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Vilmoraconitine, a novel skeleton C_{19}-diterpenoid alkaloid from *Aconitum vilmorinianum*

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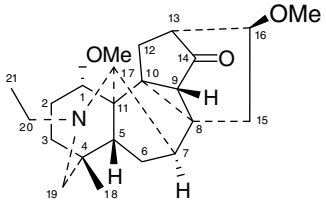
ABSTRACT

A novel C_{19}-diterpenoid alkaloid vilmoraconitine (**1**) was isolated from the roots of *Aconitun vilmorinianum*. Its structure was mainly determined by MS, 2D NMR, and X-ray methods. This is the first aconitine-type C_{19}-diterpenoid alkaloid with one three-membered ring at C-8, C-9, and C-10.

Aconitum L. (Ranunculaceae) is a large genus with about 300 species and distributes in the temperate regions of the Northern Hemisphere. The Southwestern China, particularly the Hengduan Mountains, is one important center of diversity and speciation of this genus. About 76 *Aconitum* species in China have been used as poisonous and medicinal plants, which are mainly used for treatment of rheumatoid arthritis and various types of pains. *Aconitum* species, producing highly toxic diterpenoid and norditerpenoid alkaloids, have been paid much more attention for their constituent complex structures, potential bioactivity, and noteworthy physiology. Pharmacologically, they can be developed as analgesic, cardiotonic, anti-inflammatory, anti-rheumatic, and anti-arrhythmic agents. *Aconitum vilmorinianum* Kom is wildly found in Yunnan, Guizhou, Sichuan provinces of China, and used for treatment of rheumatism and pains in folk herbs. Previous studies on this plant have led to discover about 20 diterpenoid alkaloids, which are mainly of the aconitine-type C_{19}-diterpenoid alkaloids.[1] As a part of an ongoing phytochemical investigation of *Aconitum* species, a novel skeleton C_{19}-diterpenoid alkaloid named vilmoraconitine (**1**) was isolated from the $CHCl_3$ extracts of the roots of this plant, which were collected in October 2004 in Yunnan province of China. Vilmoraconitine is the first aconitine-type C_{19}-diterpenoid alkaloid with one three-membered ring at C-8, C-9, and C-10. Described herein are the isolation and structural determination of vilmoraconitine mainly by 2D NMR spectroscopy and X-ray analysis.



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The air-dried and powdered roots (5 kg) of *A. vilmorinianum* were soaked in 5% NH_4OH and then extracted with $CHCl_3$ at room temperature for 3 times. After removal of $CHCl_3$ reduced pressure, a brown viscous syrup left. This syrup was dissolved in 300 ml EtOAc and then partitioned with 800 ml 2% HCl solution for 4 times. The acidic aqueous solution was basified with saturated Na_2CO_3 to pH 9, and then extracted with 2500 ml EtOAc for 5 times to give 19.2 g of a crude alkaloid. The crude alkaloid (19.2 g) was separated by chromatography on a column of silica gel (200 g, 200–300 mesh) into 8 fractions [I (0.3 g), II (5 g), III (5 g), IV (0.2 g), V (0.3 g), VI (2.7 g), VII (1.0 g), and VIII (3.5 g)] with a gradient of acetone in 0.2% diethylamine–petroleum ether (60–90 °C) solution. Fraction I (0.3 g) was further chromatographed over a silica gel column (60 g, petroleum ether–acetone–diethylamine, 500:25:1) and purified by recrystallization from *n*-hexane and acetone (20:1) to yield 30 mg colorless prism of compound **1**.

Vilmoraconitine (**1**)[2] was isolated as colorless prism. It gave a position reaction to Dragendorff's reagent. Its molecular formula of $C_{23}H_{33}NO_3$ was established by HR +TOF MS (found 372.2527

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$[M+H]^+$, calcd 372.2538 $[M+H]^+$) with 8 degrees of unsaturation. DEPT experiments indicated the presence of 4 methyls, 7 methylenes, 7 methines, and 5 quaternary carbons. Its ^1H and ^{13}C NMR spectra displayed the presence of one *N*-ethyl (δ_H 1.02, 3H, t, J = 7.19 Hz; 2.58–2.64, 2.34–2.38 each 1H, m; δ_C 13.3 q, 50.2 t), one quaternary methyl (δ_H 0.73, 3H, s; δ_C 26.2 q), two methoxyl (δ_H 3.34, 3.25, each 3H, s; δ_C 55.7 q, 55.6 q), and one ketocarbonyl (δ_C 211.2 s) functional groups. A comparison of the ^1H and ^{13}C NMR spectra of **1** with those of vilmorrianine D (**2**),[3] an aconitine-type C_{19}-diterpenoid alkaloid, showed that **1** exhibited some characteristic NMR features of the same type alkaloid.[4] The possible structure of **1** was determined by the extensive analysis of 1D and 2D NMR spectra; the still uncertain structure details were established by single-crystal X-ray analysis.

H–H COSY spectrum of **1** revealed the presence of one isolated methylene (–CH_2–, **1a**, AB spin system), one ethyl (CH_3–CH_2–, **1b**), one (–CH–CH_2–CH_2–, **1c**), one (–CH–CH_2–CH–, **1d**), and one (–CH–CH–CH_2–, **1e**) segments (Fig. 1) on the basis of the correlations of the proton signals at δ_H 2.49 (1H, d, J = 11.49 Hz, H-19a) with δ_H 2.00–2.23 (1H, overlapped, H-19b); at δ_H 1.02 (3H, t, J = 7.19 Hz, H-21) with δ_H 2.58–2.64, 2.34–2.38 (each 1H, m, H-20); at δ_H 2.18–2.20, 1.97–2.00 (each 1H, m, H-2) with δ_H 3.43 (1H, dd, J = 10.76, 6.30 Hz, H-1), 1.58–1.62, 1.15–1.19 (each 1H, m, H-3); at δ_H 1.37–1.42, 1.07–1.11 (each 1H, m, H-6) with δ_H 1.21 (1H, d, J = 6.71 Hz, 5-H) and 2.22–2.23 (1H, overlapped, H-7); at δ_H 3.60–3.63 (1H, m, H-16) with δ_H 2.46 (1H, t, J = 4.77 Hz, H-13) and 2.25 (1H, dd, J = 14.74, 6.78 Hz, H-15a), respectively.

In the HMBC spectrum (Fig. 1), δ_H 3.34 (3H, s, H-1-OMe) showed cross-peaks with δ_C 79.9 (C-1), δ_H 3.43 (1H, dd, J = 10.76, 6.30 Hz, H-1), 1.21 (1 H, d, J = 6.71 Hz, H-5) with δ_C 45.0 (C-10), 77.2 (C-17), δ_H 1.58–1.62, 1.15–1.19 (each 1H, m, H-3) with δ_C 48.6 (C-5), 56.9 (C-19), δ_H 1.37–1.42, 1.07–1.11 (each 1H, m, H-6) with δ_C 41.0 (C-8), δ_H 3.50 (1H, br s, H-17), 2.58–2.64, 2.34–2.38 (each 1H, m, H-20) with δ_C 56.9 (C-19), which led to the establishment of partial structure **1f** (Fig. 1). The cross-peaks between δ_H 2.22–2.23 (1H, overlapped, H-7), 2.12 (1H, s, H-9) and δ_C 30.4 (C-15), δ_H 2.15–2.16 (2H, overlapped, H-12) and δ_C 211.2 (C-14), δ_H 2.46 (1H, t, J = 4.77 Hz, H-13) and δ_C 45.0 (C-10), δ_H 3.60–3.63 (1H, m, H-16) and δ_C 41.0 (C-8), 211.2 (C-14), δ_H 3.25 (3H, s, H-16-OMe) showed cross-peaks with δ_C 79.7 (C-16) suggested the possible structure of **1** should be as **1g** (Fig. 1).

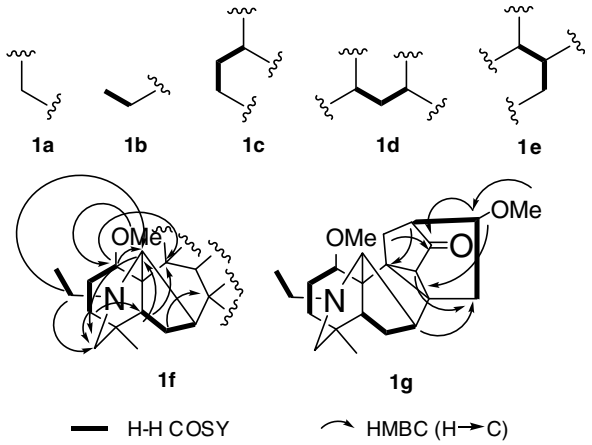

Figure 1. Segments and key COSY and HMBC correlations of **1**.

The analysis of single-crystal X-ray diffraction[5] of **1** not only confirmed the presence of one three-membered ring at C-8, C-9, and C-10, but also established the relative stereochemistry of **1** as the carbon–carbon bond between C-8 and C-10 in α orientation (Fig. 2). Finally, the structure of **1** was elucidated as 8-dehydroxyl-14-dehydro-8,9,10-cyclopropyl-vilmorrianine D, named vilmoraconitine.

Table 1
^1H and ^{13}C NMR data and HMBC correlations of **1**[a]

Position	δ_H (mult, J in Hz)	δ_C (mult)	HMBC (H→C)
1	3.43 (1H, dd, 10.76, 6.30)	79.9 d	10, 17
2a	2.18–2.20 (1H, m)	24.8 t	
2b	1.97–2.00 (1H, m)		4, 11
3a	1.58–1.62 (1H, m)	37.9 t	5
3b	1.15–1.19 (1H, m)		19
4		35.0 s	
5	1.21 (1H, d, 6.71)	48.6 d	7, 10, 17, 18, 19
6a	1.37–1.42 (1H, m)	26.4 t	4, 8, 11
6b	1.07–1.11 (1H, m)		4, 8, 17
7	2.22–2.23 (1H, overlapped)	42.6 d	15
8		41.0 s	
9	2.12 (1H, s)	39.9 d	15
10		45.0 s	
11		51.3 s	
12	2.15–2.16 (2H, overlapped)	29.5 t	14
13	2.46 (1H, t, 4.77)	46.7 d	10, 15
14		211.2 s	
15a	2.25 (1H, dd, 14.74, 6.78)	30.4 t	
15b	2.01 (1H, dd, 14.74, 2.91)		10, 12
16	3.60–3.63 (1H, m)	79.7 d	8, 14
17	3.50 (1H, br s)	77.2 d	5, 6, 19
18	0.73 (3H, s)	26.2 q	3, 5, 19
19a	2.49 (1H, d, 11.49)	56.9 t	3, 5, 17
19b	2.00–2.23 (1H, overlapped)		18
20a	2.58–2.64 (1H, m)	50.2 t	17, 19
20b	2.34–2.38 (1H, m)		17, 19
21	1.02 (3H, t, 7.19)	13.3 q	
1-OMe	3.34 (3H, s)	55.7 q	1
16-OMe	3.25 (3H, s)	55.6 q	16

[a] Data were recorded in $CDCl_3$ on Brucker DRX-500 MHz spectrometer (^1H, ^{13}C, HSQC, COSY, HMBC); chemical shifts (δ) are given in parts per million with references to the most downfield signal of $CDCl_3$ (δ 7.26 ppm) for ^1H and to the center peak of the downfield signal of $CDCl_3$ (δ 77.0 ppm) for ^{13}C.

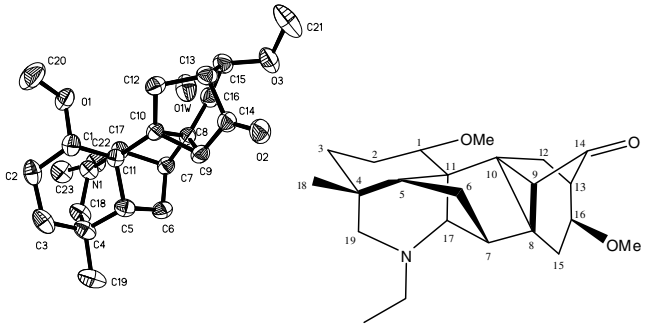

Figure 2. X-ray structure of **1** showing relative configuration.

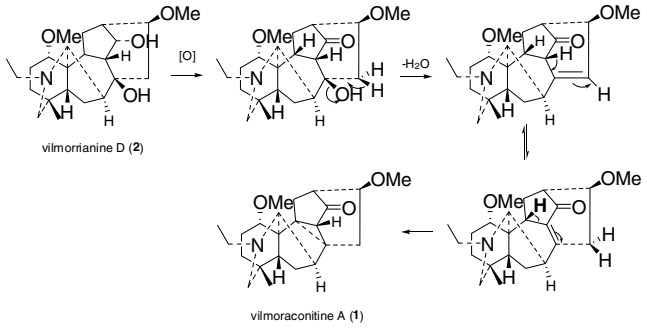

Figure 3. A possible biogenetic relationship between **1** and **2**.

According to the literature, vilmoraconitine was the first aconitine-type C_{19}-diterpenoid alkaloid with one three-membered ring at C-8, C-9, and C-10. A possible biosynthetic pathway was proposed in Figure 3, in which vilmorrianine D was also isolated form this plant by us.

Acknowledgments

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Supplementary data

^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, UV, IR, MS spectra, and crystallographic data of vilmoraconitine (**1**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.008.

References and notes

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2. Vilmoraconitine (**1**): $C_{23}H_{33}NO_3$, colorless prism (*n*-hexane–acetone, 20:1), mp 86–87 °C; $[\alpha]_D^{17.9}$ +17.50 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (logε) 202.4 (3.82) nm; IR (KBr) ν_{max} 3518, 3469, 2934, 2892, 2819, 1716, 1465, 1358, 1145, 1104, 1081, 985 cm^{-1}; NMR data can be found in Table 1; EI-MS *m/z* (70 eV, rel. int., %) 372 $[M+H]^+$ (4), 371 $[M]^+$ (9), 370 $[M-H]^+$ (16), 356 $[M-Me]^+$ (81), 341 $[M+H-OMe]^+$ (48), 326 $[341-Me]^+$ (39), 312 (61), 310 $[341-OMe]^+$ (49), 298 (26), 284 (28), 268 (22), 250 (42), 181 (38), 167 (46), 153 (41), 141 (55), 128 (58), 115 (58), 91 (49), 71 (100); HR+TOF MS found 372.2527 $[M+H]^+$, calcd for $C_{23}H_{34}NO_3$ 372.2538 $[M+H]^+$.
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4. Pelletier, S. W.; Joshi, B. S. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New York, 1991; Vol. 7, pp 297–564.
5. A colorless prism of dimensions 0.60 × 0.60 × 1.20 mm was used for X-ray diffraction on a MAC DIP-2030 K diffractometer with Mo Kα radiation and graphite monochromator by maximum 2θ value of 50.0°. The total number of independent reflections was 2023, of which 1947 were observed ($|F|^2 \geqslant 2\sigma|F|^2$). Crystallographic data for **1**: molecular formula $(C_{23}H_{33}N_1O_3)(H_2O)_{0.5}$, M = 371.51 (excluded the solvent), orthorhombic system, space group: $C222_1$, a = 12.7300 (9) Å, b = 13.1310 (8) Å, c = 24.6860 (8) Å , V = 4126.5 (4) Å^3, Z = 8, d = 1.225 g/cm^3. The structure was solved by the direct method and expanded using difference Fourier techniques, refined by the program and method (SHELXS-97).[6] Hydrogen atoms were fixed at calculated positions. The final indices were R_1 = 0.038, wR_2 = 0.105, S = 1.043. Crystallographic data (excluding structure factors) for the structures in this Letter have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 676509. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0) 1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).
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