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ALKALOIDS OF *Aconitum tuberosum*. STRUCTURE OF TUBERACONITINE AND TUBERMESACONITINE

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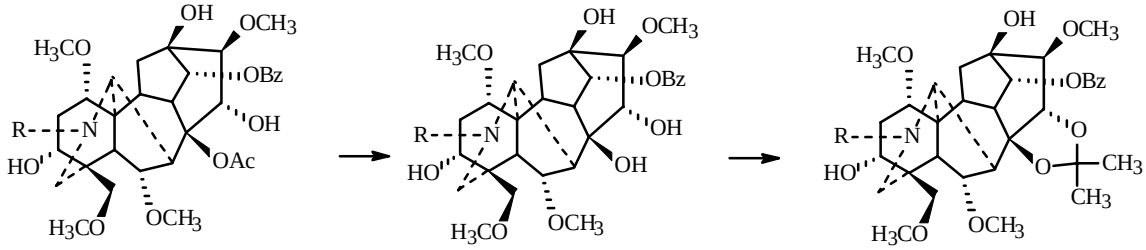
Total alkaloids of Aconitum tuberosum *roots afforded neoline and two new alkaloids, tuberaconitine and tubermesaconitine. The structures of the new alkaloids were proved based on spectral data and chemical transformations from aconitine and mesaconitine.*

Key words: *Aconitum tuberosum*, new diterpenoid alkaloids, tuberaconitine, tubermesaconitine.

Roots of *Aconitum tuberosum* Host. collected in Yugoslavia afforded the known alkaloids mesaconitine, aconitine, flaconitine and the new alkaloid tuberanine [1]. In continuation of the study of alkaloids of this plant, we isolated the known alkaloid neoline and two new alkaloids, tuberaconitine (**1**) and tubermesaconitine (**2**).

Tuberaconitine (**1**) is an amorphous base of composition $C_{35}H_{49}NO_{10}$. Its IR spectrum contains absorption bands of hydroxyl, ester, ether, and aromatic ring. The PMR of **1** exhibits signals for N-ethyl, two tertiary C-methyls, four methoxyls, a monosubstituted aromatic ring, and several methine protons with a geminal O substituent. Spectral data of the alkaloid and aconitine are consistent with similar structures. Thus, a 1H doublet at 3.37 ppm (J = 5 Hz) belongs to H-16α and indicates that a C-16 β-methoxyl is present. A 2H doublet with splitting constant 9 Hz for methylene protons of C-18 geminal to the methoxyl is observed at 3.51 ppm. The signal for H-3β of the geminal hydroxyl is observed at 3.68 ppm (1H, dd, J_1 = 10 Hz, J_2 = 7 Hz). The hydroxyl on C-13 resonates at 3.75 ppm (1H, br.s). Atom C-6 contains an α-methoxyl according to the signal of the proton geminal to it at 3.99 ppm (1H, d, J = 6.5 Hz). Signals of H-15β (4.40 ppm, 1H, dd, J_1 = 5.5 Hz, J_2 = 2.5 Hz) and H-14β (4.82 ppm, 1H, d, J = 5 Hz) geminal to the benzoyloxy are also present. The structures of aconitine (**3**) and tuberaconitine (**1**) are different; **1** contains an acetonide instead of the acetoxyl in **3**. This conclusion was reached by comparing the structural formulas and was confirmed by the presence of protons for two tertiary C-methyls in **1**. The chemical shift and multiplicity of the H-15β signal indicates that the acetonide occupies the 8,15-positions and has the α-orientation in the 15-position.



3: R = C_2H_5
4: R = CH_3

5: R = C_2H_5
6: R = CH_3

1: R = C_2H_5
2: R = CH_3

The mass spectrum of **1** confirms the above data. Strong peaks for $[M - 58]^+$ and $[M - 74]^+$ appear after loss of acetone (**1a**) and acetonide (**1b**) from the 8,15-acetonide (Scheme 1). Further fragmentation is due to loss of methoxyl from C-1 (**1c** and **1d**). A parallel pathway of mass-spectrometric decomposition is loss of methoxyl from C-1 (**1e**) and water from the C-3 hydroxyl (**1f**).

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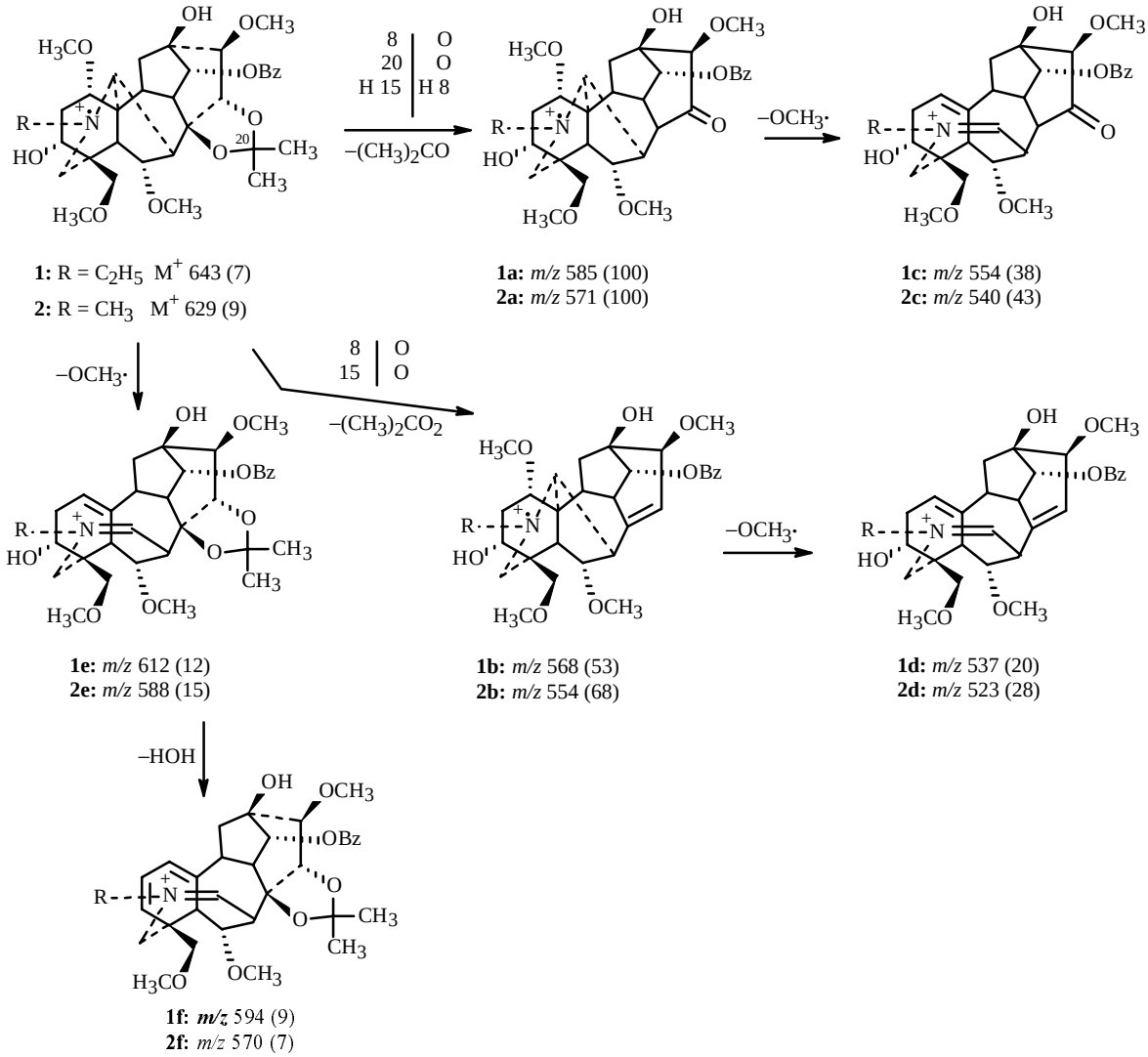

Scheme 1. Principal mass-spectrometric fragmentation pathways of tuberaconitine (**1**) and tubermesaconitine (**2**) (relative peak heights are given in parentheses).

The data indicate that tuberaconitine has structure **1**. We confirmed this by transforming aconitine (**3**) into tuberaconitine. The literature method [2] was used to prepare benzoylaconine (**5**) from **3**.

Benzoylaconine reacts with acetone in the presence of an acid catalyst to give the acetonide, which is identical to **1**.

Tubermesaconitine (**2**) is an amorphous base of composition $C_{34}H_{47}NO_{10}$. The IR spectrum of **2** exhibits absorption bands for hydroxyl, ester, ether, and aromatic ring. According to the PMR spectrum the alkaloid contains two tertiary C-methyls, N-methyl, four methoxyls, and a monosubstituted benzene ring. Comparision of the empirical formulas and functional groups of **2** and **1** shows that the alkaloids differ in the nature of the N substituent. An N-methyl occurs in **2** whereas **1** has N-ethyl. Signals for methine protons in the PMR spectrum of **2** indicate that the remaining substituents in the two alkaloids have identical locations and stereochemistry. The presence of the α-benzoyloxy group on C-14 is confirmed by the signal for the gembenzoyloxy proton H-14β (4.83 ppm, d, J = 5 Hz). The signal for H-15β appears at 4.45 ppm (1H, dd, J_1 = 5.5 Hz, J_2 = 2.5 Hz) and indicates that the acetonide bonded to C-15 has the α-configuration. The signal for the C-13 hydroxy proton appears at 3.87 ppm as a broad singlet. The signal for H-6β, geminal to a methoxyl, is observed at 4.00 ppm (d, J = 6.5 Hz).

The mass-spectrometric fragmentation of **2** (Scheme 1) is consistent with structure **2** and is very analogous to that of **1**.

The proposed structure for **2** was confirmed by correlation with mesaconitine (**4**). Boiling **4** in dioxane—water gave

benzoylmesaconine (**6**), which forms a product with TLC behavior and an IR spectrum identical to those of **2** upon storage in acetone with acid present.

EXPERIMENTAL

PMR spectra were obtained on a Tesla BS-567A (100 MHz) instrument with HMDS (= 0) internal standard ($CDCl_3$ solvent).

Mass spectra were measured in an MX-1310 spectrometer with direct sample introduction into the ion source. IR spectra were recorded on a Perkin—Elmer System 2000 Fourier IR spectrometer in KBr pellets.

Column chromatography used KSK silica gel and deactivated aluminum oxide. The purity of the compounds was monitored on silica-gel plates using $CHCl_3$—CH_3OH (10:1 and 50:1) (1) and ethylacetate—ether (1:1 and 4:3) (2).

Separation of Total Alkaloids. A portion of the total alkaloids from the $CHCl_3$ extract (1 g) [1] was dissolved in H_2SO_4 (5%), washed three times with $CHCl_3$, basicified with $NaHCO_3$, extracted with ether (250 mL), basicified with Na_2CO_3 until basic, and exhaustively extracted with $CHCl_3$. The solvent was evaporated. The mixture of alkaloids (0.43 g) was chromatographed over a silica-gel column. Elution by $CHCl_3$—CH_3OH (100:1) afforded mesaconitine (0.06 g) [3a]; by $CHCl_3$—CH_3OH (40:1), tubermesaconitine (0.04 g) and tuberaconitine (0.05 g); by $CHCl_3$—CH_3OH (25:1), neoline (0.07 g) [3b].

Tuberaconitine (1). IR spectrum (ν, cm^{-1}): 3530, 2980, 2830, 1720, 1615, 1380, 1245, 1200, 1140, 1100, 1035, 980, 845.

PMR spectrum (δ, ppm, J/Hz): 1.05 (3H, t, J = 6, N–CH_2CH_3), 1.20 (3H, s, C–CH_3), 1.36 (3H, s, C–CH_3), 3.12, 3.23, 3.25, 3.70 (3H each, s, 4×OCH_3), 3.37 (1H, d, J = 5, H-16α), 3.51 (2H, d, J = 9, 2H-18), 3.68 (1H, dd, J_1 = 10, J_2 = 7, H-3β), 3.75 (1H, br.s, 13-OH), 3.99 (1H, d, J = 6.5, H-6β), 4.40 (1H, dd, J_1 = 5.5, J_2 = 2.5, H-15β), 4.82 (1H, d, J = 5, H-14β), 7.50 and 7.97 (5H, m, Ar-H).

Correlation of 3 and 1. A solution of **5** (0.07 g) prepared from **3** by the literature method [2] in acetone (10 mL) was treated with $HClO_4$ (0.2 mL) and held at room temperature for 60 h. Acetone was removed. The solid was dissolved in water, basicified with Na_2CO_3, and extracted with $CHCl_3$. The solvent was removed. The solid was purified over a column of deactivated aluminum oxide with elution by a mixture of ethylacetate—methanol (25:1) to give a product (0.03 g) that was identical to tuberaconitine according to TLC and IR spectrum.

Tubermesaconitine (2). IR spectrum (ν, cm^{-1}): 3490, 2930, 2855, 1710, 1590, 1430, 1385, 1310, 1255, 1245, 1130, 1110, 1055, 975, 955, 830.

PMR spectrum (δ, ppm, J/Hz): 1.20 and 1.36 (3H each, s, 2×C–CH_3), 2.30 (3H, s, N–CH_3), 3.11, 3.24, 3.25, 3.67 (3H each, s, 4×OCH_3), 3.55 (2H, d, J = 7, 2H-18), 4.00 (1H, d, J = 6.5, H-6β), 4.45 (1H, dd, J_1 = 5.5, J_2 = 2.5, H-15β), 4.83 (1H, d, J = 5, H-14β), 7.50 and 8.00 (5H, m, Ar-H).

Mass spectral data are shown in Scheme 1.

Correlation of 4 and 2. A solution of **6** (0.1 g) prepared from **4** by the literature method [2] in acetone (15 mL) was treated with $HClO_4$ (0.3 mL) and left at room temperature for 72 h. Solvent was removed. The solid was dissolved in water, basicified with Na_2CO_3, and extracted with $CHCl_3$. The solvent was removed. The solid was purified over a column of deactivated aluminum oxide with elution by a mixture of ethylacetate—methanol (30:1) to give **2** (0.025 g).

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