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ALKALOIDS OF *Aconitum septentrionale* SEEDS

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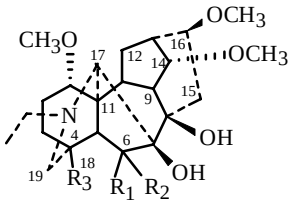
The alkaloids atisine, lappaconitine, and leucostine were isolated from Aconitum septentrionale *L. seeds. The last was examined by ^{1}H and ^{13}C NMR.*

Key words: *Aconitum septentrionale*, leucostine, ^{1}H and ^{13}C NMR spectra.

Seeds of *Aconitum septentrionale* L. yield the known alkaloids atisine and lappaconitine in addition to an amorphous base of composition $C_{23}H_{35}O_6N$ (**1**). The ^{13}C NMR spectrum of **1** contains 23 signals: 10 singlets and triplets, 13 doublets and quartets. According to the ^{1}H NMR spectrum **1** contains an N–Et group and three methoxyls.

The mass spectrum of **1** is typical of C_{18}- and C_{19}-diterpene alkaloids [1]. The base peak occurs at *m*/*z* 390 $[M - 31]^+$. The ^{1}H NMR has a 1H triplet at 3.68 ppm with SSCC 4.7 Hz (H-14$_\beta$). These data are consistent with methoxyls on C-1 [1] and C-14 [2]. The ^{13}C NMR spectrum has a singlet at 220.0 ppm that corresponds to a carbonyl that is situated in a 5-membered ring on C-6 according to the IR spectrum (1744 cm^{-1}). This is confirmed by the appearance of the signal for C-5 at 57.3 ppm [2]. The ^{13}C NMR spectrum also contains singlets at 85.7 and 75.3 ppm and a triplet at 34.8 ppm that are characteristic of C-7 and C-8 in a diol system and a C-15 methylene C atom [2]. A doublet at 81.8 ppm and a quartet at 56.4 ppm place the remaining methoxyl on C-16.

The above data suggest that **1** is a C_{18}-diterpene alkaloid without an oxygen substituent on C-4.



1 - 3

1. $R_1 + R_2 = O$, $R_3 = H$
2. $R_1 = OH\beta$, $R_2 = H\alpha$, $R_3 = H$
3. $R_1 + R_2 = O$, $R_3 = CH_2OCH_3$

A comparison of the ^{13}C NMR spectrum of **1** with those of umbrophine (**2**) [3] and dehydroacosanine (**3**) [4] (Table 1) indicates that the chemical shifts of C atoms in rings *A*, *C*, and *D* are similar in **1** and **2**. For **1** and **3**, chemical shifts are similar in rings *B*, *C*, and *D*.

The signals of C-10 and C-13 for **2** and **3** are switched according to the literature [5]. The switch is confirmed by $^{1}H—^{13}C$ COSY and $^{1}H—^{1}H$ COSY spectra of **1**. Thus, C-13 with a chemical shift of 37.5 ppm is correlated with the proton with a chemical shift of 2.4 ppm in the $^{1}H—^{13}C$ COSY spectrum (Fig. 1). The proton has a cross-peak with the proton on C-14 in the $^{1}H—^{1}H$ COSY spectrum. The C-14 proton has the lowest field triplet (J = 4.7 Hz) in the ^{1}H NMR spectrum. The protons on C-2, C-3, C-12, and C-15 are geometrically stereotopic. Proton H-15$_\beta$ appears as a doublet of doublets at 1.67 ppm with a geminal constant $J_{H\alpha—H\beta}$ = 14.3 Hz and $J_{15—16}$ = 5.4 Hz. The C-1 proton couples with H-2$_\alpha$ and H-2$_\beta$ with J = 9.2 and 6.2 Hz, respectively.

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TABLE 1. Chemical Shifts of C Atoms in ^{13}C NMR Spectra of **1**, Umbrophine (**2**), and Dehydroacosanine (**3**)

Atom	**1**	**2**	**3**
C-1	84.2	86.1	83.4
C-2	26.0	26.3	26.2
C-3	29.4	30.8	32.6
C-4	35.0	36.4	39.0
C-5	57.3	46.2	56.1
C-6	220.0	80.3	219.6
C-7	85.7	89.7	84.8
C-8	75.3	76.4	75.5
C-9	45.6	47.5	45.8
C-10	45.9	37.8*	37.7*
C-11	43.8	48.7	43.5
C-12	28.4	29.3	28.3
C-13	37.5	45.9*	45.8*
C-14	83.3	84.8	83.9
C-15	34.8	35.2	34.3
C-16	81.8	82.5	81.9
C-17	63.2	63.2	63.0
C-18	-	-	76.8
C-19	50.8	50.3	52.7
N-CH_2	49.2	49.7	50.7
CH_3	14.1	13.6	15.3
1′	56.1	56.1	56.3
14′	57.8	57.9	57.7
16′	56.4	56.3	57.6
18′	-	-	58.2

*Places should be switched according to the literature [5].

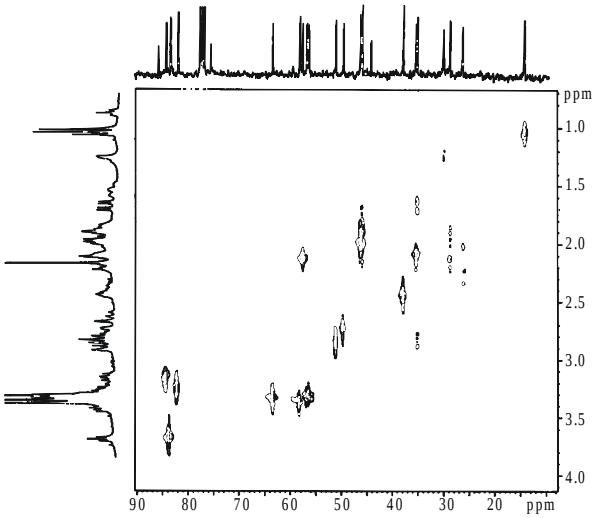

Fig. 1. ^{13}C—^{1}H COSY spectrum of **1**.

The data suggest that the alkaloid has the structure **1**. The same structure has been reported in the literature for leucostine [6]. Considering the unavailability of the original literature and the absence of any reported properties of this alkaloid, we cannot confirm that these alkaloids are identical.

EXPERIMENTAL

IR spectra were recorded on a Specord M80 instrument; NMR, on a Bruker AM-300 in $CDCl_3$ with TMS as an internal standard. Melting points were determined on a Kofler apparatus. For column chromatography we used KSK silica gel (100-160 μm, Chemapol, Czech Rep.) and for chromatographic monitoring, $CHCl_3$—C_6H_6—CH_3OH—NH_3 (4:4:2.5:0.1).

Isolation of Alkaloids. Defatted seeds (5 kg) were treated with acetone—water (1:1) and then methanol. The total alkaloids (13.07 g) were obtained, converted to the sulfate salts, and separated by fractional basicification with Na_2CO_3 and NaOH into two fractions at pH 8 (9.4 g) and 12 (3.67 g). The latter fraction was treated with acetone to give atisine (212.15 mg, mp 297°C) that was identical to an authentic sample. The former fraction was treated with a small quantity of $CHCl_3$ to give a base soluble in $CHCl_3$ (5.9 g). The solid (3.5 g) was chromatographed on a silica-gel column (1:150) with elution by benzene, benzene—$CHCl_3$ (0.5-10% by vol.), $CHCl_3$, $CHCl_3$—CH_3OH (0.5-25%), and CH_3OH. A total of 14 fractions was obtained. Fraction 6 (1% $CHCl_3$—CH_3OH) was treated with acetone to give crystalline lappaconitine (83.5 mg) that was identical to an authentic sample. The $CHCl_3$-soluble bases (5.9 g) were converted to the sulfates, washed with $CHCl_3$, and separated according to base strength by fractional basicification with Na_2CO_3 and NaOH. They were extracted at pH 3 with benzene (yield 21.8 mg); at pH 7, benzene (785.0 mg) and $CHCl_3$ (660.8 mg); at pH 10, $CHCl_3$ (2.9 g). Treatment with acetone of the fraction obtained at pH 7 gave crystals of lappaconitine (204.9 mg). The fraction obtained at pH 10 was chromatographed on a silica-gel column (1:100) with elution by benzene, benzene—methanol (0.5-25% by vol.), and methanol. A total of 10 fractions was collected. Fraction 2 (0.5% benzene—methanol, 128.0 mg) was rechromatographed on silica gel (1:100) with elution by $CHCl_3$ and $CHCl_3$—CH_3OH (1-3% by vol.) to give amorphous **1** (70 mg).

High resolution mass spectrum (*m*/*z*): M 390.2284. Calc. for $C_{22}H_{32}NO_5$ $[M - 31]^+$: 390.2280. Mass spectrum, *m*/*z* (*I*, %): M^+ 421 (13), 406 (12), 403 (28), 391 (32), 390 (100), 376 (14), 362 (33).

^1H NMR spectrum (300 MHz, ppm, J, Hz): 1.03 (3H, t, J = 7.1, N–CH_2–C**H**$_3$), 1.53 (1H, m, H-3$_\alpha$), 1.67 (1H, dd, $J_{H\alpha—H\beta}$ = 14.3, $J_{15—16}$ = 5.4, H-15$_\beta$), 1.8-2.05 (3H, m, H-3$_\beta$, H-9, H-10), 2.07-2.28 (5H, m, H-12, H-5, H-4, H-2$_\beta$), 2.30-2.50 (3H, m, H-2$_\alpha$, 2H-13), 2.55-2.90 (5H, m, 2H-19, N–C**H**$_2$–CH_3, H-15$_\alpha$), 2.85 (2H, br. s, OH-7, OH-8), 3.14 (1H, dd, H-1, $J_{1—2\alpha}$ = 9.2, $J_{1—2\beta}$ = 6.2), 3.20-3.48 (2H, m, H-16, H-17), 3.27, 3.32, 3.37 (3H each, s, OMe), 3.68 (1H, t, H-14, $J_{9—14}$ = 4.7, $J_{13—14}$ = 4.7).

IR spectrum (ν, cm^{-1}): 3488, 2928, 1744, 1448, 1376, 1096, 880.

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