

STUDIES ON THE ALKALOIDS FROM *ACONITUM KARAKOLICUM* RAP.

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Abstract – A new C_{20} -diterpene alkaloid, karakomine (**1**) along with two known compounds, songorine (**2**) and neoline (**3**), have been isolated from the roots of *Aconitum karakolicum* Rap. The structure of karakomine was determined by 1D and 2D nmr spectroscopic analyses.

Aconitum plants are frequently used for treatment of rheumatism, neuralgia, fracture, etc. in China. *A. karakolicum* Rap. (Ranunculaceae) collected in the Xinjiang Uygur Autonomous Region of China is a folk medicine in both of China and Russia. Chemical investigation of this plant has been done by Sultankhodzhaev and Song, who reported the isolation of several alkaloids.^{1a,1b} The continuation of our work on the constituents of the plant has led to isolation of a new C_{20} -diterpene alkaloid, karakomine (**1**) and two known compounds, songorine (**2**)² and neoline (**3**).² We wish to report here the structural elucidation of **1** by analyses of its ¹H and ¹³C nmr spectra.

Karakomine (**1**), $[\alpha]_D^{25} -18.7^\circ$ ($c = 0.27$, EtOH), was obtained as a yellowish amorphous powder. The high resolution ms (M^+ 359.2430, calcd 359.2457) of **1** indicated the molecular formula, $C_{22}H_{33}NO_3$. The ir spectrum of **1** showed the presence of hydroxyl group (3450 cm^{-1}) and cyclopentanone ring (1740 cm^{-1}) which was further supported by the signal at $\delta 221.9$ in ¹³C nmr of **1**. The ¹H nmr spectrum of **1** revealed the presence of an NCH_2CH_3 group [$\delta 1.07$ (3H, dd, $J=4.4, 7.3$ Hz), $\delta 2.43$ and 2.54 (1 H each, m)], a tertiary methyl [$\delta 0.78$ (3H, s, H-18)], a secondary methyl [$\delta 1.066$ (3H, d, $J = 7.3$ Hz, H-17)] and two methine protons [$\delta 3.89$ (1H, dd, $J = 5.9, 6.6$ Hz, H-1) and $\delta 4.43$ (1H, dd, $J = 7.5, 8.1$ Hz, H-12)] attached to the oxygen-bearing carbons. The ¹³C nmr DEPT spectrum of **1** indicated the presence of three methyl, seven methylene, eight methine and four quaternary carbons (see Table 1). These spectral data and the molecular

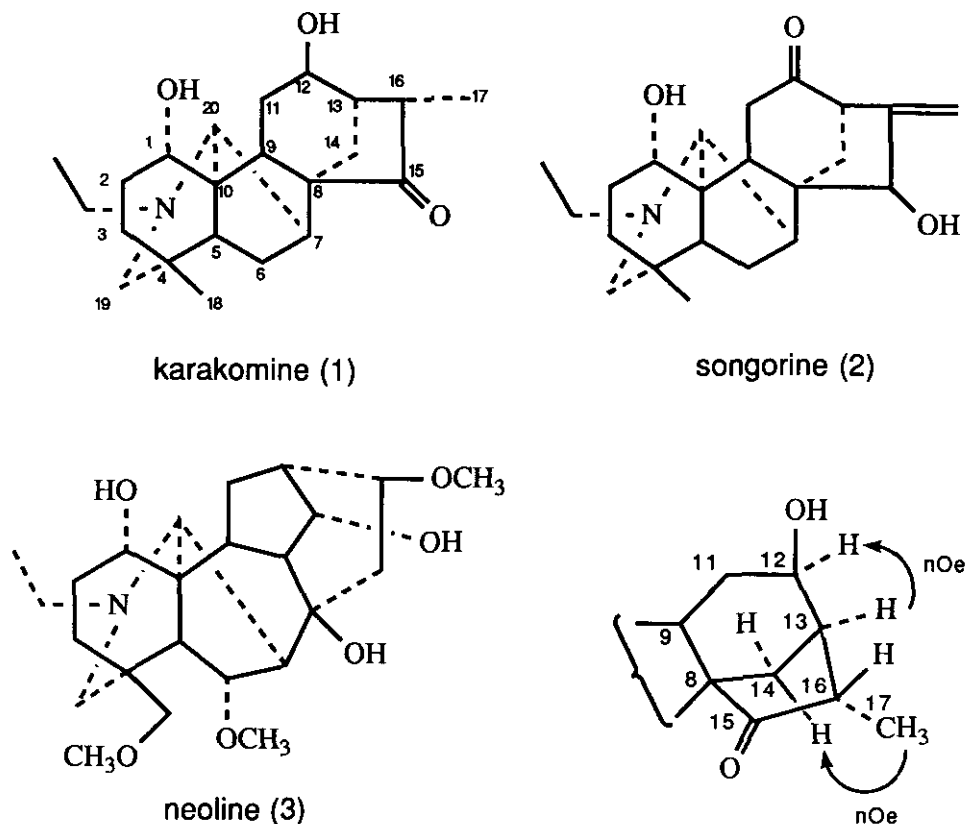


Fig. 1

formula of **1** suggested that the compound (**1**) was a C_{20} -diterpene alkaloid.^{3,4} However, the 1H nmr spectrum of **1** showed the presence of a secondary methyl group at δ 1.07 instead of usual exomethylene group at C-16. The appearance of the fragment ion peak at m/z 246 (12%)⁵ in the ms spectrum indicated that the rings A and B were the same as those of songorine (**2**). Thus, the structural differences between **1** and **2** should be in the rings C and D. The location and the configuration of the other hydroxyl group was presumed to be the 12β position by the 1H nmr decoupling experiment ($J_{12-13} = 8.8$ Hz).^{3,4} This was confirmed by the observation of nOe between H-12 and H-13 and the long-range correlation between H-16 and C-12 in the HMBC spectrum of **1**. In the HMBC spectrum, the proton signal at δ 1.07 assignable to the methyl group at C-16 exhibited the long-range correlation with the signal (δ 221.9) due to carbonyl carbon which showed cross peaks with the proton signals at δ 1.69 (H-9), δ 2.17 (H-13) and δ 1.48 (H-

Table 1. The nmr data for karakomine (1)

¹³ C nmr (100 MHz)		¹ H nmr (400 MHz)	¹ H- ¹³ C long-range correlation (HMBC)
carbon	δ _C	δ _H	
1	69.0	3.89 dd (5.9, 6.6)	H-2, H-3
2	31.5	1.84 m, 1.95 m	H-1, H-3
3	35.0	1.36 m, 1.63 m	H-2, H-3, H-6, H-18
4	33.4		H-6, H-18, H-19
5	47.7	1.33 d (8.1)	H-6, H-18, H-20
6	22.7	1.47 dd (4.4, 14.7) 2.79 dd (8.1, 14.7)	H-5, H-20
7	39.7	2.22 brd (4.4)	H-5, H-6
8	54.0		H-6, H-9, H-13
9	43.0	1.69 m	H-1, H-12, H-14
10	52.6		H-1, H-7, H-20
11	30.6	1.68 m, 2.40 m	H-9, H-13
12	67.5	4.43 dd (7.5, 8.1)	H-13, H-14, H-16
13	40.4	2.17 dd (4.4, 8.1)	H-11, H-14, H-17
14	30.0	1.48 dd (4.4, 12.5) 1.79 dd (2.2, 12.5)	H-9
15	221.9		H-9, H-13, H-17
16	38.9	2.89 dq (2.2, 7.3)	H-12, H-14, H-17
17	16.4	1.07 d (7.3)	H-13, H-14
18	26.3	0.78 s	H-3, H-19
19	58.1	2.24, 2.40 ABq (10.3)	H-3, H-5 H-18, H-20
20	66.0	3.23 brs	H-1, H-5, H-6, H-9
N-CH ₂	50.9	2.43 m, 2.54 m	H-19, CH ₃ CH ₂ -N
 CH ₃	13.6	1.07 t (7.3)	CH ₃ CH ₂ -N

The numbers in parentheses are *J* values in Hz.

14). Thus, the carbonyl group was allocated at C-15 position. Finally, the configuration of the methyl group at C-16 was assigned to be α -orientation with the observation of the long-range coupling between H-16 and H-14 α ($J = 2.2$ Hz) and the results of nOe experiments. (see Fig. 1) These spectral data were satisfactorily explained by the structure 1. Further studies on the minor alkaloids of this plant are now under progress.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage equipped with a microscope and are uncorrected. Optical rotation was measured on a JASCO DIP-181 digital polarimeter. Mass spectra were recorded on a MAT-711 and Hitachi M-2000. The 1D and 2D nmr spectra were measured on a JEOL GSX-400 and a Bruker AM-400 spectrometers. The ir spectrum was recorded on a Perkin-Elmer 599B.

Extraction and Isolation of Alkaloids

Air dried and powdered roots of *A. Karakolicum* Rap. (20 kg) were extracted with 95% ethanol (100 l) by cold percolation at 25 °C for three days. The solvent was evaporated *in vacuo* to give approx. 2.1 kg of oily mass which was dissolved in 2% HCl (6 l). The aqueous phase was progressively basified with concentrated ammonia and extracted with CH₂Cl₂ to afford the crude alkaloid portions at pH 5 (60 g) and at pH 8 (34 g), respectively. The pH 8 portion was chromatographed over silica gel to afford neoline (3, 450 mg, elution with CH₂Cl₂ : EtOH = 10 : 1) and songorine (2, ca. 1.8 g, elution with CH₂Cl₂ : EtOH = 5 : 1). After removal of neoline by filtration, the filtrate was concentrated to give a syrup which was subjected to alumina column chromatography. Elution with petroleum ether yielded karakomine (1, 56 mg).

REFERENCES AND NOTE

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