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ALKALOIDS OF *Aconitum tuberosum*

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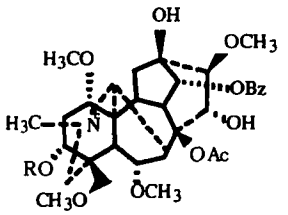
The new alkaloid tuberanine has been isolated from the roots of Aconitum tuberosum *together with the known alkaloids mesaconitine, aconitine, and flaconitine. The structure of tuberanine has been established on the basis of its spectral characteristics and correlations with mesaconitine. It has been shown that the roots of this plant can serve as a source of raw material for obtaining the alkaloid mesaconitine.*

We have investigated the alkaloid composition of the roots of *Aconitum tuberosum* Host. gathered in Yugoslavia and supplied by I. Dolzhanskii, a representative of the French firm Latoxan.

Chloroform extraction of the roots yielded 0.48% of total alkaloids. From this mixture, together with the known mesaconitine [1], aconitine [2], and flaconitine [3], we isolated a new alkaloid, which has been called tuberanine (**1**). The main alkaloid quantitatively proved to be mesaconitine, the yield of which was 0.18% on the weight of the air-dry plant. Mesaconitine belongs to the group of aconitine-like neurocardiotoxins causing cardiac arrhythmia and can be used together with aconitine for creating an experimental model of cardiac arrhythmia [4, 5].

Tuberanine (**1**) has the composition $C_{35}H_{47}NO_{12}$, mp 253—255°C (acetone). In the IR spectrum of the alkaloid there were absorption bands of hydroxy and ester groups, ether bonds, and an aromatic ring. In the PMR spectrum of (**1**) there were the signals of N-methyl, two acetoxy, and four methoxy groups and of the protons of a monosubstituted benzene ring and also the signals of a number of methine protons geminal to oxygen substituents. The mass spectrum of tuberanine contained an intense peak of an ion with *m/z* 642 and the 100% peak of an ion with *m/z* 582. Its composition and spectral characteristics showed similarity to those of mesaconitine (**2**) and indicated that tuberanine was an alkaloid of the aconitine type [1]. A comparison of the empirical formulas and functional compositions of the two alkaloids (**1**) and (**2**) showed that tuberanine contains an additional acetoxy group.



1. R=$COCH_3$
2. R=H

In the PMR spectrum of tuberanine, just as in that of mesaconitine, the signal of H-14β was observed at 4.80 ppm in the form of a doublet with a splitting constant of 5 Hz, which showed the presence of one OH group at C-9 or C-13 of the lycoctonine skeleton [6]. The signal of one of the acetoxy groups in the spectrum of (**1**) was found in an unusually strong field at 1.32 ppm, which permitted it to be placed at C-8 and the benzyloxy group at C-14 [4]. The PMR spectrum of tuberanine also had the H-6β signal in the form of a doublet at 4.00 ppm (J = 6.5 Hz), witnessing the presence of an α-methoxy group at C-6. The signal of the H-15β proton was observed at 4.37 ppm in the form of a doublet of doublets with splitting constants of

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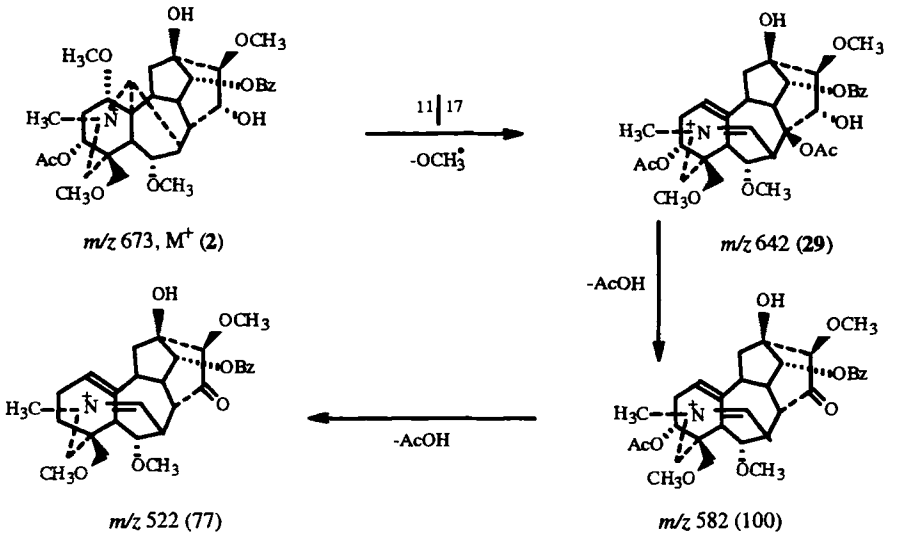
5 and 2.5 Hz, while the signal of the hydroxy group geminal to it was found at 4.26 ppm (d, J = 2.5 Hz). The presence of these signals showed that tuberanine and mesaconitine have the same substituents at C-15 and C-16. The second acetoxy group in tuberanine is located at C-3, as was confirmed by the detection of the H-3β signal in the weak field of the PMR spectrum of the alkaloid (4.83 ppm, dd, J = 10 and 5 Hz) as compared with that of mesaconitine.

Consequently, tuberanine is 3-acetylmesaconitine. This conclusion has been confirmed by its mass spectrum.

The scheme shows the main direction of the mass-spectrometric fragmentation of tuberanine with the participation of the methoxy group at C-1 and of the acetoxy groups at C-8 and C-3.



Scheme of the main route of the mass -spectrometric decomposition of tuberanine (a relative intensities of the ions, %, are given in parentheses).

For a definitive confirmation of the structure of tuberanine, we acetylated mesaconitine (**2**) with acetic anhydride in the presence of pyridine and obtained 3-acetylmesaconitine [5], identical with tuberanine. This is the first time that 3-acetylmesaconitine has been isolated from a plant.

EXPERIMENTAL

IR spectra were taken on a Perkin-Elmer model 2000 Fourier spectrometer in tablets with KBr; PMR spectra on a Tesla BS 567A 100 MHz instrument with HMDS as internal standard (solvent $CDCl_3$); and mass spectra on a MKh 1310 spectrometer with a system for the direct injection of the specimen into the ion source.

For chromatography we used type KSK silica gel and deactivated alumina. The individuality of the substances was checked by TLC using as sorbents silica gel and alumina in the systems: 1) chloroform—methanol (10:1 and 50:1); and 2) ethyl acetate—ether (4:3).

Isolation and Separation of the Total Alkaloids. Air-dry comminuted roots of *Aconitum tuberosum* were wetted with 5% aqueous sodium bicarbonate and were extracted five times with chloroform. The combined chloroform extracts were evaporated in vacuum to volume of 2 liters and were shaken with 5% aqueous sulfuric acid until the alkaloids had been extracted completely. The acid solution was filtered and was washed with chloroform three times. Then it was cooled and was alkalinized with sodium bicarbonate and the alkaloids were exhaustively extracted with chloroform. After the solvent had been distilled off, 0.032 g of washing fraction and 4.8 g of alkaline fraction were obtained.

The alkaline chloroform fraction (4.8 g) was treated with methanol, and 1.8 g of chromatographically homogeneous mesaconitine was separated off. Part of the mother solution (1.2 g) was chromatographed on a column of silica gel (50 g) and eluted by chloroform with the subsequent addition of methanol. Elution with chloroform-methanol (100:1) led to the isolation

of 0.05 g of aconitine, 0.11 g of mesaconitine, 0.06 g of tuberanine, and 0.07 g of flaconitine.

Tuberanine (1). IR spectrum (ν, cm^{-1}): 3520, 2975, 2931, 2878, 2825, 1734, 1710, 1602, 1493, 1451, 1382, 1318, 1281, 1244, 1200, 1153, 1097, 1049, 1027, 986, 959, 895, 840, 770, 728, 717, 653, 615, 570, 528.

PMR spectrum (δ, ppm): 1.32 (3H, s, $OCOCH_3$-8), 1.99 (3H, s, $OCOCH_3$-3), 2.27 (3H, s, N-CH_3), 3.12, 3.12, 3.20, 3.67 (each 3H, s, 4×OCH_3), 3.86 (1H, s, OH-13), 4.02 (3H, d, J=7 Hz, H-6β), 4.26 (1H, d, J=2.5 Hz, OH-15), 4.37 (1H, dd, J=5 and 2.5 Hz, H-15β), 4.80 (1H, d, J=5 Hz, H-14β), 4.83 (1H, dd, J=10 and 5 Hz, H-3β), 7.44 and 7.95 (5H, m, ArH).

Mass spectrum *m/z* (%)^ 673 [M^+ (2)], 658 (2), 642 (29), 613 (20), 600 (18), 582 (100), 555 (54), 522 (77), 508 (9), 494 (14), 492 (12), 490 (7), 105 (53).

Acetylation of Mesaconitine. To 0.07 g of mesaconitine were added 2 ml of acetic anhydride and 3 drops of pyridine, and the mixture was kept at room temperature for 10 h. Then the excess of acetic anhydride and the pyridine were evaporated off, the residue was dissolved in 5 ml of water, and this solution was made alkaline with sodium bicarbonate and extracted with ether. After evaporation and elimination of the solvent with the help of acetone, 0.04 g of 3-acetylmesaconitine was isolated, and this was shown to be identical with tuberanine by comparison of their TLC characteristics and their IR spectra and by a mixed melting point.

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