Fitoterapia 91 (2013) 280–283

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

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

Da-Ke Zhao [a], Hong-Lian Ai [b], Shu-Hui Zi [b], Li-Mei Zhang [b], Sheng-Chao Yang [b], Hua-Chun Guo [b], Yong Shen [b,*], Yan-Ping Chen [b], Ji-Jun Chen [c,*]

[a] School of Agriculture, Yunnan University, Kunming 650091, PR China
[b] College of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming 650201, PR China
[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China

ARTICLE INFO

Article history:
Received 30 April 2013
Accepted in revised form 16 September 2013
Available online 29 September 2013

Keywords:
Diterpenoid alkaloids
Weisaconitines A–D
Analgesic activity
Aconitum weixiense
Ranunculaceae

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

* Corresponding authors. Tel./fax: +86 871 65227213.
E-mail addresses: pharmsy@sina.com (Y. Shen), chenjj@mail.kib.ac.cn (J.-J. Chen).

0367-326X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2013.09.013

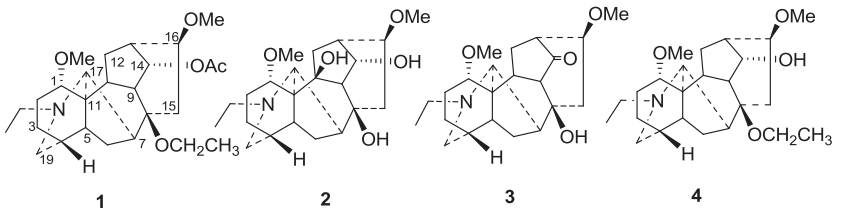

Fig. 1. The structures of compounds **1**–**4**, isolated from *Aconitum weixiense*.

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	–	–	–

Table 2
^{1}H NMR data (400 MHz, in $CDCl_3$) of compounds **1**–**4**.

Position	1	2	3	4
1	3.18 (*dd*, *J* = 10.2, 6.4)	3.77 (*dd*, *J* = 10.0, 6.2)	3.19 (*dd*, *J* = 10.4, 6.0)	3.18 (*dd*, *J* = 10.2, 6.4)
2a	1.43 (*m*)	1.32 (*m*)	1.37 (*m*)	1.59 (*m*)
2b	2.07 (*m*)	2.02 (*m*)	2.13 (*m*)	2.12 (*m*)
3a	2.02 (*m*)	1.36 (*m*)	1.75 (*m*)	1.76(*m*)
3b	2.43 (*m*)	1.70 (*m*)	1.84 (*m*)	2.04 (*m*)
4	1.63 (*m*)	1.73 (*m*)	1.58 (*m*)	1.79 (*m*)
5	2.08 (*m*)	1.75 (*m*)	1.73 (*m*)	1.70 (*m*)
6a	1.44 (*m*)	1.96 (*m*)	1.63 (*m*)	1.28 (*m*)
6b	2.09 (*m*)	2.14 (*m*)	1.94 (*m*)	1.85 (*m*)
7	2.10 (*m*)	2.16 (*s*)	2.23 (*m*)	215 (*m*)
9	2.57 (*m*)	2.12 (*d*, *J* = 4.2)	2.31 (*m*)	2.21 (*m*)
10	2.38 (*m*)	2.06 (*m*)	1.97 (*m*)	1.78 (*m*)
12a	1.92 (*m*)	2.35 (*m*)	1.86 (*m*)	1.76 (*m*)
12b	2.15 (*m*)	2.47 (*m*)	1.98 (*m*)	2.13 (*m*)
13	2.18 (*m*)	1.92 (*m*)	2.47 (*m*)	2.44 (*m*)
14	4.74 (*t*, *J* = 4.3)	4.73 (*t*, *J* = 4.2)	–	4.00 (*t*, *J* = 4.5)
15a	2.07 (*m*)	2.01 (*m*)	2.09 (*m*)	1.71 (*m*)
15b	2.40 (*m*)	2.43 (*m*)	2.47 (*m*)	1.99 (*m*)
16	3.35 (*m*)	3.36 (*m*)	3.34 (*m*)	3.35 (*m*)
17	2.90 (*br. s*)	2.94 (*br. s*)	2.79 (*br. s*)	2.83 (*br. s*)
19a	2.48 (*d*, *J* = 10.2)	2.34 (*d*, *J* = 10.4)	2.38 (*d*, *J* = 10.2)	2.44 (*d*, *J* = 10.6)
19b	2.57 (*d*, *J* = 10.2)	2.57 (*d*, *J* = 10.4)	2.53 (*d*, *J* = 10.2)	2.52 (*d*, *J* = 10.6)
NCH_2CH_3	2.41 (*m*)	2.40 (*m*)	2.43 (*m*)	2.38 (*m*)
	2.55 (*m*)	2.45 (*m*)	2.47 (*m*)	2.42 (*m*)
NCH_2CH_3	1.06 (*t*, *J* = 7.0)	1.04 (*t*, *J* = 7.0)	1.07 (*t*, *J* = 6.9)	1.06 (*t*, *J* = 7.1)
OCH_2CH_3-8	3.18 (*m*)	–	–	3.25 (*m*)
OCH_2CH_3-8	1.12 (*t*, *J* = 6.8)	–	–	1.09 (*t*, *J* = 6.9)
OMe-1	3.26 (*s*)	3.25 (*s*)	3.24 (*s*)	3.23 (*s*)
OMe-16	3.34 (*s*)	3.32 (*s*)	3.32 (*s*)	3.33 (*s*)
COMe-14	2.00 (*s*)	–	–	–

2.4. Analgesic activity in mice

The analgesic activity was performed according to the previous reports [9,10]. Aspirin, purchased from Nanjing Baijingyu Pharmaceutical Co. Ltd, China (purity > 98.5%, No. 2001101006) was used as the positive control. The test animal, Kunming mice (body weight 20–24 g), was obtained from Animal Experiment Center of Sichuan University, China. Acetic acid (CH_3COOH) was provided by Chengdu Chemical Reagent Factory Co. Ltd, China (purity > 99.5%, No. 20110301).

3. Results and discussion

Weisaconitine A (**1**) was obtained as colorless gum and its molecular formula was determined to be $C_{26}H_{41}NO_5$ based on ESIMS ($[M + H]^+$; *m/z* 448) and HRESIMS (*m/z* 448.3047 $[M + H]^+$, calc. 448.3058) analyses, indicating 7 degrees of unsaturation. The IR spectrum showed the absorption band for ester carbonyl (1739 cm^{-1}). The ^{1}H and ^{13}C NMR data displayed the presence of one N-ethyl group (δ_H 2.41, 2.55, each 1H, m; 1.06, 3H, t, *J* = 7.0 Hz; δ_C 49.3 t, 12.9 q), one O-ethyl group (δ_H 3.18, 2H, m; 1.12, 3H, t, *J* = 6.8 Hz; δ_C 56.3 t, 16.0 q), an acetyl group (δ_H 2.00, 3H, s; δ_C 170.8 s, 21.2 q), and two OMe groups (δ_H 3.34, 3.26, each 3H, s; δ_C 56.6 q,56.1 q). Compound **1** exhibited 26 resonances in the ^{13}C NMR (DEPT) spectrum (Table 1) attributing to 5 methyls, 8 methylenes, 10 methylidynes, and 3 quaternary C-atoms. The above spectral data suggested that compound **1** might be a C_{18}-diterpenoid alkaloid. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of compound **1** were almost identical with those of dolaconine [7] except that the O-ethyl group signal appeared in compound **1** and the signal due to C-8 was downfield shifted from δ_C 73.7 in dolaconine to δ_C 77.7 in **1**, suggesting that only one O-ethyl group locates at C-8. This was further confirmed by the cross-peaks between the signal at δ_H 3.18 (2H, m, OCH_2CH_3) and C-8 in the HMBC spectrum (Fig. 2). The full NMR data (Tables 1 and 2) assignments of compound **1** were achieved according to the HSQC, HMBC, COSY and ROESY spectral analyses.

Compound **1** had the same relative configuration as dolaconine, supported by their almost same ^{1}H and ^{13}C NMR data, and ROESY spectrum (Fig. 3). Thus, the structure of compound **1** was determined as 8-O-ethyldolaconine, named weisaconitine A (**1**).

Weisaconitine B (**2**), colorless powder, was assigned to have a molecular formula of $C_{22}H_{35}NO_5$ in agreement with ESIMS ($[M + H]^+$ *m/z* 394) and HRESIMS (394.2588 $[M + H]^+$; calc. 394.2588) analyses. The IR spectrum showed absorption attributable to hydroxyl (3436 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of compound **2** (Tables 1 and 2) displayed 22 carbon signals due to 3 methyls, 7 methylenes, 9 methylidynes and 3 quaternary C-atoms. In the ^{1}H NMR spectrum, the signals for 2 OMe singlets at δ_H 3.32, 3.25 (each3H, s) were observed, together with one NCH_2CH_3 at δ_H 1.04 (t, *J* = 7.0 Hz), suggesting that compound **1** be characteristic of the C_{18}-diterpenoid alkaloid. The careful analyses of ^{1}H NMR and ^{13}C NMR spectroscopic data indicated that the structure of **2** was similar to genicunine B [8] except that one quaternary methyl (δ_H 0.76, 3H, s; δ_C 26.2 q) at C-4 disappeared in **2**, and the chemical shift locating at C-4 was downfield shifted from δ_C 34.3 (s) in genicunine B to δ_C 36.2 (d) in **2**. The long-range correlations between H-4

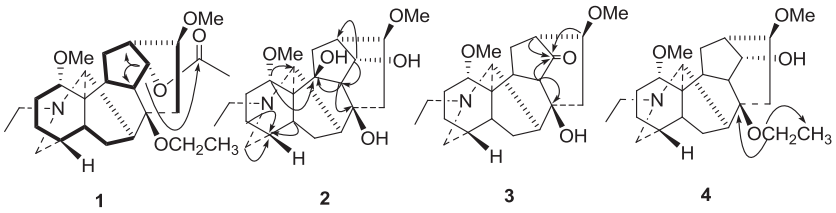

Fig. 2. Selected ^{1}H, ^{1}H-COSY ( ) and HMBC (→) correlations of compounds **1**–**4**.

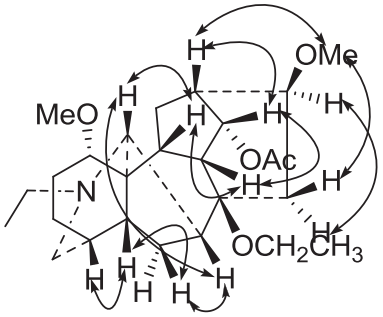

Fig. 3. Selected ROESY correlations of compound **1**.

and C-3, C-5 and C-19 in the HMBC spectrum confirmed its structure (Fig. 2). Accordingly, compound **2** was established to be 4-demethylgenicunine B.

Weisaconitine C (**3**) was elucidated as $C_{22}H_{33}NO_4$ by ESIMS ($[M + H]^+$ *m/z* 376) and HRESIMS (376.2483 $[M + H]^+$; calc. 376.2482) experiments. The IR spectrum showed absorption of C═O (1745 cm^{-1}). The ^{1}H NMR data displayed the presence of two OMe signals at δ_H 3.32, 3.24 (each 3H, s) and one N-ethyl signal at δ_H 1.07 (t, $J = 6.9$ Hz), indicating the feature of the C_{18}-diterpenoid alkaloid. Comparing the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of compound **1** with those of aconosine [7] showed high similarity except for a C═O signal at δ_C (216.5, s) at C-14 instead of an OH group. This was further confirmed by the HMBC (Fig. 2) correlations from H-9, H-10, H-13 and H-16 to C-14 (δ_C 216.5, s, C═O). Consequently, weisaconitine C (**3**) was elucidated as 14-oxoaconosine (**3**).

Weisaconitine D (**4**) had a molecular formula $C_{24}H_{39}NO_4$, in agreement with ESIMS ($[M + H]^+$ *m/z* 406) and HRESIMS (406.2948 $[M + H]^+$; calc. 406.2952) analyses. Its ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) suggested that compound **1** might be a C_{18}-diterpenoid alkaloid, bearing an O-ethyl, a N-ethyl and two OMe groups. Compound **4** differing from aconosine [7] in the substitution of C-8 at where one O-ethyl group (δ_H 3.25, m, 2H; 1.09, t, $J = 6.9$, 3H; δ_C 55.9 t, 16.1 q) instead of one OH group, was present, which was also confirmed by the HMBC correlation between δ_H 3.25(2H, m, O-ethyl) and C-8. Hence, compound **4** was defined as 8-O-ethylaconosine (**4**).

The analgesic activity was investigated by CH_3COOH-induced writhing response in mice [9,10]. Different doses of compound **4** with 50, 100, 200 mg/kg were administered *o.p.*, and showed writhing inhibitions of 24%, 26%, 34%, as compared to the reference drug aspirin (63%) at a dose of 200 mg/kg. Compounds **1**–**3**, trace diterpenoid alkaloids with 7, 10 and 13 mg respectively, were not performed for the test of the analgesic activity.

Conflict of interest

There is no conflict of interest.

Acknowledgments

This work was financially supported by the State Key Laboratory of Phytochemistry and Plant Resources in West China, KIB, CAS (No. P2012-KF04), the National Natural Science Foundation of China (21362045) and the project of Sciences and Technology Department of Yunnan Province (No. 2012CG007). The authors are grateful to the staff of the analytical group of State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for the measurements of all spectra.

References

[1] Xiong J, Tang NH, Ji CJ, Lu Y, Gong NB. Vilmoraconitine, a novel skeleton C19-diterpenoid alkaloid from *Aconitum vilmorinianum*. Tetrahedron Lett 2008;49:4851–3.
[2] Xiao PG, Wang FP, Gao F, Yan LP, Chen DL, Liu Y. A pharmacophylogenetic study of *Aconitum* L. (Ranunculaceae) of China. Acta Phytotaxon Sin 2006;44:1–46.
[3] Wang FP, Chen QH. The C_{19}-diterpenoid alkaloids. In: Cordell GA, editor. Alkaloids: chemistry and biology, vol. 69. New York: Elsevier Science; 2010. p. 1–577.
[4] Wang FP, Chen QH, Liang XT. The C_{18}-diterpenoid alkaloids. In: Cordell GA, editor. Alkaloids: chemistry and biology, vol. 67. New York: Elsevier Science; 2009. p. 1–78.
[5] Wang FP, Liang XT. C_{20}-diterpenoid alkaloids. In: Cordell GA, editor. Alkaloids: chemistry and biology, vol. 59. New York: Elsevier Science; 2002. p. 1–288.
[6] Yunnan Institute of Botany. Flora of Yunnanica, Tomus 1, vol. 11. Beijing: Science Press; 1977 87.
[7] Ding LS, Chen WX. Study on the chemical constituents of *Aconitum campylorrhynchum*. Acta Pharmacol Sin 1990;25:441–4.
[8] Wang FP, Li ZB, Wang JZ, Jian XX, Chen DL. New norditerpenoid alkaloids of *Aconitum geniculatum*. Acta Chim Sin 2000;58:576–9.
[9] Xu SY, Bian RL, Chen X. Methodology of pharmacological experiment. Beijing: People's Medical Publishing House; 2002 882–904.
[10] Tang XC, Zhu MY, Feng J, Wang YE. Study on pharmacological actions of lappaconitine hydrobromide. Acta Pharmacol Sin 1983;18:579–84.