STUDIES ON THE ALKALOIDS FROM *ACONITUM KARAKOLICUM* RAP. PART II

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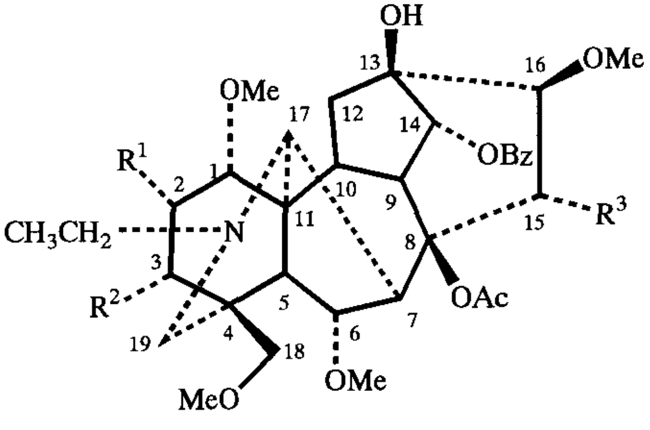
Abstract — A novel C_{19}-diterpenoid alkaloid, karaconitine (**1**) and four known alkaloids, 3-deoxyaconitine (**2**), aconitine (**3**), indaconitine (**4**) and chasmaconitine (**5**), have been isolated from the roots of *Aconitum karakolicum* Rap. The structure of karaconitine (**1**) was determined to be 2α-hydroxy-3-deoxyaconitine by the analyses of its spectral data.

In the previous paper,[1] we reported the isolation of a new C_{20}-diterpenoid alkaloid, named karacomine, and two known compounds songorine and neoline, from *A. karakolicum*. Continuous investigation on the constituents of this plant has resulted in the isolation of a new C_{19}-diterpenoid alkaloid, karaconitine (**1**), and four known alkaloids, 3-deoxyaconitine (**2**),[2,3] aconitine (**3**),[3] indaconitine (**4**)[4] and chasmaconitine (**5**).[5] In this paper we report here the structural elucidation of karaconitine (**1**) by spectroscopic means.



karaconitine (1) : R^1= OH, R^2= H, R^3= OH
3-deoxyaconitine (2) : R^1= R^2= H, R^3= OH
aconitine (3) : R^1= H, R^2= R^3= OH
indaconitine (4) : R^1 = H, R^2= OH, R^3= H
chasmaconitine (5) : R^1= R^2= R^3= H

Table 1. The ^{1}H nmr data for karaconitine (**1**) and the ^{13}C nmr data for karaconitine (**1**), 3-deoxyaconitine (**2**) and aconitine (**3**).

^{1}H nmr (400 MHz, δ, CDCl$_3$)		^{13}C nmr (100 MHz, δ, CDCl$_3$)			
H	karaconitine (1)	C	(1)	(2)^a	(3)^a
1	3.14, d (4.5)	1	85.9	85.5	83.7
2	4.02, m	2	62.3	26.7	34.1
3α	2.02, dd (14.8, 3.6)	3	42.3	37.0	71.0
3β	1.71, dd (14.8, 2.0)	4	38.8	39.4	43.4
5	2.25, d (6.0)	5	48.8	49.5	47.2
6	4.00, d (6.0)	6	82.3	83.7	82.2
7	2.92, s	7	45.5	45.6	44.5
9	2.97, m	8	91.7	92.5	92.2
10	2.25, m	9	45.0	44.9	45.2
12α	2.78, m	10	40.7	41.4	41.4
12β	2.30, m	11	52.5	50.3	50.1
14	4.90, d (4.4)	12	38.6	37.0	36.2
15	4.46, dd (4.4, 3.2)	13	73.9	74.4	74.2
16	3.26, d (4.4)	14	78.8	79.2	79.1
17	3.06, s	15	78.7	79.3	78.9
18α	3.68, d (8.8)	16	90.2	90.5	90.3
18β	2.97, d (8.8)	17	60.0	61.0	60.6
19α	2.58, d (11.2)	18	78.9	79.2	77.1
19β	2.51, d (11.2)	19	51.4	53.6	48.8
$NCH_2C\underline{H}_3$	1.15, t (7.2)	$NCH_2\underline{C}H_3$	12.1	13.7	13.2
$NC\underline{H}_2CH_3$	2.88, m	$N\underline{C}H_2CH_3$	48.8	49.5	47.2
$NC\underline{H}_2CH_3$	2.24, m				
13-OH	3.87, s	14-O$\underline{C}$OPh	166.1	166.0	165.9
15-OH	4.35, d (3.2)	8-O$\underline{C}$OCH$_3$	172.4	172.6	172.2
8-OCOCH$_3$	1.41, s	8-OCO$\underline{C}$H$_3$	21.3	21.7	21.3
1-OMe	3.33, s	1-OMe	56.1	56.3	55.6
6-OMe	3.19, s	6-OMe	58.9	59.3	59.0
16-OMe	3.72, s	16-OMe	61.0	61.4	60.9
18-OMe	3.28, s	18-OMe	58.3	58.2	57.9
aromatic	8.03 d, (7.2)	aromatic	129.8	130.0	130.2
protons	7.46 t, (7.2)	carbons	129.6	129.9	129.6
	7.58 t, (7.2)		128.6	128.8	128.5
			133.3	133.4	133.1

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

Karaconitine (**1**), mp 222-224°, $[\alpha]_D$ +7.96° (c 0.47, MeOH). Its molecular formula, $C_{34}H_{47}NO_{11}$, was derived from the FAB mass spectral data (m/z 646, M^++1). The ir spectrum of **1** exhibited the absorption bands due to hydroxyl (3480 cm^{-1}) and ester (1730 cm^{-1}) groups and aromatic ring (1620, 1455 and 715 cm^{-1}). The ^1H nmr spectrum of **1** showed the following signals, δ 1.15 (3H, t, J = 7.2 Hz, N-$CH_2C\underline{H}_3$), δ 3.19, 3.28, 3.33, 3.72 (3H each, s, OCH_3 x 4) and δ 7.46-8.03 (5H, m, $OCOC_6H_5$). The above spectral data suggested that the compound (**1**) should be a C_{19}-diterpenoid alkaloid. The downfield signal at δ 4.90 (1H, d, J = 4.4 Hz, H-14β) and the upfield signal at δ 1.41 (3H, s, $OCOCH_3$) indicated that the benzoyloxy and the acetoxyl groups should be located at C-14 and C-8, respectively. The four methoxyls could be assigned at C-1, C-6, C-16 and C-18 respectively, because the ether protons on their carbons were observed at δ 3.14 (1H, d, J = 4.5 Hz, H-1β), 4.00 (1H, d, J = 6.0 Hz, H-6β), 3.26 (1H, d, J = 4.4 Hz, H-16α), 2.97 and 3.68 (1H each, ABq, J = 8.8 Hz, H-18). These assignments were confirmed by the carbon signals at δ 85.9 (C-1), 82.3 (C-6), 90.2 (C-16) and 78.9 (C-18) and the analysis of HMBC spectrum. From consideration of the ir absorption band at 3480 cm^{-1}, molecular formula and three downfield carbon signals at δ 62.3, 73.9 and 78.7, karaconitine (**1**) should have three hydroxyl groups in the molecule. The chemical shift of the methoxyl protons (δ 3.72) at C-16 suggested the presence of hydroxyl groups at C-13 and C-15.[6] Comparing the chemical shift of C-15 (δ 78.7) with those of C-15 bearing α-OH (δ 78.5 - 79.0) and C-15 bearing β-OH (δ 68.0),[7] the hydroxyl group at C-15 could be assigned to α-configuration. When the ^{13}C nmr spectrum of **1** was compared with those[8] of 3-deoxyaconitine (**2**) and aconitine (**3**), the chemical shift pattern of **1** was very close to that of **2** except the appearance of a new $\underline{C}H$ signal at δ 62.3 (C-2) in place of a $\underline{C}H_2$ signal at δ 26.3 in **2** and the downfield shift of C-3 signal (δ 37.0 → 42.3) (see Table 1). If the downfield shift of C-3 was ascribed to the β-effect of a hydroxyl group, the remaining hydroxyl group should be situated at C-2 instead of usual C-3 position. This was further confirmed by the analysis of HMBC spectrum.

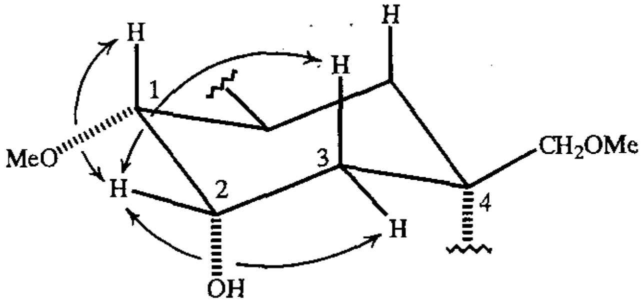


Figure 1

The stereochemistry of the hydroxyl group at C-2 could be assigned to α-configuration by the coupling constants of H-1 [δ 3.14 (d, J = 4.5 Hz)], H-3α [δ 2.02 (dd, J = 3.6, 14.8 Hz)] and H-3β [δ 1.71 (dd, J = 2.0, 14.8 Hz)] and the analysis of NOESY spectrum of **1** (Figure 1). Thus, the structure of karaconitine was established to be 2α-hydroxy-3-deoxyaconitine (**1**). This is the first report on the isolation of C_{19}-diterpenoid alkaloid bearing oxygen substituent at C-2 instead of C-3.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage equipped with a microscope and uncorrected. Optical rotation was measured on a JASCO DIP-181 digital polarimeter. Mass spectra were recorded on MAT-711 and Hitachi M-2000. The nmr spectra were measured on a JEOL GSX-400 and a Brucker AM-400 spectrometers. The ir spectra were 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% of 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 5 portion (60 g) was chromatographed over silica gel to afford aconitine (**3**, 5.0 g, elution with petroleum ether : acetone = 3 : 1). After removal of aconitine by filtration, the filtrate was concentrated to give a syrup which was subjected to silica gel column chromatography. Elution with petroleum ether : acetone = 2 : 1 yielded 3-deoxyaconitine (**2**, 3.0 g) and chasmaconitine (**5**, 75 mg). After removal of **2** and **5** by filtration, the mother liquors were concentrated and then the residue was chromatographed over silica gel eluted with CH_2Cl_2 : MeOH = 20 : 1 to give karaconitine (**1**, 100 mg) and indaconitine (**4**, 500 mg).

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