A New Flavonol From *Saussurea involucrata* With Anti-Inflammatory Activity

Sheng Pan¹ and Zi-Guan Zhu²

**Abstract**

A new flavonol named 6-(2''3''-epoxy-3''-methylbutyl)-resokaempferol (1), together with five known compounds (2-6) were isolated from the EtOAc-soluble extract of the aerial part of *Saussurea involucrata*. Their structures were elucidated on the basis of spectroscopic methods. All compounds were evaluated for their anti-inflammatory effects by measuring the production of nitric oxide (NO) and TNF-α in vitro. Among them, compound 1 showed potential inhibitory activity on the production of NO and TNF-α in LPS-induced RAW 264.7 cells with IC₅₀ values of 48.0 ± 1.5 and 41.4 ± 1.7 µM, respectively.

**Keywords**

*Saussurea involucrata*, flavonol, Asteraceae, NO, TNF-α

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*Saussurea involucrata* (Kar. et Kir.) Sch.-Bip (Asteraceae), called “Tianshan Snow Lotus” or “Xinjiang Xuelian” in China, is one of the precious medicinal herbs that is widely distributed in Central Asia.¹ As an important Chinese traditional medicine, *S. involucrata* is used to treat cough,² rheumatoid arthritis,³,⁴ dysmenorrhoea,⁵ altitude sickness and stomachache.⁶ Published studies have showed that *S. involucrata* possesses a number of bioactive compounds including lignans,⁷ flavonoids,⁸ coumarins,⁹ sesquiterpene lactones,⁹ steroids and phenylpropanoids.¹⁰ Some of these compounds displayed a wide range of biological activities such as anti-inflammatory,¹¹ anti-aging,¹¹ anti-oxidative, anti-fatigue,¹² and anti-tumor effects.² During the course of our search for novel lead compounds with anti-inflammatory effect from Chinese traditional medicines, a new flavonol named 6-(2''3''-epoxy-3''-methylbutyl)-resokaempferol (1), together with five known compounds (2-6), were isolated from the whole plant of *S. involucrata*. Herein we report the isolation, structural elucidation and anti-inflammatory activities of these compounds.

**Results and Discussion**

Compound 1 was isolated as a yellow amorphous powder, and its molecular formula was determined to be C₂₀H₁₈O₆ by HREIMS (Supplemental Figure S4) from the ion at m/z 354.1023. The IR (KBr) spectrum showed absorptions attributable to hydroxyl (3357 cm⁻¹) and carbonyl (1743 cm⁻¹) groups. The ¹H NMR spectroscopic data of 1 (Table 1, Supplemental Figure S1) showed six aromatic protons at δ_H 7.67 (s, H-5), 6.57 (s, H-8), 7.76 (d, J = 8.5 Hz, H-2'5') and 6.90 (d, J = 8.5 Hz, H-3'5') at δ_H 2.90 (t, J = 6.0 Hz, H-2''), a methylene at δ_H 2.74-2.83 (m, H-1'') and two methyls at δ_H 1.26 (s, H-4'') and 1.35 (s, H-5''). The ¹³C NMR (Table 1, Supplemental Figure S2) displayed twenty carbons, including a carbonyl carbon at δ_C 182.1 (C-4), twelve aromatic carbons at δ_C 164.9-100.1, two olefinic carbons at δ_C 145.7 (C-2) and 133.8 (C-3), and two methyl carbons at δ_C 25.1 (C-4'') and 19.2 (C-5''). When combined with the corresponding downfield region (δ_C 182.1-100.1) in the ¹¹C NMR spectrum of 1 and the comparison of NMR data of 1 with those of kaempferol,¹³ the flavonol skeleton of 1 was deduced. The structure of the 2''3''-epoxy-3''-methylbutyl moiety at C-6 could be deduced by the key HMBC correlations of δ_H 2.74-2.83 (H-1'') to δ_C 128.5 (C-5), 121.9 (C-6) and 63.5 (C-2''), δ_H 2.90 (H-2'') to δ_C 121.9 (C-6), 28.9 (C-1'') and 58.7 (C-3''), and δ_H 1.26 (H-4'') to δ_C 63.5 (C-2'') and 58.7 (C-3'') (Figure 1 and Supplemental Figure S3). In addition, the comparison of NMR data between 1 and (2'3''-7,4'-hydroxyl-6''-(2''3''-epoxy-3''-methylbutyl) flavanone further confirmed the structure of 1.¹⁴ Because of the small amount of 1 available, the absolute configuration of C-2'' was not determined. Thus, the structure of com-

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¹Spine Surgery, Jilin Central Hospital, China  
²Department of Hand and Reconstructive Surgery, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou, China

**Corresponding Author:** Zi-Guan Zhu, Department of Hand and Reconstructive Surgery, Zhejiang Provincial People’s Hospital, People’s Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014, China.  
Email: zhuziguanzz@163.com

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Table 1. $^1$H and $^{13}$C NMR Spectral Data of Compound 1 in Acetone-$D_6$ ($^1$H: 500 MHz, $^{13}$C: 125 MHz).

| No. | $\delta_C$   | $\delta_H$   |
|-----|--------------|--------------|
| 2   | 145.7        |              |
| 3   | 133.8        |              |
| 4   | 182.1        |              |
| 5   | 128.5        | 7.67, s      |
| 6   | 121.9        |              |
| 7   | 164.9        |              |
| 8   | 100.1        | 6.57, s      |
| 9   | 163.7        |              |
| 10  | 115.1        |              |
| 1'  | 122.8        |              |
| 2'  | 129.0        | 7.76, d, (8.5)|
| 3'  | 116.2        | 6.90, d, (8.5)|
| 4'  | 158.7        |              |
| 5'  | 116.2        | 6.90, d, (8.5)|
| 6'  | 129.0        | 7.76, d, (8.5)|
| 1'' | 28.9         | 2.74-2.83, m  |
| 2'' | 63.5         | 2.90, t (6.0) |
| 3'' | 58.7         |              |
| 4'' | 25.1         | 1.26, s      |
| 5'' | 19.2         | 1.35, s      |

Figure 1. Key HMBC correlations of compound 1.

Compound 1 was determined as 6-(2'',3''-epoxy-3''-methylbutyl)resokaempferol.

Five known compounds (2-6) were also obtained from S. involucrata, and identified as quercetin (2),\textsuperscript{15} quercetin 3-O-rhamnoside (3),\textsuperscript{15} isorhamnetin 3-O-rutinoside (4),\textsuperscript{15} kaempferol 7-O-glucopyranoside (5),\textsuperscript{2} and chrysoeriol 7-O-glycoside (6)\textsuperscript{2} by comparing their NMR data with those in the literature (Figure 2).

S. involucrata possesses a significant anti-inflammatory effect. Thus, in this study, all isolates were evaluated for their anti-inflammatory activities \textit{in vitro}. Nitric oxide (NO) and TNF-\(\alpha\) are two key signals to reflect the occurrence of inflammatory diseases in the body such as tissue injury, ulcerative colitis and osteoarthritis. Therefore, the anti-inflammatory activities of compounds 1-6 were tested by evaluating the production of NO and TNF-\(\alpha\) in LPS-induced RAW 264.7 cells (Table 2); dexamethasone and silybin were used as positive control. Compounds 1, 3-5 and 6 displayed moderate anti-inflammatory activities by inhibiting the production of NO and TNF-\(\alpha\) in RAW264.7 cells. Compound 1 showed potential inhibitory activity on the production of NO and TNF-\(\alpha\) with IC\(_{50}\) values of 48.0 ± 1.5 and 41.4 ± 1.7 \(\mu\)M, respectively. Furthermore, compound 4 also showed inhibitory activity on NO production with an IC\(_{50}\) value of 20.1 ± 1.3 \(\mu\)M.

Investigations on S. involucrata have resulted in the isolation of various flavonoid derivatives, including flavonoid glycosides and flavonols; these compounds exhibited moderate to high anti-inflammatory effect. In this article, we report a novel flavonol with potent anti-inflammatory activity from the dried aerial part of S. involucrata. Further investigation of the chemical constituents of S. involucrata will be undertaken.

**Experimental**

**General**

Optical rotations were measured with a Perkin-Elmer 241 polarimeter, UV spectra with a JASCO V-650 spectrophotometer (JASCO, Tokyo, Japan), IR spectra with a Perkin-Elmer FT-IR System BX spectrophotometer, and nuclear magnetic resonance (NMR) spectra with a Varian Unity Inova 500 MHz spectrometer (Varian Unity Inova, Phoenix, USA) using TMS as the internal standard. Mass spectra were obtained on a QTOF2 high resolution mass spectrometer (Micromass, Wythenshawe, UK). Column chromatography (CC) was performed on silica gel 200-300 mesh (Yantai Xinde Chemical Co., Ltd, Yantai, China) and RP-18 (Merck, Darmstadt, Germany). TLC was performed with silica gel GF\(_{254}\) glass plates (Qingdao Marine Chemical Co. Ltd., Qingdao, China). HPLC was carried out using a Shimadzu LC-6AD instrument with a SPD-20A detector (Shimadzu, Kyoto, Japan), and an ODS column (250 mm × 10 mm i.d., 5 \(\mu\)m, YMC-ODS-A).

**Plant Material**

The dried aerial parts of S. involucrata were collected in tianshan mountains, Xinjiang, China, and authenticated by Professor Qiang Zhao (College of Pharmacy, Hangzhou Medical College). A voucher specimen of the plant (No. 20190315) was deposited at Hangzhou Medical College, Hangzhou, China.

**Extraction and Isolation**

The dried aerial parts of S. involucrata (5.0 kg) were extracted 3 times with 95% EtOH under reflux and concentrated under vacuum to obtain a crude extract (2.1 kg). This was suspended in H\(_2\)O and partitioned successively with light petroleum (PE), CH\(_3\)Cl\(_2\), EtOAc and n-BuOH. The anti-inflammatory effects of these four fractions were further evaluated through measuring the inhibition of NO...
production in LPS-induced RAW 264.7 cells. The EtOAc-soluble fraction (97.5 g) was subjected to silica gel column chromatography using a gradient of CH₂Cl₂-MeOH, and was separated into 12 fractions (Fr.1-Fr.12). Fr.3 (4.4 g) was chromatographed over silica gel, eluted with a gradient of PE-EtOAc, and was separated into 15 fractions (Fr.3.1-Fr.3.15). Fr.3.4 (215.3 mg) was chromatographed on a Sephadex LH-20 column (MeOH) to give compounds 2 (10.3 mg) and 4 (6.4 mg). Fr.3.5 (312.6 mg) was further purified by HPLC using a gradient solvent system of 45 to 55% MeOH in H₂O over 70 minutes to yield compounds 1 (2.8 mg, t_R = 33.4 minutes), 3 (6.2 mg, t_R = 42.5 minutes), 5 (5.8 mg, t_R = 50.3 minutes) and 6 (7.1 mg, t_R = 61.4 minutes).

6-(2''3''-Epoxy-3''-methylbutyl)-resokaempferol (1). A yellow amorphous powder; [α]_{D} = −10.5 (c 0.1, MeOH); UV (MeOH) \( \lambda_{	ext{max}} \) (log ε): 230 (3.89), 351 (3.22) nm; IR (KBr disc) \( \nu_{	ext{max}} \): 3357, 2930, 1743, 1610, 1447 cm⁻¹; ¹H (500 MHz) and ¹³C NMR (125 MHz) spectral data in acetone-\( d_{6} \), see Table 1; HREIMS: \( m/z \) 354.1023 [M]+ (calcd for C₂₀H₁₈O₆, 354.1103).

Anti-inflammatory Assays

In vitro anti-inflammatory activities of compounds 1-6 were evaluated by monitoring the production of NO and TNF-α in RAW264.7 cells.¹⁶ Briefly, RAW264.7 cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS), 1% antibiotics and L-glutamine, and seeded in a 96-well chamber (3 x 10⁴ cells/well). Next, cells were treated with different concentrations of compounds 1-6 after 24 hours incubation. Then RAW264.7 cells were exposed to LPS (1 μg/mL) for another 24 hours. The concentration of NO was measured using a Griess kit (Beyotime Biotechnology, Shanghai, China), with dexamethasone as the positive control. The concentrations of TNF-α were determined by an ELISA kit (R&D company, Minnesota, USA) according to the manufacturer's instructions. Finally, the level of TNF-α was determined from a standard curve with silybin used as a positive control.

Table 2. Anti-inflammatory Activities of Compounds 1-6.

| Compound | IC₅₀(μM) | NO | TNF-α |
|----------|----------|----|-------|
| 1        | 12.0 ± 1.1 | 41.4 ± 1.7 |
| 2        | >100      | >100 |
| 3        | 41.6 ± 0.9 | >100 |
| 4        | 201.1 ± 1.3 | 50.7 ± 1.4 |
| 5        | 67.0 ± 1.0 | >100 |
| 6        | >100      | >100 |
| Dexamethasone⁶ | 8.2 ± 0.4 | - |
| Silybin²   | -         | 62.7 ± 1.1 |

IC₅₀ values represent the means ± SEM of three parallel measurements. *Positive control.

Declaration of Conflicting Interests

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ORCID ID

Zi-Guan Zhu https://orcid.org/0000-0001-7449-9754

Supplemental Material

Supplemental material for this article is available online.

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