

**TWO NEW C₁₉-DITERPENOID ALKALOIDS FROM *ACONITUM*
NAGARUM VAR. *LASIANDRUM***

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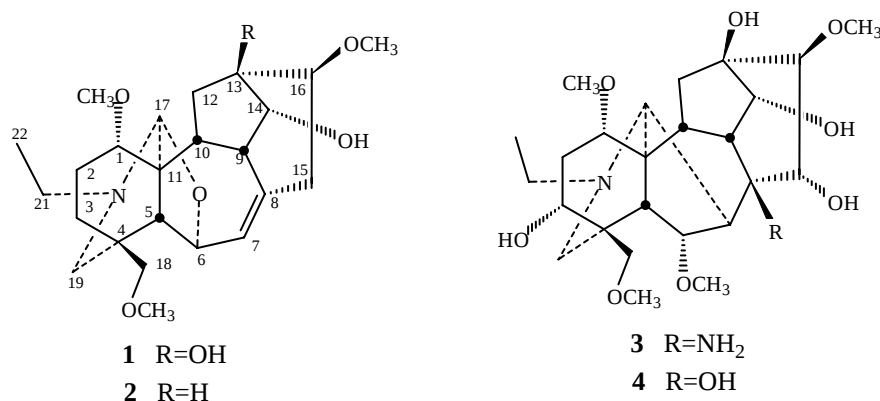
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Abstract—Further investigation on the phytochemistry of the plant *Aconitum nagarum* var. *lasiandrum* led to isolate two C₁₉-diterpenoid alkaloids, francheline (1) and lasianine (3). Their structures were established on the basis of the spectral data.

The plant *Aconitum nagarum* var. *lasiandrum* (Ranunculaceae) growing in the Xuanwei district of Yunnan 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., songorine³ 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, C₂₄H₃₇NO₆, was established based on HR-ESI-MS and 2D-NMR. NMR and MS spectra showed that it was the franchetidine-type C₁₉-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 methoxys (δ_{H} 3.29, 3.31, 3.42, each 3H, s). Its ¹H- and ¹³C-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-14 β , 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

^1H - ^{13}C long-range correlations (HMBC) between 1-OCH₃ (δ_{H} 3.31, s) and C-1 (δ_{C} 86.4, d), 16-OCH₃ (δ_{H} 3.42, s) and C-16 (δ_{C} 85.8, d), 18-OCH₃ (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), H₂-12 (δ_{H} 1.93, m, 2.00, m), H₂-15 (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 franchetine, thus, was assigned to be **1** by careful analysis of the ^1H - and ^{13}C -NMR and 2D-NMR (^1H - ^1H COSY, HMQC, and HMBC) spectra.



Lasianine (**3**) was obtained as colorless needle crystals. The formula C₂₅H₄₂N₂O₈ 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: C₁₉H₁₉N (NCH₂CH₃×1, OCH₃×4, OH×4, NH₂×1)]. 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), H₂-2 (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 ^1H , 100 MHz for ^{13}C , CDCl_3)

No.	1			2
	δ_{H} ($J=\text{Hz}$)	δ_{C}	HMBC ($\text{H}\rightarrow\text{C}$)	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 ^1H , 100 MHz for ^{13}C , CD_3OD)

No.	3			4⁹
	δ_{H} ($J=\text{Hz}$)	δ_{C}	HMBC ($\text{H}\rightarrow\text{C}$)	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-OCH₃ (δ_H 3.26, s) and C-1 (δ_C 83.9, d), the 6-OCH₃ (δ_H 3.37, s) and C-6 (δ_C 85.3, d), the 16-OCH₃ (δ_H 3.58, s) and C-16 (δ_C 93.4, d), the 18-OCH₃ (δ_H 3.29, s) and C-18 (δ_C 75.6, t) in the HMBC of **3**. The ¹³C-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-NH₂ group. Structure of lasianine was therefore established as (**3**). All the ¹H- and ¹³C-NMR spectral data obtained for lasianine (Table 2) supported structure (**3**). Lasianine (**3**) is the third natural aconitine-type C₁₉-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 GF₂₅₄, CHCl₃-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- H₂O^{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 OCH₃, OEt, OH, *etc.*, atlackes on C-8 in **A** to give compounds **6**, **7**, and **8** respectively. Clearly, in our case (NH₄OH-ion exchange resin), there has a NH₄⁺ instead of a :NH₂, thus, the lasianine (**3**) can not be artificially produced.

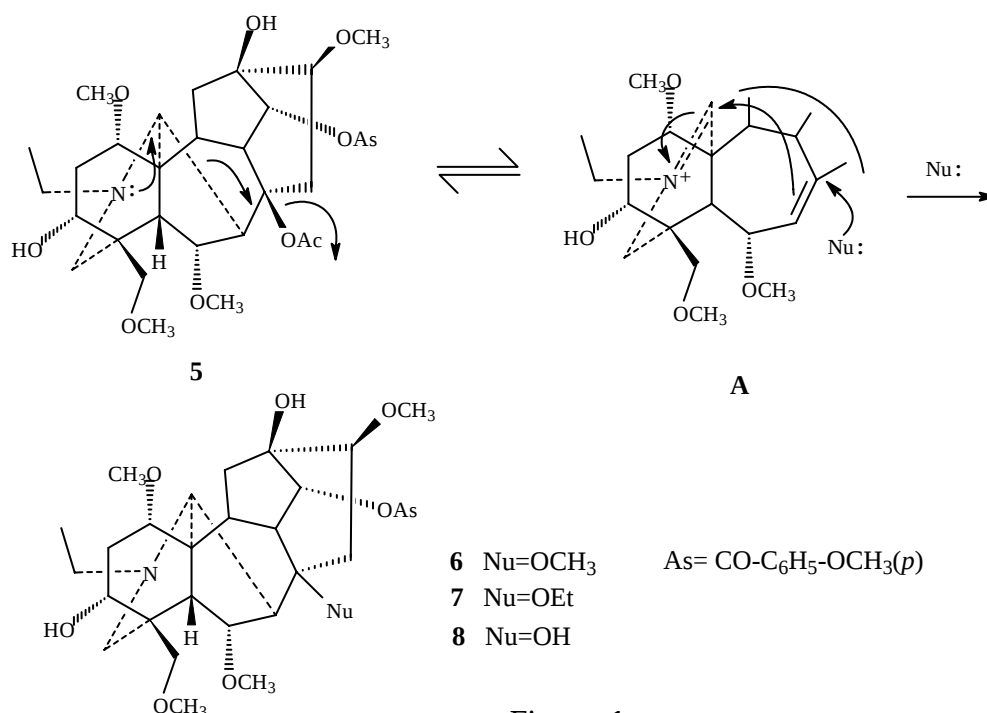


Figure 1

EXPERIMENTAL

General Experimental procedure. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200 SXY spectrophotometer. ¹H- and ¹³C-NMR spectra were measured on a Varian Unity INOVA 400/45 NMR spectrometer in CDCl₃ or CD₃OD 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 GH₂₅₄ 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, 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.

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 H₂O. The air-dried resin was then alkalinized with 10% aqueous NH₄OH (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), $n\text{-BuOH}$ (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 $\text{CHCl}_3\text{-MeOH}$ (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 $\text{CHCl}_3\text{-MeOH-NH}_4\text{OH}$ (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: $\text{CH}_3\text{OH-H}_2\text{O-NH}_4\text{OH}$ (5:3:1-6:1:1), Waters 2410 refraction detector) provided lasianine (**3**) (27 mg).

Franechline (1). White amorphous powder, mp $86\sim 88^\circ\text{C}$; $[\alpha]_{\text{D}}^{20} - 146.8^\circ$ (c 0.5, CHCl_3). IR (KBr) cm^{-1} : 3427, 2926, 1661, 1455; ^1H - 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 [$\text{M}+\text{H}$] $^+$, calcd for $\text{C}_{24}\text{H}_{38}\text{NO}_6$, 436.2699.

Lasianine (3). Colorless needle crystals, mp $134\sim 136^\circ\text{C}$; $[\alpha]_{\text{D}}^{20} + 12.9^\circ$ (c 0.4, MeOH). IR (KBr) cm^{-1} : 3424, 2931, 1632, 1454, 1099; ^1H -and ^{13}C -NMR: see Table 2; EI-MS m/z (%): 499 [$\text{M}+\text{H}$] $^+$ (100); 467 (M-OCH_3) (9); 449 (14); 417 (13); HR-ESI-MS m/z : 499.3032 [$\text{M}+\text{H}$] $^+$, calcd. for $\text{C}_{25}\text{H}_{43}\text{N}_2\text{O}_8$, 499.3019.

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