16-Demethoxymethyllycaconitine, a new norditerpenoid alkaloid from *Delphinium cuneatum*

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A new norditerpenoid alkaloid has been isolated from the roots of *Delphinium cuneatum*. On the basis of ^{1}H, ^{13}C NMR, IR, and mass spectra, the structure of the alkaloid was established as 1α,6β,14α-trimethoxy-7,8-dihydroxy-18-(2″-methyl)succinylanthranoyloxyaconane (16-demethoxymethyllycaconitine). The roots of *Delphinium cuneatum* also contain the known alkaloid methyllycaconitine and *N*,*N*′-di(methoxycarbonyl)-3,4-diaminotoluene.

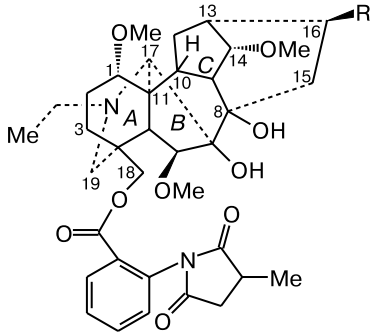
Key words: *Delphinium cuneatum*, roots; norditerpenoid alkaloids.

Diterpenoid alkaloids have long attracted attention of chemists and pharmacologists due to complexity of their structures and a broad spectrum of physiological activities. The main sources of these alkaloids are plants of the genera *Aconitum* and *Delphinium*.[1]

Our study was concerned with the roots of *Delphinium cuneatum* collected at the Belebeyevskoye table (Bashkortostan, the Aslykul′/Kandrykul′ locality) during the blooming period. To the best of our knowledge, published data on the alkaloid composition of this plant are missing.

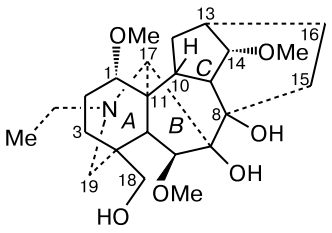
The alkaloids altogether isolated by extraction with aqueous acetone was separated according to the basicity into two large fractions (**A** and **B**) of moderately basic (**A**, pH 10) and highly basic (**B**, pH 12) alkaloids. Each of them was separated into narrower fractions with pH 6, 7, 9, and 12.

Column chromatography of the pH 6 fraction obtained from fraction **B** (pH 6(**B**)) gave alkaloid **1**, which had not been described previously. According to IR spectroscopy, its molecule contains an ester group (ν = 1720 cm^{-1}); this is confirmed by alkaline hydrolysis of the alkaloid, resulting in amino alcohol **2**. According to the high-resolution mass spectrum, the molecular weight of compound **2** is equal to 437.277, which corresponds to the molecular formula $C_{24}H_{39}NO_6$. A comparison of the molecular masses of the starting alkaloid (*m*/*z* 652 $[M]^+$) and amino alcohol and analysis of the spectroscopic data (^{1}H NMR, ^{13}C NMR, mass, and IR spectra) indicate that methylsuccinylanthranilic acid is the esterifying acid in compound **1**. The data from ^{1}H and ^{13}C NMR spectra indicate that molecules **1** and **2** contain three methoxy groups and an *N*-ethyl group; their mass spectra are similar to those typical of C_{19} diterpenoid alkaloids.[2] The fact that the $[M - 31]^+$ peak is the most abundant in the mass spectra of alkaloids **1** and **2** suggests the presence of methoxy group in position 1.[3] The ratio of the intensities



1, 3

R = H (**1**), OMe (**3**); R = R′ = H (**4**), R = R′ = OMe (**5**)



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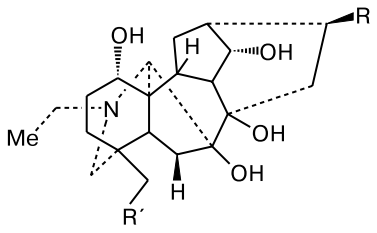

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of the $[M - 15]^+$ and $[M - 31]^+$ ion peaks equal to 33 : 100 in the mass spectra of C_{19}-diterpenoid alkaloids containing a MeO group at C(1) is typical of the C(6)(OMe)—C(7)(OH)—C(8)(OH) fragment.[4] The characteristic doublets with small spin-spin coupling constants of the α-protons at the C(6) atom (δ 3.77 and 3.72, respectively, J = 1.6 Hz), observed in the ^{1}H NMR spectrum of alkaloid **1** and the product of its hydrolysis **2** also attest to the presence of a β-OMe group at C(6), while the triplets at δ 3.47 (**1**) and 3.50 (**2**) (J = 4.2 Hz) suggest the presence of an α-OMe group at the C(14) atom.[5] The signals established reliably in the ^{1}H NMR spectra include singlets for the protons at C(17) (δ 3.11 (**1**) and 3.12 (**2**)), two doublets for diastereotopic protons at C(19) (δ 2.42 and 2.71 (**1**), δ 2.43 and 2.68 (**2**)), three singlets in the region of δ 3.20—3.40 for the MeO group (in both spectra), and a doublet (δ 2.28) for the Me group of the succinimide fragment (in the spectrum of **1**).

The JMODCH ^{13}C NMR spectrum of alkaloid **1** contains eight signals for methine C atoms, three signals for methoxy groups (δ 56.0, 57.3, and 58.5), eleven signals for the methylene C atoms and C atoms containing no attached protons in the main carbon skeleton, and also the signals corresponding to the methylsuccinylanthranyl residue and the *N*-ethyl fragment (δ 51.1 and 14.2). This is consistent with the above considerations concerning the alkaloid structure.

The structure of compound **1** was finally established based on comparison of the chemical shifts of alkaloids **1** and **2** in the ^{13}C NMR spectra with those of known compounds, *viz.*, methyllycaconitine (**3**),[6] cardiopetalidine (**4**),[7] and virescenine (**5**)[8] (Table 1), and calculations according to additive schemes using α-, β-, and γ-increments of substituents. A comparison of the ^{13}C NMR spectra of alkaloid **1** and methyllycaconitine showed that the chemical shifts of the signals for rings *A* and *B* differ by less than 1 ppm; substantial changes in the chemical shifts are observed for signals of the C(13), C(15), and C(16) atoms. According to the data from the ^{13}C NMR spectrum, the molecule of **1** has one methylene group more than methyllycaconitine and does not have the methoxy group at C(16). The pattern of changes of the chemical shifts of the C(13), C(15), and C(16) atoms observed in the ^{13}C NMR spectra on going from cardiopetalidine (**4**), devoid of any substituent in position 16, to virescenine (**5**), which has a methoxy group in this position, is similar to that observed on going from compound **1** to compound **3**. Transition from **5** to **4** or from **3** to **1** results in upfield shifts equal to 4.9 and 6.1 ppm, respectively, for C(13) and 9.3 and 8.2 ppm, respectively, for C(15).

Thus, the isolated alkaloid **1** has the structure of methyllycaconitine devoid of the methoxy group at C(16), *i.e.*, it is 16-demethoxymethyllycaconitine.

The fraction of alkaloids pH 7(**B**) was additionally separated according to basicity into five fractions (**C**) with pH 3, 6, 7, 9, and 12. Methyllycaconitine (**3**) was isolated from the fraction pH 6(**C**) by column chromatography on silica gel.

Column chromatography of the fraction pH 12(**A**) gave *N*,*N*′-di(methoxycarbonyl)-3,4-diaminotoluene.

Table 1. ^{13}C NMR chemical shifts of 16-demethoxymethyllycaconitine (**1**), 16-demethoxylycoctonine (**2**), methyllycaconitine (**3**),[6] cardiopetalidine (**4**),[7] and virescenine (**5**)[8]

Atom	δ				
	1	**2**	**3**	**4**	**5**
C(1)	83.1	83.2	83.9	72.7	72.4
C(2)	25.5	25.6	26.0	29.6	28.5
C(3)	32.2	31.8	32.0	31.9	29.3
C(4)	37.8	39.0	37.6	33.5	37.7
C(5)	43.0*	43.2*	43.2	47.5	41.9
C(6)	90.9	90.8	90.8	34.0	33.5
C(7)	89.8	89.9	88.5	87.2	86.1
C(8)	77.6	77.7	77.4	78.4	76.2
C(9)	50.7	50.2	50.3	48.0	48.0
C(10)	46.3*	46.5*	46.1	43.8	43.6
C(11)	48.9	48.8	49.0	50.2	49.4
C(12)	29.1	29.2	28.7	32.0	26.9
C(13)	31.9	31.9	38.0	34.8	39.7
C(14)	84.9	85.3	83.9	75.8	75.5
C(15)	25.4	25.5	33.6	26.7	36.0
C(16)	22.6	22.7	82.5	24.9	81.9
C(17)	64.9	65.4	64.5	64.0	64.9
C(18)	69.6	68.0	69.5	27.4	78.7
C(19)	52.5	52.9	52.3	59.4	55.8
$\underline{C}H_3—CH_2—N$	14.2	14.4	14.0	13.5	13.9
$CH_3—\underline{C}H_2—N$	51.1	51.3	50.9	50.5	50.5
$C(1)—O\underline{C}H_3$	56.0	56.1	55.7	—	—
$C(14)—O\underline{C}H_3$	58.5	58.3	58.1	—	—
$C(16)—O\underline{C}H_3$	—	—	56.3	—	56.4
$C(6)—O\underline{C}H_3$	57.3	57.3	57.8	—	—
$C(18)—O\underline{C}H_3$	—	—	—	—	59.4
C=O	164.2	—	164.1	—	—
C(1′)	127.1	—	127.1	—	—
C(2′)	133.0	—	133.0	—	—
C(3′)	129.3	—	129.4	—	—
C(4′)	133.6	—	133.6	—	—
C(5′)	131.1	—	131.0	—	—
C(6′)	130.0	—	130.0	—	—
C(1″)	179.8	—	179.8	—	—
C(2″)	35.3	—	35.3	—	—
C(3″)	37.0	—	37.0	—	—
C(4″)	175.8	—	175.8	—	—
C(5″)	16.4	—	16.4	—	—

* The assignment may be changed.

Experimental

IR spectra were recorded on UR-20 and Specord M-80 spectrometers in Nujol. Mass spectra (EI, 70 eV) were recorded on Varian MAT-CH5 and MX-1310 mass spectrometers by the

peak superposition procedure. ^{1}H and ^{13}C NMR spectra were measured on Bruker AM-300 and Bruker AMX-III 300 instruments in $CDCl_3$ using Me_4Si as the internal standard.

Extraction from the roots of *Delphinium cuneatum*. Ground air-dried roots (760 g) were extracted at ~20 °C with 70% aqueous acetone (7×). The combined extracts (11.7 L) were concentrated and the aqueous residue (4 L) was alkalified with sodium carbonate to pH 10 and extracted with chloroform. A 10% solution of KOH was added to the aqueous solution to pH 12 and extracted again with chloroform. The first chloroform extract (**A**, pH 10) was concentrated to 2 L and extracted with 5% H_2SO_4 (15×100 mL). The acidic extract was alkalified successively to pH 6, 7, 9, and 12, each solution being extracted with benzene. The yields of fractions were 2.16 (pH 6(**A**)), 1.64 (pH 7(**A**)), 0.19 (pH 9(**A**)), and 0.36 g (pH 12(**A**)). The second chloroform extract (**B**, pH 12) was processed in a similar way. The yields of fractions were 0.63 (pH 6(**B**)), 5.57 (pH 7(**B**)), 0.52 (pH 9(**B**)), and 0.17 g (pH 12(**B**)).

A part of the fraction pH 7(**B**) (5.37 g) was treated with CCl_4, the insoluble part of alkaloids (0.1 g) was separated, and the soluble part was repeatedly extracted with 5% H_2SO_4. The acidic extract (broad fraction **C**) was successively alkalified, each solution being extracted with benzene. The yields of alkaloid fractions were 0.4 (pH 3(**C**)), 3.43 (pH 6(**C**)), 0.28 (pH 7(**C**)), 0.3 (pH 9(**C**)), and 0.04 g (pH 12(**C**)).

16-Demethoxymethyllycaconitine (1). The alkaloids of the fraction pH 6(**B**) (0.3 g) were chromatographed on a column with SiO_2 (40/100), using benzene—MeOH mixtures with MeOH concentrations increasing from 0.5 to 10% (v/v) for elution. From the fractions eluted with benzene—3% MeOH, 0.059 g of alkaloid **1** was obtained. MS, m/z (I_{rel} (%)): 652 $[M]^+$ (12.5), 637 (33.5), 621 (100), 216 (58), 188 (19). ^{1}H NMR ($CDCl_3$), δ: 1.02 (t, 3 H, C<u>H</u>$_3$—CH_2—N, J = 6.9 Hz); 1.20—1.60 (m, 7 H, H(2)$_a$, 2 H(3), H(10), 2 H(12), H(16)$_a$); 1.65—1.90 (m, 3 H, 2 H(15), H(16)$_b$); 2.00—2.25 (m, 4 H, H(2)$_b$, H(5), H(9), H(13)); 2.28 (d, 3 H, H(5″), J = 6.2 Hz); 2.42 (d, 1 H, H(19)$_a$, ^2J = 11.7 Hz); 2.71 (d, 1 H, H(19)$_b$, ^2J = 11.7 Hz); 2.77 (q, 2 H, CH_3—C<u>H</u>$_2$—N, J = 6.9 Hz); 2.90—3.10 (m, 4 H, H(17), H(2″), H(3″)); 3.28, 3.38, 3.39 (all s, each 3 H, 3 OMe); 3.47 (t, 1 H, H(14), J = 4.2 Hz); 3.52 (m, 1 H, H(1)); 3.77 (d, 1 H, H(6), J = 1.6 Hz); 3.85 (s, 2 H, 2 OH); 4.05, 4.08 (both d, 2 H(18), ^2J = 12.3 Hz); 7.28, 8.02 (both dd, each 1 H, H(3′), H(6′), ^3J = 7.6 Hz, ^4J = 1.6 Hz); 7.55, 7.67 (both td, each 1 H, H(4′), H(5′), ^3J = 7.6 Hz, ^4J = 1.6 Hz).

16-Demethoxylycoctonine (2). A 5% solution of KOH (5 mL) in MeOH was added to a solution of 16-demethoxymethyllycaconitine (**1**) (0.014 g) in 5 mL of MeOH and the mixture was stirred for 2 h at 50 °C until the initial compound **1** completely disappeared (TLC). The solvent was distilled off *in vacuo*, water was added to the residue, and the mixture was extracted with benzene (6×5 mL). The extract was concentrated to give 0.009 g of 16-demethoxylycoctonine (**2**) (quantitative yield). High-resolution mass spectrum, found: m/z 437.277 $[M]^+$. $C_{24}H_{39}NO_6$. Calculated: M = 437.2777. Found: m/z 422.254 $[M - Me]^+$. $C_{23}H_{36}NO_6$. Calculated: M = 422.2543. Found: m/z 406.259 $[M - OMe]^+$. $C_{23}H_{36}NO_5$. Calculated: M = 406.2594. MS, m/z (I_{rel} (%)): 437 $[M]^+$ (7.2), 422 $[M - Me]^+$ (39.8), 406 $[M - OMe]^+$ (100). ^{1}H NMR ($CDCl_3$), δ: 1.03 (t, 3 H, C<u>H</u>$_3$—CH_2—N, J = 6.8 Hz); 1.20—1.55 (m, 6 H, 2 H(3), H(10), 2 H(12), H(16)$_a$); 1.60—1.90 (m, 4 H, H(2)$_a$, 2 H(15), H(16)$_b$); 1.90—2.10 (m, 3 H, H(2)$_b$, H(5), H(13)); 2.15 (dd, 1 H, H(9), J = 11.2 Hz, J = 1.9 Hz); 2.49 (d, 1 H, H(19)$_a$, ^2J = 10.8 Hz); 2.72 (d, 1 H, H(19)$_b$, ^2J = 10.8 Hz); 2.77 (q, 2 H, CH_3-C<u>H</u>$_2$—N, J = 6.8 Hz); 3.05 (d, 1 H, H(18)$_a$, ^2J = 11.3 Hz); 3.11 (s, 1 H, H(17)); 3.18, 3.26, 3.37 (all s, each 3 H, 3 OMe); 3.28 (m, 1 H, H(1)); 3.38 (t, 1 H, H(14), J = 4.3 Hz); 3.42 (d, 1 H, H(6), J = 1.6 Hz); 3.53 (d, 1 H, H(18)$_b$, ^2J = 11.3 Hz); 3.70 (s, 3 H, 3 OH).

Methyllycaconitine (3). The alkaloids of the fraction pH 6(**C**) (0.15 g) were chromatographed on a column with SiO_2 (40/100) using benzene—MeOH mixtures with MeOH concentrations increasing from 0.5 to 10% (v/v) for elution. Concentration of the fractions eluted with benzene—5% MeOH gave 0.047 g of chromatographically pure methyllycaconitine, whose spectroscopic characteristics (^{1}H and ^{13}C NMR) were identical to those reported in the literature.[6]

***N,N′*-Di(methoxycarbonyl)-3,4-diaminotoluene.** The alkaloids of the fraction pH 12(**A**) (0.3 g) were chromatographed on a column with SiO_2 (40/100) using benzene—MeOH mixtures with MeOH concentrations increasing from 1 to 20% (v/v) and then MeOH for elution. The fractions eluted with MeOH were rechromatographed on SiO_2 (40/100) using $CHCl_3$—MeOH mixtures (1—20% v/v) as eluents to give 0.16 g of *N,N′*-di(methoxycarbonyl)-3,4-diaminotoluene, m.p. 168—171 °C. MS, m/z (I_{rel} (%)): 238 $[M]^+$ (32), 206 (28.5), 147 (100). IR, ν/cm^{-1}: 1690, 1712 (NHCOO). ^{1}H NMR (CD_3OD), δ: 2.17 (s, 3 H, C(1)Me); 3.70, 3.72 (both s, each 3 H, 2 OMe); 4.84 (s, 2 H, 2 NH); 7.06—7.51 (m, 3 H, Ar). ^{13}C NMR (CD_3OD), δ: 17.3 (q, C(1)—<u>Me</u>); 52.5, 52.8 (both q, 2 OMe); 116.4 (d, C(2)); 117.1 (d, C(5)); 131.6 (d, C(6)); 137.5, 138.0, 138.5 (all s, C(1), C(3), C(4)); 156.6 (s, C(3)NH<u>C</u>=O); 157.5 (s, C(4)NH<u>C</u>=O).

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