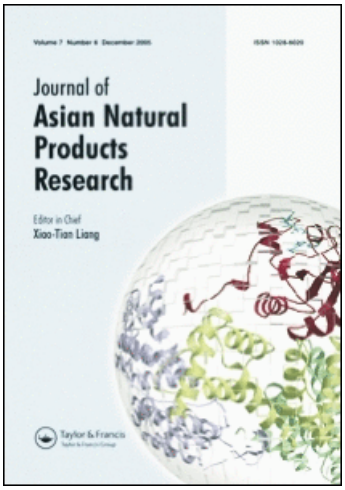

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Structure of lasiansine from *Aconitum nagarum* var. *lasiandrum*

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A new C_{19}-diterpenoid alkaloid, lasiansine (**1**), was isolated from the roots of *Aconitu nagarum* var. *lasiandrum* (Ranunculaceae) together with six known diterpenoid alkaloids. The structure of **1** was elucidated by spectral methods (^{1}H-NMR, ^{13}C-NMR, 2D-NMR, HRMS, IR), and the ^{13}C-NMR spectrum of 16-epipyroaconine (**3**) and the single-crystal X-ray analysis of its derivative (**5**) are reported for the first time.

Keywords: Ranunculaceae; *Aconitum nagarum* var. *lasiandrum*; C_{19}-Diterpenoid alkaloid; Lasiansine; 16-Epipyroaconine

1. Introduction

The plant *Aconitum nagarum* var. *lasiandrum* (Ranuculaceae), which grows in Xuanwei prefecture of Yunnan province, is used as a folk medicine to treat rheumatism and neuralgia [1]. *A. nagarum* var. *lasiandrum* has been reported to contain eighteen diterpenoid alkaloids: aconitine, 3-deoxyaconitine, neoline, nagarine, aconifine [2], 14-acetylneoline, songorine [3], flavaconitine, virescenine, denudatine, songoramine [4], vilmorrianine A, karakoline, sachaconitine, talatizidine, isotalatizidine, chasmanine, and yunaconitine [5]. Our studies on the plant led to the isolation of a new C_{19}-diterpenoid alkaloid, lasiansine (**1**), as well as six additional known alkaloids: 16-epipyroaconine (**3**), talatisamine, 15α-hydroxyneoline, 1-epiaconine, 12-epi-19-dehydronapelline, and 12-epinapelline. Here we report the isolation and structural elucidation of the new alkaloid, and ^{13}C-NMR data for 3 and single-crystal X-ray analysis of its derivative (**5**) (figure 1).

2. Results and discussion

Compound **1** has the molecular formula $C_{24}H_{39}NO_7$, established from its HR-FABMS ([$M^+ + H$] at *m/z* 454.2808) and ^{13}C-NMR spectrum. The NMR and MS spectra of **1** showed that it was a C_{19}-diterpenoid alkaloid [6]. The ^{1}H- and ^{13}C-NMR spectra showed the

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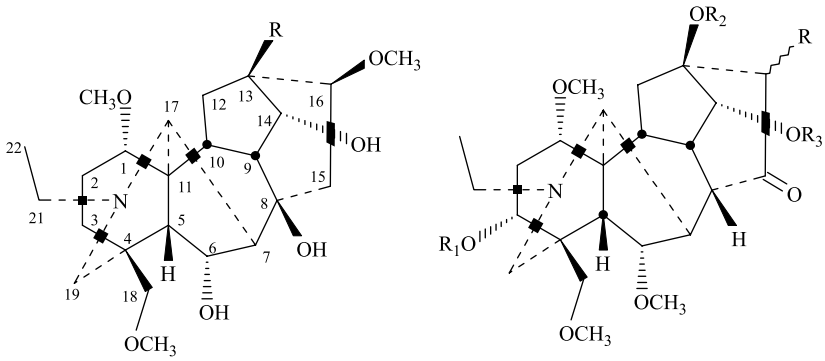

1 R = OH
2 R = H

3 R = –OCH$_3$ $R_1 = R_2 = R_3 = H$
4 R = –OCH$_3$ $R_1 = R_2 = R_3 = H$
5 R = –OCH$_3$ $R_1 = R_2 = R_3 = Ac$

Figure 1. Structures of compounds **1**–**5**.

presence of an *N*-ethyl (δ_H 1.09, 3H, t, $J = 7.2$ Hz; 2.55, 2H, m; δ_C 13.5, q, 49.1, t) and three methoxyls (δ_H 3.23, 3.31, 3.40, each 3H, s; δ_C 56.0 q, 59.1 q, 57.8 q). Its IR (3422 cm^{-1}) and the ^{13}C-NMR spectrum (δ_C 71.8, d, 73.9, s, 76.6, s, 79.1, d) showed the presence of two secondary hydroxyl groups and two tertiary hydroxyl groups. Three methoxyl groups could be located at C-1, C-18 and C-16 due to the HMBC correlations between 1-OCH$_3$ (δ_H 3.23, s) and C-1 (δ_C 85.6, d), 18-OCH$_3$ (δ_H 3.31, s) and C-18 (δ_C 80.8, t), and 16-OCH$_3$ (δ_H 3.40, s) and C-16 (δ_C 84.1, d). Two secondary hydroxyl groups were assigned to C-6 and C-14 based on the HMBC correlations between C-6 (δ_C 71.8, d) and H-5 (δ_H 2.00, hidden), H-7 (δ_H 2.01, hidden), H-17 (δ_H 3.09, s), and C-14 (δ_C 79.1, d) and H-9 (δ_H 2.34, m), H-16 (δ_H 3.36, d, $J = 8.4$ Hz). The remaining hydroxyl groups in **1** could be located at C-8 and C-13 due to the HMBC correlations between C-8 (δ_C 73.9, s) and H-6 (δ_H 4.71, d, $J = 6.8$ Hz), H-7, H-9, H-10 (δ_H 1.90, m), H-14 (δ_H 3.97, d, $J = 5.2$ Hz), H-15 (δ_H 2.25, m, 2.49, m), H-16, H-17 (table 1). Comparison of the MS and NMR spectra of 1 with those of 6-epiforsticine (**2**), a known alkaloid isolated from *Aconitum hemsleyanum* var. *pengzhouense* and *A. kuzsnezoffii* [7], showed that it had an additional hydroxyl group. The ^{13}C-NMR spectra of **1** and **2** are very similar except for C-9, C-10, C-12, C-13, C-14, C-15 and C-16, indicating that the additional hydroxyl group is located on C-13 [8]. This assignment was further confirmed by the HMBC correlations between H-9, H-10, H-12 (δ_H 2.26, m, 2.52, m), H-14, H-15, H-16 and C-13 (δ_C 76.6, s) (table 1). The structure of lasiansine was therefore established as **1**.

In a further investigation of the plant *Aconitum nagarum* var. *lasiandrum*, we also isolated a rare known alkaloid, 16-epi-desbenzoyl-pyroaconitine (**3**) [9,10], but, owing to the deficiency of the NMR data and the difficult differentiation between **3** and its epimer **4** [9], we had to make a careful study of the 2D-NMR spectrum of **3** (table 1, figure 2). Its structure was finally confirmed by a single-crystal X-ray analysis (figure 3) of the derivative **5**.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Perkin–Elmer 341 polarimenter. IR spectra were obtained using a Nicolet FT-IR 200 SXY spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were

Table 1. NMR data for lasiansine (**1**) and 16-epipyroaconine (**3**) (^{1}H: 400 MHz, ^{13}C: 100 MHz, $CDCl_3$).

Carbon	***1***				***3***	
	δ_C	δ_H *mult (J=Hz)*	$^1H-^1H$ *COSY*	*HMBC*	δ_C	δ_H *mult (J=Hz)*
1	85.6 d	3.00 dd (10.8, 6.4)	2●-H, 2●-H	C-2, C-10, C-11, C-17, 1-OCH_3	83.4 d	2.97 dd (9.6, 6.0)
2	25.7 t	1.94 m (●)	1-H, 2●-H, 3-H	C-1, C-11	32.9 t	2.04 m (●)
		2.27 m (●)	1-H, 2●-H, 3-H	C-1		2.27 m (●)
3	35.2 t	1.50 td (11.6, 3.6) (●)	2-H, 3●-H, 19-H	C-2, C-4, C-19	71.6 d	3.65 dd (10.0, 4.8)
		1.68 dt (12.8, 3.6) (●)	2-H, 3●-H, 19-H	C-1, C-2		
4	39.0 s	●	●	●	43.6 s	●
5	50.5 d	2.00 (hidden)	6-H, 17-H	C-1, C-4, C-6, C-7, C-10, C-11, C-17, C-18, C-19	48.1 d	1.96 d (6.4)
6	71.8 d	4.71 d (6.8)	5-H, 17-H	C-4, C-5, C-7, C-8, C-17	84.0 d	3.90 d (6.8)
7	56.1 d	2.01 (hidden)	17-H	C-5, C-6, C-8, C-9, C-17	41.6 d	2.71 (hidden)
8	73.9 s	●	●	●	44.7 d	2.01 (hidden)
9	50.6 d	2.34 m	10-H, 14-H	C-8, C-10, C-11, C-12, C-13, C-14, C-15	48.9 d	2.44 m
10	42.1 d	1.90 m	9-H, 12●-H	C-8, C-11, C-12, C-13, C-17	40.5 d	2.72 (hidden)
11	50.4 s	●	●	●	51.1 s	●
12	36.2 t	2.26 m (●)	10-H, 12●-H, 14-H	C-10, C-13, C-16	33.6 t	1.63 t (12.8) (●)
		2.52 m (●)	12●-H	C-11, C-13, C-16		2.68 m (●)
13	76.6 s	●	●	●	78.3 s	●
14	79.1 d	3.97 d (5.2)	9-H, 16-H	C-8, C-9, C-13, C-16	76.4 d	4.16 d (4.8)
15	40.1t	2.25 m (●)	15●-H, 16-H	C-8, C-13, C-16	212.3 s	●
		2.49 m (●)	15●-H, 16-H	C-7, C-8, C-9, C-13, C-16		
16	84.1 d	3.36 d (8.4)	14-H, 15-H	C-8, C-12, C-13, C-14, 16-OCH_3	85.8 d	3.82 brs
17	62.5 d	3.09 s	5-H, 6-H, 7-H	C-1, C-6, C-7, C-8, C-10, C-11, C-19	61.8 d	2.90 s
18	80.8 t	3.36 ABq (8.4)	18-H	C-3, C-4, C-19, 18-OCH_3	76.6 t	3.61 ABq (9.6)
		3.78 ABq (8.4)	18-H	C-3, C-4, C-5, C-19, 18-OCH_3		3.68 ABq (9.6)
19	54.0 t	2.57 m (●)	5-H, 19-H	C-3, C-4, C-17	49.0 t	2.40 ABq (11.6) (●)
		2.78 d (10.8) (●)	3●-H, 19-H	C-3, C-4, C-18, C-21		2.86 ABq (10.8) (●)
21	49.1 t	2.55 m	22-H	C-17, C-19, C-22	47.6 t	2.45 m
22	13.5 q	1.09 t (7.2)	21-H	C-21	13.0 q	0.98 t (7.2)
1-OCH_3	56.0 q	3.23 s	●	C-1	55.8 q	3.16 s
6-OCH_3	●	●	●	●	57.7 q	3.21 s
16-OCH_3	57.8 q	3.40 s	●	C-16	62.0 q	3.67 s
18-OCH_3	59.1 q	3.31 s	●	C-18	59.1 q	3.23 s

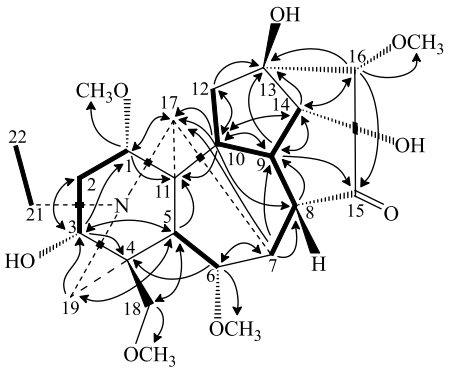

Figure 2. (thick black lines) $^{1}H-^{1}H$ COSY (W-type coupling: H-12/H-16) and (→) selected HMBC correlations of **3**.

recorded using a Varian Unity INOVA 400/45 NMR spectrometer with $CDCl_3$ and TMS as the internal standard. EI-MS and HR-MS were measured from a VG Auto spec 3000 or Finnegan MAT 90 instrument. Silica gel GH_{254} and H (Qingdao Ocean Chemical Factory, China) were used for TLC and column chromatography, respectively. Spots on TLC were detected with modified Dragendorff's reagent. A polyvinyl sulfonic ion-exchange resin (H-form, cross linking 1 × 1, Chemical Factory of Nankai University, China) was used for the extraction of total alkaloids.

3.2 Plant material

The plant *Aconitum nagarum* var. *lasiandrum* was obtained from Xuanwei prefecture, Yunnan province, China, and authenticated by Professor W.T. Wang of the Beijing Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (No. 2009216) has been deposited.

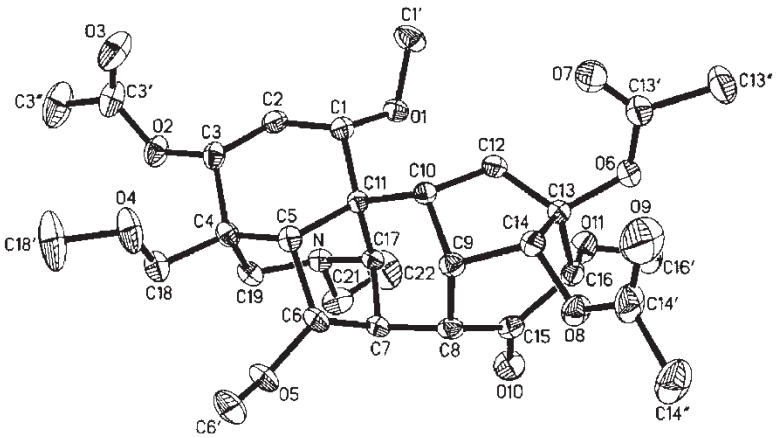

Figure 3. ORTEP drawing of compound **5**.

3.3 Extraction and isolation

Powdered roots (16.3 kg) of *Aconitum nagarum* var. *lasiandrum* were percolated with 3● HCl (250 l) according to the method reported in the literature [11]. Wet resin (dry weight 1.8 kg) was added to the percolate, followed by repeated washing on a suction filter with deionized H_2O. The air-dried resin was then alkalized with 10% aqueous NH_4OH (4.5 l) and continuously extracted with methanol. Evaporation under reduced pressure gave the residue (130 g), to which 5% HCl (2.6 l) was added. The solution was filtered, and then made basic to pH 10 with concentrated NH_4OH. The alkaline solution was extracted sequentially with $CHCl_3$ (4 l) and n-BuOH (3 l) to give the crude alkaloids ● (38 g) and ● (80 g), respectively.

The crude alkaloid ● (80 g) was chromatographed on a silica gel H (500 g) column eluting with $CHCl_3$–MeOH (30:1–1:2) to afford six parts, A (10.3 g), B (10.1 g), C (12.7 g), D (19.8 g), E (24.8 g), and F (8.2 g). Part A was subjected to a silica gel H (150 g) column eluting with petroleum–acetone–diethylamine (85:15:1–60:40:1) to give fractions A-1 (980 mg) and A-2 (505 mg). Fraction A-1 was chromatographed repeatedly on a silica gel H (30 g) column eluting with petroleum–acetone–diethylamine (90:10:1) to yield compound 9 (364 mg) and A-1-1 (557 mg). Fraction A-1-1 was chromatographed on a silica gel H (16 g) column eluting with petroleum–acetone (9:1–3:2) to afford compound **6** (412 mg). Part C was subjected to a silica gel H (120 g) column eluting with petroleum–acetone–diethylamine (87:13:1–50:50:1) to yield compounds **10** (782 mg) and **7** (4.02 g). Part E was chromatographed repeatedly on a silica gel H (240 g) column eluting with petroleum–acetone–diethylamine (60:40:1–20:80:1) to give compound **8** (1.12 g) and E-1 (466 mg) and E-2 (596 mg). Part E-1 was subjected to a silica gel H (14 g) column eluting with petroleum–acetone–diethylamine (70:30:1–50:50:1) to give compound 3 (228 mg). Part E-2 was chromatographed repeatedly on a silica gel H (18 g) column eluting with petroleum–acetone–diethylamine (70:30:1–50:50:1) to yield compound **1** (90 mg).

3.3.1 Lasiansine (1). Amorphous white powder. $[\alpha]_D^{20}$ + 5.5 (0.50, $CHCl_3$); mp 94–96°C; ^{1}H- and ^{13}C-NMR see table 1; IR (KBr) ν_{max} (cm^{-1}): 3422, 2925, 1646, 1450, 1110; EI-MS *m/z* (%): 454 (M + H, 100), 422 (M–OCH_3, 10), 404 (8); HR-FABMS *m/z* 454.2808 $[M + H]^+$ (calcd for $C_{24}H_{40}NO_7$, 454.2804 $[M + H]^+$).

3.3.2 16-Epipyroaconine (3). White amorphous powder. $[\alpha]_D^{20}$ − 106.8 (0.50, $CHCl_3$); mp 96–98°C; ^{1}H- and ^{13}C-NMR see table 1; IR (KBr) ν_{max} (cm^{-1}): 3415, 1706, 1450, 1099; EI-MS *m/z* (%): 482 (M + H, 67), 450 (M–OCH_3, 30), 432 (M–OCH_3–H_2O, 100), 400 (37); HR-ESIMS *m/z* 482.2749 $[M + H]^+$ (calcd for $C_{25}H_{40}NO_8$, 482.2753 $[M + H]^+$).

3.3.3 Compound (5). Colorless orthorhombic crystals from cyclohexane–acetone were mounted on a P_4 four-circle diffractometer and exposed to graphite-monochromated Mo Kα irradiation. The unit cell parameters are $a = 9.356(1)$ Å, $b = 12.347(2)$ Å, $c = 27.425(8)$ Å in space group $P_{2_12_12_1}$. Of the 4053 scans measured with $1.57 < Q < 27.48°$, 3661 were independently observed at the level of $F_0 > 4\sigma(F_0)$. The structure was determined by the direct method using the program SHELXTL and the method of atomic squares on F_2. The final *R* indices [$I > 2\sigma(I)$] were $R_1 = 0.0414$, $\omega R_2 = 0.0907$.

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