THE STRUCTURE OF KARAKOLIDINE

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UDC 547.944/945

From the tubers of *Aconitum karakolicum* we have isolated a new base which we have called karakolidine (I) [1, 2]. Karakolidine has the composition $C_{22}H_{33}O_5N$ (M^+ 393.2515) and contains N-ethyl, tertiary C-methyl, and methoxy groups. By acetylation we established the presence in the base of four hydroxy groups. Consequently, the developed formula of karakolidine is $C_{18}H_{20}(N{-}C_2H_5)(CH_3)(OCH_3)(OH)_4$. Of the four hydroxy groups, two are secondary, as is confirmed by the formation of a diacetyl derivative (I, $R_1 = R_3 = COCH_3$; $R_2 = R_4 = H$) on acetylation with acetic anhydride at room temperature [3] and by the formation of a didehydro derivative (II) on Kiliani oxidation. The two remaining hydroxy groups are apparently tertiary, since they are acetylated only when the base is heated with acetyl chloride.

The spectral characteristics of karakolidine are close to those of the diterpene alkaloid karakoline, the structure of which has been proved previously [2]. The results of a comparison of the developed formulas of these alkaloids showed that karakolidine differs from karakoline by the presence of an additional hydroxy group. When karakolidine tetraacetate was subjected to pyrolysis with subsequent saponification of the reaction products, two substances were formed. The main one was pyrokarakolidine, $C_{22}H_{33}NO_4$ (III), which, according to its NMR spectrum, contains N-ethyl, tertiary C-methyl, and methoxy groups. The spectrum also shows a signal at 5.37 ppm in the form of a doublet with a splitting constant of 6.5 Hz due to one olefinic proton. In the UV spectrum there is an absorption maximum at 240 nm (log ε 3.69) which disappears on acidification and reappears on neutralization. The isomerization of pyrokarakolidine in methanol in the presence of perchloric acid gave isopyrokarakolidine, $C_{22}H_{33}NO_4$ (IV), with the same functional composition. However, its NMR spectrum has the signal of two olefinic protons in the form of a multiplet at 5.09 ppm. The nature of the splitting of the olefinic protons is similar to the corresponding pattern in the spectra of isopyro compounds of the aconitine series [2, 4]. The UV spectrum of this product lacks the absorption maximum observed in the spectrum of pyrokarakolidine. The course of pyrolysis and the properties of the products formed confirm that karakolidine belongs to alkaloids with a lycoctonine skeleton and shows the presence of a hydroxy group at C_8 and a methoxy group at C_{15} [2, 4, 5]. Pyrolysis led to the elimination of a molecule of acetic acid with the formation of the $\Delta^{8,16}$ double bond. On subsequent saponification, pyrokarakolidine (III) was obtained, and the allyl isomerization of this in an acid medium gave isopyrokarakolidine with $\Delta^{15,16}$ (IV). The presence of a splitting constant of the olefinic proton (6.5 Hz) in pyrokarakolidine showed the β-orientation of the methoxy group at C_{15} [4-7].

The second substance formed on pyrolysis was, according to its chromatographic behavior, identical with isopyrokarakolidine. As mentioned above, the oxidation of karakolidine with Kiliani's solution led to the formation of didehydrokarakolidine $C_{22}H_{31}O_5N$ (II), the IR spectrum of which contained the absorption bands of carbonyl groups in five-membered (1760 cm^{-1}) and six-membered or larger (1690 cm^{-1}) rings. The carbonyl group in the six-membered ring obviously arose as a consequence of the oxidation of the hydroxy group at C_1, since in the mass spectrum of karakolidine the maximum peak is that of the ion M − 17 arising in the splitting off of the substituent from C_1 [8]. This is confirmed by the presence in the NMR spectrum of karakolidine tetraacetate of a one-proton signal in the form of a quadru-

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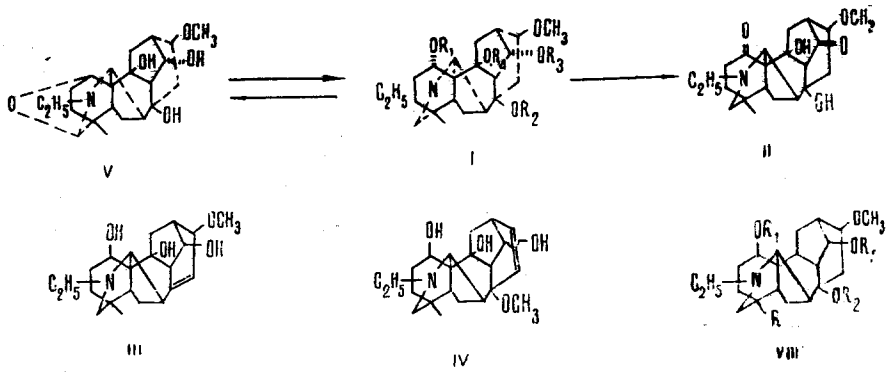
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 481-486, July-August, 1975. Original article submitted May 7, 1974.

plet at 5.31 ppm (J_1 = 10 Hz, J_2 = 7 Hz), which is characteristic for a β-proton geminal to a C_1 acetoxy group [2, 6]. Finally, the presence of a hydroxy group at C_1 and its α-configuration are shown by the formation of anhydrohydroxykarakolidine $C_{22}H_{33}O_5N$ (V) on the oxidation of the alkaloid with potassium permanganate by Marion's method [2, 9, 10] and by the Adams reduction of the compound obtained to the initial compound.

Scheme 1



The absorption band at 1760 cm^{-1} in the IR spectrum of didehydrokarakolidine shows that the remaining secondary hydroxy group is located in the five-membered ring and, consequently, may be present in position 6, 10, or 12. In the NMR spectrum of karakolidine the signal of the proton geminal to the group under consideration appears in the form of a triplet with an intensity of one proton at 4.65 ppm (J = 4.5 Hz) and in the spectrum of karakolidine tetraacetate it undergoes a downfield shift (5.01 ppm). The value of the spin–spin coupling constant excludes positions 6 and 12 for this hydroxy group and coincides with that for a β-proton at C_{10} [2, 4, 6]. The appearance of the signal in the form of a triplet shows the absence of substituents at C_9 and C_{11} in karakolidine. In a comparison of the NMR spectra of karakoline and karakolidine it can be seen that the signal under discussion in the spectrum of karakolidine is displaced downfield by 0.49 ppm in comparison with the same signal in the spectrum of karakoline. It has been shown previously for the case of the alkaloids deltaline (eldeline) and acetyldelpheline that the displacement is caused by the descreening influence of a C_{13} hydroxy group [11].

In view of the lycoctine skeleton of karakolidine and the absence of aminomethyl and secondary amino groups from its molecule, the ethyl group in the base must be attached to nitrogen and the tertiary C-methyl group at C_4. From what has been said above, it follows that karakolidine has structure (I) ($R_1 = R_2 = R_3 = R_4 = H$).

In the mass spectrum of pyrokarakolidine (III), the maximum peak is that of the ion M − 31 (A), instead of the expected peak $M^+ - OH$ arising through the splitting out of a hydroxyl radical from C_1 [8]. In the spectrum of isopyrokarakolidine (IV), however, the ejection of this hydroxy group does lead to the maximum peak. To explain the direction of the mass-spectrometric decomposition of the pyro products, we performed the pyrolysis of karakolidine [2], and after partial hydrolysis we isolated pyrokarakoline (VI), and also monoacetylpyrokarakoline (VII). In the mass spectra of these products, the peaks of the ions $M^+ - 31$ are again the maximum peaks. Consequently, in the pyro products the main direction of fragmentation is connected with the cleavage of the C_7–C_{17} bond and the ejection of the methoxy radical from C_{15}. Such a direction of fragmentation apparently depends on the pronounced strain in the molecule of the pyro product introduced by the $\Delta^{8,16}$ double bond. It must be noted that in the mass spectra of (III) and (VI) the peaks of the ion M − OR_1 (B) do not exceed 2%, while in monoacetylpyrokarakoline (VII) they amount to 85%.

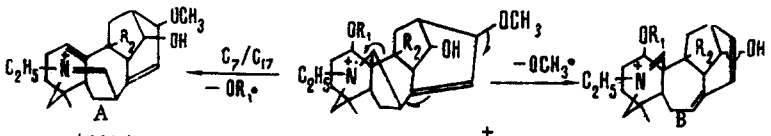
The results of a study of the mass spectra of compounds containing ester groups in positions 1 and 8 (Table 1) showed that their fragmentation under the action of electron impact has some peculiarities. The fragmentation pathways of alkaloids with an acetoxy group at C_8 and a methoxy group at C_1 have been considered previously. It was shown that the intensity of the $M^+ - 60$ peak arising through the splitting out of a molecule of acetic acid depends on the substitution of ring D and on the temperature [12]. It can be seen from Table 1 that when acetoxy groups are present simultaneously and C_1, and also when there is a

TABLE 1

Substance	Relative intensities					
	M^+	$M-OR_1$	$M-OR_1-R_2OH$	M^+-OR_2	M^+-R_2OH	$M-OR_1CH_3OH$ or $M-R_2OH-OCH_3$
Isotalatisidine triacetate VIII ($R_1=R_2=R_3=OCOCH_3$; $R=CH_2OCH_3$)	1,8	100*	20	—	5	4
Karakoline triacetate VIII ($R_1=R_2=R_3=OCOCH_3$; $R=CH_3$)	0,7	100*	11	—	3,4	5
Acetyldibenzoylkarakoline VIII ($R_1=R_3=OCOC_6H_5$; $R_2=OCOCH_3$; $R=CH_3$)	0,3	100	31	11	16	10
Diacetylmonobenzoylkarakoline VIII ($R_1=OCOC_6H_5$; $R_2=R_3=OCOCH_3$; $R=CH_3$)	0,4	100	29	11	10	13

*The peak of the ion $M^+ - OR_1$ is composite, consisting of the peaks of the ions $M^+ - OR_1$ and $M^+ - OR_2$.

Scheme 1



III. (1,9%) — III. $R_1=H$; $R_2=OH$. M^+(73%) — III. (100%)

VI. (1,3%) — VI. $R_1=R_2=H$; M^+(46%) — VI. (100%)

VII. (85%) — VII. $R_1=COCH_3$; $R_2=H$; M^+(57%) — VII. (100%)

benzoyloxy group at C_1, the main direction of fragmentation is connected with the splitting out of the acyloxy radical from C_1 and the subsequent elimination of a molecule of acetic acid with the formation of the $\Delta^{8,16}$ double bond. In the mass spectra of the triacetates of karakoline and isotalatisidine, this direction of fragmentation is practically the only one and does not depend on the temperature, in contrast to the spectra of the compounds studied previously [11]. In the mass spectrum of karakolidine tetraacetate (I, $R_1 = R_2 = R_3 = R_4 = COCH_3$), besides the peaks $M^+ - 59$ (100%) and $M^+ - 59 - 60$ (79%), there is also the peak of the ion $M^+ - 179$ (M − 59 − 60 − 60) (19%), arising on the ejection of a molecule of acetic acid at the expense of the acetoxy group at C_{13}.

The formation of the ions M − 59, M − 60, and M − 91 in the spectra of acetyldibenzoylkarakoline and diacetylmonobenzoylkarakoline takes place by the pathways proposed previously [12]. The features of the fragmentation of compounds with a $\Delta^{8,16}$ double bond described above permit us to consider that the further decomposition of the ion $M^+ - R_2OH$ arising at the expense of the acetoxy group at C_8 can take place in two directions: the first direction is accompanied by the ejection of a methoxyl radical from C_{13}, and the second by the splitting off of the substituent from C_1.

EXPERIMENTAL METHOD

The melting points are uncorrected. The mass spectra were taken on an MKh-1303 instrument fitted with a system for direct introduction into the ion source; the NMR spectra were taken on a JNM-4H-100/100 MHz instrument in deuterochloroform with HMDS as internal standard (the values are given in the δ scale). Chromatography was performed in a thin layer of type KSK silica gel in the benzene–methanol (4: 1) system.

Karakolidine (I), mp 222-224°C (methanol). NMR spectrum, ppm: 0.87 (3H, singlet), 1.07 (3H, triplet), 3.28 (3H, singlet), 4.65 (1H, triplet).

Didehydrokarakolidine (II). A solution of 0.16 g of karakolidine in 2 ml of 2% sulfuric acid and 0.32 g of Kiliani's solution was left at ∿ 20°C for 16 h, and it was then made alkaline with sodium carbonate and the precipitate that deposited was filtered off. From the alkaline solution chloroform extracted an oily product which was separated according to basic

strengths into five fractions. The acetone treatment of fraction 3 yielded 0.02 g of a product with mp 179-181°C, mol. wt. 389.

Karakolidine Tetraacetate. A mixture of 0.2 g of karakolidine and 3 ml of acetyl chloride was kept in a sealed tube at 40-45°C for 80 h. The excess of acetyl chloride was evaporated off. The residue was dissolved in water, the solution was made alkaline with sodium carbonate in the presence of ice, and the reaction product was extracted with ether. This gave a homogenous product in the form of a powder with M^+ 561. NMR spectrum, ppm: 1.92 (3 h, singlet), 1.99 (3H, singlet), 2.01 (3H, singlet), 2.06 (3H, singlet), 5.01 (1H, triplet; J = 4.5 Hz), 5.31 (1H, quartet, J_1 = 10 Hz, J_2 = 7 Hz).

Pyrokarakolidine (III). Karakolidine tetraacetate (0.17 g) was heated in vacuum at 185-195°C for 8 min. The reaction mixture was cooled and was dissolved in 5 ml of 5% methanolic KOH, and the solution was boiled for 45 min. Then the methanol was evaporated off and the residual aqueous solution was made alkaline with sodium carbonate. The reaction product was extracted with ether. The residue obtained after the evaporation of the dried extract gave, on treatment with ether, a powder which was separated off and boiled in a mixture of ether and acetone (20:1). The insoluble material was separated off. The mother solution deposited 0.045 g of a product with mp 174-186°C (decomp), M^+375.

Isopyrokarakolidine (IV). A solution of 0.035 g of pyrokarakolidine in 5 ml of methanol and 0.2 ml of perchloric acid was boiled for 2 h. The methanol was distilled off, the residue was dissolved in 2 ml of water, the solution was made alkaline with sodium carbonate, and the reaction product was extracted with ether. The substance obtained after the elimination of the ether was treated with a mixture of hexane and chloroform (20:1), and 0.011 g of (IV) separated out with mp 119-122°C. The mother liquor showed one spot identical with that of isopyrokarakolidine, mol. wt. 375.

Anhydrohydroxykarakolidine (V). Karakolidine (0.1 g) was oxidized by the method of Achmatowicz et al. [9]. The reaction product was extracted with ether, then with chloroform. The chloroform fraction was separated preparatively in a layer of silica gel in the benzene—methanol (4:1) system. This gave an amorphous substance (V). Yield 0.02 g. Mass spectrum: M^+ 391, $M^+ - 56$ (100%) [10].

The Adams hydrogenation of 9 mg of (V) gave 5 mg of karakolidine.

Pyrolysis of Karakolidine Triacetate. Karakolidine triacetate (0.98 g) was heated in vacuum at 185-195°C for 7 min. The reaction mixture was cooled, dissolved in 15 ml of a 5% solution of KOH in methanol, and the resulting solution was boiled for 2 h. The methanol was evaporated off, water added, and the reaction product was extracted with ether. This yielded 0.13 g of pyrokarakoline, mp 151-153°C [from ether—acetone (5:1)], mol. wt. 359. NMR spectrum, ppm: 0.78 (3H, singlet), 1.03 (3H, triplet), 3.33 (3H, singlet), 5.43 (1H, doublet).

The mixture of substances from the mother solution left after the extraction of the pyrokarakoline was separated preparatively in a layer of silica gel in the benzene—methanol (4:1) system. The resulting product was treated with hexane, and 0.045 g of (VII) separated out in the form of a white powder with mol. wt. 401. NMR spectrum, ppm: 0.74 (3H, singlet), 1.07 (3H, triplet), 1.90 (3H, singlet), 3.30 (3H, singlet), 4.80 (1H, quartet; J_1 = 10 Hz, J_2 = 7 Hz), 5.39 (1H, doublet).

Diacetylmonobenzoylkarakoline. A mixture of 0.75 g of monobenzoylkarakoline [3] and 2 ml of acetyl chloride was kept in a sealed tube at room temperature for 6 days. The excess of acetyl chloride was evaporated off, the residue was dissolved in water, the solution was made alkaline with sodium carbonate in the presence of ice, and the reaction product was extracted with ether. The extract was dried, and the precipitate that deposited (0.03 g) was separated off, M^+ 565.

SUMMARY

As the result of a study of chemical transformations and spectral characteristics, for the new alkaloid karakolidine a lycoctonine skeleton with N-ethyl and C_4-methyl groups, hydroxy groups at C_1 (α), C_8, C_{10} (α), and C_{13}, and a methoxy group at C_{15} (β) has been established.

Some peculiarities of the fragmentation of derivatives of karakoline and karakolidine under the action of electron impact have been found.

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