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KARACONITINE, A NEW C_{19}-NORDITERPENOID ALKALOID FROM *Aconitum karakolicum* ROOTS

S. K. Usmanova[1,2]* and H. A. Aisa[1]

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The known alkaloids aconitine, benzoylaconine, and songorine and the new C_{19}-norditerpenoid alkaloid karaconitine were isolated from roots of Aconitum karakolicum *Rapaics (Ranunculaceae). The structure of karaconitine was established using ESI-mass spectra, 1D (^{1}H and ^{13}C) and 2D NMR (HSQC, HMBC) and DEPT spectral data, and comparison of its ^{13}C NMR spectrum with that of aconine.*

Keywords: *Aconitum karakolicum*, Ranunculaceae, C_{19}-norditerpenoid alkaloids, karaconitine, aconitine, benzoylaconine, songorine.

In continuation of research on the alkaloid composition of roots of *Aconitum karakolicum* Rapaics (Ranunculaceae), we isolated in addition to the known alkaloids aconitine, benzoylaconine, and songorine [1] a new base **1** of formula $C_{24}H_{39}NO_9$ with mp 155–157°C that we called karaconitine.

The mass spectrum of **1** showed peaks for ions with *m/z* 485 $[M]^+$, 470 $[M – 15]^+$, and 454 $[M – 31]^+$. The proton spectrum had resonances for *N*-methyl, four methoxyls, and protons geminal to three hydroxyls. Its DEPT spectrum gave resonances for 24 C atoms including 4 singlets, 11 doublets, 4 triplets, and 5 quartets (Table 1). An analysis of the 1D PMR, ^{13}C NMR, and DEPT; 2D HSQC and HMBC; and mass spectral data showed that two of the four singlets at δ 78.57 and 76.02 ppm were due to C atoms of two tertiary hydroxyls. The results indicated that karaconitine had the functional formula $C_{19}H_{19}(NCH_3)(OCH_3)_4(OH)_5$ and was a C_{19}-norditerpenoid alkaloid of the aconitine series.

The HSQC spectrum exhibited correlation peaks between singlets for protons of four methoxyls (δ 3.67, 3.51, 3.38, and 3.31 ppm) and resonances for four C atoms (δ 55.48, 58.86, 61.49, and 59.29 ppm). The methoxyl proton resonances in the HMBC spectrum gave cross peaks with resonances of atoms at δ 89.92 (C-16), 80.75 (C-6), 80.16 (C-1), and 78.20 (C-18) ppm, respectively. Therefore, the methoxyls were located on C-1, C-6, C-16, and C-18. The appearance of a strong peak for $[M – 31]^+$ in the mass spectrum of **1** confirmed that a methoxyl was located on C-1 [2].

A proton resonance at δ 3.01 (s, 3H) in the HSQC spectrum of **1** correlated with the resonance of an atom at 41.86 ppm and correlated in the HMBC spectrum to a doublet for a C atom at δ 67.55 (C-17) and a triplet for the atom with δ 52.29 (C-19).

The resonance of H-16 in the HMBC spectrum of **1** gave cross peaks with resonances of adjacent C atoms with δ 78.57 (C-13), 78.19 (C-14), 80.96 (C-15), and 16-OCH_3. The chemical shifts (CSs) of C-13, C-14, and C-15 suggested that hydroxyls were located in these positions. Resonances of C-14 and C-15 in the HSQC spectrum showed cross peaks with resonances for H-14 (3.93, d, 1H, J = 5.2) and H-15 (4.77, d, 1H, J = 6.0). The CSs, multiplicity, and SSCC of resonances for H-14 and H-15 were due to the presence of α-oriented OH groups on C-14 and C-15. These results were consistent with hydroxyls on C-13, C-14, and C-15. The presence of an OH group on C-13 caused a weak-field shift of the resonance for C-12 to the region 33.5–38.0 ppm [3]. The resonance for C-12 was observed in the ^{13}C NMR spectrum at 36.23 ppm and confirmed that C-13 had an OH group.

The DEPT spectrum of **1** showed a singlet for the C atom at 76.02 ppm. The CS of this resonance indicated that C-8 contained a tertiary OH group. The resonance for C-8 was observed in the region δ 75.5–76.5 ppm with simultaneous occurrence of an α-oriented hydroxyl on C-15 [3]. Therefore, C-8 was substituted with a hydroxyl.

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1) Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, No. 40-1, Beijing South Road, Urumqi, China, 830011, e-mail: haji@ms.xjb.ac.cn; 2) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: skusmanova@mail.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 242–243, March–April, 2011. Original article submitted June 25, 2010.

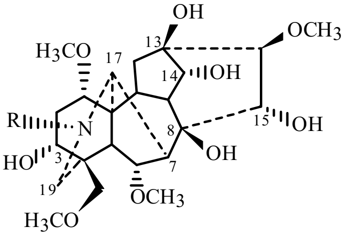
TABLE 1. PMR (599.95 MHz) and ^{13}C NMR (150.87 MHz) Spectra of **1** ($CDCl_3$, δ, ppm, TMS, J/Hz) and ^{13}C NMR Spectrum of **2**

C atom	δ_C (**1**)	δ_C (**2**)	HSQC (**1**)	C atom	δ_C (**1**)	δ_C (**2**)	HSQC (**1**)
1	80.16	84.1	3.48 (1H, m)	14	78.19	80.6	3.93 (1H, d, J = 5.2)
2	29.07	35.5	1.51 (1H, m),	15	80.96	78.5	4.77 (1H, d, J = 6.0)
			2.33 (1H, d, J = 16.0)	16	89.92	91.8	3.07 (1H, d, J = 5.4)
3	70.21	71.9	4.25 (1H, m)	17	67.55	60.8	3.00 (1H, s)
4	43.73	43.3		18	78.20	77.4	3.87 (1H, d, J = 8.8)
5	43.93	49.0	2.21 (1H, d, J = 7.2)				3.34 (1H, d, J = 8.4)
6	80.75	83.0	4.29 (1H, d, J = 7.2)	19	52.29	48.3	3.56 (1H, d, J = 12.6)
7	47.18	51.3	3.11 (1H, s)				3.27 (1H, d, J = 12.6)
8	76.02	76.4		$N\text{-}CH_3$	41.86		3.01 (3H, s)
9	46.32	50.1	2.26 (1H, t, J = 5.2)	$1\text{-}OCH_3$	55.62	55.7	3.38 (3H, s)
10	40.26	42.4	2.14 (1H, m)	$6\text{-}OCH_3$	58.53	58.0	3.51 (3H, s)
11	50.02	50.5		$16\text{-}OCH_3$	60.48	61.9	3.67 (3H, s)
12	36.23	37.4	2.33 (1H, d, J = 16.0)	$18\text{-}OCH_3$	59.31	59.1	3.31 (3H, s)
			1.62 (1H, m)	$N\text{-}\underline{CH_2}CH_3$		46.2	
13	78.57	78.7		$N\text{-}CH_2\underline{CH_3}$		13.4	

The proton spectrum of **1** exhibited a resonance at 4.25 ppm (m, 1H) that correlated in the HSQC spectrum with the resonance of the C atom at 70.21 ppm. Therefore, a hydroxyl was placed on C-3, which was consistent with the CS of C-4, which was observed as a result of the *β*-effect of the C-3 hydroxyl at δ 43.73 ppm [3].

The H-2 resonance exhibited an HMBC correlation with C-3; of H-7, through three bonds with C-11 and C-15.

Thus, based on the spectral data, the new base **1**, which we called karaconitine, was a C_{19}-norditerpenoid alkaloid of the aconitine series and had the structure **1**.



1: R = CH_3; **2:** R = CH_2CH_3

A comparison of the CSs for C atoms of karaconitine and aconine [4] showed that their CSs were similar with the exception of those for C-1, C-2, C-5, C-6, C-7, C-9, C-17, and C-19 in the spectrum of **1**. These differences were explained by the fact that **1** has an *N*-methyl instead of an *N*-ethyl.

EXPERIMENTAL

Melting points were determined on a Yanaco MP-S3 block (Micromelting Point Apparatus, Yanaco Mfg. Co.). Mass spectra were measured in a 2690-ZQ 4000 Water-alliance LC-MS spectrometer. NMR spectra were taken on a Varian-Inova instrument (600 MHz, $CDCl_3$). The purity of the isolated compounds was monitored by TLC on silica gel plates using $CHCl_3$:MeOH (5:1, 7:1), acetone:MeOH (10:1), and acetone. Spots were detected by I_2 vapor and spraying with Dragendorff's solution. Column chromatography used silica gel (200–300 mesh, Qindao Ocean Chemical Factory).

Isolation of Alkaloids. Mother liquors of alkaloids were separated into two parts based on TLC analysis. These were fractions containing alkaloids with R_f values greater than that of aconitine (A) and fractions including all alkaloids with R_f values less than that of aconitine (B). Part B was chromatographed over a column of silica gel with elution by petroleum ether:EtOAc (10:1, 5:1, 1:1), EtOAc, and EtOAc:MeOH (10:1, 5:1, 1:1). Fractions with identical R_f values were combined to give six principal fractions. The third fraction was chromatographed over Al_2O_3 with elution by hexane:acetone (5:1, 1:1). The initial hexane:acetone (5:1) eluates contained aconitine; later ones (1:1), songorine. Aconitine was identified by direct comparison with an authentic sample; songorine, by comparison of its PMR and ^{13}C NMR spectra with those published [5].

The fourth fraction was worked up with MeOH to afford benzoylaconine, the crystals of which were washed with acetone. The acetone rinsings were chromatographed over silica gel with elution by EtOAc and EtOAc:MeOH (5:1, 1:1). A total of 11 fractions was collected. Fractions 9–10 were treated with acetone to afford **1** (0.05 g), mp 155–157°C.

Mass spectrum (ESI-P, *m*/*z*): 486 $[M]^+$, 470 $[M - 15]^+$, 454 $[M - 31]^+$, 436, 422, 404, 148, 132, 104.

Table 1 presents PMR and ^{13}C NMR spectral data.

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