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TWO NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM NAGARUM* VAR. *LASIANDRUM*

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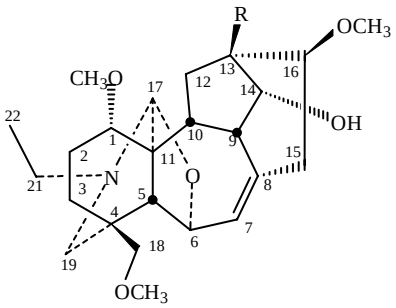
Abstract—Further investigation on the phytochemistry of the plant *Aconitum nagarum* var. *lasiandrum* led to isolate two C_{19}-diterpenoid alkaloids, francheline (**1**) and lasianine (**3**). Their structures were established on the basis of the spectral data.

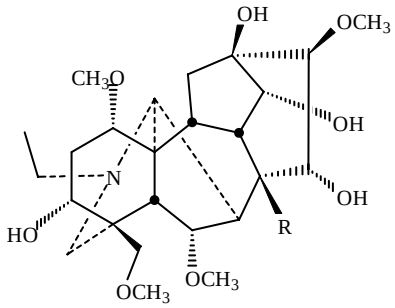
The plant *Aconitum nagarum* var. *lasiandrum* (Ranuculaceae) growing in the Xuanwei district of Yunna province of China is used for folklore medicine to treat rheumatism and neuralgia.[1] The roots of *A. nagarum* var. *lasiandrum* has been reported to contain many diterpenoid alkaloids belong to the aconitine-type, e.g., aconitine, 3-deoxyaconitine, neoline, nagarine, aconifine;[2] 14-acetylneoline,[3] flavaconitine,[4] vilmorrianine A, karakoline, sachaconitine, talatizidine, isotalatizidine, chasmanine, and yunaconitine;[5] the lycoctonine- type, e.g., virescenine;[4] the denudatine-type, e.g., denudatine as the major alkaloid,[4] as well as the napelline-type, e.g., songorine[3] and songoramine.[4] Considering the contribution of this plant to both local folk medicine and chemotaxonomy,[4] our investigation on its roots led to isolate two additional new alkaloids, francheline (**1**) and lasianine (**3**). The present report describes the isolation and structural elucidation of these alkaloids.

The new base, francheline (**1**), was obtained as amorphous powder substance. Its molecular formula, $C_{24}H_{37}NO_6$, was established based on HR-ESI-MS and 2D-NMR. NMR and MS spectra showed that it was the franchetine-type C_{19}-diterpenoid alkaloid.[6] The NMR spectra showed the presence of an *N*-ethyl (δ_H 0.99, 3H, t, J=7.2 Hz; 2.42, 2.60, each 1H, m; δ_C 49.0 t, 13.0, q), and three methoxyls (δ_H 3.29, 3.31, 3.42, each 3H, s). Its ^{1}H- and ^{13}C-NMR spectra also showed the distinctive *N*,*O*-mixed acetal moiety (δ_H 4.32, br s, 1H; δ_C 92.5, d), and a trisubstituted double bond (δ_H 5.71, d, J=5.6 Hz, 1H; δ_C 128.7, d, 136.6, s). One-proton wide singlet signal at δ_H 4.04 was assigned to be H-14β, indicating the appearance of the hydroxyl group at C-14. Three methoxyl groups could be located at C-1, C-16, and C-18 due to the

^{1}H-^{13}C long-range correlations (HMBC) between 1-OCH_3 (δ_H 3.31, s) and C-1 (δ_C 86.4, d), 16-OCH_3 (δ_H 3.42, s) and C-16 (δ_C 85.8, d), 18-OCH_3 (δ_H 3.29, s) and C-18 (δ_C 79.1, t) in the HMBC of **1**. Comparison of the MS and NMR spectra of **1** with those of 14-debenzoylfranchetine (**2**)[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, and C-14 (Table 1), indicating that the additional hydroxyl group was located on C-13.[8] This assignment was further confirmed by the multibond correlations between the H-9 (δ_H 2.13, m), H-14 (δ_H 4.04, br s), H_2-12 (δ_H 1.93, m, 2.00, m), H_2-15 (δ_H 2.59, m), H-16 (δ_H 3.31, m) and the C-13 (δ_C 77.9, s) (Table 1) in the HMBC of **1**. The structure of francheline, thus, was assigned to be **1** by careful analysis of the ^{1}H- and ^{13}C-NMR and 2D-NMR (^{1}H-^{1}H COSY, HMQC, and HMBC) spectra.



1 R=OH
2 R=H



3 R=NH_2
4 R=OH

Lasianine (**3**) was obtained as colorless needle crystals. The formula $C_{25}H_{42}N_2O_8$ was confirmed by HR-ESI-MS and 2D-NMR spectral data. The NMR spectra of lasianine (**3**) exhibited an *N*-ethyl group [δ_H 1.11 (3H; t, *J*=7.2 Hz), 2.47, 2.81 (each 1H, m); δ_C 48.4 t, 13.5 q], four methoxyl groups (δ_H 3.26, 3.29, 3.37, 3.58, each 3H, s; δ_C 55.9, q, 59.1, q, 58.3, q, 61.7, q) and a primary amino group [an even number of molecular weight (MW=498) and the expanded formula: $C_{19}H_{19}N$ ($NCH_2CH_3\times1$, $OCH_3\times4$, $OH\times4$, $NH_2\times1$)]. Its IR (3424 cm^{-1}) and ^{13}C NMR spectra (δ_C 70.8, d, 77.6, s, 80.2, d, 82.4, d) also showed the presence of three secondary hydroxyl groups and one tertiary hydroxyl group. Three secondary hydroxyl groups were assigned to C-3, C-14, and C-15 based on the correlations between the C-3 (δ_C 70.8, d) and H-1 (δ_H 3.15, dd, *J*=6.4, 7.6 Hz), H_2-2 (δ_H 1.98, m, 2.33, m), the C-14 (δ_C 80.2, d) and H-9 (δ_H 2.13, m), H-16 (δ_H 3.09, d, *J*=6.4 Hz), as well as the C-15 (δ_C 82.4, d), and H-7 (δ_H 2.15, m),

Table 1 NMR spectral data of francheline (**1**) (400 MHz for ^{1}H, 100 MHz for ^{13}C, $CDCl_3$)

No.	**1**			**2**
	δ_H (J=Hz)	δ_C	HMBC (H→C)	δ_C
1	3.24 dd (10.8, 6.4)	86.4 d	C-2, C-10, C-11, C-17, 1-OCH_3	86.6
2	1.93 m (α)	24.3 t	C-3, C-4, C-11	24.3
	2.48 m (β)		C-1, C-3	
3	1.52 ddd (12, 4.4, 2.4)	32.7 t	C-2, C-4, C-5, C-19	32.7
	1.77 ddd (13.6, 4.8, 2.0)		C-1, C-2, C-4, C-5, C-18, C-19	
4	—	37.2 s	—	37.3
5	2.21 s	47.3 d	C-3, C-4, C-6, C-7, C-10, C-11, C-17, C-18, C-19	48.0
6	4.39 d (6.0)	74.7 d	C-4, C-5, C-7, C-8, C-11, C-17	74.9
7	5.71 d (5.6)	128.7 d	C-5, C-6, C-9, C-15	128.3
8	—	136.6 s	—	137.4
9	2.83 br s	45.5 d	C-7, C-8, C-10, C-12, C-13, C-14, C-15	44.3
10	2.42 m	46.3 d	C-1, C-5, C-8, C-9, C-11, C-12, C-14, C-17	49.4
11	—	50.3 s	—	50.3
12	1.93 m (β)	38.7 t	C-9, C-10, C-11, C-13, C-14, C-16	29.3
	2.00 m (α)		C-9, C-10, C-13, C-16	
13	—	77.9 s	—	40.3
14	4.04 br s	82.4 d	C-8, C-9, C-13, C-16	77.4
15	2.59 m (β)	38.9 t	C-7, C-8, C-9, C-13, C-16	38.6
	3.09 m (α)		C-7, C-8, C-16	
16	3.31 m	85.8 d	C-13, C-14, C-15, 16-OCH_3	85.1
17	4.32 br s	92.5 d	C-1, C-5, C-6, C-10, C-11, C-19, C-21	92.4
18	3.06 ABq (9.2)	79.1 t	C-3, C-4, C-5, C-19, 18-OCH_3	79.2
	3.16 ABq (9.2)		C-3, C-4, C-5, C-19, 18-OCH_3	
19	2.04 ABq (11.0) (β)	52.0 d	C-3, C-4, C-5, C-17, C-18, C-21	52.0
	2.44 ABq (11.0) (α)		C-3, C-4, C-5, C-17	
21	2.42 m	49.0 t	C-17, C-19, C-22	49.0
	2.60 m		C-17, C-19, C-22	
22	0.99 t (7.2)	13.0 q	C-21	13.1
1-OCH_3	3.31 s	57.1 q	C-1	57.1
16-OCH_3	3.42 s	57.7 q	C-16	56.1
18-OCH_3	3.29 s	59.4 q	C-18	59.3

Table 2 NMR spectral data of lasianine (**3**) (400 MHz for ^{1}H, 100 MHz for ^{13}C, CD_3OD)

No.	**3**			**4**[9]
	δ_H (J=Hz)	δ_C	HMBC (H→C)	δ_C
1	3.15 dd (7.6, 6.4)	83.9 d	C-3, C-10, C-11, C-17, 1-OCH_3	84.1
2	1.98 m (α)	34.9 t	C-1, C-3, C-4, C-11	35.5
	2.33 m (β)		C-1, C-3, C-4, C-11	
3	3.76 dd (9.6, 4.8)	70.8 d	C-2, C-4	71.9
4	—	44.4 s	—	43.2
5	2.06 br s	49.7 d	C-6, C-11, C-17, C-19	49.0
6	4.13 d (6.4)	85.3 d	C-4, C-5, C-8, C-17, 6-OCH_3	83.0
7	2.15 m	46.7 d	C-5, C-11, C-15, C-17	51.3
8	—	61.0 s	—	76.4
9	2.13 m	50.3 d	C-8, C-10, C-12, C-13, C-14, C-15	50.1
10	1.94 m	43.2 d	C-5, C-8, C-9, C-11, C-12, C-13, C-17	42.4
11	—	51.4 s	—	50.5
12	1.92 m (β)	38.3 t	C-9, C-10, C-13, C-16	37.4
	2.51 m (α)		—	
13	—	77.6 s	—	78.8
14	3.81 d (5.2)	80.2 d	C-8, C-9, C-13, C-16	80.6
15	4.23 d (6.4)	82.4 d	C-8, C-9, C-16	78.5
16	3.09 d (6.4)	93.4 d	C-8, C-12, C-13, C-14, C-15, 16-OCH_3	91.8
17	3.06 s	62.8 d	C-5, C-6, C-7, C-8, C-10, C-11, C-19, C-21	60.8
18	3.35 ABq (8.4)	75.6 t	C-3, C-4, C-5, 18-OCH_3	77.4
	3.70 ABq (8.4)		C-3, C-4, C-5, 18-OCH_3	
19	2.44 (hidden) (β)	50.0 t	C-3	48.3
	2.78 (hidden) (α)		C-4, C-21	
21	2.47 m	48.4 t	C-22	46.2
	2.81 m		C-17, C-22	
22	1.11 t (7.2)	13.5 q	C-21	13.4
1-OCH_3	3.26 s	55.9 q	C-1	55.7
6-OCH_3	3.37 s	58.3 q	C-6	58.0
16-OCH_3	3.58 s	61.7 q	C-16	61.9
18-OCH_3	3.29 s	59.1 q	C-18	59.1

H-9, H-16, in the HMBC of **3**. The remained hydroxyl group in **3** could be located at C-13 by showing

the correlations between C-13 (δ_C 77.6, s) and H-9, H-10 (δ_H 1.94, m), H-12 (δ_H 1.92, m), H-14 (δ_H 3.81, d, *J*=5.2 Hz), H-16. The four methoxyl groups in **3** were put on C-1, C-6, C-16, and C-18 due to the correlations between the 1-OCH_3 (δ_H 3.26, s) and C-1 (δ_C 83.9, d), the 6-OCH_3 (δ_H 3.37, s) and C-6 (δ_C 85.3, d), the 16-OCH_3 (δ_H 3.58, s) and C-16 (δ_C 93.4, d), the 18-OCH_3 (δ_H 3.29, s) and C-18 (δ_C 75.6, t) in the HMBC of **3**. The ^{13}C-NMR spectra (Table 2) of lasianine (**3**) and aconine (**4**)[9] are similar, except for C-7, C-8, C-15, C-16, C-17, C-18, and C-21 which are caused by replacing the hydroxyl group with the amino group at C-8. This implied the presence of an amino group at C-8 in **3**. Apparently, Dreiding model observation and very rigid framework of lasianine ruled out another possibility of α-configuration of 8-NH_2 group. Structure of lasianine was therefore established as (**3**). All the ^{1}H- and ^{13}C-NMR spectral data obtained for lasianine (Table 2) supported structure (**3**). Lasianine (**3**) is the third natural aconitine-type C_{19}-diterpenoid alkaloid possessing the 8-amino group at C-8. Mild treatments of the extracts and column fractions throughout the isolation products, TLC comparison (silica gel GF_{254}, $CHCl_3$-MeOH=9:1) of the crude ethanol extracts with the authentic sample (lasianine, **3**) as well as refluxing aconitine with a mixture of dioxane-concentrated ammonia for 4 h have precluded the possibility of substitution reactions to occur at C-8 in **4**. In fact, our studies showed that refluxing the aconitine-type alkaloids having the 8-OAc group, as yunaconitine (**5**), with MeOH, EtOH, dioxane, and diglyme- H_2O[11,12] afforded the corresponding 8-OR-containing compounds **6**, **7**, **8** respectively. Mechanically, these compounds were formed by a process as showed in Fingure **1**. First, Grob fragmentation of **5** produced the intermediate **A**, and then, the nucleophilic species, such as OCH_3, OEt, OH, *etc*., atlackes on C-8 in **A** to give compounds **6**, **7**, and **8** respectively. Clearly, in our case (NH_4OH-ion exchage resin), there has a NH_4^+ instead of a :NH_2, thus, the lasianine (**3**) can not be artificially produced.

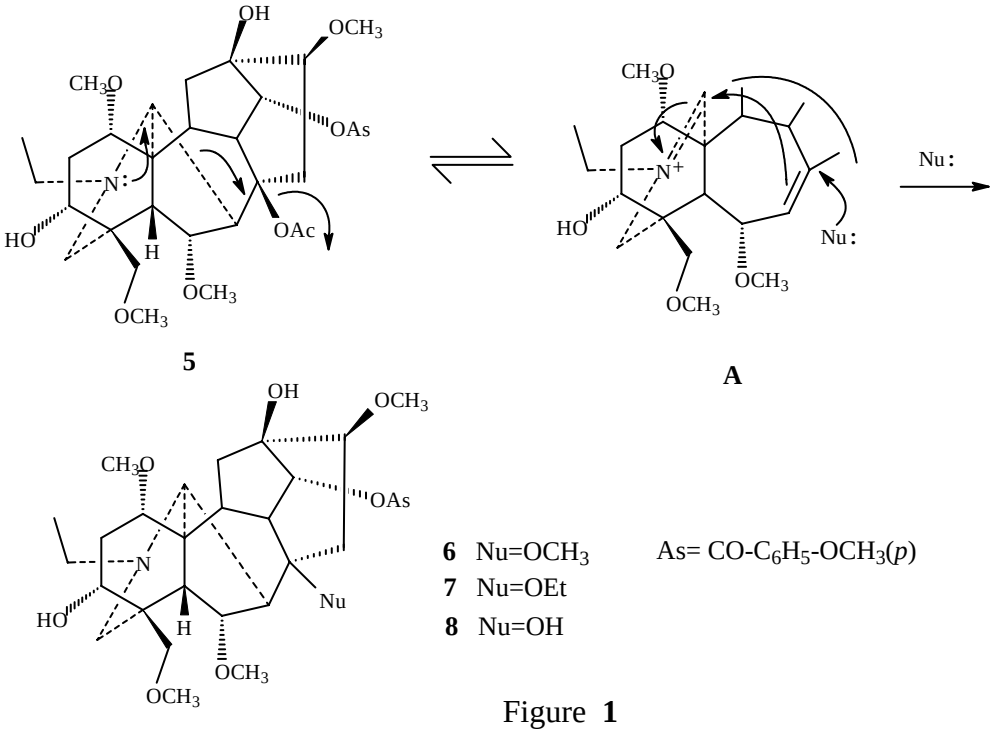


Figure **1**

EXPERIMENTAL

General Experimental procedure. Optical rotations were recorded on a Perkin-Elmer 341 polarimenter. IR spectra were obtained on a Nicolet FT-IR 200 SXY spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured on a Varian Unity INOVA 400/45 NMR spectrometer in $CDCl_3$ or CD_3OD with TMS as the internal standard. EI-MS and HR-ESI-MS were measured from a VG Auto spec 3000 or Finnegan MAT 90 instrument. Silica gel GH_{254} and H (Qindao Sea 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.

Plant material. The *Aconitum* Stapf *nagarum* var. *lasiandrum* was collected in Xuanwei district, Yunna 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.

Extraction and Isolation. According to method reported in the literature,[13] powdered roots (16.3 kg) of *Aconitum nagarum*. var. *lasiandrum* were percolated with 0.05 mol HCl (250 L). 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 (45 L) and continuously extracted with

methanol. Evaporation on reduced pressure gave the residue (130 g), to which 5% HCl (2.6 L) added, and filtrated, basified with concentrated. NH_4OH to pH 10. The alkaline solution was extracted sequentially with $CHCl_3$ (4 L), n-BuOH (3 L) to give the crude alkaloids Ⅰ (38 g) and Ⅱ (80 g), respectively.

The crude alkaloid Ⅱ (80 g) was chromatographed on a silica gel H 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 silica gel H column chromatographed eluting with petroleum-acetone-diethylamine (85:15:1-60:40:1) to give fractions A-1 (457 mg), A-2 (980 mg), A-3 (2.98 g), A-4 (1.70 g), and A-5 (505 mg). Fraction A-3 was chromatographed repeatedly on a silica gel H eluting with petroleum-acetone-diethylamine (85:15:1-50:50:1) to yield francheline (**1**) (45 mg). Fraction E was chromatographed on a silica gel column eluting with petroleum-acetone-diethylamine (40:60:1-20:80:1) to afford fractions E-1 (2.2 g), E-2 (3.5 g), E-3 (7.0 g), and E-4 (6.5 g). E-3 was subjected to silica gel column chromatography eluting with $CHCl_3$-MeOH-NH_4OH (93:7:0.5-40:60:0.5) to yield fractions E-3-1 (700 mg), E-3-2 (466 mg), E-3-3 (3.23 g), E-3-4 (1.12 g), and E-3-5 (1.35 mg). E-3-4 was purified by HPLC (RP-C18, 10 μm, 1.0 × 20 cm; mobile phase: CH_3OH-H_2O-NH_4OH (5:3:1-6:1:1), Waters 2410 refraction detector) provided lasianine (**3**) (27 mg).

Franechline (1). White amorphous powder, mp 86~88℃; $[\alpha]_D^{20}$ - 146.8° (c 0.5, $CHCl_3$). IR (KBr)cm^{-1}: 3427, 2926, 1661, 1455; ^{1}H- and ^{13}C-NMR: see Table 1; EI-MS *m/z* (%): 436 (M^++1) (100); 404 (M-OCH_3) (9); 360 (34); HR-ESI-MS *m/z*: 436.2696 $[M+H]^+$, calcd for $C_{24}H_{38}NO_6$, 436.2699.

Lasianine (3). Colorless needle crystals, mp 134~136℃; $[\alpha]_D^{20}$ + 12.9° (c 0.4, MeOH). IR (KBr)cm^{-1}: 3424, 2931, 1632, 1454, 1099; ^{1}H-and ^{13}C-NMR: see Table 2; EI-MS *m/z* (%): 499 $[M+H]^+$ (100); 467 (M-OCH_3) (9); 449 (14); 417 (13); HR-ESI-MS *m/z*: 499.3032 $[M+H]^+$, calcd. for $C_{25}H_{43}N_2O_8$, 499.3019.

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