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ALKALOIDS FROM ROOTS OF *Aconitum monticola*

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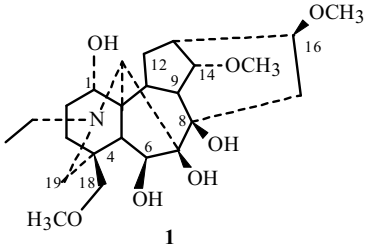
Plants of the genus *Aconitum* L. belong to one of the most valuable alkaloid-bearing plant families, Ranunculaceae. One of the representatives of this genus, *Aconitum monticola* Steinb., forms large thickets of industrial value on slopes of the Altai (136.3 t per 146.5 ha) and also grows in Dzhungar Alatau [1, 2]. The plant is widely used in folk medicine to treat rheumatoid arthritis, neuralgia, deforming osteoarthritis, athetoid spasm, and many other diseases [3]. The alkaloid composition of roots of *A. monticola* collected in Dzhungar Alatau has been studied [4, 5].

Herein we communicate results from a study of the component composition of the aerial part of *A. monticola* collected during fruiting in Katon-Karagai Region of East-Kazakhstan Oblast, Republic of Kazakhstan. Extraction of air-dried ground roots that were treated preliminarily with Na_2CO_3 solution (5%) by EtOH produced an extract from which a crystalline compound with mp 188–189°C (acetone:H_2O) separated upon condensation. According to physicochemical constants and spectral data, the compound was identified as saccharose [6]. The extract was subsequently separated into pure components over a column of Al_2O_3 (act. II) upon elution by benzene, benzene:EtOH (with a gradient of increasing polarity), and EtOH. Evaporation of solvent and recrystallization isolated pure compounds that were identified on the basis of mp, IR, PMR, ^{13}C NMR, and mass spectra and a comparison with literature data as the diterpene alkaloids songorine [7], songoramine [7], and monticamine [5]. Furthermore, the high-molecular-weight alcohols octadecyl and triacontanol were isolated from the benzene effluent.

Elution of the column by benzene:EtOH (9:1) isolated crystalline compound **1**, $C_{24}H_{39}NO_7$, *m/z* 453 (20) $[M]^+$, mp 207–210°C (EtOH), $[\alpha]_D^{20}$ +51.2° (EtOH). The yield was 4.04% of total alkaloids and 0.45% of the air-dried root weight.

The structure of **1** was established using spectral data. The PMR and ^{13}C NMR spectra showed the presence of three methoxyls (δ_H 3.32, 3.33, 3.36 ppm; δ_C 84.90, 82.60, 78.80 ppm; and δ_C 56.10, 57.70, and 59.60 ppm) and an ethyl group on a N atom [δ_H 1.06 (t) and 2.85 (m) ppm]. One of the methoxyls (δ_C 59.60) was situated on C-18 (δ_C 78.80). The other two were on C-14 and C-16 (from NMR spectra recorded in single-resonance mode and 2D ^1H–^{13}C COLOC spectra). According to ^{13}C NMR data (DEPT) and the mass spectrum, 453 (20) $[M]^+$, **1** contained four hydroxyls, one of which was situated on C-1 (δ_C 72.81 ppm) [8]. The others were located on C-6,7,8 [δ_C 80.60 (d), 88.10 (s), 79.20 (s), respectively]. Proton H-6 in the PMR spectrum was located at 4.45 ppm and had $J_{H6H5} \sim 1$ Hz. This was consistent with the α-orientation and the β-orientation of the C-6 hydroxyl. Based on these data, **1** was identified as 6-*O*-demethyldelsoline. It is noteworthy that delsoline was isolated previously from *A. monticola* [9]. Compound **1** was isolated from this plant for the first time. It was also isolated by Chinese researchers from *A. excelsum* [10] and called by them excelsine, which was erroneous because this name was used earlier for an alkaloid with a different structure [11].



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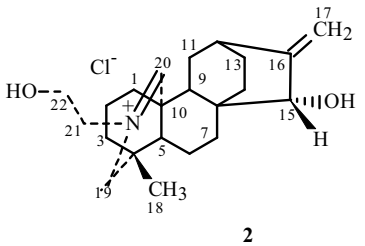

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Elution of the column by EtOH produced **2** as colorless crystals with mp 296–300°C (EtOH), $[\alpha]_D^{20}$ +22.6° (EtOH). PMR and ^{13}C NMR spectral data indicated that the structure included a methyl at δ 1.09 ppm (s) (a quartet at δ 24.79 ppm in the ^{13}C NMR spectrum) and a terminal methylene on C-16. This was characteristic of alkaloids with the atisane skeleton [δ 5.06, 5.11 (s) in the PMR spectrum; δ 110.08 (t) and 154.64 (s) in the JMod ^{13}C NMR spectrum]. Proton H-15 appeared in the PMR spectrum as a singlet at 3.75 ppm. A weak-field shift of C-15 (δ 74.75) indicated that this C atom contained a hydroxyl. A weak-field shift and magnetic non-equivalence of the H-19 protons (δ 3.61 and 3.72) were characteristic. The resonance of C-19 was shifted to weak field (δ 59.72) (compared with the position of the corresponding resonance in spectra of atisane-type alkaloids) [8]. The resonance of C-20 bound to a four-coordinate N atom ($C=N^+$) was observed at δ 183.44 ppm in the ^{13}C NMR spectrum. The IR spectrum of **2** exhibited absorption bands characteristic of hydroxyl stretching vibrations (3376, 3262 cm^{-1}), an N=C bond (1677), and a terminal methylene $C=CH_2$ (1651). An analysis of the IR, PMR, and ^{13}C NMR spectra identified **2** as atisinium chloride, which was isolated from *A. monticola* for the first time. The yield of **2** was 1.71% of the total alkaloids and 0.13% of the air-dried root weight.

An x-ray structure analysis of **2** agreed with the molecular structure of atisinium chloride that was published by Pelletier et al. [12].

The availability and facile isolation of atisinium chloride (**2**) allowed its biological activity to be studied. The cytotoxic activity of **2** was evaluated in a survival test of *Artemia salina* (Leach) brine shrimp larvae. According to the results, the compound exhibited cytotoxic activity. The LD_{50} value was 117.34 μg/mL. The test found antibacterial activity for atisinium chloride against the Gram-positive test strains *Bacillus subtilis* and *Staphylococcus aureus* and moderate antifungal activity against the yeast fungus *Candida albicans*.

Thus, the higher fatty alcohols octadecyl and triacontanol, saccharose, and the diterpene alkaloids atisinium chloride, 6-*O*-demethyldelsoline, songorine, songoramine, and monticamine were isolated and identified from roots of *A. monticola* growing in Kazakhstan. Atisinium chloride and 6-*O*-demethyldelsoline were isolated from this *Aconitum* species for the first time. Antibacterial activity was found for atisinium chloride.

UV spectra were recorded on a Helios-β V-4.60 spectrophotometer in the range 190-500 nm; IR spectra, in KBr pellets on a Nicolet Avatar-320 spectrophotometer. PMR and ^{13}C NMR spectra were recorded on Bruker AC-300 [operating frequency 300.13 (^1H) and 75.47 MHz (^{13}C)] and AV-600 [operating frequency 600.30 (^1H) and 150.96 MHz (^{13}C)] relative to TMS internal standard. A DFS Thermo Scientific high-resolution mass spectrometer with ionizing-electron energy 70 eV (vaporizer temperature 270–300°C) was used to record mass spectra and to determine molecular weights and elemental compositions. HPLC of alkaloids was performed on a Hewlett–Packard Agilent 1100 instrument with a column (4.6 × 150 mm) packed with Sorbax C_{18}-CB phase (5 μm). The mobile phase was CH_3CN. Detection was made at wavelength 224 nm.

Isolation of Alkaloids. Ground subterranean organs of *A. monticola* (442 g) were wetted with Na_2CO_3 solution (5%), dried, and extracted completely with EtOH. Upon standing the extract developed a crystalline powdery precipitate of saccharose. The extraction was performed five times until the reaction with silicotungstic acid was negative. The resulting extracts were combined and evaporated to afford a total thick extract (11.4%). The extract was worked up with H_2SO_4 (2%) and extracted with $CHCl_3$. The acidic solution was cooled, made basic with Na_2CO_3, and extracted with $CHCl_3$. The resulting total alkaloids were chromatographed over Al_2O_3 (act. II) at a 1:60 total:sorbent ratio. Elution of the column by benzene isolated the crystalline compounds octadecyl alcohol and triacontanol and the base songoramine with mp 198–200°C, $C_{22}H_{29}NO_3$, yield 0.028% (of air-dried raw material weight). Its physicochemical characteristics were identical to those published [8]. Elution by benzene:EtOH (9:1) produced several fractions. Fraction 1 produced after recrystallization from EtOH songorine with mp 203–205°C, $C_{22}H_{31}NO_3$, yield 0.032% (of air-dried raw material weight), the properties of which agreed with those in the literature [8].

Fraction II yielded the crystalline compound 6-*O*-demethyldelsoline (**1**), $C_{24}H_{39}NO_7$, mp 207–210°C (EtOH), $[\alpha]_D^{20}$ +51.2° (EtOH). The yield was 4.04% of total alkaloids and 0.45% of the air-dried root weight. Mass spectrum (*m*/*z*, I_{rel}, %): 454 (6), 453 (20) $[M]^+$, 438 (48), 436 (100), 406 (39), 376 (14). Found [M] 453.2731; calc. [M] 453.2744. IR spectrum (KBr, cm^{-1}): 3534 (OH).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.06 (3H, t, J = 7.0, $NCH_2C\underline{H}_3$), 1.41 (1H, m, H-2α), 1.57–1.71 (5H, m, H-5, H-15β, H-12α, H-3β, H-2β), 1.81 (1H, m, H-3α), 1.90–2.11 (2H, m, H-12β, H-9), 2.41 (1H, m, H-10), 2.50 (2H, t, J = 8.0, H-19), 2.80–2.96 (3H, m, H-17, NCH_2), 3.05 (1H, br.s, 6-OH), 3.15 (2H, m, H-13, H-18α), 3.21–3.30 (3H, m, H-16, H-18β, 1-OH), 3.32, 3.33, 3.36 (9H, 3 s, $3CH_3O$), 3.65 (2H, m, H-1, 7-OH), 3.68 (1H, dd, J = 6.4, 8.1, H-14), 3.7 (1H, br.s, 8-OH), 4.5 (1H, br.s, H-6).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 11.36 (N<u>C</u>H$_3$), 27.30 (C-3), 29.0 (C-2), 30.62 (C-12), 36.50 (C-15), 36.80 (C-10), 37.61 (C-4), 43.22 (C-9), 44.0 (C-13), 48.90 (C-11), 49.31 (C-5), 50.40 (NCH_2), 56.10 (16-<u>C</u>H$_3$O), 57.70 (14-<u>C</u>H$_3$O), 59.60 (18-<u>C</u>H$_3$O), 57.81 (C-19), 66.55 (C-17), 72.82 (C-1), 78.80 (C-18), 79.20 (C-8), 80.61 (C-6), 88.05 (C-7), 82.61 (C-16), 84.90 (C-14).

Elution of the column by benzene:EtOH (2%) isolated monticamine with mp 163–166°C, $C_{22}H_{33}NO_5$. Yield 0.05% (of air-dried raw material weight). The physicochemical properties were identical with those published [8].

Elution of the column by EtOH isolated fractions containing the crystalline compound atisinium chloride (**2**), which was very soluble in EtOH and H_2O. R_f 0.15 ($CHCl_3$:EtOH, 9:1), mp 296–300°C (EtOH), $[\alpha]_D^{20}$ +22.6° (EtOH), $C_{22}H_{34}ClNO_2$. IR spectrum (KBr, ν, cm^{-1}): 3376 (OH), 3262 (NH), 2930, 2862, 1677 (C=N), 1651 (C=CH_2), 1450, 1417, 1369, 1340, 1222, 1190, 1067 (C–O), 999, 894.

PMR spectrum ($CDCl_3$ + CD_3OD, δ, ppm, J/Hz): 1.01 (1H, m, H-3β), 1.09 (3H, s, CH_3-18), 1.06–1.12 (1H, m, H-2β), 1.19 (1H, m, H-13α), 1.41 (2H, m, H-5, H-6α), 1.44 (1H, m, H-1α), 1.50–1.61 (3H, m, H-3α, H-7α, H-14α), 1.68-1.80 (5H, m, H-1β, H-2α, H-6β, H-11α, H-13β), 1.82–1.95 (2H, m, H-11β, H-14β), 2.04 (1H, dd, $J_{7\alpha,7\beta}$ = 12.8, $J_{7,6}$ = 4.1, H-7β), 2.17 (1H, m, H-9), 2.48 (1H, m, H-12), 3.61 (2H, dd, J = 7.0, 1.8, H-19β), 3.75 (1H, s, H-15), 3.72 (1H, d, J = 6.8, H-19α), 4.01 (2H, m, H-21), 4.09 (2H, m, H-22), 4.40 (2H, br.s, 15- and 22-OH), 5.06, 5.11 (2H, 2 s, CH_2-17), 8.8 (1H, br.s, H-20).

^{13}C NMR spectrum ($CDCl_3$ + CD_3OD, δ, ppm): 19.09 (C-6), 19.65 (C-2), 24.79 (C-18), 25.44 (C-14), 27.89 (C-13), 30.42 (C-11), 33.54 (C-4), 34.99 (C-7), 35.62 (C-12), 37.54 (C-8), 39.83 (C-9), 41.14 (C-1,3), 45.08 (C-5), 46.46 (C-10), 57.82 (C-21), 59.72 (C-19), 64.03 (C-22), 74.75 (C-15), 110.08 (C-17), 154.64 (C-16), 183.44 (C-20).

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