UDC 547-944

THE STRUCTURE OF BREVICARINE

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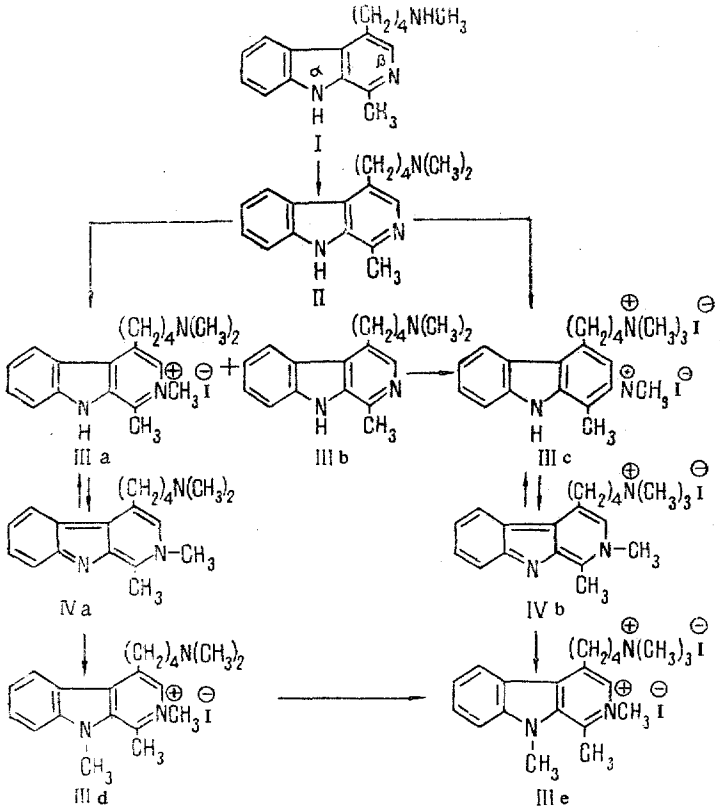
Khimiya Prirodnykh Soedinenii, Vol. 5, No. 5, pp. 397-404, 1969

The alkaloid brevicarine $C_{17}H_{21}N_3$ (I) has been isolated from Carex brevicollis DC, family Cyperaceae as a satellite of the main alkaloid of this plant—brevicolline [1]. At present, the herb C. brevicollis is subjected to industrial treatment for the preparation of brevicolline, which is used as a new medicinal agent [2], and brevicarine accumulates in considerable amounts in the wastes from this production. The wastes are the methanol-insoluble residues obtained at the stage of the crystallization of the brevicolline, which contain brevicarine sulfate together with mineral salts and other impurities. The amount of "residue" is 40% of the technical total of the alkaloids and from H it is possible to obtain about 20% of pure brevicarine.

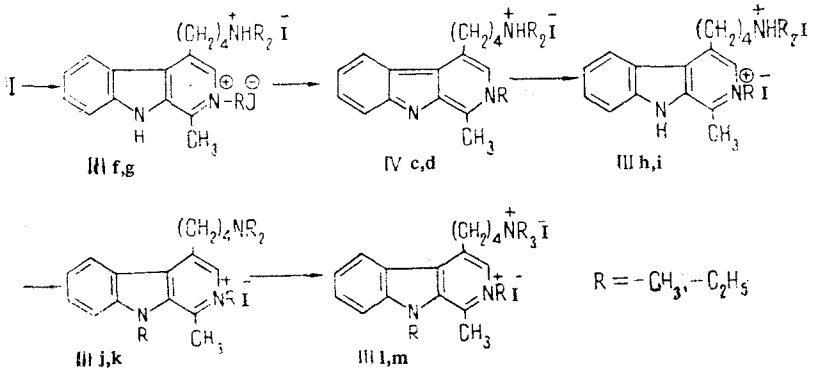
Brevicarine, when crystallized from organic solvents (acetone, aqueous ethanol, or a mixture of acetone and chloroform) forms a stable hydrate with the composition $C_{17}H_{21}H_3 \cdot 1.5\ H_2O$, mp 61° C. The water of crystallization is eliminated by prolonged drying in vacuum.

Brevicarine forms salts with two equivalents of acid, is readily acetylated by acetic anhydride, and gives methylbrevicarine (II) on reaction with formalin and formic acid.

Depending on the reaction conditions and the amount of alkylating agent, brevicarine forms a series of N-alkyl halides (IIIa-IIIm) from which anhydrobases (IVa-IVd) can be obtained. These reactions confirm the hypothesis put forward previously that brevicarine is a β-carboline derivative [3] and is related to brevicolline [4], the methiodides of which behave absolutely in the same manner. The scheme of the methylation of brevicarine (I) is given below.



The alkylation of brevicarine with methyl iodide and ethyl iodide in various sequences and the treatment of the intermediate substances with dilute alkali has given a series of products of different degrees of alkylation, thanks to which it has been possible to establish the sequence of substitution. The reactions were carried out with heating, and therefore even in the first stage the di(alkyl iodide) derivatives IIIf and IIIg were formed. It was possible to perform the decomposition with alkali in stages. In the first stage the anhydrobases (IVc and IVd) were formed, as could be judged from the pronounced yellow coloration of these compounds.

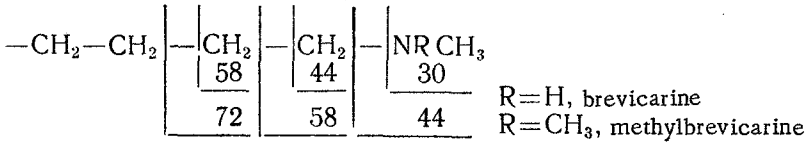


The proposed structure of brevicarine (I) is also confirmed by the results of a study of its mass and NMR spectra* (the UV and IR spectra have been given previously [1]).

The molecular ion in the mass spectrum of brevicarine (Fig. 1) is located at m/e 267, corresponding to its chemical composition. Other ions in the region of high mass numbers are M −15, M −30, M − 44, M − 57, M −58, M − −71, and M − 72. In the region of low mass numbers the peak at m/e 44 has the highest intensity (probably corresponding to $CH_3-\overset{+}{N}H=CH$), and there is a small peak with m/e 29.

In the spectrum of methylbrevicarine, the molecular ion (m/e 295) and, correspondingly, all the ions obtained have masses 14 mass units greater than those mentioned.

This nature of the mass spectra of the compounds studied can be explained by the fragmentation of an unbranched N-alkylamine chain through the successive cleavages of carbon-carbon bonds in analogy with the processes taking place on electron impact in the molecules of aliphatic amines [5].



The remaining part of the molecule is represented by low-intensity peaks, which can be explained by the stability of this aromatic system. Nevertheless, signals with m/e 182, 167, and 149 show fragmentation processes occurring here and, in all probability, they are similar to the transformations of correspondingly substituted pyridine compounds [5a].

All 21 of the hydrogen atoms present in the molecule are reflected in the NMR spectrum of brevicarine (Fig. 2). The two three-proton signals at 2.35 and 2.70 ppm belong, respectively, to $>N-CH_3$ and $=N-CH_3$ groupings

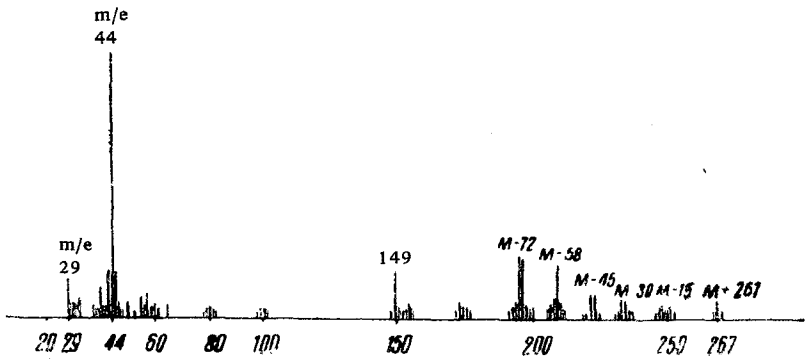


Fig. 1. Mass spectrum of brevicarine.

*The authors thank Dr. Dolejš (Czechoslovakian Academy of Sciences) for recording the mass spectra and Prof. Yu. N. Sheinker for recording and assisting in the interpretation of the NMR spectrum (Moscow, VNIKhFI [All-Union Chemical and Pharmaceutical Scientific Research Institute]).

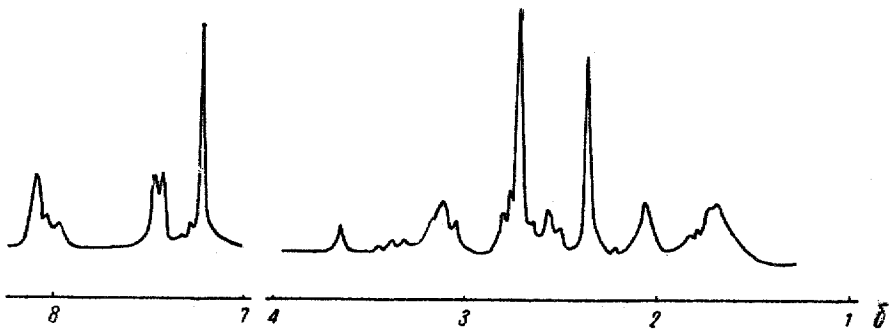


Fig. 2. NMR spectrum of brevicarine.

similar to those in brevicolline [6], the position of the N–CH_3 signal being somewhat shifted probably because in this case this group is present in an aliphatic chain and not in a ring as in the case of brevicolline. In the region of methylene protons there are four two-proton signals at 1.67, 2.05, 2.55, and 3.10 ppm characterizing all the methylene groups of the aliphatic residue. The proton of the methylamine grouping obviously falls in the region of the methylene protons at 1.67 ppm. This can explain the broadening of this signal at the bottom. The signal of the indole NH is shown unsharply at 10.10 ppm. In the region of aromatic protons, the signal at 8.08 ppm is due to the α-hydrogen of the pyridine nucleus; the presence of such a proton necessarily leads to the assumption that the substituents in the pyridine moiety of the molecule occupy the 1, 4 positions. The four protons of the phenyl fragment are characterized by signals in the 7.4–7.5 ppm (three-proton units) and 7.95–8.05 ppm (one proton unit) regions.

Experimental

All the melting points are uncorrected and were determined on a Kofler block. The mass spectrum was recorded on a MKh-1303 mass spectrometer and the NMR spectrum on a JNM-4H-100/100 MHz instrument. Chromatography was carried out in thin layers of nonfixed Al_2O_3 (activity grade 2.5–3) in the chloroform–6% methanol system, and on paper by the ascending method in the butan-1-ol–acetic acid–water (4 : 1 : 5) system.

Isolation of brevicarine*. The insoluble residue obtained at the stage of crystallization of brevicarine from methanol in the experimental factory (100 g) was dissolved in 300 ml of hot water, and the solution was filtered and decolorized with 2–3 g of activated carbon. The filtered solution was cooled with ice and, with vigorous stirring, brought to pH 12 by the gradual addition of 40% caustic soda solution. The brevicarine that separated out in the form of a cream-colored precipitate was filtered off with suction and carefully washed with hot water. The completeness of the precipitation of the brevicarine was checked by the addition of alkali to the wash waters. The air-dried brevicarine was crystallized from an eightfold volume of acetone. By concentrating the mother solution it was possible to isolate the alkaloid completely. Yield 18–20 g; mp 61° C (hydrate), 112° C (anhydrous), R_f 0.311 (in a thin layer of Al_2O_3), 0.32 (on paper) There was no depression of the melting point with the sample obtained previously [1]. Their UV and IR spectra also coincided.

Brevicarine dihydrochloride was obtained by the addition of HCl to an ethanolic solution of brevicarine. $C_{17}H_{21}N_3 \cdot 2HCl$, mp 195–196° C (ethanol).

Brevicarine dinitrate was obtained similarly. Mp 202° C (decomp.) (ethanol).

Found, %: C 51.36; H 5.97; N 17.93. Calculated for $C_{17}H_{21}N_3 \cdot 2HNO_3$, %: C 51.90; H 5.89; N 17.80.

Brevicarine picrate was obtained by mixing ethanolic solutions of the base and picric acid, mp 210–212° C (decomp.).

Acetylbrevicarine was obtained by heating brevicarine with acetic anhydride for 1 hr. The excess of acetic anhydride was evaporated off in vacuum and the residue was recrystallized from acetone. Yield 53%, mp 154° C.

Found, %: C 73.42; H 7.45; N 13.82. Calculated for $C_{19}H_{23}ON_3$, %: C 73.78; H 6.44; N 13.59.

N_γ-Methylbrevicarine (II). A mixture of 1.2 g of brevicarine, 2 ml of formic acid, and 4 ml of formalin was heated in the boiling water bath for 30 min. The reaction mixture was cooled and diluted with 10 ml of water, and the base was precipitated with 12% caustic soda solution. The precipitate was filtered off. Lustrous acicular crystals, mp 74–78° C (from 50% methanol), 128–129° C (from benzene). Yield quantitative.

*A. F. Sholl took part in this section of the work.

Found, %: C 76.86; H 8.33; N 14.77. Calculated for $C_{18}H_{23}N_3$, %: C 76.86; H 8.24; N 14.93.

N_γ-Methylbrevicarine dihydrochloride was obtained by the addition of concentrated HCl to an ethanolic solution of the base. Mp 218–220° C (aqueous ethanol).

Found, %: C 55.31; H 7.88; N 10.32; Cl 18.18. Calculated for $C_{18}H_{23}N_3 \cdot 2HCl \cdot 2H_2O$, %: C 55.24; H 7.67; N 10.70; Cl 18.16.

N_γ-Methylbrevicarine N_β-methiodide (IIIa). A solution of 0.6 g of methylbrevicarine in a mixture of 2 ml of acetone and 1 ml of methanol was treated with 0.2 ml of CH_3I. The yellow precipitate that deposited after 10–15 min was filtered off. Mp 251–252° C (decomp.) (from water), R_f 0.51 (Al_2O_3 of activity grade 3, chloroform + 10% of methanol); R_f 0.39 (on paper).

Found, %: C 51.06; H 6.60; N 9.40; I 29.03. Calculated for $C_{19}H_{26}N_3I \cdot H_2O$, %: C 51.70; H 6.34; N 9.51; I 28.80.

Methylbrevicarine N_γ-methiodide (IIIb). A solution of 0.5 g of methylbrevicarine in 10 ml of acetone was treated with 0.2 ml of CH_3I. The voluminous precipitate that deposited instantaneously was filtered off and washed with acetone. Yield 0.5 g (76.8%), mp 256–258° C, R_f on paper 0.25.

Found, %: N 10.07. Calculated, %: N 9.92.

N_β, N_γ-Dimethylbrevicarine (IVa). A solution of 0.35 g of IIIa in 10 ml of 50% ethanol was treated with a few drops of conc NaOH solution, and the yellow precipitate that deposited was recrystallized from aqueous ethanol. Mp 140–143° C (decomp.).

Found, %: N 14.19. Calculated for $C_{19}H_{25}N_3$, %: N 14.23.

N_α, N_γ-Dimethylbrevicarine N_β-methiodide (IIId). At room temperature, 0.3 ml of CH_3I was added to a solution of 0.6 g of IVa in ethanol. After some time, a yellowish precipitate deposited. Mp 222–224° C (decomp.) (from aqueous ethanol).

Found, %: C 54.82; H 6.65; N 9.60; I 28.32. Calculated for $C_{20}H_{28}N_3I$, %: C 54.92; H 6.40; N 9.61; I 29.00.

N_γ-Methylbrevicarine N_β, N_γ-dimethiodide (IIIc). A solution of 1.5 g of methylbrevicarine in 45 ml of ethanol was treated with 5 ml of CH_3I. The mixture was heated in the water bath under reflux. After 10–15 min, a white precipitate deposited. Mp 280–282° C (decomp.) (from methanol). Yield 2.2 g (~70%).

The same product was obtained by heating a mixture of 0.5 g of brevicarine with an excess of CH_3I in an autoclave at 130° C for 3 hr. Yield 66.3%. Mp 281–282° C (decomp.) (from ethanol).

Found, %: C 42.89; H 5.51, 7.75; I 44.31. Calculated for $C_{20}H_{29}N_3I_2$, %: C 42.47; H 5.13; N 7.43; I 44.97.

N_β, N_γ-Dimethylbrevicarine N_γ-methiodide (IVb). A few drops of 10% NaOH solution was added to a hot solution of 1.5 g of IIIc in 35 ml of aqueous ethanol. The solution became bright yellow. On cooling a yellow precipitate deposited. Mp 188–190° C (decomp.) (from ethanol).

Found, %: N 9.65. Calculated for $C_{20}H_{28}N_3I$, %: N 9.61.

N_α, N_γ-Dimethylbrevicarine N_β, N_γ-dimethiodide (IIIe). A solution of 0.5 g of IVb in hot ethanol was treated with 0.3 ml of CH_3I and the mixture was heated in the water bath under reflux for some time. On cooling, a yellow precipitate deposited. Yield 0.5 g (75.5%). Mp 184–186° C (decomp.) (from ethanol).

Found, %: C 43.22; H 5.53; N 7.25; I 42.28. Calculated for $C_{21}H_{31}N_3I_2$, %: C 43.52; H 5.35; N 7.23; I 43.86.

Brevicarine N_β, N_γ-dimethiodide (IIIf, R = CH_3). A solution of 1.35 g of brevicarine in 45 ml of CH_3OH was treated with 3 ml of CH_3I and heated in the water bath under reflux. After 15 min, a voluminous precipitate of brevicarine dimethiodide deposited. Yield quantitative. Mp 270–271° C (from ethanol). R_f 0.34 (on paper).

Found, %: C 41.11; H 5.16; N 7.54; I 45.42. Calculated for $C_{19}H_{27}N_2I_3$, %: C 41.37; H 4.90; N 7.62; I 46.09.

N_β-Methylbrevicarine N_γ-methiodide (IVc, R = CH_3). A solution of 1.2 g of IIIf in aqueous ethanol was treated with 10% NaOH solution. Bright yellow precipitate. Yield 65.4%, mp 186–188° C (decomp.) (from ethanol), R_f 0.324 (on paper).

Found, %: N 9.04. Calculated for $C_{19}H_{26}N_3I \cdot 2H_2O$, %: N 9.15.

N_α-Ethylbrevicarine N_β, N_γ-dimethiodide (IIIh). A mixture of 1 g of IVc, 4 ml of toluene, 4 ml of nitrobenzene, and 3 ml of C_2H_5I was heated for 15 min. The white precipitate was washed with ether and recrystallized from water. Yield 0.8 g (63.5%), mp 290–292° C (decomp.), R_f 0.33 (on paper).

Found, %: C 43.96; H 5.58; N 7.40; I 44.04. Calculated for $C_{21}H_{31}N_3I_2$, %: C 43.52; H 5.52; N 7.25; I 43.86.

N_γ-Methyl-N_α-ethylbrevicarine N_β-methiodide (IIIj). A solution of 0.5 g of IIIh in ethanol was treated with a 10% solution of NaOH. The bright yellow precipitate of the methiodide was recrystallized from ethanol. Mp 180° C (decomp.).

Found, %: C 55.32; H 6.71; N 9.19; I 27.98. Calculated for $C_{21}H_{30}N_3I$, %: C 55.87; H 6.65; N 9.31; I 28.15.

N_γ-Methyl-N_α-ethylbrevicarine N_β,N_γ-dimethiodide (IIIl). A solution of 0.2 g of IIIj in ethanol was heated with CH_3I. Mp 178–179° C (decomp.) (ethanol).

Found, %: N 7.08. Calculated for $C_{22}H_{33}N_3I_2$, %: N 7.08.

The alkyl iodide derivatives of brevicarine with different sequences of alkyl radicals were obtained similarly: brevicarine N_β,N_γ-diethiodide (IIIg), mp 243–245° C (aqueous ethanol); N_β-ethylbrevicarine N_γ-ethiodide (IVd), mp 256–258° C (aqueous ethanol); N_α-methylbrevicarine N_β,N_γ-diethiodide (IIIm), mp 208–210° C (aqueous ethanol); N_γ-ethyl-N_α-methylbrevicarine N_β-ethiodide (IIIk), mp 195–197° C (aqueous ethanol); N_α,N_γ-dimethylbrevicarine N_β,N_γ-diethiodide, mp 170° C (ethanol).

The compositions of all the compounds given were confirmed by elementary analysis.

Conclusions

1. The alkylation reaction of brevicarine have been studied in detail.

The structural formula I proposed for brevicarine [1-methyl-4-(4-methylaminobutyl)-β-carboline] has been confirmed by NMR and mass spectroscopy.

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22 April 1968

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