THE STRUCTURE OF KARAKOLINE

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On studying the tubers of Aconitum karakolicum [1, 2] collected in the Kirghiz SSR (Kungei Alatau range) in the period of the withering of the epigeal part, we isolated songorine [3], napelline, a base with mp 159-160°C, and the new alkaloids karakoline and a base with mp 222-224°C. The total amount of combined alkaloids was 1.23% of the weight of the dry plant.

Karakoline has the composition $C_{22}H_{35}O_4N$ (mol. wt. 377.2546) and contains N-ethyl, tertiary C-methyl, methoxy, and three hydroxy groups. The acetylation of karakoline with acetic anhydride in the presence of pyridine readily gave a diacetate (I, $R_1=R_3=Ac$; $R_2=H$), while the completely acetylated product, the triacetate (I, $R_1=R_2=R_3=Ac$) was formed only when the base was heated with acetyl chloride. This permitted the assumption that in the alkaloid two of the hydroxy groups are secondary and one is tertiary. The mass spectrum of karakoline is characteristic for alkaloids with the lycoctonine skeleton [4]. Chemical reactions also show that this alkaloid belongs to this group.

In the mass spectrum of the base, the peak of the ion M−17 is the maximum peak, showing the presence of a hydroxy group at C_1 [4]. The oxidation of karakoline by potassium permanganate using Marion's method [5] led to a compound containing the grouping of an internal ether of an α-carbinolamine, $C_{22}H_{33}O_4N$ (II). In its mass spectrum, the maximum peak is that of the ion M−56 arising through the loss of a molecule of acrolein, which is characteristic for such compounds [6]. On hydrogenation according to Adams, (II) readily gave the initial base. The formation of the internal α-carbinolamine ether is possible if the hydroxy group at C_1 has the α orientation [5]. When the alkaloid was oxidized with chromium trioxide in acetone, a didehydro derivative, $C_{22}H_{31}O_4N$ (III) was obtained in the IR spectrum of which absorption bands appeared at 1665 cm^{-1} (carbonyl in a six-membered or larger ring) and 1745 cm^{-1} (carbonyl in a five-membered ring). The unusually low position of the absorption band of the six-membered ketone in the IR spectrum of the product becomes normal (1710 and 1750 cm^{-1}) in the IR spectrum of its hydrochloride. This shows that in the oxidation product there is an interaction of the carbon atom of the carbonyl group with the electron pair of the nitrogen which disappears in the salts [7, 8]. These results confirm the presence of a secondary hydroxy group at C_1.

The secondary hydroxy group in the five-membered ring can be located in positions 6, 10, or 12. The presence in the NMR spectrum of the base of a one-proton triplet at 4.16 ppm (J = 4.5 Hz) excludes position 6 for the hydroxy group [8, 9]. The choice between positions 10 and 12 was made by an analysis of the NMR spectrum of acetyldibenzoylkarakoline (I, $R_1=R_3=Br$; $R_2=Ac$). In its NMR spectrum the protons of the acetyl group appear in an unusually high field (1.30 ppm). This phenomenon is observed when a benzoyloxy group is located at C_{10} and an acetoxy group at C_8 and is due to the influence of the anisotropic field of the benzene ring on the protons of the acetoxy group [10]. Thus, the secondary hydroxy group in the five-membered ring must be located at C_{10} and the tertiary group at C_8. What has been said, and also a triplet with a coupling constant of 4.5 Hz from the proton at C_{10}, shows the α orientation of the geminal hydroxy group [8, 9].

The results of an analysis of the mass spectrum of acetyldibenzoylkarakoline enabled the methoxy group to be assigned to C_{15}. In the mass spectrum of this compound there were peaks of the ions M−59 and M−91. The first arises through the splitting out of an acetoxyl radical from C_8. The subsequent ejec-

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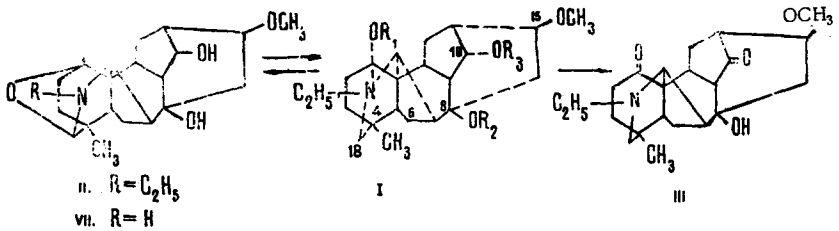
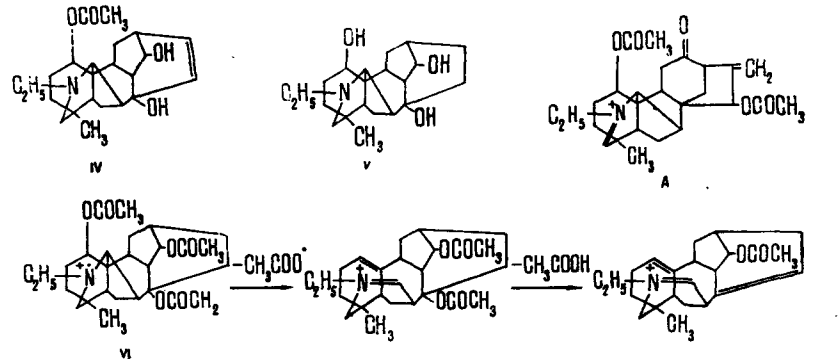
tion of a molecule of methanol leads to the formation of the M−91 ion. Such fragmentation is possible when there is an acetoxy group at C_8 and a methoxy group at C_{15} [11].

Pyrolysis of karakoline triacetate in vacuum with subsequent saponification of the reaction product for 45 min gave a substance with the composition $C_{23}H_{33}O_4N$. Its NMR spectrum had the signal of one acetyl group and also the signals of a N-ethyl group, a tertiary C-methyl group and two olefinic protons, but lacked the signal of a methoxy group. The presence of the signals of two olefinic protons, the nature of which is similar to the corresponding pattern in isopyroacetyltalatisamine [9] shows that the reaction product is an isopyro compound. The absence of a methoxyl from it shows that, in addition to the elimination of acetic acid and an allyl rearrangement, demethylation has taken place with the formation of a new hydroxy group at C_8. The unsaponifiable acetoxy group is located at C_1 according to the following facts. The maximum peak in the mass spectrum of the substance is that of the M−59 ion arising through the ejection of the substituent at C_1 [4]. In addition, its NMR spectrum has a quartet with an intensity of one proton with its center at 4.83 ppm, which can be due only to the proton at C_1 geminal to the acetoxy group [8]. Thus, pyrolysis gave demethylisopyroacetylkarakoline (IV). The following transformations confirm the correctness of structure (IV).

The pyrolysis product was hydrogenated over a platinum catalyst. After saponification for two hours, a crystalline substance (V) was formed which differed from the initial substance by 40 amu and the IR spectrum of which did not contain the absorption band of an ester carbonyl. Acetylation of the latter gave a triacetate (VI), which shows the formation of an additional hydroxy group. The location of this group at C_8 was confirmed by its mass spectrum. In the mass spectrum of (VI) the peaks with the greatest intensity are those of the ions M−59 and M−119 (M−59−60). The detachment of an acetoxy radical from C_1 gives the M−59 ion and the subsequent splitting off of acetic acid at the expense of the acetoxy group at C_8 is responsible for the peak of the M−119 ion [11].



Consequently, on the basis of the results of pyrolysis it can be stated that karakoline belongs to the alkaloids with a lycoctonine skeleton and in it there is a hydroxy group at C_8 and a methoxy group at C_{15}. In the case of the alkaloid delphinine it has been shown strictly that the process of isomerization of "pyro" derivatives into "isopyro" compounds is an allyl rearrangement [12]. The ease of this rearrangement in the pyrolysis of karakoline triacetate shows the β configuration of the methoxy group at C_{15} [13, 14, 8].



It remains to determine the position of the tertiary C-methyl group. The majority of known alkaloids with a lycoctonine skeleton have a methyl, methoxymethylene, or hydroxymethylene substituent at C_4. Consequently, in view of biogenetic considerations, the tertiary C-methyl group may be located at C_4. If this is the case, ring A of anhydrohydroxykarakoline (II) and of the alkaloid songoramine [3] must possess identical structures, while the acetylation of songoramine with acetic anhydride in the presence of pyridine gives a diacetate of the anhydronium salt (A) as a result of the cleavage of the ether bridge [3]; in its NMR spectrum the signals of the C-methyl group and of the N-ethyl group are shifted considerably in the downfield direction ($\Delta\delta$ 0.52 ppm and 0.58 ppm, respectively).

Anhydrohydroxykarakoline was acetylated under similar conditions, but no cleavage of the ether bridge took place. Monoacetylanhydrohydroxykarakoline with the acetyl group at C_{10} was obtained. In view of this, we recorded the NMR spectra of the latter and of songoramine in trifluoroacetic acid, i.e., under conditions in which an anhydronium salt is formed and observed the same pronounced shift of the signals of the groups mentioned ($\Delta\delta$ 0.67 ppm for the C-methyl group and 0.51 ppm for the N-ethyl group) which can obviously serve as a proof of the presence of the methyl group at C_4.

The oxidation of karakoline by Marion's method for 30 min gave N-deethylanhydrohydroxykarakoline (VII). This confirms the presence of a N-ethyl group in karakoline [5]. On the basis of the facts given above, the structure and configuration of karakoline are expressed by formula (I; $R_1=R_2=R_3=H$).

The quartet found in the NMR spectrum of (IV) is also to be seen in all the derivatives of karakoline containing an acetoxy or benzoyloxy group at C_1. This quartet is due to the $-CH_2-CHOR-C$ grouping (ABX system, $J_{AX}=10$ Hz; $J_{BX}=7$ Hz) [8]. The dihedral angles calculated for these constants are 140-145° and 30-35°. Such angles agree well with the distorted chair conformation of ring A. Consequently, in solutions the benzoates and acetates are obviously present in the distorted chair conformation.

The acetyl group in demethylisopyroacetylkarakoline (IV) is difficult to remove by saponification. In order to determine whether this is characteristic for an acetoxy group at C_1 in alkaloids with a lycoctonine skeleton, we saponified karakoline diacetate, talatisidine diacetate, and lappaconidine tetraacetate. The course of saponification was followed chromatographically. It was found that no less than 2 h is necessary for the complete hydrolysis of the acetates mentioned, while with saponification of talatisamine diacetate and lappaconine triacetate, which do not contain acetyl groups at C_1, the initial bases are obtained with quantitative yield in 45 min. In the time during which talatisamine diacetate and lappaconine triacetate are saponified completely, karakoline diacetate gives approximately equal yields of the initial base and of the crystalline monoacetate (I; $R_1=Ac$; $R_2=R_3=H$). The difficulty of saponifying off the acetoxy group at C_1 is apparently due to the steric factor and does not depend on its orientation (karakoline and talatisidine).

EXPERIMENTAL

The melting points are uncorrected. The homogeneity of the substances was established by chromatography in a thin layer of type ShSK silica gel in the benzene–methanol (4:1) system. The mass spectra were taken on an MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source, and the NMR spectra on a JNM-4H-100/100 MHz instrument in deuterochloroform with HMDS as internal standard, the signal of which was taken as zero (figures given in the δ scale).

Separation of the Combined Alkaloids. The air-dry tubers of Aconitum karakolicum (45 kg) were moistened with 5% sodium carbonate solution and extracted with chloroform (eight decantations). The combined chloroform extracts were shaken with 5% sulfuric acid solution. The acid solution was washed, made alkaline with sodium carbonate, with cooling, and exhaustively extracted with chloroform. This gave 553 g of combined alkaloids from which, on treatment with acetone, 319 g of crystals was obtained. Part of the crystalline fraction (100 g) was separated into eleven fractions of differing basicity. From fractions 1-4 by treatment with acetone, 38.3 g of karakoline was obtained with mp 179-181°C. After recrystallization from acetone, mp 183-184°C, $[\alpha]_D^{17}-10.0°$ (c 0.3; CH_3OH).

NMR spectrum, ppm: 0.84 ($\rangle C-CH_3$, singlet) 1.07 ($N-CH_2-CH_3$, triplet), 3.29 (OCH_3, singlet), 4.16 (1H, triplet). IR spectrum, cm^{-1}: 3550, 3000-3400 (OH group).

Fractions 7 and 8 were dissolved in 40 ml of methanol and the solution was made weakly acidic with an ethanolic solution of hydrogen chloride. The crystalline hydrochloride that deposited (9.8 g), after two recrystallizations from ethanol, had mp 257-259°C. The base obtained from the hydrochloride was identified as songorine. The mother liquor from the hydrochloride, after drying, was treated with water and the hydrochloride that deposited was separated off (0.22 g), mp 274-286°C (decomp.). The melting point of the base from the hydrochloride (0.18 g) was 159-160°C (acetone). Fraction 10 was treated with acetone, giving 0.19 g of crystals. The crystals were recrystallized twice from acetone giving 0.1 g of a base with mp 222-224°C.

The mother liquors from fractions 9-10 were combined and separated into 12 fractions of differing basicity. Fractions 8-9 were treated with acetone, which precipitated 0.12 g of a base with mp 163-165°C identified by its IR spectrum and by a mixed melting point as napelline.

Karakoline Diacetate. A solution of 0.1 g of karakoline in 3 ml of acetic anhydride and 0.5 ml of pyridine was left at room temperature for 4 days and, after the usual working up, it gave a product with mp 119-122°C (from hexane). Yield 0.07 g, mol. wt. 461. NMR spectrum: 1.97 ppm (6H, singlet).

Karakoline Triacetate. A mixture of 0.6 g of karakoline and 10 ml of acetyl chloride was kept in a sealed tube at 40-43°C for 56 h. This gave 0.54 g of a product with mp 165-169°C (from acetone), mol. wt. 503. NMR spectrum, ppm: 1.97 (6H, singlet) and 1.87 (3H, singlet).

Anhydrohydroxykarakoline (II). A solution of 0.22 g of potassium permanganate in 180 ml of a 50% mixture of acetone and water was added to 0.2 g of karakoline in 10 ml of an 80% mixture of acetone and water. The resulting mixture was shaken for 5 min and the excess of potassium permanganate was decomposed with sodium sulfite. The manganese dioxide was separated off and the acetone was evaporated off from the water bath. The resulting aqueous solution was made alkaline with potassium carbonate and was extracted first with ether and then with chloroform. The solvent was distilled off from the ethereal extract and the residue crystallized; mp 165-195°C (from acetone). Yield 0.07 g, mol. wt. 375.

The chloroform extract gave 0.085 g of the initial karakoline.

Didehydrokarakoline (III). A mixture of 0.2 g of karakoline, 30 ml of acetone, 0.3 g of chromium trioxide, and 10 ml of acetone was left at room temperature for 72 h. The acetone was evaporated off, the residue was dissolved in 2% sulfuric acid, and the excess of chromium trioxide was decomposed with sodium sulfite. The solution was made alkaline with sodium carbonate, and the precipitate that deposited was filtered off and washed with water. The filtrate was extracted with chloroform and the oily product remaining after the distillation of the solvent was separated into six fractions of different basicity. By treatment with ether, fractions 2-3 gave 0.025 g of a product with mp 179-181°C, mol. wt. 373. IR spectum, cm^{-1}: 1665, 1745.

Dibenzoylkarakoline. To 0.2 g of karakoline in 3 ml of dry pyridine was added 1 ml of benzoyl chloride and the mixture was left at room temperature for 48 h. The solvent was distilled off in vacuum, the residue was dissolved in water, and the solution was made alkaline with sodium carbonate and was extracted with chloroform. The extract was distilled to give, after drying, an amorphous homogeneous product. IR spectrum: 1710 cm^{-1}.

Acetyldibenzoylkarakoline. A mixture of 0.12 g of dibenzoylkarakoline and 3 ml of acetyl chloride was left at room temperature for 12 days. Then the solution was evaporated, and the residue was made alkaline with sodium carbonate and was extracted with ether. The product was purified on a column of alumina. On elution with benzene–ethanol (50:1), fractions 1-4 gave a homogeneous product which, on treatment with hexane, yielded a white powder with mol. wt. 627. NMR spectrum, ppm: 7.97 and 7.41 (10H, multiplet), 1.30 (3H, singlet).

Demethylisopyroacetylkarakoline (IV). Karakoline triacetate (0.3 g) was heated in vacuum at 200-210°C for 12 min. After the mixture had cooled, it was dissolved in 10 ml of a 5% methanolic solution of KOH and boiled under reflux for 45 min. The product obtained after the usual working up was chromatographed on a column of alumina. On elution with benzene–methanol (50:1), fractions 4-11 yielded a crystalline product with mp 145-147°C (acetone). Yield 0.09 g. Mol. wt. 387; NMR spectrum, ppm: 1.91 (3H, singlet), 4.83 (1H, quartet), 5.63 (2H, multiplet).

Dihydrodemethylisopyrokarakoline (V). Demethylisopyroacetylkarakoline (0.07 g) was subjected to Adams hydrogenation in 20 ml of methanol in the presence of five drops of perchloric acid. Then the catalyst was separated off, the reaction product was dissolved in 5 ml of methanolic KOH, and the resulting solution was boiled under reflux for 2 h. After the usual working up, a product (0.03 g) was obtained with mp 172-174°C (from acetone), mol. wt. 347.

Triacetyldihydrodemethylisopyrokarakoline (VI). A mixture of 0.01 g of (V) and 1 ml of acetyl chloride was kept in a sealed tube at 40-45°C for 10 h. The usual working up gave a homogeneous oily product with mol. wt. 473.

Monoacetylanhydrohydroxykarakoline. A solution of 0.04 g of anhydrohydroxykarakoline in 2 ml of acetic anhydride and 0.2 ml of pyridine was left for 6 days, and after the usual working up an oily product was obtained with mol. wt. 417. NMR spectrum, ppm: 2.01 (3H, singlet), 4.80 (1H, triplet).

N-Deethylanhydrohydroxykarakoline. Under the conditions described above for the preparation of compound (II), 0.1 g of karakoline was oxidized for 30 min. A chloroform extract yielded a product with mp 220-222°C (acetone). Yield 0.025 g, mol. wt. 347.

Saponification of Karakoline Diacetate. A mixture of 0.12 g of karakoline diacetate and 5 ml of 5% ethanolic KOH was boiled under reflux for 45 min, giving 0.04 g of karakoline. The mother liquor after the separation of the karakoline was carefully dried, and on treatment with hexane it yielded 0.035 g of karakoline monoacetate with mp 97-99°C. NMR spectrum, ppm: 1.96 (3H, singlet). The new mother liquor was dried, the residue was dissolved in 3 ml of 5% methanolic KOH, and the solution was boiled under reflux, samples being taken for TLC every 30 min. The spot of the monoacetate disappeared after saponification for 2 hours. After this process, 0.02 g of karakoline was isolated with mp 181-183°C.

Saponification of Talatisidine Diacetate. The saponification was performed as in the preceding experiment. After 45 min, according to TLC, there was no talatisidine diacetate (R_f 0.85) left in the reaction mixture; two other spots had appeared with R_f 0.57 and 0.43. The latter was identical with that of talatisidine. The reaction mixture was then sampled every 30 min. The spot with R_f 0.57 had disappeared after 2 h. The reaction product was worked up in the usual way, giving 0.017 g of talatisidine with mp 216-219°C (acetone).

Saponification of Talatisamine Diacetate. A mixture of 0.2 g of talatisamine and 8 ml of 5% methanolic potassium hydroxide was boiled under reflux for 45 min. Then the mixture was worked up in the usual way, giving 0.011 g of talatisamine with mp 135-138°C. According to TLC, the mother liquor contained only talatisamine.

Saponification of Lappaconine Triacetate. A mixture of 0.01 g of the substance and 0.5 ml of 5% methanolic KOH was boiled under reflux for 45 min. After the usual working up, the product showed on TLC a single spot identical with that of karakoline.

Saponification of Lappaconidine Tetraacetate. Saponification was performed as in the preceding experiment. According to GLC, after 45 min the lappaconidine tetraacetate had disappeared, and two spots had appeared with R_f 0.49 and 0.41. The latter was identical with that of lappaconidine. The spot with R_f 0.49 disappeared after hydrolysis for 2 h.

SUMMARY

We have isolated from the tubers of Aconitum karakolicum collected in the Kirghiz SSR, Kungei Alatau range, songorine, napelline, a base with mp 159-160°C, and the new alkaloids karakoline and a base with mp 222-224°C.

The results of a study of the chemical reactions and spectral characteristics of karakoline have shown that it consists of a lycoctonine skeleton with N-ethyl and C_4-methyl groups, hydroxyls at C_1, C_8, and C_{10}, and a methoxy group at C_{15}.

LITERATURE CITED

1. M. N. Sultankhodzhaev, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 399 (1972).
2. M. N. Sultankhodzhaev, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 127 (1973).
3. M. S. Yunusov, Ya. V. Rashkes, S. Yunusov, and A. S. Samatov, Khim. Prirodn. Soedin., 101 (1970).
4. M. S. Yunusov, Ya. V. Rashkes, V. A. Tel'nov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 515 (1969).
5. O. Achmatowicz, Y. Tsuda, and Leo Marion, Can. J. Chem., 43, 2336 (1965).
6. M. S. Yunusov, Ya. V. Rashkes, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 85 (1972).
7. A. D. Kuzovkov, Zh. Obshch. Khim., 31, 1389 (1961).
8. S. W. Pelletier, L. H. Keith, and P. C. Parthasarathy, J. Amer. Chem. Soc., 89, 4146 (1967).
9. M. S. Yunusov and S. Yu. Yunusov, Khim. Prirodn. Soedin., 90 (1970).
10. Y. Tsuda and Leo Marion, Can. J. Chem., 41, 1634 (1963).
11. M. S. Yunusov, Ya. V. Rashkes, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 626 (1971).
12. K. Wiesner, F. Bickelhaupt, and D. Babin, Exper., 15, 93 (1959).
13. L. Fieser and M. Fieser, Organic Chemistry, 3rd Ed., Reinhold, New York (1956).
14. O. Achmatowicz, Y. Tsuda, Leo Marion, T. Okomota, Natsume Mitsutaka, Chang Hong Hsi, and K. Kajima, Can. J. Chem., 4, 43 (1965).