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Four new C_{18}-diterpenoid alkaloids with analgesic activity from *Aconitum weixiense*

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ABSTRACT

Four new C_{18}-diterpenoid alkaloids, weisaconitines A–D (**1**–**4**), were isolated from *Aconitum weixiense*. Based on extensive UV, IR, MS, 1D and 2D NMR analyses, their structures were elucidated as 8-O-ethyldolaconine (**1**), 4-demethylgenicunine B (**2**), 14-oxoaconosine (**3**), and 8-O-ethylaconosine (**4**). The analgesic activity of compound **4** was studied with CH_3COOH-induced writhing model in mice. Compound **4** showed writhing inhibitions of 24% (50 mg/kg), 26% (100 mg/kg) and 34% (200 mg/kg), respectively, as compared to the reference drug aspirin (63%) at a dose of 200 mg/kg.

1. Introduction

Aconitum L. (Ranunculaceae), annual or perennial herbs, mainly distribute in the temperate regions of the northern hemisphere. There are about 300 species all over the world, of which about 76 *Aconitum* spp. have been used as poisonous and medicinal plants in China [1]. The diterpenoid alkaloids, including 'C_{18}-, C_{19}- and C_{20}-diterpenoid alkaloids', are main chemical constituents with analgesic, cardiotonic, anti-inflammatory, and anti-arrhythmic activities [2–5]. Three diterpenoid alkaloids, 3-acetylaconitine, lappaconitine, and crassicauline A, used as analgesic drugs taking effects on sodium channel, are clinically employed for the treatment of various pains in China [3].

The roots of *Aconitum weixiense* W. T. Wang, endemic to Weixi county of Yunnan province in China, are utilized to treat pains and rheumatism by natives [6]. Furthermore, we observed that the plant was severely grazed by cattle, but causing no death to them. Therefore, it was indicative that *A. weixiense* was nontoxic, being prominently different from other *Aconitum* spp.. To find safer and biologically active substances, the roots of *A. weixiense* were phytochemically investigated to afford four new C_{18}-diterpenoid alkaloids, weisaconitines A–D (**1**–**4**, Fig. 1). Compound **4** showed an analgesic activity by CH_3COOH-induced writhing model in mice. This paper described their isolation, structural elucidation and analgesic activity.

2. Experimental

2.1. General experimental procedures

Optical rotations were determined on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). UV spectra were measured on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan). IR (KBr) spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, California, USA). 1D and 2D NMR were recorded on Bruker AM-400, Bruker DRX-500 or AVANCE III-600 spectrometers (Bruker, Bremerhaven, Germany). Mass spectra were run on a VG

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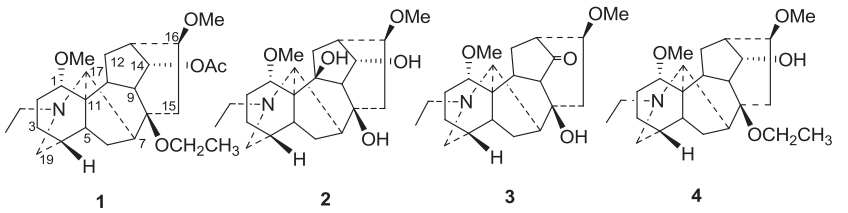

Fig. 1. The structures of compounds **1**–**4**, isolated from *Aconitum weixiense*.

Spec-3000 spectrometer (VG, Manchester, UK) and Waters AutoSpec Premier P776 (Waters, USA). Silica gel (200–300 mesh) for column chromatography and TLC plates (GF_{254}) were obtained from Qingdao Makall Chemical Company (Makall, Qingdao, China). Al_2O_3 for column chromatography was purchased from Shanghai Wusi Chemical Reagents Company, Ltd. (Wusi, Shanghai, China). Fractions were visualized by silica gel plates sprayed with Dragendorff's reagent.

2.2. Plant material

The roots of *A. weixiense* W. T. Wang were collected at Weixi county of Yunnan Province, P. R. China, in October, 2012, and authenticated by Prof. Li-Gong Lei (Kunming Institute of Botany, Chinese Academy of Sciences). The voucher specimen (No. YNS2012-26) had been deposited in the Yunnan Research Center on Good Agricultural Practice for Dominant Chinese Medicinal Materials, College of Agriculture and Biotechnology, Yunnan Agricultural University.

2.3. Extraction and isolation

The air-dried roots of *A. weixiense* (3 kg) were powdered and extracted three times with MeOH for 2 h under reflux. Being removed solvent under reduced pressure, the crude extract was dissolved with 3 L 1.5% HCl solution. After filtration, the acidic solution was basified to pH 9.0 with ammonia (25%) and extracted with $CHCl_3$ to obtain crude alkaloidal extract (65 g). The alkaloidal extract was subjected to silica column chromatography (Si CC, 800 g, 8 × 50 cm) and eluted with petroleum ether–acetone–diethylamine (100:1:1, 80:1:1, 50:1:1, 25:1:1, 25:5:1, 25:10:1, 25:25:1, 10:20:1, *v*/*v*, each 1 L) gradient to afford five fractions (A–E). Fr. B (25 g) was further separated to obtain five subfractions (B1–B5) by Si CC with petroleum ether–diethylamine (50:1, 40:1, 30:1, 20:1, each 500 mL) as the eluent. Fr. B2 (2.7 g) was performed on Al_2O_3 CC (2.0 × 25 cm, 30 g) and eluted with petroleum ether–EtOAc (15:1, each 100 mL) to yield compound **1** (7 mg). Fr. B3 (5.8 g) was applied to Al_2O_3 CC (4 × 22 cm, 112 g) with an eluent of petroleum ether–EtOAC (10:1, each 500 mL), and then purified through Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to produce compounds **3** (10 mg) and **4** (1.8 g). Fr. D (15 g) was further separated to obtain five subfractions (D1–D5) by Si CC with petroleum ether–acetone–diethylamine (15:1:1, 10:1:1, 10:2:1, 10:5:1, *v*/*v*, each 500 mL) as the eluent. Fr. D3 (2.1 g) was applied to Al_2O_3 CC (2.0 × 25 cm, 30 g) with an eluent of petroleum ether–EtOAc (1:3, each 100 mL), and further purified through Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to provide compound **2** (13 mg). All obtained compounds had a degree of purity >90%, based on the TLC method in three different solvent systems exhibiting one spot with Dragendorff's reagent, and NMR spectra with the smooth baseline and no impurity peak.

Weisaconitine A (**1**): Colorless gum; $[\alpha]_D^{13.5}$: −0.90 (*c* 0.20, MeOH); IR (KBr) ν_{max}: 2925, 1739, 1063 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 448.3047 ($[M + H]^+$, $C_{26}H_{42}NO_5^+$, calcd for 448.3058).

Weisaconitine B (**2**): Colorless powder; $[\alpha]_D^{15.9}$: −11.37 (*c* 0.20, MeOH); IR (KBr) ν_{max}: 3436, 2924, 1059 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 394.2588 ($[M + H]^+$, $C_{22}H_{36}NO_5^+$, calcd for 394.2588).

Weisaconitine C (**3**): Colorless powder; $[\alpha]_D^{13.5}$: −35.47 (*c* 0.10, MeOH); IR (KBr) ν_{max}: 3432, 2922, 1745, 1064 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 376.2483 ($[M + H]^+$, $C_{22}H_{34}NO_4^+$, calcd for 376.2482).

Weisaconitine D (**4**): Colorless powder; $[\alpha]_D^{16.1}$: −16.07 (*c* 0.20, MeOH); IR (KBr) ν_{max}: 3431, 2924, 1064 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 406.2948 ($[M + H]^+$, $C_{24}H_{40}NO_4^+$, calcd for 406.2952).

Table 1
^{13}C NMR data (100 MHz, in $CDCl_3$) of compounds **1**–**4**.

Position	1	2	3	4
1	85.8 d	78.6 d	85.5 d	86.2 d
2	28.3 t	29.6 t	27.9 t	29.8 t
3	36.7 t	34.6 t	37.2 t	35.8 t
4	35.8 d	36.2 d	36.0 d	36.6 d
5	44.8 d	41.3 d	45.1 d	45.6 d
6	27.3 t	26.0 t	26.1 t	28.4 t
7	46.7 d	45.2 d	46.0 d	45.9 d
8	77.7 s	72.4 s	83.0 s	77.9 s
9	44.3 d	56.1 d	55.4 d	45.5 d
10	38.2 d	81.1 s	46.2 d	40.7 d
11	49.0 s	53.8 s	50.2 s	50.2 s
12	26.5 t	37.7 t	25.4 t	26.4 t
13	40.9 d	38.1 d	43.8 d	39.2 d
14	75.0 d	74.1 d	216.5 s	75.2 d
15	29.6 t	40.1 t	29.2 t	30.4 t
16	81.3 d	81.6 d	86.4 d	82.7 d
17	61.1 d	64.4 d	64.2 d	62.9 d
19	51.0 t	50.0 t	49.6 t	49.5 t
$\underline{NCH_2CH_3}$	49.3 t	49.7 t	48.8 t	48.8 t
$N\overline{CH_2CH_3}$	12.9 q	13.6 q	13.6 q	13.5 q
$O\underline{CH_2CH_3}$-8	56.3 t	–	–	55.9 t
$O\overline{CH_2CH_3}$-8	16.0 q	–	–	16.1 q
OMe-1	56.1 q	56.0 q	56.1 q	56.3 q
OMe-16	56.6 q	56.4 q	56.4 q	56.5 q
$\underline{C}OMe$-14	170.8 s	–	–	–
$\overline{CO}Me$-14	21.2 q	–	–	–