ALKALOIDS OF THE CULTIVATED SPECIES Aconitum tauricum

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Mesaconitine, hypaconitine, and the new alkaloid taurenine have been isolated from the roots of the cultivated species Aconitum tauricum, and the structure of taurenine has been established.

Over a long period, investigations have been pursued in the Polar-Alpine Botanical Garden (PABS) on the introduction of various species of the genus Aconitum into the subarctic. Specimens in the form of seeds and live plants have been brought in from various ecologo-geographic zones [1].

The alkaloid compositions of many Aconitum species growing in nature and, all the more, under cultivation have not been studied. It therefore seemed desirable to include within the circle of investigations all the available species of plants containing diterpene alkaloids, which, as is known, possess a broad spectrum of physiological activity.

One of the objects of investigation was the cultivated plant Aconitum tauricum, belonging to the section Napellus DC. – an alpine meadow European species that is widely distributed, particularly in the eastern alps. Its main growth sites in nature are high mountain pastures and sections with thin stony soils in the subalpine, and more rarely, the alpine zone at a height of 700-2000 m above sea level.

A. tauricum has been cultivated in the PABS nurseries and tree plantations of populated areas of Murmansk province since 1970. As the starting material we used seeds gathered in the natural growth sites (Austrian alps) and received from the Wolkenstein Botanical Garden. PABS is located in the central part of the Khibini Mountains, typical features of the climate of which are largely similar to those of the alpine zone of high mountain regions.

With a short vegetation season the plants do not succeed in entering the fruit-ripening phase, and their propagation is achieved by the vegetative method. When climatic conditions are favorable, however, the productivity of individual plants increases considerably: a voluminous phytomass (through the development of lateral shoots) and numerous second-order peduncles, and also a more powerful root system, are formed, and all this taken together distinguishes cultivated specimens from plants growing in nature.

We have studied the alkaloid composition of A. tauricum roots gathered in the period of incipient withering of the epigeal part (1989). The total amount of alkaloids in them was 0.77% on the weight of the air-dry raw materials.

By column chromatography and rechromatography and on the basis of salt solubilities, from the total alkaloids we isolated the known bases hypaconitine and mesaconitine and a new alkaloid with the composition $C_{26}H_{41}NO_8$, mp 100-102°C, which we have called taurenine (I).

The IR spectrum of (I) showed absorption bands of hydroxy groups at 3490, 3410, and 3325 cm^{-1} and of an ester carbonyl at 1720 cm^{-1}.

The PMR spectrum of taurenine contained the signals of the following protons: of the methyl of a N-ethyl group at 1.08 ppm (3H, t, J = 7.5 Hz); of an acetyl group at 2.00 ppm (3H, s); and of three methoxy groups at 3.21, 3.26, and 3.41 ppm (singlets, 3H each); there were also a two-proton signal at 4.01 ppm (2H, m) and two one-proton signals at 4.32 ppm (1H, dd, J_1 = 6 Hz; J_2 = 3 Hz) and 4.47 ppm (1H, d, J = 3 Hz).

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When (I) was subjected to alkaline hydrolysis, an amino alcohol $C_{24}H_{39}NO_7$ (II), mp 205-207°C, was isolated. The mass spectrum of (II) was similar to the mass spectra of diterpene alkaloids of the C-19 series. The maximum peak was that of the $(M - 17)^+$ ion resulting from the splitting out of a hydroxy group from C-1 [2].

The presence in its mass spectrum of the peaks of the ions $(M - 56)^+$ and $(M - 87)^+$ showed that in (II) there was an α-hydroxy group at C-1 and a methoxy group at C-18 [3].

The PMR spectrum of (II) contained signals from the methyl of a N-ethyl group at 1.13 ppm (3H, t, J = 7 Hz) and from three methoxy groups at 3.34, 3.35, and 3.45 ppm (singlets, 3H each), and also from geminal protons: at 4.11 ppm (1H, t, J = 4.4 Hz, C-14–β-H), 4.13 ppm (1H, d, J = 6.6 Hz, C-6–β-H), and 4.41 ppm (1H, d, J = 6.4 Hz, C-15–β-H).

Below are given the chemical shifts of the carbon atoms in the ^{13}C NMR spectra of taurenine (I), the amino alcohol (II), 15-α-hydroxyneoline (III), nagarine (IV), and delphidine (V):

C	I	II	III	IV	V
1	72,3	72,2	72,1	72,2	72,0
2	29,7	29,6	29,3	29,5	29,5
3	29,7	30,7	29,9	29,8	29,9
4	38,2	38,2	38,1	38,1	38,2
5	43,8	44,2	44,1	44,2	46,1
6	84,7	84,1	84,1	83,4	84,1
7	47,0	49,5	49,5	52,9	48,2
8	92,2	79,4	79,1	74,5	85,4
9	43,6	46,7	46,7	48,4	44,0
10	41,5	40,8	40,7	42,1	40,8
11	49,4	48,7	49,3	49,6	49,9
12	29,7	30,2	29,9	30,6	29,5
13	43,6	43,8	43,6	44,2	44,0
14	75,1	76,0	75,6	74,8	75,0
15	76,0	79,1	78,6	68,1	38,4
16	89,0	90,4	90,5	83,9	82,4
17	62,5	62,7	62,6	62,1	63,0
18	80,0	80,3	80,0	80,2	79,3
19	56,5	56,8	56,9	56,9	56,8
N–CH_2	48,7	48,6	48,5	48,0	48,4
CH_3	13,2	13,2	13,0	13,0	13,0
C(6)′	58,3	58,1	58,5	57,9	58,1
C(16)′	58,3	57,6	57,5	58,1	56,6
C(18)′	59,2	59,2	59,1	59,2	59,1
C=O	172,8	—	—	—	169,9
CH_3	22,6	—	—	—	22,5

By comparing the composition, physical constants, and spectral properties of (II) with those of the known bases it was established that the amino alcohol (II) was identical to the alkaloid 15-α-hydroxyneoline (III) [4].

It remained to determine the position of the acetoxy group in taurenine, since in (III) there are four hydroxy groups, at C-1, C-8, C-14, and C-15. There were no signals above 4.7 ppm in the PMR spectrum of (I), which showed the absence of acetoxy groups at C-1, C-14, or C-15 [5].

The ^{13}C NMR spectrum of taurenine contained 23 signals: four singlets, 11 doublets, four triplets, and four quartets. The assignment of the signals was made on the basis of the ^{13}C NMR spectra of (I) and (II) obtained under conditions of complete and incomplete decoupling of carbon–proton interactions and a comparison of the results obtained with the ^{13}C NMR spectra of the diterpene alkaloids 15-α-hydroxyneoline (III) [4], nagarine (IV) [6], and delphidine (V) [7].

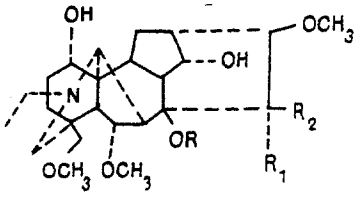
The chemical shifts in (I) and (V) differed considerably for the C-8, C-15, and C-16 carbon atoms.

In delphidine, just as in the structurally close alkaloids delstaphisine and delstaphisagrine, the C-8 quaternary carbon atom, bearing an acetoxy group, experiences resonance at 85 ppm. If, however, there is a hydroxy group at C-15 as, for example, in aconitine and mesaconitine [7], a C-8 quaternary carbon atom with an acetoxy substituent resonates at 92 ppm (β-effect of the C-15–α-OH).

The presence in the ^{13}C NMR spectrum of (I) of a signal in the form of a singlet at 92.2 ppm permits the acetoxy group in taurenine to be placed at C-8. In (I) the C-16 carbon

atom also experiences a characteristic downfield shift from the C-15-α-OH group [4]. The molecular ion of (I) (495) has a low intensity (0.9%), while an intense peak with m/z 435 is due to the presence of the acetoxy group at C-8 [8].

On the basis of the above facts, taurenine has the structure (I).



I. R=Ac, R_1=OH, R_2=H
II, III. R=R_2=H, R_1=OH
IV. R=R_1=H, R_2=OH
V. R=Ac, R_1=R_2=H

The epigeal part of A. tauricum gathered in the phase before the budding of the plant (1989) contained a total of 0.38% of alkaloids, the main one, quantitatively, being hypaconitine (0.2%).

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer; mass spectra on a MKh-1310 mass spectrometer fitted with a system for direct introduction into the ion source; and the PMR spectrum of (I) on a Tesla BS-567 A instrument and that of (II) on a Bruker AM 300 in deuterochloroform with HMDS as internal standard (values given on the δ scale). The ^{13}C NMR spectrum of (I) was taken on a Tesla BS-567 A spectrometer, and that of (II) on a Bruker AM 300 in deuterochloroform. The chemical shifts are given relative to the internal standard TMS.

For TLC we used KSKG silica gel (0-80 mesh) and alumina "for chromatography" (0-80 mesh). Solvent systems: chloroform–methanol (20:1); and chloroform–benzene–methanol (15:5:0.5).

For column chromatography we used deactivated alumina "for chromatography." Melting points are uncorrected.

Extraction of A. tauricum. The roots of the plant (0.66 kg) were moistened with 5% aqueous sodium carbonate solution and were extracted with chloroform at room temperature (6 times). The usual method of working up the extract gave an ether fraction (4.24 g, fraction A) and a chloroform fraction (0.89 g, fraction B), the total yield of alkaloids being 5.13 g.

Mesaconitine and Hypaconitine. Fraction A was treated with acetone, and a crystalline mixture of two bases was separated off (2.14 g, fraction C). The acetone mother liquor contained 1.94 g of bases (fraction D). Fraction C was recrystallized twice from acetone. This gave a crystalline fraction (0.74 g, fraction E) and a mother liquor (1.34 g, fraction F).

Fractions E and F, separately, were dissolved in 10% aqueous hydrochloric acid, and the solutions were extracted with ether–benzene (1:1, 3 × 80 ml) and with benzene (2 × 80 ml). The acid solution was then made alkaline with sodium carbonate and was extracted with ether.

The extracts obtained from the alkaline solutions contained mesaconitine, while the acid extracts, after appropriate treatment, yielded hypaconitine.

Mesaconitine, mp 207-208°C (M^+ 631). It was identified by a mixed melting point with an authentic sample, by TLC, and by a comparison of IR spectra.

Hypaconitine, mp 189-190°C (M^+ 615), yield 0.84 g; mp of hypaconitine hydrobromide 177-178°C. It was identified by comparing its ^{13}C NMR spectrum with that given in the literature.

Taurenine. a) Fraction D was chromatographed on a column of alumina (1:75) with elution by benzene and by benzene–methanol (0.5-10%).

The 2% benzene–methanol fractions (0.79 g) were rechromatographed on a column of alumina (1:100) with elution by the same solvents, and fractions were obtained that contained a base (0.56 g) having R_f values different from those of mesaconitine and hypaconitine, which were present as impurities.

b) Fraction B (0.89 g) was chromatographed on a column of alumina (1:75) with elution by benzene and benzene–methanol (0.5-10% of methanol), and another 0.31 g of the same base was obtained.

The combined fractions (0.87 g) were dissolved in 10% aqueous hydrochloric acid, and the solution was diluted with water to a total volume of 100 ml and was extracted with chloroform (4 × 75 ml).

The acidic chloroform extract was washed with 2% aqueous sodium carbonate and then with water, and, after it had been dried over anhydrous sodium sulfate, the solvent was distilled off. The residue (0.43 g) was chromatographed on alumina (1:25) with elution by chloroform. This gave 0.21 g of taurenine, mp 100-102°C [petroleum ether–chloroform (1:2)], M^+ 495(0.9%), 480(1.6), 478(4.6), 435(43), 420(100), 418(21.4), 404(18), 390(53.5), 60(8.0).

Amino Alcohol of Taurenine (II). Taurenine (0.05 g) was heated in 10 ml of a 2% aqueous methanolic solution of KOH on the water bath for one hour. After the solvent had been distilled off, the residue was dissolved in water and the solution was extracted with ether (3 × 50 ml). An amino alcohol with mp 205-207°C (0.04 g) was obtained. IR: 3545, 3470, 3320 cm^{-1} (OH). M^+ 453(18.5), 438(32.5), 436(100), 420(9), 397(6.0), 366(12.0%).

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SYNTHESIS OF A NUMBER OF COMBINED ANALOGUES OF SUBSTANCE P AND LITORIN

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With the aim of obtaining new biologically active compounds, we have synthesized nine combined peptides (I)-(IX) consisting of combinations of the C-terminal tripeptide litorin and the hydrophobic central fragments of substance P, and also modified analogues of them. The synthesis of these compounds was achieved by the methods of classical peptide chemistry with the condensation of their N-terminal moieties with the C-terminal tripeptide H-His-Phe-Met-NH_2.

At the present time, when the localization of neuropeptides in the organism has been basically established, the problem remains of elucidating their role in the functioning of the nervous system. An important place among such peptides is occupied by peptides of the tachykinin series – in particular, substance P (SP) and bombesin-like peptides. They possess a broad spectrum of biological action both on the central nervous system and on other vitally important functions of the organism, frequently exerting an effect on identical processes. Attention is attracted by the remarkable coincidence of the distribution in the brain of the bombesin-like and SP-like immunoreactivities [1]. It is known that some syn-

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