STUDIES ON THE ALKALOIDS FROM *ACONITUM KARAKOLICUM* RAP.

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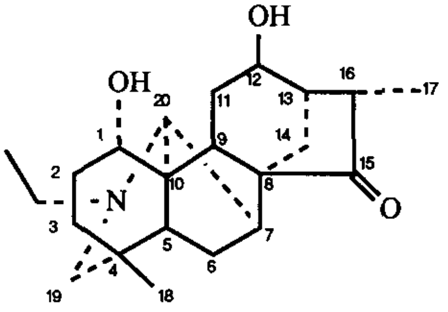
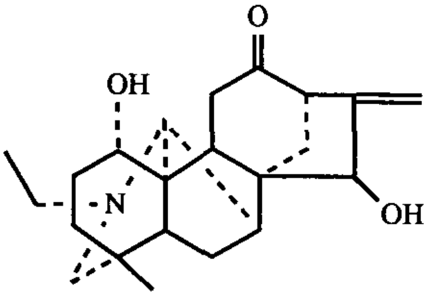
Abstract – A new C_{20}-diterpene alkaloid, karakomine (**1**) along with two known compounds, songorine (**2**) and neoline (**3**), have been isolated from the roots of *Aconitum karakolicum* Rap. The structure of karakomine was determined by 1D and 2D nmr spectroscopic analyses.

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 ^{13}C nmr spectra.

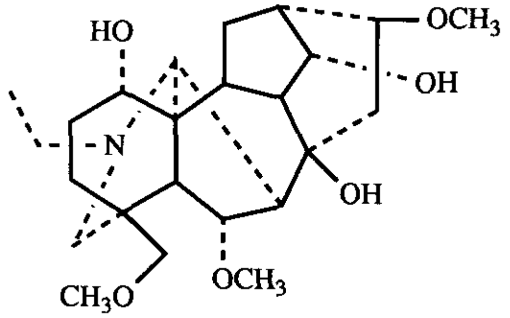
Karakomine (**1**), $[\alpha]_D$ -18.7° (c = 0.27, EtOH), was obtained as an yellowish amorphous powder. The high resolution ms (M^+ 359.2430, calcd 359.2457) of **1** indicated the molecular formula, $C_{22}H_{33}NO_3$. The ir spectrum of **1** showed the presence of hydroxyl group (3450 cm^{-1}) and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



karakomine (1)



songorine (2)



neoline (3)

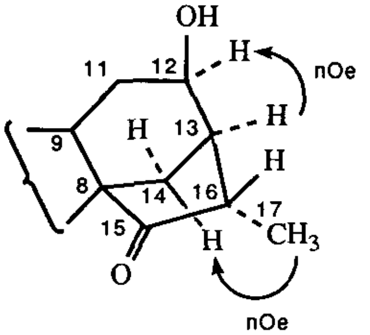


Fig. 1

formula of **1** suggested that the compound (**1**) was a C_{20}-diterpene alkaloid.[3,4] However, the ^{1}H 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 ^{1}H nmr decoupling experiment ($J_{12\text{-}13}$ = 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, $C\underline{H_3}CH_2$-N
CH_3	13.6	1.07 t (7.3)	$CH_3C\underline{H_2}$-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-14α (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 Brucker 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 CH_2Cl_2 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 CH_2Cl_2 : EtOH = 10 : 1) and songorine (**2**, ca. 1.8 g, elution with CH_2Cl_2 : 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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