A new norditerpenoid alkaloid acsonine from the roots of *Aconitum kusnezoffii* Reichb.

E. G. Zinurova,[a] T. V. Khakimova,[a] L. V. Spirikhin,[a] M. S. Yunusov,[a] P. G. Gorovoi,[b] and G. A. Tolstikov[c]*

[a]*Institute of Organic Chemistry, Ufa Research Center of the Russian Academy of Sciences, 71 prosp. Oktyabrya, 450054 Ufa, Russian Federation. Fax: +7 (347 2) 35 6066. E-mail: chemorg@anrb.ru*

[b]*Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 159 prosp. 100-letiya Vladivostoka, 690022 Vladivostok, Russian Federation. Fax: +7 (423 2) 31 4050*

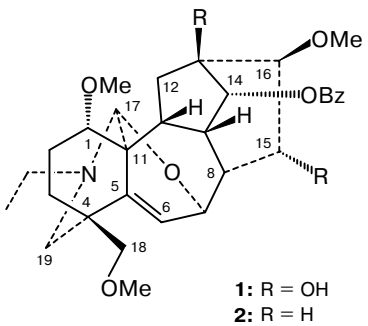
[c]*N. N. Vorozhtsov Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, 9 prosp. Akad. Lavrent'eva, 630090 Novosibirsk, Russian Federation. Fax: +7 (383 2) 34 4752. E-mail: osadchii@nioch.nsc.ru*

The known norditerpenoid alkaloid mesaconitine and a new alkaloid acsonine **1** were isolated from the roots of *Aconitum kusnezoffii* Reichb. The structure of **1** was established based on spectroscopic data.

Key words: *Aconitum kusnezoffii* Reichb., norditerpenoid alkaloids, mesaconitine, acsonine.

The known norditerpene alkaloid mesaconitine and new amorphous compound **1** with the composition $C_{31}H_{41}NO_8$ called acsonine were isolated from the roots of *Aconitum kusnezoffii* Reichb., gathered in the environs of the village Lipovtsy (Far East, Primorskii division, Oktyabr′skii region). Previously, the norditerpene alkaloids aconitine, deoxyaconitine, hypaconitine, beivutine, and mesaconitine and the diterpene alkaloids lepenine and denudatine have been isolated from this plant.[1]

Base **1** exhibits a mass spectrum typical of norditerpene alkaloids;[2] as follows from the ^1H and ^{13}C NMR spectra, this compound contains an $N—CH_2—CH_3$ group, three methoxy groups, two hydroxy groups, a benzoyloxy group, and one oxygen atom as an ether group. The ^{13}C NMR spectrum recorded with C—H coupling modulation contains 6 singlets, 6 triplets, 15 doublets, and 4 quartets. The singlet at δ 138.3 and the doublet at δ 123.8 in the ^{13}C NMR spectrum point to the presence of a trisubstituted double bond. Relying on these results, this base can be classified as a C_{19}-diterpene alkaloid. All the assignments presented below (Table 1) were based on COSY $^1H—^{13}C$ and $^1H—^1H$ spectra.



1: R = OH
2: R = H

The low-field doublet at δ 83.2 in the ^{13}C NMR spectrum and the signal at δ 5.11 (d, J = 5.1 Hz) in the ^1H NMR spectrum are consistent with the presence of the benzoyloxy group at C(14) with C(13) bearing an oxygen function.[3] The singlet at δ_C 76.4 prompts the conclusion that this function is a hydroxy group. The presence of the hydroxy group at C(13) is confirmed by the lowest-field position (δ_C 94.2) of the doublet for the C(16) atom, which carries the methoxy group.[3] The H(16) proton at δ 2.94 (d, J = 5.7 Hz) is connected with H(15) (δ 5.10, J = 5.7 Hz), which is geminal with respect to the hydroxy group. The signal for the C(15) atom shows itself at δ 74.6. The second low-field doublet

Table 1. ^{13}C NMR spectra for acsonine (**1**) and franchetine (**2**)[6]

Atom	δ		Atom, group	δ	
	1	**2**		**1**	**2**
C(1)	86.3	86.1	C(16)	94.2	85.2
C(2)	24.3	24.3	C(17)	92.6	92.2
C(3)	32.7	38.4	C(18)	78.9	79.1
C(4)	37.1	37.0	C(19)	52.0	52.1
C(5)	138.3	136.1	$N—CH_2$	49.1	48.9
C(6)	123.8	127.7	Me	13.2	13.0
C(7)	74.2	78.4	MeOC(1)	57.2	55.8
C(8)	47.5	49.5	MeOC(16)	61.5	56.2
C(9)	42.7	42.7	MeOC(18)	59.4	59.2
C(10)	47.4	47.8	C=O	166.6	166.2
C(11)	50.5	50.4	Ar:		
C(12)	38.9	29.8	C(1′)	129.8	130.6
C(13)	76.4	38.2	C(2′), C(6′)	129.8	129.7
C(14)	83.2	74.9	C(3′), C(5′)	128.4	128.3
C(15)	74.6	32.6	C(4′)	133.4	132.6

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at δ_C 92.6 may be due to the presence of the oxygen bridge C(7)—O—C(17)—N; this signal corresponds to the C(17) atom. In the ^{1}H NMR spectrum, the signal for H(17) is a singlet at δ 4.37. The direct carbon—proton coupling constant is 163.65 Hz, which confirms the presence of two heteroatoms at C(17).[4] The second site of attachment of the oxygen bridge is the C(7) atom, which is responsible for the doublet at δ_C 74.2. The H(7) proton (a doublet at δ 4.56 (J = 6.3 Hz)) is connected with the olefinic proton (δ 6.15, J = 6.3 Hz), indicating that the double bond is located between C(5) and C(6).

The doublet at δ_C 86.3 is due to the C(1) atom linked to a methoxy group. The presence of the methoxy group at C(1) is confirmed by one of the most intense peaks, $[M - 31]^+$, in the mass spectrum of **1**.[5]

The triplet at δ 78.9 in the ^{13}C NMR spectrum is due to the C(18)-methylene group to which a methoxy group is attached. The protons of the methylene group are inequivalent; in the ^{1}H NMR spectrum, they are manifested at δ 3.03 and 3.15 (both d, each 1 H, J = 9.1 Hz).

The structure **1** proposed for acsonine is unusual; it has a double bond between the C(5) and C(6) atoms and an oxygen bridge between C(7) and C(17) and has no oxygen function at C(8). Only one norditerpene alkaloid franchetine (**2**),[6] isolated from *Aconitum franchetii* and having a similar structure, has been documented. These specific structural features of acsonine and franchetine are apparently correlated and are due to the specific features of the metabolism of C_{19}-diterpene alkaloids in the plant.

Experimental

NMR spectra were recorded on a Bruker AM-300 instrument in $CDCl_3$ using Me_4Si as the internal standard. The molecular masses and elemental compositions were determined using a high-resolution mass spectrometer (Finnigan MAT 8200). Melting points were determined using a Koffler hot stage. Column chromatography was carried out using silica gel KSK 100—160 μm; for TLC, silica gel LSL 5/40 μm with 13% gypsum (Chemapol, Czech Republic) was used. The following solvent systems were employed for monitoring: (1) $CHCl_3$—C_6H_6—MeOH—NH_4OH (4 : 4 : 3 : 0.1); (2) Et_2O—C_6H_{14}—MeOH—NH_4OH (5 : 2 : 2 : 0.1). Freshly distilled solvents were used. The pH was checked based on the color of the universal indicator paper strips.

The isolation of alkaloids. Air-dry crushed roots of *Aconitum kusnezoffii* Reichb. (1.15 kg) were subjected to exhaustive extraction with an acetone—water mixture (7 : 3). Acetone was evaporated from the extracts, the aqueous solution was acidified with 20% H_2SO_4 and washed with $CHCl_3$. The acidic solution was alkalified with Na_2CO_3 to pH 8 and then with a 5% solution of NaOH to pH 12 and the alkaloids were successively extracted with chloroform to give fractions I (7.01 g) and II (1.80 g). The first fraction was treated with acetone; this resulted in the isolation of 580 mg of crystalline mesaconitine, m.p. 208 °C.[7] Acetone was distilled off from the mother liquor, the residue (6.43 g) was dissolved in 200 mL of 5% H_2SO_4, and the components were separated according to their basicities. The acidic solution (pH 3) was extracted with benzene (3×200 mL) and then with chloroform (3×200 mL). This gave two fractions, 226 mg and 402 mg. The aqueous layer was alkalified with Na_2CO_3 to pH 7 and with NaOH to pH 12, the bases being extracted with chloroform (3×200 mL) at each pH value. The yields of the fractions were 3.21 g (pH 7) and 2.59 g (pH 12). Treatment of the fraction obtained at pH 12 with acetone afforded 1.18 g of crystalline mesaconitine. The mother liquor after separation of mesaconitine (1.41 g) was subjected to column chromatography on silica gel; alkaloids were eluted with dichloroethane, a dichloroethane—methanol (0.5—30% v/v) mixture, and methanol. This gave 13 fractions in total. Fraction 3 (0.5% methanol in dichloroethane) (200 mg) was dissolved in 20 mL of 5% H_2SO_4, and the components were separated according to their basicities as described above. This operation was repeated for the "pH 3—chloroform" fraction (98 mg) obtained upon separation. This gave 30 mg of amorphous base **1**.

Found: m/z 524.2599 $[M - 31]^+$. $C_{31}H_{41}NO_8$. Calculated: M − OMe = 524.2648. MS, m/z (I_{rel} (%)): 555 $[M]^+$ (14), 540 (38), 524 (52), 434 (19), 252 (15), 251 (15), 149 (14), 122 (11), 105 (100).

^{1}H NMR (300.13 MHz, δ, J/Hz): 1.05 (t, 3 H, N—CH_2—C$\underline{H}_3$, J = 7.2 Hz); 2.94 (d, 1 H, H(16), J = 5.7 Hz); 3.03, 3.15 (both d, each 1 H, 2 H(18), J = 9.1 Hz); 3.27, 3.36, 3.68 (all s, each 3 H, OCH_3); 4.45 (s, 1 H, H(17)); 4.56 (d, 1 H, H(7), J = 6.3 Hz); 5.10 (d, 1 H, H(15), J = 5.7 Hz); 5.11 (d, 1 H, H(14), J = 5.1 Hz); 6.15 (d, 1 H, H(6), J = 6.3 Hz); 7.45 (t, 2 H, $CO_2C_6\underline{H}_5$, J = 7.3 Hz); 7.57 (t, 1 H, $CO_2C_6\underline{H}_5$, J = 7.3 Hz); 8.08 (d, 2 H, $CO_2C_6\underline{H}_5$, J = 7.3 Hz).

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