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ALKALOIDS OF THE EPIGEAL PART OF Aconitum karakolicum

STRUCTURE OF KARASAMINE AND OF 1-BENZOYLKARASAMINE

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The known alkaloids phenyl-β-naphthylamine, karakoline, neoline, delsosine, monticamine, songorine, napelline acetylnapelline, napelline-n-oxide, and isoboldine and two new alkaloids, which have been called karasamine (I) and 1-benzoylkarasamine (II), have been isolated from the epigeal part of Aconitum karakolicum Rapaics. The structure of the new alkaloids have been shown by the preparation of the mono- and triacetates (IV) and (VI) of karasamine and its carbinolamine derivative (V), and also by the direct passage from karakoline (III) to karasamine (I) and 1-bezoylkarasamine (II) and by a study of their spectral characteristics.

We have investigated the alkaloids of the epigeal part of Aconitum karakolicum Rapaics collected in the valley of the R. Irisu, Kirghiz SSR in the budding period. The total alkaloid content amounted to 1.06% on the weight of the dry plant. Separation of the combined alkaloids yielded phenyl-β-naphthylamine [1] karakoline, neoline, delsoline, monticamine, songorine, apelline, acetylnapelline [2, 3], napelline N-oxide [4], the aporphine alkaloid isoboldine, and two new alkaloids which have been called karasamine (I) and 1-benzoylkarasamine (II) [5].

Karasamine (I) has the composition $C_{23}H_{37}NO_4$ (M^+ 391.2764 HRMS), mp 110-112°C (acetone). Its IR spectrum has absorption bands of hydroxy groups at 3180 and 3590 cm^{-1} and of ether bonds at 1100 cm^{-1}. According to the NMR spectra, the alkaloid contains a N-ethyl, a tertiary C-methyl, and two methoxy groups. Acetylation showed the presence of two hydroxy groups. The spectral characteristics of the alkaloid are close to those of the diterpene alkaloid karakoline (III), and a comparison of developed formulas showed that karasamine differed from karakoline by the presence of a methoxy group in place of a hydroxy group. In the mass spectrum of karasamine, the maximum peak was that of the $M^+ - 17$ ion, which shows the presence of a hydroxy group at C-1 [6]. When (I) was acetylated with acetic anhydride in the presence of pyridine, a monoacetyl derivative (IV) was obtained in the NMR spectrum of which there was a one-proton quartet at 4.82 ppm J_1 = 10 Hz, J_2 = 7 Hz, which is characteristic for a proton geminal to a C-1 acetoxy group [7, 8]. In the mass spectrum of (IV) the maximum peak was that of an ion $M^+ - 59$, which indicated that the acetoxy group was present at C-1 [7].

When (I) was oxidized with potassium permanganate in aqueous acetone, an anhydroepoxy derivative (V) was formed. These results indicate the presence in karasamine of α-oriented hydroxy group at C-1. The acetylation of karasamine with acetic anhydride in the presence of p-toluenesulfonic acid led to the diacetate (VI). The maximum peaks in the mass spectrum of the diacetate were those of the ions $M^+ - 59$ (100%) and $M^+ - 59 - 60$ (93%), arising on the successive ejection of an acetoxy radical from C-1 and of a molecule of acetic acid at the expense of the acetoxy group at C-8 [7].

The facts given permit us to consider that the additional methoxy group in karasamine is located at C-14. To confirm this, we methylated karakoline (III) with methyl iodide in dioxane and obtained the C-14 monomethyl ether of karakoline, which was identical with karasamine according to a mixed melting point with an authentic sample and to the results

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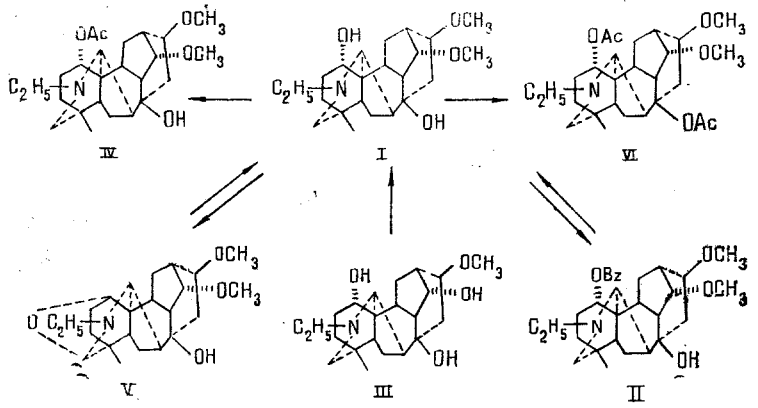
of a comparison of TLC and spectral characteristics. It must be mentioned that of the two secondary hydroxy groups in karakoline that at C-1 is methylated with considerably greater difficulty, which is apparently explained by the existence of a hydrogen bond between the C-1 hydroxy group and the free electron pair at the nitrogen atom. On this basis, the structure of karasamine may be represented by formula (I).

Alkaloid (II), mp 206–208°C (acetone) had the composition $C_{30}H_{41}NO_5$. Its IR spectrum showed absorption bands of an ester carbonyl at 1710 cm^{-1}, of hydroxy groups at 3400 cm^{-1}, and of ether bonds at 1100 cm^{-1}. In the NMR spectrum, the signals of N-ethyl, tertiary C-methyl, and two methoxy groups and of five aromatic protons of a monosubstituted aromatic ring were observed. On alkaline hydrolysis, benzoic acid and an amino alcohol identical with karasamine according to a mixed melting point with an authentic sample and a comparison of spectral characteristics were obtained.



The position of the benzoyloxy group was found on the basis of the following facts. The NMR spectrum (II) contained a one-proton quartet at 5.18 ppm (J_1 = 10 Hz, J_2 = 7 Hz), which is characteristic for a β-C-1 proton geminal to a benzoyloxy group [7, 8] and which disappeared in the spectrum of the amino alcohol. The mass spectrum of the alkaloid likewise confirmed the presence of a benzoyl group at C-1, since the maximum peak in it was that of a $M^+ - 121$ (m/z 374) ion resulting from the ejection of a benzoyloxy radical from C-1 [7]. When karasamine was benzoylated with benzoyl chloride in pyridine, alkaloid (II) was obtained, which confirmed what has been said above; consequently, (II) was 1-O-benzoylkaramine.

EXPERIMENTAL

Melting points are corrected. Mass spectra were recorded on MKh-1310 and MKh-1303 instruments fitted with systems for direct introduction into the ion source; NMR spectra on a JNM-4H-100/100 MHz instrument in deuteromethane and deuterochloroform with HMDS as internal standard (values are given in the δ scale); and IR spectra on a UR-20 spectrophotometer in tablets with KBr. KSK silica gel and alumina (Brockmann activity grade II, neutral) were used for chromatography.

Isolation and Separation of the Combined Alkaloids. The air-dry comminuted epigeal part of Aconitum karakolicum (101 kg) was moistened with 5% sodium carbonate solution and extracted with chloroform. The concentrated chloroform extract was shaken with 5% sulfuric acid until the alkaloids had been extracted completely. The acid solution was washed with chloroform, made alkaline with sodium carbonate, and extracted first with ether and then exhaustively with chloroform. A total of eight extractions was performed, and after the solvents had been distilled off the chloroform used for washing the acid solution yielded 7 g, the ethereal fraction 950 g, and the chloroform extracted fraction 132 g of material.

The material from the washing chloroform fraction (1 g) was chromatographed on a column of alumina, and elution with petroleum ether gave 0.09 g of phenyl-β-methylamine.

The ether-extracted material was treated with acetone, and 115 g of karakoline was separated off. The mother solution was separated according to basicity into 20 fractions. Fractions 1-5 were treated with acetone, and another 4.7 g of karakoline was separated off. The mother solution was chromatograph ed on a column on silica gel. Elution was begun with chloroform, and methanol was gradually added. Chloroform elution yielded 3.7 g of karakoline,

chloroform–methanol (25:1) 2.3 g of neoline, and chloroform–methanol (10:1) 4.2 g of acetylnapelline. Fractions obtained by elution with chloroform–methanol (100:1) were rechromatographed on a column of alumina, and elution with ether–methanol (50:1) yielded 0.27 g of delsoline. The addition of ethanolic hydrogen chloride to fractions 6-12 led to the separation of 136 g of songorine hydrochloride. The residues from the mother solutions were dissolved in water and the solution was washed with ether and then with chloroform, after which it was made alkaline with sodium carbonate and extracted with ether and with chloroform. The washing chloroform fraction was chromatographed on a column of alumina, and elution with ether–methanol (50:1) gave 0.17 g of 1-O-benzoylkarasamine (II). With the aid of acetone, the ethereal fraction yielded 4.7 g of acetylnapelline. The mother liquor, when chromatographed on a column of alumina, gave 0.32 g of karasamine (I), 4.7 g of acetylnapelline, 0.17 g of monticamine, 7.9 g of songorine, and 9.3 g of napelline. The chloroform fraction when chromatographed on a column of silica gel with elution by benzene–methanol (25:1) gave 0.025 g of isoboldine. Fraction 20 from the separation according to base strength, was treated with acetone and gave 4.7 g of napelline N-oxide.

Karasamine (I). $C_{23}H_{37}NO_4$, mp 110-112°C (acetone). IR spectrum, cm^{-1}, 3180 and 3590 (OH group), 1100 (–C–O–C). NMR spectrum, ppm: 0.81 (3 H, singlet); 1.03 (3 H, triplet); 3.22 and 3.30 (3 H each, singlets). Mass spectrum: M^+ 391 (9%); $M^+ - 17$ (100%); m/z 358 (38%).

Karasamine Monoacetate (IV). A solution of 0.09 g of karasamine in 2 ml of acetic anhydride and one drop of pyridine was left at room temperature for 7 h. The excess of acetic anhydride was evaporated off, the residue was dissolved in water, and the solution was made alkaline with sodium carbonate and was extracted with ether. After the solvent had been distilled off, 0.09 g of a homogeneous product was obtained with M^+ 433. NMR spectrum, ppm 0.73 (3 H, singlet); 1.07 (3 H, triplet); 1.96 (3 H, singlet); 3.23 and 3.29 (3 H each, singlets); 4.82 (1 H, quartet, J_1 = 10 Hz, J_2 = 7 Hz). Ir spectrum: 1720 cm^{-1}.

Karasamine Diacetate (VI). A solution of 0.05 g of karasamine in 2 ml of acetic anhydride was treated with 0.015 g of p-toluenesulfonic acid and was heated at 90°C for 2 h. Then the product was worked up in the manner described above, giving 0.04 g of karasamine diacetate with M^+ 475. NMR spectrum, ppm: 0.71 (3 H, singlet); 1.02 (3 H, triplet); 1.93 and 1.97 (3 H each, singlets); 3.24 and 3.29 (3 H each, singlets); 4.83 (1 H, quartet, J_1 = 10 Hz, J_2 = 7 Hz).

Anhydroepoxykarasamine (V). To a solution of 0.15 g of potassium permanganate in 200 ml of 50% mixture of acetone and water was added 0.12 g of karasamine in 5 ml of an 80% mixture of acetone and water, and the resulting mixture was shaken for 10 minutes. The excess of potassium permanganate was decomposed with sodium sulfite, the manganese dioxide was separated off, and the acetone was distilled off from the aqueous mother liquor. The aqueous solution was acidified with sodium carbonate and was extracted first with ether and then with chloroform. The ethereal fraction was purified on a column of alumina. This gave 0.027 g of anhydroepoxykarasamine (V), with mp 137-139°C (ether), $M^+ - 56$ (100%).

Passage from Karakoline to Karasamine,. A solution of 1 g of karakoline (III) in 40 ml of dry dioxane was treated with 0.27 g of sodium hydride and the mixture was stirred for 1 h, after which 3 ml of freshly distilled methyl iodide was added to it and it was stirred for 6 h, being heated to 50°C from time to time. After this, the solid matter was filtered off and the excess of dioxane was evaporated off. The residue was dissolved in 2% sulfuric acid, and the solution was washed with ether, made alkaline with sodium carbonate, and extracted with chloroform. The chloroform extract, after the solvent had been distilled off, was chromatographed on a column of alumina. This gave 0.37 g of 14-O-methylkarakoline, identical with karasamine according to a mixed melting point with an authentic sample and to results of a comparison of spectral characteristics.

1-O-Benzoylkarasamine (II). $C_{30}H_{41}NO_5$, mp 206-208°C (acetone). IR spectrum, cm^{-1}: 3400 (OH group); 1710 (ester carbonyl); 1100 (C–O–C). PMR spectrum, ppm, 0.74 (3 H, singlet); 1.19 (3 H, triplet); 3.21 and 3.34 (3 H each, singlet); 7.38 and 7.95 (5 H, multiplet); 5.18 (1 H, quartet, J_1 = 10 Hz, J_2 = 7 Hz). M^+ 495, m/z 354 (100%).

Alkaline Hydrolysis of 1-O-Benzoylkarasamine, A solution of 0.09 g of 1-O-benzoylkarasamine in 3 ml of a 5% solution of KOH in methanol was boiled for 3 h. The methanol was evaporated off, and the residue was dissolved in 2% sulfuric acid and extracted with ether. Then the acid solution was made alkaline with sodium carbonate and extracted with ether again.

The washing ethereal fraction, after the solvent had been distilled off, yielded 0.007 g of benzoic acid, and the main ethereal extract gave 0.039 g of karasamine.

Benzoylation of Karasamine. A solution of 0.09 g of karasamine in 3 ml of pyridine was treated with 0.05 g of benzoyl chloride and the mixture was kept at room temperature for 4 h. The excess of pyridine was distilled off in a rotary evaporator, the residue was dissolved in water, and the solution was made alkaline with sodium carbonate and was extracted with ether. After the solvent had been distilled off, with the aid of acetone 0.053 g of 1-O-benzoylkarasamine was isolated.

SUMMARY

The alkaloids of the epigeal part of Aconitum karakolicum Rapaics. have been studied, and the known alkaloids phenyl-β-naphthylamine, karakoline, neoline, delsoline, monticamine, songorine, napelline, acetylnapelline, napelline N-oxide, and isoboldine and two new alkaloids – karasamine and 1-O-benzoylkarasamine have been isolated.

On the basis of a spectral characteristics and chemical transformations, structures have been proposed for karasamine and 1-O-benzoylkarasamine.

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ISOLATION AND STUDY OF THE PROPERTIES OF AN INTERFERON-LIKE INHIBITOR OF VIRUSES FROM NORMAL HUMAN BLOOD SERUM

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A protein with a molecular weight of 17,400 daltons and an isoelectric point at pH 4.9 has been isolated from the blood serum of healthy donors by successive ion-exchange chromatography of QAE-Sephadex A-50, affinity chromatography on DNA-cellulose, and polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate. The protein isolated, like interferon, suppresses the development of the cytopathogenic action of the viruses of vesicular stomatitis and murine ecephalomyocarditis in cultures of human cells of the L-41 and M-19 lines. The amino acid composition of the protein isolated differs from those of various fractions of human interferons.

The development of the state of resistance of a higher organism to viral infection is brought about not only by the induction of interferon but also by the presence (or induction) of other nonspecific viral inhibitors [1]. The detection and partial characterization of

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