Diterpenoid Alkaloids from the Roots of *Aconitum sinomontanum* and Their Evaluation of Immunotoxicity

Jiao Zhang¹, Yuze Li², Yuwen Cui³, Pu Jia², Zhenggang Yue⁴, Bei Song⁵* and Xiaomei Song¹*

¹School of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang 712046, China
²The College of Life Sciences, Northwest University, Xi’an 710069, China
³Department of Pharmacy, Xi’an Medical University, Xi’an 710021, China
⁴Shaanxi Collaborative Innovation Center of Chinese Medicinal Resource Industrialization, Shaanxi University of Chinese Medicine, Xianyang 712046, China
⁵The Second Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang 712046, China

(Received May 17, 2018; Revised July 16, 2018; Accepted July 18, 2018)

**Abstract:** One new C₁₈-diterpenoid alkaloid, along with four known diterpenoid alkaloids have been isolated from the roots of *Aconitum sinomontanum*. Their structures were established as sinomontanine I (1), delcosine (2), lepenine (3), napelline (4), and kirinine B (5) by extensive spectroscopic techniques and chemical methods. The immunosuppressive effects of compounds 1–4 were evaluated in vitro through ConA-induced or LPS-induced splenocyte proliferation, with IC₅₀ values of 8.909 μM, 1.515 μM, 5.078 μM, and 1.167 μM (ConA-induced), or 3.661 μM, 4.417 μM, 5.129 μM, and 1.830 μM (LPS-induced), and compounds 1–4 showed a significant cytotoxic effect with CC₅₀ values of 447.5 μM, 702.2 μM, 310.6 μM and 794.1 μM, respectively. The CC₅₀/IC₅₀ value of 2 and 3 suggested that these compounds were potential immunosuppressive agents for the treatment of autoimmune diseases characterized by arthritis, such as rheumatoid arthritis.

**Keywords:** *Aconitum sinomontanum* Nakai; diterpenoid alkaloids; immunotoxicity; LPS; ConA. © 2018ACG Publications. All rights reserved.

---

**1. Introduction**

The plant *Aconitum sinomontanum* Nakai, a species in the *Aconitum* genus of Ranunculaceae, is widely distributed in the west of China and used as a folk medicine in Shaanxi province, known as “Ma-Bu-Qi” [1]. Phytochemical studies revealed that *Aconitum sinomontanum* mainly contained C₁₈, C₁₉ and C₂₀ diterpenoid alkaloids [2]. Diterpenoid alkaloids are a very important family of natural products that feature structural complexity and various bioactivities, such as anti-inflammatory [3–4], analgesic, antiarrythmic, anti-epileptiform, anticancer, anti-parasite and anesthetic activities [5–6]. Most natural diterpenoid alkaloids were isolated from the genera *Aconitum* [7], *Consolida* [8] and *Delphinium* (Ranunculaceae) [9] and the genus *Spiraea* (Rosaceae) [10]. As part of our research project to explore more bioactive lead compounds from

* Corresponding authors: E- Mail: songxiaom@126.com; songbei168@126.com Phone: +86-136-3673-3632
the medicinal herbs in the Qinba mountains of China, the chemical constituents and pharmacological studies of *Aconitum sinomontanum* were studied, and one new C_{18}-diterpenoid alkaloid sinomontanine I (1), along with four known diterpenoid alkaloids, delcosine (2) [11], lepenine (3) [12], napelline (4) [13], and kirinine B (5) [12] were isolated (Figure 1). Since the roots of *Aconitum sinomontanum* were commonly used to treat rheumatism and fracture, the isolated compounds 1–4 were evaluated in vitro through ConA- or LPS-induced splenocyte proliferation models [14], and suggested that these compounds may be become potential immunosuppressive agents.

![Chemical Structures of compounds 1-5](image)

**Figure 1.** Chemical Structures of compounds 1-5

2. **Materials and Methods**

2.1. **Material**

The roots of *Aconitum sinomontanum* Nakai. were collected from the Qinba mountains of Shaanxi Province of China in July 2016, and identified by senior experimentalist Jitao Wang. A voucher specimen (herbarium No. 20160739) has been deposited in the Medicinal Plants Herbarium (MPH), Shaanxi University of Chinese Medicine, Xianyang, China.

Optical rotation indices were determined in methanol on a Rudolph Autopol II digital polarimeter (Rudolph, Hackettstown, NJ, USA). ESI-MS was performed on a Quattoro Premier instrument (Waters, Milford, MA, USA). The HR-ESI-MS spectra were recorded on an Agilent Technologies 6550 Q-TOF (Santa Clara, CA, USA). 1D and 2D-NMR spectra were recorded on Bruker-AVANCE 400 instrument (Bruker, Rheinstetten, Germany) with TMS as an internal standard. The analytical HPLC was performed on a Waters e2695 Separations Module coupled with a 2998 Photodiode Array Detector and an Accurasil C-18 column (4.6 mm × 250 mm, 5 μm particles, Ameritech, Chicago, IL, USA). Semipreparative HPLC was performed on a system comprising an LC-6AD pump equipped with an SPD-20A UV detector (Shimadzu, Kyoto, Japan) and an Ultimate XB-C18 (10 mm × 250 mm, 5 μm particles) or YMS-Pack-ODS-A (10 mm × 250 mm, 5 μm particles). Silica gel was purchased Qingdao Haiyang Chemical Group Corporation (Qingdao, China).

2.2. **Extraction and Isolation**

The air-dried and powdered underground parts of *Aconitum sinomontanum* Nakai (15.0 kg) were extracted with 80% EtOH at 80°C for three times (each time 5Kg, 40 L for 1.5 h). After removal of EtOH solvent under reduced pressure, the extract (6 L) was dispersed in water (4.5 L), adjusted with 9% HCl...
solution to pH 0.8, and extracted with petroleum ether (PE). The acidic water solution was alkalinized to pH 10.26 with 25% ammonia solution, extracted with CHCl₃ six times, and evaporated under pressure to give crude alkaloids (800 g). The crude alkaloids (795 g) were chromatographed on silica gel column, eluting with gradient solvent system (PE/acetic acid/diethylamine, 50:1:0.1–1:1:0.1) to give 4 fractions (Fr.1–Fr.4). Fr.4 (40 g) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 1.0 mL·min⁻¹) with CH₃OH/H₂O (30:70) as mobile phase to obtained compound 1 (0.1579 g; tₑ = 110.3 min), compound 2 (3.1825 g; tₑ = 38.5 min), compound 3 (6.1585 g; tₑ = 70.2 min), compound 4 (1.008 g; tₑ = 82.8 min), and compound 5 (0.2749 g; tₑ = 95.6 min). See more detailed spectrums in the supplementary materials.

2.3. Spectroscopic Data

Snomontanine I (1): A white amorphous powder, IR (KBr) νmax: 3127, 2946, 2835, 1454, and 1028 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) and ¹³C-NMR (100 MHz, CDCl₃) spectral data, see Table 1; HR-ESI-MS: m/z 440.2653 [M + H]+ (calcd. for C₂₂H₃₈N₂O₇, 440.2648) and NMR data (Table 1). The ¹H-NMR spectrum (Table 1) of 1 showed the presence of an ethylenamino group protons at δH1.08 (3H, t, J=7.3), δH2.81 (1H, m), δH2.97 (1H, m); and three OMe protons at δH3.33 (3H, s), 3.36 (3H, s), and 3.39 (3H, s); A signal at δH 3.61 (1H, dd, J=4.1, 4.4) indicated the presence of Hβ-C(14) [15]. The ¹³C-NMR spectrum (Table 1) displayed 23 carbon resonances. Among them, resonances at δC 56.5, 57.9 and 58.3 were attributed to three OMe groups, and the NMR features of the remained 20 resonances were characteristic to a ranaconitine-type C₁₈-diterpenoid alkaloids [16]. In which δC 50.0 and δC 13.8 were attributed to a N-Et group; δC 70.4, 72.6, 78.7 and 88.2 were attributed to four oxygenated carbons associated withhydroxyl groups. The assignments of the NMR signals associated with 1 were derived from ¹H-¹H COSY, HSQC, HMBC, and NOESY experiments. The structure of 1 was further established by HMBC (Figure 2). In the HMBC spectrum , correlations of H-3 (δH 1.83, 2.15), H-5 (δH 1.76), H-17 (δH 2.75), H-20 (δH 2.81, δH 2.98) to C-19 (δC 61.3) suggested that C-19 was involved in the N-CH₂-CH₂ group; correlations of OCH₃ (δH 3.36) to C-6 (δC 90.3), OCH₃ (δH 3.39) to C-14 (δC 84.7), OCH₂(δH 3.33) to C-16 (δC 83.2) suggested that three methoxyl groups were linked at C-6, C-14 and C-16, respectively; correlations of H-3 (δH 1.83, 2.15), H-5 (δH 1.76), H-19 (δH 2.70) to δC 70.4 suggested that δC 70.4 was assigned as C-4, and a hydroxyl group should be located at C-4 combined with literature data [15]; correlation of OH (δH 4.12, s) to C-8 (δC 78.7) suggested that a hydroxyl group should be located at C-8, which was further confirmed by the HMBC correlations observed from H-6, H-14, H-9 and H-15 to C-8. The ¹³C-NMR spectrum of 1 was very similar to that of the known compound 2 except the signals of C-4 and C-14 and signals of C-atoms close to C-4 and C-14. In the ¹³C-NMR of 1, C-4 signal was at 70.4 and that of C-3 at 35.0, C-5 at 52.4, compared to 29.4, 37.6 and 44.0 of compound 2, respectively, indicating that C-4 of 1 had an O-containing substituent; in addition, C-14 signal appeared at 84.7 and that of C-13 at 38.2, compared with 75.8 and 45.3 of 2, suggested that a methoxyl group was linked at C-14, consistent with the above inference, so suggested that the remaining two hydroxyl groups were linked at C-1 and C-7. Meanwhile, in the NOE (Figure 2), the α-orientation of 1-OH was confirmed by the correlation between H-1 (δH 3.64) and H-10 (δH 1.97) [17].The NOE correlations of Hᵐ⁻1/H-3, Hᵐ⁻1/H-5, H⁻1/H⁻10, H⁻1/H⁻17, H⁻10/H⁻14, and H⁻14/H⁻9, indicated β-orientation of H-9, H-10 and H-17; the NOE correlations of H⁻6/H⁻17 and H⁻6/H⁻9 indicated α-axial of H-6 and H-16, and β-orientation of 6-OCH₃ and 16-OCH₃. By comparison with the previously reported data [15], 4-OH, 7-OH and 8-OH were deduced to be β-orientation. Moreover, the NOE correlations of H⁻1/H⁻3 and H-5 while no correlations between H-2 and H-5 indicated 1 had ring A (C-1, C-2, C-3, C-4, C-5, and C-11) in the chair conformation. Thus, according to the Organic compound system nomenclature, compound 1 was assigned
the name as 1α,4β,7β,8β-tetrahydroxy-6β,14α,16β-trimethoxy-19-en- ranacontine, namely sinomontanine I.

Table 1. $^1$H NMR, $^{13}$C NMR, $^1$H–$^1$H COSY, HSQC and HMBC data for compound 1

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_C$</th>
<th>$\delta_H$</th>
<th>$^1$H–$^1$H COSY</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72.7</td>
<td>3.64 (t,4.1,6.2)</td>
<td>H-2</td>
<td>35.0 (C-3), 50.6 (C-11)</td>
</tr>
<tr>
<td>2</td>
<td>29.8</td>
<td>1.68 (m,H-2a)</td>
<td>H-1,H-3</td>
<td>35.0 (C-3), 50.6 (C-11), 70.4 (C-4)</td>
</tr>
<tr>
<td>3</td>
<td>35.0</td>
<td>1.83 (m,H-3a)</td>
<td>H-2</td>
<td>29.8 (C-2), 52.4 (C-5), 61.3 (C-19), 70.4 (C-4)</td>
</tr>
<tr>
<td>4</td>
<td>70.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>52.4</td>
<td>1.76 (br s)</td>
<td>H-6</td>
<td>38.2 (C-10), 50.6 (C-11), 61.3 (C-19), 65.3 (C-17), 70.4 (C-4), 88.2 (C-7)</td>
</tr>
<tr>
<td>6</td>
<td>90.3</td>
<td>4.12 (s)</td>
<td>H-5</td>
<td>50.6 (C-11), 52.4 (C-5), 70.4 (C-4), 78.7 (C-8), 88.2 (C-7)</td>
</tr>
<tr>
<td>7</td>
<td>88.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>78.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>43.6</td>
<td>2.92 (m)</td>
<td>H-10,H-14</td>
<td>30.7 (C-12), 33.7 (C-15), 38.2 (C-13), 43.9 (C-10), 78.7 (C-8), 84.7 (C-14)</td>
</tr>
<tr>
<td>10</td>
<td>43.9</td>
<td>1.97 (m)</td>
<td>H-9,H-12</td>
<td>30.7 (C-12), 43.6 (C-9), 50.6 (C-11), 65.3 (C-17), 78.7 (C-8)</td>
</tr>
<tr>
<td>11</td>
<td>50.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>30.7</td>
<td>1.62 (m,H-12a)</td>
<td>H-10,H-13</td>
<td>43.6 (C-9), 43.9 (C-10), 50.6, (C-11), 83.2 (C-16), 84.7 (C-14)</td>
</tr>
<tr>
<td>13</td>
<td>38.2</td>
<td>2.39 (m)</td>
<td>H-12,H-14</td>
<td>30.7 (C-12), 43.6 (C-9), 43.9 (C-10), 83.2 (C-16), 84.7 (C-14)</td>
</tr>
<tr>
<td>14</td>
<td>84.7</td>
<td>3.61 (dd,4.1,4.4)</td>
<td>H-13,H-15</td>
<td>43.6 (C-9), 43.9 (10), 78.7 (C-8), 83.2 (C-16)</td>
</tr>
<tr>
<td>15</td>
<td>33.7</td>
<td>1.73 (m,H-15a)</td>
<td>H-16</td>
<td>38.2 (C-13), 43.6 (C-9), 78.6 (C-8), 83.2 (C-16), 88.2 (C-7)</td>
</tr>
<tr>
<td>16</td>
<td>83.2</td>
<td>3.25 (m)</td>
<td>H-15</td>
<td>30.7 (C-12), 43.6 (C-9), 84.7 (C-14)</td>
</tr>
<tr>
<td>17</td>
<td>65.3</td>
<td>2.75 (m)</td>
<td>H-5</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>61.3</td>
<td>2.70 (m,2H)</td>
<td></td>
<td>35.0 (C-3), 50.6 (C-11), 65.3 (C-17), 70.4 (C-4)</td>
</tr>
<tr>
<td>20</td>
<td>50.0</td>
<td>2.81 (m,H-20a)</td>
<td></td>
<td>61.3 (C-19), 65.3 (C-17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.98 (m,H-20b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>13.8</td>
<td>1.08 (t,3H,7.3)</td>
<td></td>
<td>50.0 (C-20)</td>
</tr>
<tr>
<td>6-OCH$_3$</td>
<td>58.3</td>
<td>3.36 (s)</td>
<td></td>
<td>83.2 (C-6)</td>
</tr>
<tr>
<td>14-OCH$_3$</td>
<td>57.9</td>
<td>3.39 (s)</td>
<td></td>
<td>84.7 (C-14)</td>
</tr>
<tr>
<td>16-OCH$_3$</td>
<td>56.5</td>
<td>3.33 (s)</td>
<td></td>
<td>83.2 (C-16)</td>
</tr>
</tbody>
</table>

*400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR in CDCl$_3$ in ppm, $J$ in Hz*
The known compounds were identified by comparison of their spectral data with those described in the literature, and identified to be delcosine(2) [11], lepeneine(3) [12], napelline(4) [13] and kirinine B (5) [12].

Figure 2. Key 1H-1H COSY (H↔H), HMBC (H→C) and NOESY (H↔H) correlations of compound 1

3.2. Immunosuppressive Effects Assay

In order to be better used A.Sinomontanum in the world, the evaluation of immunotoxicity based on substance is inevitable. Therefore, lipopolysaccharide (LPS) and concanavalin A (ConA) induced splenic lymphocyte proliferation test were used to evaluate the immunotoxicity of the compounds[18]. The immunosuppressive effects of compounds 1–4 were evaluated in vitro through ConA-induced or LPS-induced splenocyte proliferation, which was concentration-dependently suppressed by compounds 2 and 3 (Figure 3.b,c), with IC_{50} values of 4.417 μM and 5.129 μM (LPS-induced) or 1.515 μM and 5.078 μM(ConA-induced), respectively. However, compounds 2 and 3 showed a significant cytotoxic effect (Figure 3.a), with CC_{50} values of 702.2 μM and 310.6 μM, respectively. The CC_{50}/IC_{50} value of 2 and 3 suggested that these compounds may become potential immunosuppressive agents.

Figure 3. Cytotoxicity on splenocytes and inhibition on ConA-induced or LPS-induced splenocyte proliferation of compounds 1–4. *

*a Cytotoxicity of compounds 1–4 on BALB/c mice splenocytes; b Inhibition of compounds 1–4 on LPS-induced splenocyte proliferation; c Inhibition of compounds 1–4 on ConA-induced splenocyte proliferation.

*Results are mean ± S.D. *P < 0.05, **P < 0.01, ***P < 0.001, treatment group versus control
Acknowledgements

This project was financially supported by the National Natural Science Foundations of China (grant no. 81503195); Innovative Research Team in TCM Material Foundation and Key Preparation Technology (grant no. 2012KCT-20); and the project was supported by the Open Research Fund of Key Laboratory of Basic and New Herbal Medicament Research, Shaanxi university of Chinese medicine (No.2017KF02,17JS030).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Jiao Zhang: 0000-0002-0308-6810
Yuze Li: 0000-0001-7571-3214
Yuwen Cui: 0000-0001-9153-6406
Pu Jia: 0000-0001-6245-7303
Zhenggang Yue: 0000-0001-6296-8509
Bei Song: 0000-0003-1970-7359
Xiaomei Song: 0000-0003-1906-1578

References

Diterpenoid alkaloids from the roots of Aconitum sinomontanum