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ORIGINAL ARTICLES

Diterpenoid alkaloids from a Tibetan medicinal plant *Aconitum richardsonianum* var. *pseudosessiliflorum* and their cytotoxic activity

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Abstract: The chemical constituents from *Aconitum richardsonianum* var. *pseudosessiliflorum* were investigated. The roots of this plant were extracted three times with 90% EtOH at the room temperature. The ethanol extracts were combined and concentrated under reduced pressure to yield residue, which was suspended in water and successively partitioned with chloroform. The chloroform extraction was isolated and purified by silica gel and Sephadex LH-20 column chromatography. Six compounds were isolated and elucidated as delelatine (**1**), isodelpheline (**2**), 3-acetylaconitine (**3**), isoatisine (**4**), nordhagenine A (**5**) and yunaconitine (**6**). Compounds 1 - 5 were obtained from *Aconitum Brunneum* for the first time. Compound (**1**) showed significant cytotoxic activities (IC_{50} = 4.36 μM) against the human tumor cell line P388.

keywords: *Aconitum richardsonianum* var. *pseudosessiliflorum*; Ranunculaceae; diterpene alkaloids; cytotoxic activity

1 Introduction

The genus aconitum belongs to the family Ranunculaceae and consists of approximately 400 species distributed throughout the northern hemisphere including Asia, Europe, and North America. Of the 400 species more than 200 are endemic to China [1]. Since antiquity, preparations of various species of Aconitum plants have been widely used by various civilizations as sources of both traditional medicines and arrow poisons [2]. Plants of Aconitum genus are the rich sources of diverse diterpenoid alkaloids, and structurally classified as C_{19} and C_{20}-diterpenoid alkaloids, with a wide range of fascinating bioactivities [3,4].

Aconitum richardsonianum var. *pseudosessiliflorum* is a perennial herb, mainly distributed around an altitude of 4000 meters in the northwest of China. Its roots have been used in Tibetan folk medicine for the treatments of rheumatism and neuralgia for a long time [5]. Previous investigation on the roots of this species collected in Qinghai Province resulted in the isolation of yunaconitine, aconitine, foresaconitine, talatisamine, 8-deacetyl-yunaconitine, chasmanine, chrysophanol, β-sitosterol, palmitic acid, 4, 4'-dimethoxybiphenyl, daucosterol and 1-O-p-ethoxy-cinnamoyl-Dglucopyranose [6]. In our effort to find pharmacologically and structurally interesting substances from traditional Chinese medicines [7-9], we have investigated the roots of this species, collected in Lhasa region, Tibet Autonomous Region. As a result, six known compounds (**1 - 6**) were obtained (Figure 1). We present herein the isolation and structural elucidation of these compounds, as well as their cytotoxic activities. Compounds **1 - 5** were obtained from this plant for the first time.

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2 Experimental

2.1 General

Melting points were determined on a kofler micro-hot stage melting point apparatus and uncorrected. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. ^1H and ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer in $CDCl_3$ with TMS as internal standard. HR-ESI-MS and EI-MS spectra were obtained on Bruker APEX II FT-MS and HP 5988 MS spectrometers respectively. IR spectra were taken with KBr pellets on a Nicolet NEXUS 670 FT-IR spectrometer. Silica gel (200 - 300 mesh) for CC and GF254 for TLC were obtained from the Qingdao Marine Chemical Factory (Qingdao, P.R. China). Size-exclusion chromatography was performed using Sigma Lipophilic Sephadex LH-20.

2.2 Plant materials

The roots of the plants *Aconitum richardsonianum* var. *pseudosessiliflorum* were collected in the Lhasa area, Tibet Autonomous Region, People's Republic of China, in August 2008. Voucher specimens (2008D006) were deposited at the Department of Botany, Northwest Normal University, and were identified by Prof. Yi-Feng Wang.

2.3 Extraction and isolation

The chopped plant materials (4 kg) were extracted with 90% EtOH (5 L) three times at the room temperature, and

dried *in vacuo* to yield the crude extract (280 g). The EtOH extract was treated with 5% HCl (1.5 L), and then the acidic solution was basified with 26% NH_4OH to pH 11 and extracted with $CHCl_3$ (1.5 L) to give crude alkaloids (150 g) after removing the solvent. This residue was sequentially separated on silica gel by eluting with step gradient of petroleum ether/acetone (100 : 0, 70 : 30, 50 : 50 and 30 : 70, v/v) to afford four parts (A – D). Fraction B (16.0 g) was repeatedly subjected to column chromatography on silica gel with petroleum ether/acetone (from 20 : 1 to 10 : 1, v/v) and Sephadex LH-20 with MeOH to give compounds **2** (6.0 mg) and **5** (7.1 mg). Fraction C (9.2 g) was chromatographed on silica gel column with petroleum ether/acetone gradient (from 5 : 1 to 1 : 1, v/v) to afford compounds **1** (7.0 mg), **3** (6.1 mg) and **4** (6.2 mg). Repeated chromatography of fraction D (3.6 g) on silica gel with petroleum ether/acetone gradient (from 3 : 1 to 1 : 1, v/v) afforded compound **6** (6.0 mg).

2.4 Cytotoxicity assay experiment

The assay was performed following a standard published procedure using microtiter plate format and sulforhodamine B standing at the end point [10]. The leukemic cells (P388) were obtained from the Department of Botany, Lanzhou University, People's Republic of China, and exposed to test compounds **1–6** for three days. Cells were grown and maintained in an incubator set at 37 ℃, 5% CO_2 and 95% humidity. RPMI-1640 media (Sigma-Aldrich, Beijing, P.R. China) supplemented with 10% fetal calf serum (FCS) and 0.5% trypsin were used to culture P388 cells.

3 Results and discussion

The compounds were identified by comparison of their ^1H and ^{13}C NMR, MS and IR spectral data with those reported in literature [6, 11-15], respectively.

Delelatine (**1**), white crystal [petroleum ether-acetone (7 : 3)], mp 85 – 87 ℃. EI-MS *m/z*: 435 $[M]^+$; IR ν_{Max}^{KBr} cm^{-1}: 3360 (OH), 1160, 1130, 1050, 930; ^1H-NMR ($CDCl_3$, 400 MHz) δ: 0.95 (3H, s, H-18), 1.05 (3H, t, J = 7.0 Hz, NCH_2CH_3), 3.29, 3.40 (each 3H, s, 2 × OCH_3), 4.22 (1H, br s, H-6α), 4.15 (1H, t, J = 4.5 Hz, H-14β), 5.10, 5.20 (each 1H, s, O-CH_2-O);[13] ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 83.9 (C-1), 26.3 (C-2), 36.8 (C-3), 34.2 (C-4), 55.9 (C-5), 78.8 (C-6), 94.0 (C-7), 81.8 (C-8), 47.8 (C-9), 42.6 (C-10), 49.7 (C-11), 27.0 (C-12), 36.5 (C-13), 73.9 (C-14), 32.2 (C-15), 81.8 (C-16), 63.9 (C-17), 25.3 (C-18), 57.5 (C-19), 49.2, 12.4 (NCH_2CH_3), 56.4 (1-OCH_3), 56.4 (16-OCH_3), 93.2 (O-CH_2-O) [11].

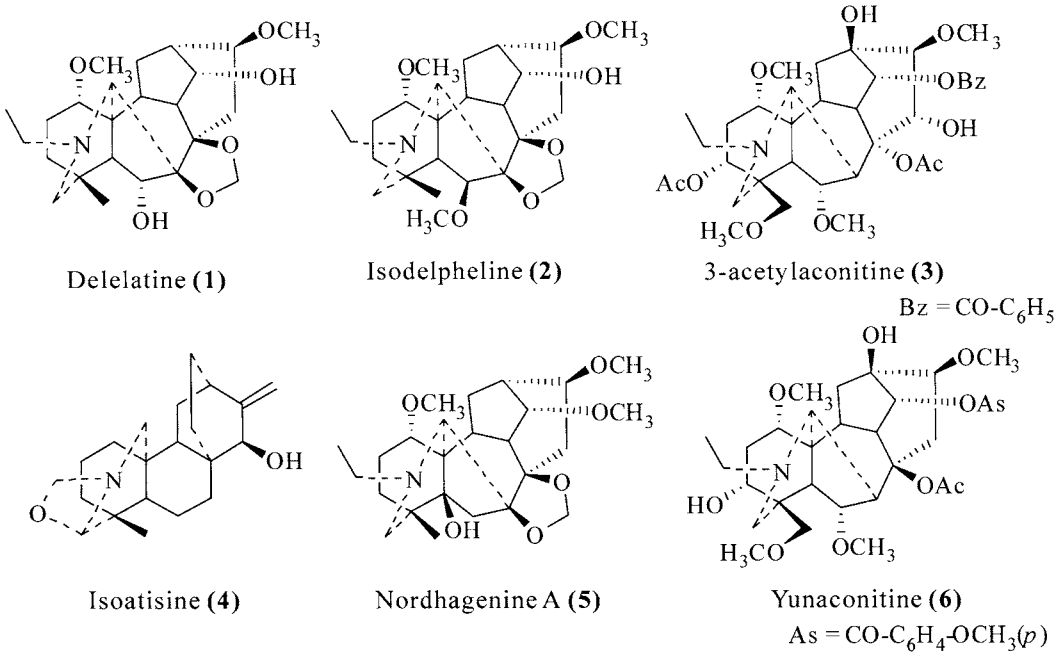

Figure 1 The structures of compounds **1–6**.

Isodelpheline (**2**), amorphous powder. EI-MS *m/z*: 449 $[M]^+$; IR ν_{Max}^{KBr} cm^{-1}: 3474 (OH), 2928, 2820, 1193, 1090, 753; ^1H-NMR ($CDCl_3$, 400 MHz) δ: 0.90 (3H, s, H-18), 1.03 (3H, t, J = 7.0 Hz, NCH_2CH_3), 3.21, 3.36, 3.74 (each 3H, s, 3 × OCH_3), 3.57 (1H, t, J = 6.4 Hz, H-1β), 3.60 (1H, d, J = 8.8 Hz, H-6α), 3.70 (1H, t, J = 6.4 Hz, H-14β), 4.41 (1H, t, J = 6.3 Hz, H-16α), 5.07, 5.16 (each 1H, s, O-CH_2-O);[13] ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 83.8 (C-1), 26.3 (C-2), 36.9 (C-3), 33.9 (C-4), 35.8 (C-5), 89.3 (C-6), 93.9 (C-7), 80.9 (C-8), 47.9 (C-9), 42.2 (C-10), 49.3 (C-11), 26.8 (C-12), 35.8 (C-13), 74.3 (C-14), 32.2 (C-15), 82.0 (C-16), 64.5 (C-17), 26.2 (C-18), 57.2 (C-19), 50.6, 14.1 (NCH_2CH_3), 55.8 (1-OCH_3), 59.0 (6-OCH_3), 56.4 (16-OCH_3), 93.9 (O-CH_2-O)[12].

3-acetylaconitine (**3**), white crystal [petroleum ether-acetone (8 : 2)], mp 195 – 197 ℃. EI-MS *m/z*: 673 $[M]^+$; IR ν_{Max}^{KBr} cm^{-1}: 3500 (OH), 1710, 1280, 1100, 1610,

1580, 720; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.67 (3H, t, J = 7.2 Hz, NCH_2CH_3), 1.40 (3H, s, 8-OAc), 2.15 (3H, s, 3-OAc), 3.15, 3.20, 3.30, 3.70 (each 3H, s, 4 × OCH_3), 4.85 (1H, d, J = 4.8 Hz, 14-βH), 4.90 (1H, dd, J = 12.0, 6.4 Hz, 3-βH), 7.30 – 8.00 (5H, m, Ar-H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 83.5 (C-1), 32.1 (C-2), 71.4 (C-3), 42.6 (C-4), 46.2 (C-5), 82.3 (C-6), 45.6 (C-7), 92.1 (C-8), 44.9 (C-9), 40.8 (C-10), 49.9 (C-11), 36.6 (C-12), 74.3 (C-13), 79.1 (C-14), 78.8 (C-15), 90.0 (C-16), 61.0 (C-17), 71.6 (C-18), 49.0 (C-19), 47.1, 13.4 (NCH_2CH_3), 56.0 (1-OCH_3), 58.5 (6-OCH_3), 60.7 (16-OCH_3), 58.8 (18-OCH_3), 172.4, 21.2 (8-OAc), 170.3, 21.3 (3-OAc), 165.8, 129.5, 128.6, 132.6 (Ar) [13].

Isoatisine (**4**), colorless crystal [petroleum ether-acetone (8 : 2)], mp 152 – 153 ℃. EI-MS m/z: 343 $[M]^+$; IR ν_{Max}^{KBr} cm^{-1}: 3455 (OH), 3090, 1657, 870. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 4.98, 4.87 (2H, s, H-17), 1.19 (3H, s, H-18), 2.71 (2H, s, H-20), 3.47 (2H, m, H-21); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 40.5 (C-1), 22.0 (C-2), 39.9 (C-3), 38.0 (C-4), 48.5 (C-5), 19.2 (C-6), 31.8 (C-7), 37.4 (C-8), 39.6 (C-9), 35.9 (C-10), 28.1 (C-11), 36.3 (C-12), 27.5 (C-13), 26.3 (C-14), 76.8 (C-15), 156.4 (C-16), 109.7 (C-17), 24.3 (C-18), 98.4 (C-19), 49.8 (C-20), 54.9 (N-CH_2-), 58.6 (O-CH_2-) [14].

Nordhagenine A (**5**), colorless crystal [petroleum ether-acetone (8 : 2)], mp 178 – 180 ℃. EI-MS *m/z*: 449 $[M]^+$; IR ν_{Max}^{KBr} cm^{-1}: 3492 (OH), 2918, 2830, 1173, 1083; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.81 (3H, s, H-18), 1.04 (3H, t, J = 7.0 Hz, NCH_2CH_3), 3.22, 3.31, 3.41 (each 3H, s, 3 × OCH_3), 3.55 (1H, t, J = 6.2 Hz, H-1β), 3.70 (1H, t, J = 4.6 Hz, H-14β), 3.90 (1H, t, J = 6.3 Hz, H-16β), 4.98, 4.86 (each 1H, s, O-CH_2-O); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 82.8 (C-1), 21.0 (C-2), 28.2 (C-3), 39.8 (C-4), 79.0 (C-5), 34.0 (C-6), 84.1 (C-7), 83.9 (C-8), 42.0 (C-9), 38.0 (C-10), 54.8 (C-11), 27.4 (C-12), 40.0 (C-13), 84.0 (C-14), 42.2 (C-15), 83.8 (C-16), 62.3 (C-17), 21.0 (C-18), 61.6 (C-19), 51.5, 14.0 (NCH_2CH_3), 56.2 (1-OCH_3), 59.0 (14-OCH_3), 57.8 (16-OCH_3), 94.5 (O-CH_2-O) [15].

Yunaconitine (**6**), amorphous powder. EI-MS *m/z*: 660 $[M+H]^+$; IR ν_{Max}^{KBr} cm^{-1}: 3498 (OH), 2928, 2856, 1715, 1356, 1379, 1278, 1067, 810; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.00 (2H, d, J = 8.8 Hz, H-2', 6'), 6.92 (2H, d, J = 8.9 Hz, H-3', 5'), 4.87 (1H, d, J = 5.2 Hz, H-14), 3.86, 3.54, 3.29, 3.25, 3.15 (each 3H, s, 5 × OCH_3), 1.33 (3H, s, COMe), 1.10 (3H, t, J = 6.9 Hz, Me-21); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 83.2 (C-1), 33.7 (C-2), 71.8 (C-3), 43.2 (C-4), 47.5 (C-5), 82.3 (C-6), 44.8 (C-7), 85.7 (C-8), 48.8 (C-9), 41.0 (C-10), 50.2 (C-11), 35.3 (C-12), 74.8 (C-13), 78.5 (C-14), 39.7 (C-15), 83.6 (C-16), 61.8 (C-17), 77.3 (C-18), 48.9 (C-19), 47.5 (C-20), 13.4 (C-21), 56.0 (1-OCH_3), 58.9 (6-OCH_3), 57.9 (16-OCH_3), 59.2 (18-OCH_3), 55.5 (4'-OCH_3), 170.0 (COMe), 21.7 (COMe), 166.3 (Ar-CO), 122.7 (C-1'), 122.7 (C-2', 6'), 113.8 (C-3', 5'), 163.6 (C-4') [6].

These compounds were evaluated for their cytotoxic activities against P388 cells using the SRB (Sulforhodamine B) method as reported previously [10], with etoposide as the positive control. The cell growth inhibitory potency of compounds **1** – **6** is expressed as IC_{50} (μM) values. As listed in Table 1, both compounds **3** and **5** exhibited moderately cytotoxic activities. Gratifyingly, compound **1** exhibited cytotoxic activities with an IC_{50} value of 4.36 μM. This further supports the traditional medicinal uses of this species. However, compounds **2**, **4** and **6** were found to be inactive against P388 cells.

Table 1 Cytotoxicity data for compounds **1** – **6** against human leukemic cells (P388)[ab]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
Delelatine (**1**)	4.36	Isoatisine (**4**)	>50
Isodelpheline (**2**)	>50	Nordhagenine (**5**)	20.0
3-acetylaconitine (**3**)	18.60	Yunaconitine (**6**)	>50
Etoposide	7.53		

[a] Results are expressed as IC_{50} values in M. [b] Compounds **2**, **4** and **6** were inactive for P388 human cell lines (IC_{50} >50 μM).

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