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

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**Abstract:** One new $C_{18}$-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 $IC_{50}$ 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 $CC_{50}$ values of 447.5 μM, 702.2 μM, 310.6 μM and 794.1 μM, respectively. The $CC_{50}/IC_{50}$ 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 $C_{18}$, $C_{19}$ and $C_{20}$ 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, antiarrhythmic, 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

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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 .

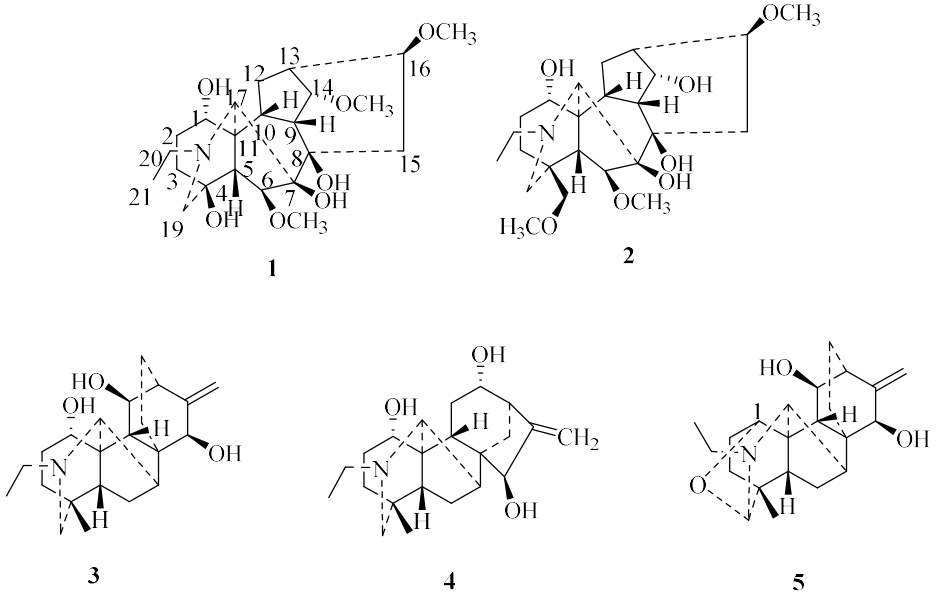


**Figure 1.** Chemical Structures of compounds **1-5**

## 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 a 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 alkalized to pH 10.26 with 25% ammonia solution, extracted with $CHCl_3$ 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/acetone/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 $mL \cdot min^{-1}$) with $CH_3OH/H_2O$ (30:70) as mobile phase to obtained compound **1** (0.1579 g; $t_R$ = 110.3 min), compound **2** (3.1825 g; $t_R$ = 38.5 min), compound **3** (6.1585 g; $t_R$ = 70.2 min), compound **4** (1.008 g; $t_R$ = 82.8 min),and compound **5** (0.2749 g; $t_R$ = 95.6 min). See more detailed spectrums in the supplementary materials.

### *2.3. Spectroscopic Data*

*Snomontanine I **(1)**:* A white amorphous powder, IR (KBr) $\nu_{max}$: 3127, 2946, 2835, 1454, and 1028 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) and $^{13}$C-NMR (100 MHz, $CDCl_3$) spectral data, see Table **1**; HR-ESI-MS: *m/z* 440.2653 $[M + H]^+$(calcd. for $C_{23}H_{38}NO_7$, 440.2648). $[\alpha]_D^{25}$ +48.6(c=0.0017, MeOH).

## 3. Results and Discussion

### *3.1. Structure Elucidation*

Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{23}H_{37}NO_7$ based on HR-ESI-MS (positive ion): m/z 440.2653 $[M + H]^+$(calcd. for $C_{23}H_{38}NO_7$, 440.2648) and NMR data (Table **1**). The $^1$H-NMR spectrum (Table **1**) of **1** showed the presence of an ethylamino group protons at $\delta_H$1.08 (3H, t, *J*=7.3), $\delta_H$2.81 (1H, m), $\delta_H$2.97 (1H, m); and three OMe protons at $\delta_H$3.33 (3H, s), 3.36 (3H, s), and 3.39 (3H, s); A signal at $\delta_H$ 3.61 (1H, dd, *J*=4.1, 4.4) indicated the presence of $H_\beta$-C(14) [15]. The $^{13}$C-NMR spectrum (Table **1**) displayed 23 carbon resonances. Among them, resonances at $\delta_C$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 $C_{18}$-diterpenoid alkaloids [16]. In which $\delta_C$50.0 and $\delta_C$13.8 were attributed to a N-Et group; $\delta_C$70.4, 72.6, 78.7 and 88.2 were attributed to four oxygenated carbons associated with hydroxyl groups. The assignments of the NMR signals associated with **1** were derived from $^1$H-$^1$H COSY, HSQC, HMBC, and NOESY experiments. The structure of **1** was further established by HMBC spectrum (Figure 2). In the HMBC spectrum , correlations of H-3 ($\delta_H$ 1.83, 2.15), H-5 ($\delta_H$ 1.76), H-17 ($\delta_H$ 2.75), H-20 ($\delta_{Ha}$ 2.81, $\delta_{Hb}$ 2.98) to C-19 ($\delta_C$ 61.3) suggested that C-19 was involved in the N-$CH_2$-$CH_3$ group; correlations of $OCH_3$ ($\delta_H$ 3.36) to C-6 ($\delta_C$ 90.3 ), $OCH_3$ ($\delta_H$ 3.39) to C-14 ( $\delta_C$ 84.7), $OCH_3$($\delta_H$ 3.33) to C-16 ($\delta_C$ 83.2) suggested that three methoxyl groups were linked at C-6, C-14 and C-16, respectively; correlations of H-3 ($\delta_H$ 1.83, 2.15), H-5 ($\delta_H$ 1.76), H-19 ($\delta_H$ 2.70) to $\delta_C$ 70.4 suggested that $\delta_C$ 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 ($\delta_H$ 4.12, s) to C-8 ($\delta_C$ 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 $^{13}$C-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 $^{13}$C-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 componud **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 NOSEY spectrum (Figure **2**) , the α-orientation of 1-OH was confirmed by the correlation between H-1 ($\delta_H$ 3.64) and H-10 ($\delta_H$ 1.97) [17].The NOE correlations of $H_\beta$-1/H-3, $H_\beta$-1/H-5, H-1$_\beta$/H-10, H-1$_\beta$/H-17, H-10/$H_\beta$-14, and $H_\beta$-14/H-9, indicated β-orientation of H-9, H-10 and H-17; the NOE correlations of H-6/$H_\beta$-17 and H-16/$H_\beta$-9 indicated α-axial of H-6 and H-16, and β-orientation of 6-$OCH_3$ and 16-$OCH_3$. 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- ranaconitine, namely sinomontanine I.

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

| Position | $\delta_C$ | $\delta_H$ | $^{1}$H-$^{1}$H COSY | HMBC |
|---|---|---|---|---|
| 1 | 72.7 | 3.64 (t,4.1,6.2) | H-2 | 35.0 (C-3),50.6 (C-11) |
| 2 | 29.8 | 1.68 (m,H-2a)<br>1.70 (m,H-2b) | H-1,H-3 | 35.0 (C-3),50.6 (C-11),70.4 (C-4) |
| 3 | 35.0 | 1.83 (m,H-3a)<br>2.15 (m,H-3b) | H-2 | 29.8 (C-2),52.4 (C-5),61.3 (C-19),<br>70.4 (C-4) |
| 4 | 70.4 | | | |
| 5 | 52.4 | 1.76 (br s) | H-6 | 38.2 (C-10),50.6 (C-11),61.3 (C-19),<br>65.3 (C-17),70.4 (C-4),88.2 (C-7) |
| 6 | 90.3 | 4.12 (s) | H-5 | 50.6 (C-11),52.4 (C-5),70.4 (C-4),<br>78.7 (C-8),88.2 (C-7) |
| 7 | 88.2 | | | |
| 8 | 78.7 | | | |
| 9 | 43.6 | 2.92 (m) | H-10,H-14 | 30.7 (C-12),33.7 (C-15),38.2 (C-13),<br>43.9 (C-10),78.7 (C-8),84.7 (C-14) |
| 10 | 43.9 | 1.97 (m) | H-9,H-12 | 30.7 (C-12),43.6 (C-9),50.6 (C-11),<br>65.3 (C-17),78.7 (C-8) |
| 11 | 50.6 | | | |
| 12 | 30.7 | 1.62 (m,H-12a)<br>2.03 (m,H-12b) | H-10,H-13 | 43.6 (C-9),43.9 (C-10),50.6, (C-11),<br>83.2 (C-16),84.7 (C-14) |
| 13 | 38.2 | 2.39 (m) | H-12,H-14 | 30.7 (C-12),43.6 (C-9),43.9 (C-10),83.2 (C-16)<br>84.7 (C-14) |
| 14 | 84.7 | 3.61(dd,4.1,4.4) | H-13,H-15 | 43.6 (C-9),43.9 (10),78.7 (C-8),83.2 (C-16) |
| 15 | 33.7 | 1.73 (m,H-15a)<br>2.60 (q,8.6,6.1,8.6) | H-16 | 38.2 (C-13),43.6 (C-9),78.6 (C-8),<br>83.2 (C-16),88.2 (C-7) |
| 16 | 83.2 | 3.25 (m) | H-15 | 30.7 (C-12),43.6 (C-9),84.7 (C-14) |
| 17 | 65.3 | 2.75 (m) | H-5 | |
| 18 | | | | |
| 19 | 61.3 | 2.70 (m,2H) | | 35.0 (C-3),50.6 (C-11),65.3 (C-17),70.4 (C-4) |
| 20 | 50.0 | 2.81 (m,H-20a)<br>2.98 (m,H-20b) | | 61.3 (C-19),65.3 (C-17) |
| 21 | 13.8 | 1.08 (t,3H,7.3) | | 50.0 (C-20) |
| 6-$OCH_3$ | 58.3 | 3.36 (s) | | 83.2 (C-6) |
| 14-$OCH_3$ | 57.9 | 3.39 (s) | | 84.7 (C-14) |
| 16-$OCH_3$ | 56.5 | 3.33 (s) | | 83.2 (C-16) |

*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] , lepenine(**3**) [12], napelline(**4**) [13] and kirinine B (**5**) [12].

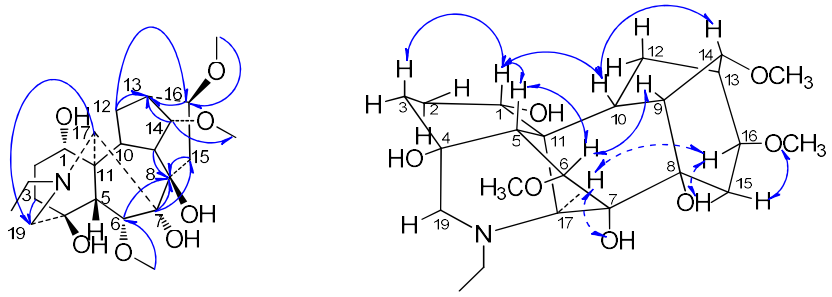

**Figure 2.** Key $^{1}$H-$^{1}$H COSY (H↔H), HMBC (H→C) and NOESY (H↔H) correlations of compound **1**

### *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 be become potential immunosuppressive agents.

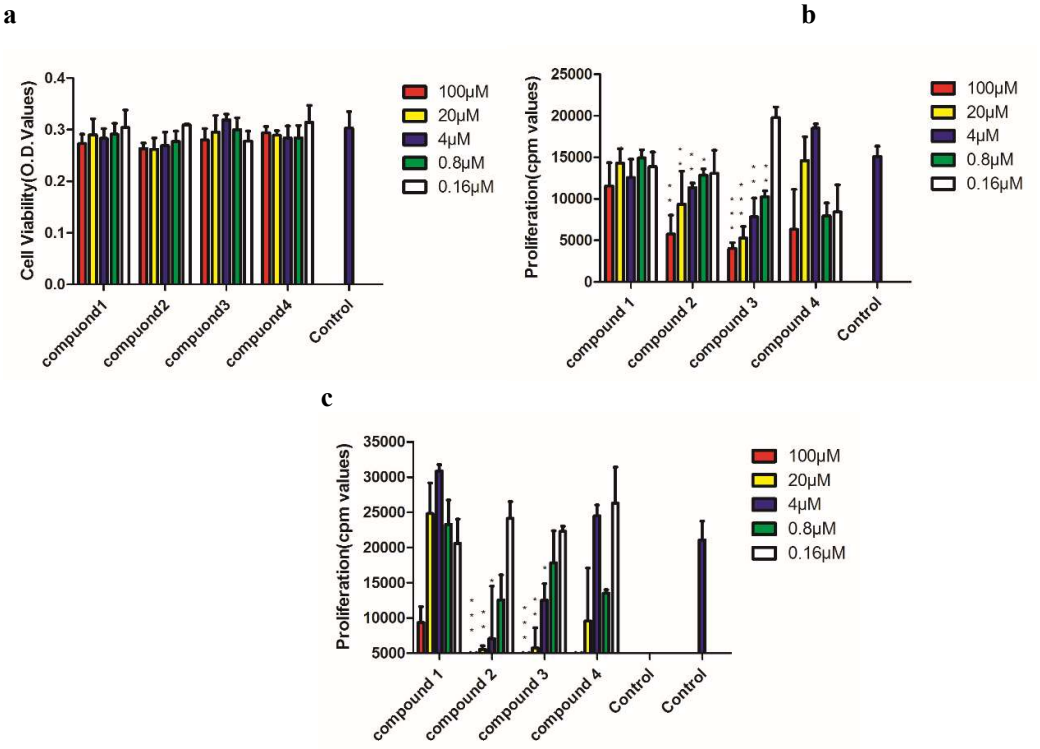


**Figure 3.** Cytotoxicity on splenocytes and inhibition on ConA-induced or LPS-induced splenocyte proliferation of compounds **1–4**.*

[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

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## Supporting Information

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

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