.8$ Hz)
		H-9β	2.25 (1H, br d, $J = 15.0$ Hz)
10	34.3, s		
11	44.5, d	H-11β	2.46 (1H, br q, $J = 7.8$ Hz)
12	180.0, s		
13	14.4, q	H-13	1.31 (3H, d, $J = 7.8$ Hz)
14	18.8, q	H-14	0.84 (3H, s)
15	109.1, t	H-15a	4.57 (1H, br s)
		H-15b	4.89 (1H, br s)

Note: [a] Chemical shifts are given in δ (ppm); assignment based on DEPT, NOESY, HMQC, and HMBC experiments.

sterol, 3-*O*-(6′-*O*-linoleoyl-β-D-glucosyl)-β-sitosterol [6], 3-*O*-(6′-*O*-palmitoyl-β-D-glucosyl)-β-sitosterol [7], (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1,3,4-octadecanetriol [8], (2*S*,3*S*,4*R*,11*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-11-octadecene [8], syringin [4], tangshenoside III [9], 1,5-di-*O*-caffeoylquinic acid [10], and benzyl alcohol glycoside [11]. Among them, compound **1** is a new compound, and syringin is the main component, which is about 0.3% (w/w) of the dry cell cultures.

Compound **1** was obtained as colorless needles. EI-MS gave the molecular ion peak at *m/z* 250 $[M]^+$. Its molecular formula was established as $C_{15}H_{22}O_3$ on the basis of the analyses of EI-MS, ^{1}H NMR, ^{13}C NMR and DEPT spectral data, and further confirmed by HR-EI-MS at *m/z* 250.1569 $[M]^+$. The IR spectrum of **1** indicated the presence of hydroxyl (3254 cm^{-1}), carbonyl of lactone (1762 cm^{-1}), and exocyclic methylene (887 cm^{-1}) groups. The ^{1}H NMR spectrum exhibited a methyl singlet signal at δ_H 0.84, a methyl doublet signal at δ_H 1.31 ($J = 7.8$ Hz), and a pair of exomethylene proton signals at δ_H 4.57 (1H, br s) and 4.89 (1H, br s). The ^{13}C NMR spectral data revealed the presence of 15 carbon signals designated to three quaternary carbons, five methines, five methylenes, and two methyl groups. The ^{1}H and ^{13}C NMR spectral data of compound **1** are shown in Table 1. All these data indicated that compound **1** was an eudesmane-type sesquiterpene, and this was further confirmed by HMQC and HMBC spectra (Figure 1). By comparing the NMR spectral data of compound **1** with

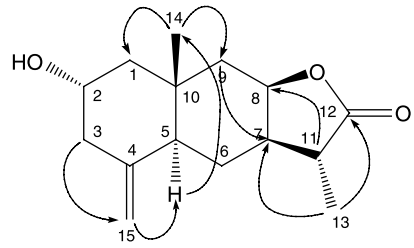
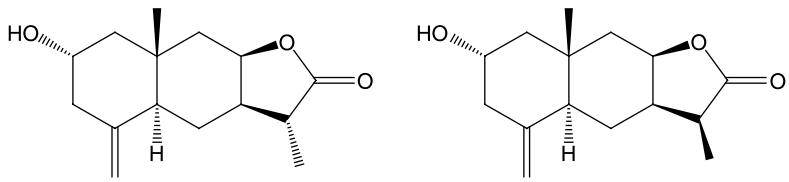


Figure 1. Key HMBC correlations of compound **1**.



1 2

Figure 2. The structures of compounds **1** and **2**.

those of 11αH-2α-hydroxy-eudesman-4(15)-en-12,8β-olide (**2**, Figure 2) [12], the ^{1}H NMR spectral data of these two compounds were similar. In the ^{1}H NMR spectrum of compound **1**, the chemical shifts of H-7 and H-11 were shifted downfield by 0.32 and 0.37 ppm, whereas H-6α, H-6β, H-8α, and H-13 were shifted upfield by 0.08, 0.13, 0.23, and 0.08 ppm, respectively, when compared with compound **2**. These implied that the configuration of H-11 in compound **1** might be different from that in compound **2**. Furthermore, according to the coupling constant value of H-5 (1H, br d, $J = 12.0$ Hz), H-5 was deduced to be an α-configuration. The above deduction was further supported by the NOESY experiment. The NOESY correlations between H-7 and H-5, H-7 and H-13 suggested H-5-, H-7-, and H-13-directed α-orientation. Thus, H-11 had an opposite orientation, namely, β-configuration (Figure 3). Therefore, the structure of **1** was elucidated as 11βH-2α-hydroxy-eudesman-4(15)-en-12,8β-olide.

The other 14 compounds were identified by comparison of their physical and spectral data with those of reported values. The obtained compounds were categorized as sesquiterpene, steroid, alkanoic acid (or alkanoate), ceramide, phenylpropanoid, etc., which have been found in the wild plant. It is notable that flavonoid, one kind constituents of the main and typical in the wild plant, has not been isolated from *in vitro* cell cultures; however, one phenylpropanoid (C_6—C_3 monomer), syringin, was isolated in a high yield (0.3%), 10 times higher than that in the wild plant. These results suggested the loss or incapacity of some enzymes responsible for flavonoid biosynthesis under this cultural condition, and the reinforcement of the phenylpropanoid biosynthesis, since these two types of biosynthesis possess the same precursors [13]. Furthermore, there have also been some reports describing the pharmacological activities of syringin, such as anti-inflammatory and analgesic [14], hypoglycemic [15], antidepressant [16], and antitumor activities [17]. As described before, the extract of the cultures of *S. involucrata* has also shown anti-inflammatory and analgesic activities *in vivo*; therefore, syringin might be one of the main bioactive ingredients in the cultures of *S. involucrata*, and could be produced massively by plant cell culture technology in the future because of its desirable yield in cell cultures.

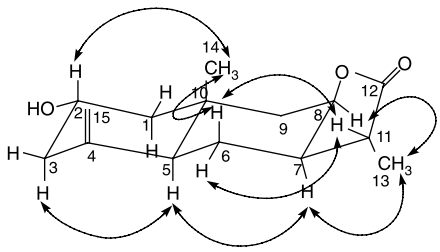


Figure 3. Key NOESY correlations of compound **1**.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined using a PE Model 343 spectrometer. IR spectra

were obtained on a Thermo Nicolet 5700 FT-IR microscope spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury-400 and Varian NMR System-600 spectrometers. Mass spectra were measured on a VGZAB-2F spectrometer. Semi-preparative high-pressure liquid chromatography (HPLC) was performed by silica column (250 mm × 10.0 mm i.d., 5 μ; Apollo Silica, Alltech Co. Ltd, Chicago, IL, USA) or C_{18} column (250 mm × 10.0 mm i.d., 5 μ; Grace Adsorbosphere, W.R. Grace & Co., Columbia, MD, USA). Column chromatography was carried out using macroporous resin (Amberlite XAD16, Rohmhaas Co., Philadelphia, PA, USA), Sephadex LH-20 (Pharmacia, Fine Chemical Co. Ltd, Uppsala, Sweden), and silica gel (200–300 mesh; Qingdao Haiyang Chemical Co. Ltd, Qingdao, China).

3.2 Material

Cell cultures were maintained in Murashige and Skoog [18] basal medium supplemented with 0.5 mg l^{-1} α-naphthalene acetic acid, 0.5 mg l^{-1} 6-benzyl aminopurine, 0.2 mg l^{-1} 2,4-dichlorophenoxyacetic acid, 30 g l^{-1} sucrose, and 5 g l^{-1} agar. The medium was adjusted to pH 5.8 with 1 mol l^{-1} NaOH before autoclaving. The cultivation was performed in 250 ml flasks in the dark at 25 ± 1°C, and subcultured at an interval of 12 days.

3.3 Extraction and isolation

Dried powder (1.4 kg) of the cultures of *S. involucrata* was extracted with 95% ethanol under reflux for 5 h, four times. The combined extract was evaporated to dryness under reduced pressure to give the residue (205 g), which was then suspended in water and extracted with petroleum ether, ethyl acetate, and *n*-butanol successively.

The petroleum ether fraction (40.28 g) was subjected to silica gel column chromatography (200–300 mesh), eluted with a gradient of petroleum ether–acetone (from 100:1 to 2:1), to furnish five fractions (A–E). Fraction E was subjected to silica gel column chromatography (300–400 mesh), further purified by normal phase semi-preparative HPLC, eluted with *n*-hexane–ethyl acetate (1:1) to obtain compound **1**, 11βH-2α-hydroxy-eudesman-4(15)-en-12,8β-olide (1.7 mg). Fractions A–D were further separated by repeated silica gel and Sephadex LH-20 column chromatography to give eight pure compounds, β-sitosterol (340.6 mg), palmitic acid (37.2 mg), hexacosanoic acid (11.2 mg), linoleic acid (21.3 mg), linoleic acid monoacylglycerol (7.0 mg), 3-*O*-(6′-*O*-linoleoyl-β-D-glucosyl)-β-sitosterol (6.7 mg), 3-*O*-(6′-*O*-palmitoyl-β-D-glucosyl)-β-sitosterol (22.7 mg), and (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1,3,4-octadecanetriol (142.5 mg).

The ethyl acetate fraction (30.15 g) was subjected to silica gel column chromatography, eluted with a gradient of $CHCl_3$–CH_3OH (from 50:1 to 4:1), and then further separated by repeated silica gel column chromatography to give two pure compounds, 3-*O*-β-D-glucosyl-β-sitosterol (183.6 mg) and (2*S*,3*S*,4*R*,11*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-11-octadecene (288.4 mg).

The *n*-butanolic fraction (96.74 g) was subjected to macroporous resin column chromatography, and eluted with EtOH–H_2O (0% → 50% → 80% → 100%) to afford four fractions. Fraction 2 eluted by 50% EtOH (23.3 g) was then subjected to silica gel column chromatography, eluted with a gradient of $CHCl_3$–CH_3OH–H_2O (from 20:1:0 to 20:7:1), and then further separated by semi-preparative HPLC and Sephadex LH-20 columns to give four pure compounds, syringin (4025.5 mg), tangshenoside III (17.1 mg), 1,5-di-*O*-caffeoylquinic acid (63.8 mg), and benzyl alcohol glycoside (4.7 mg).

*3.3.1 11βH-2α-Hydroxy-eudesman-4(15)-en-12,8β-olide (**1**)*

Colorless needles, $C_{15}H_{22}O_3$; $[\alpha]_D^{20}$ +0.031 ($c = 0.07$; $CHCl_3$); IR (ν_{max}): 3254, 1762, 887 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data are listed in Table 1; EI-MS (*m/z*): 250 $[M]^+$, 235, 232, 217, HR-EI-MS (*m/z*): 250.1569 $[M]^+$ (calcd for $C_{15}H_{22}O_3$, 250.1569).

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References

[1] B. Guo, M. Gao, and C.Z. Liu, *Plant Cell Rep.* **26**, 261 (2007).
[2] L.Q. Wu, S.X. Guo, and P.G. Xiao, *China J. Chin. Mater. Med.* **30**, 965 (2005).
[3] C.X. Fu, D.X. Zhao, X.F. Xue, Z.P. Jin, and F.S. Ma, *Process Biochem.* **40**, 3789 (2005).
[4] R.D. Chen, J.H. Zou, J.M. Jia, and J.G. Dai, *Acta Pharm. Sin.* **44**, 436 (2009).
[5] J.M. Jia, C.F. Wu, W. Liu, H. Yu, Y. Hao, J.H. Zheng, and Y.R. Ji, *Biol. Pharm. Bull.* **28**, 1612 (2005).
[6] H. Matsuda, M. Tokunaga, H. Iwahashi, S. Naruto, H. Yagi, T. Masuko, and M. Kubo, *Biol. Pharm. Bull.* **28**, 929 (2005).
[7] S. Kadota, N. Lami, Y. Tezuka, and T. Kikuchi, *Chem. Pharm. Bull.* **37**, 3214 (1989).
[8] B.N. Su, R. Misico, E.J. Park, B.D. Santarsiero, A.D. Mesecar, H.H.S. Fong, J.M. Pezzuto, and A.D. Kinghorn, *Tetrahedron* **58**, 3453 (2002).
[9] M.N. Clifford, B. Kellard, and E. Ah-sing, *Phytochemistry* **29**, 1989 (1989).
[10] I. Merfort, *Phytochemistry* **31**, 2111 (1992).
[11] H.M. Hua, X. Li, S.E. Xing, and Y.H. Fei, *Chin. Pharm. J.* **40**, 653 (2005).
[12] G. Topcu, S. Öksüz, H.L. Shieh, G.A. Cordell, J.M. Pezzuto, and B.J. Candan, *Phytochemistry* **33**, 407 (1993).
[13] M.R. Hemm, S.D. Rider, J. Ogas, D.J. Murry, and C. Chapple, *Plant J.* **38**, 765 (2004).
[14] J. Choi, K.M. Shin, H.J. Park, H.J. Jung, H.J. Kim, Y.S. Lee, J.H. Rew, and K.T. Lee, *Planta Med.* **70**, 1027 (2004).
[15] H.S. Niu, I.M. Liu, J.T. Cheng, C.L. Lin, and F.L. Hsu, *Planta Med.* **74**, 109 (2008).
[16] V.A. Kurkin, A.V. Dubishchev, V.N. Ezhkov, I.N. Titova, and E.V. Avdeeva, *Pharm. Chem. J.* **40**, 33 (2006).
[17] W. Zhang, W.D. Zhang, C. Zhang, R.H. Liu, T.Z. Li, P. Fu, and L. Shan, *Phytother. Res.* **21**, 1113 (2007).
[18] T. Murashige and F. Skoog, *Physiol. Plant* **15**, 473 (1962).