SUMMARY

The spatial structure of the diterpene alkaloid delcosine (iliensine) has been determined by x-ray structural analysis and it has been confirmed unambiguously that the OH group in ring A is present at C(1) and has the α orientation.

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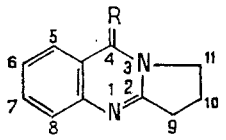
STRUCTURE OF THE PRODUCTS OF THE METABOLISM OF DEOXYPEGANINE AND OF DEOXYVASICINONE

V. N. Plugar', N. D. Abdullaev, Ya. V. Rashkes, M. R. Yagudaev, and N. Tulyaganov

UDC 577.12+547.944.1.92+543.51

The structures of the main components of the total metabolites of the anticholinesterase drug deoxypeganine (DOP) and its analog deoxyvasicinone (DOV) in the rat organism have been studied. The structures of two metabolites of DOP and DOV — 11α-hydroxydeoxyvasicinone and 2-(β-ethoxycarbonyl)ethyl-4-quinazoline — have been determined by the PMR method. On the basis of their structures and the results of measurements of the elementary compositions of ions, the fragmentation of the compounds has been elucidated. A minor component of the total metabolites has been isolated by chromato-mass spectrometry and for it the structure of 6-methoxydidehydrovasicinone is proposed. A scheme of the metabolism of DOP and DOV has been put forward.

Over a number of years, we have been studying the metabolites, excreted with the urine of animals, of the hydrochloride of deoxypeganine (DOP, I) and its derivative deoxyvasicinone (DOV, II) [1], which possess cholinotropic activity. It has been shown that the biotransformation of DOP takes place through a stage of the formation of DOV [2].



I. R = H_2 Deoxypeganine

II. R = O. Deoxyvasicinone

Mass spectrograms of the combined free metabolites of DOP and DOV are similar in the region of the spectrum above m/z 185 (Fig. 1, Ia and IIa). Even greater similarity is observed between the spectrum of the total combined metabolites of (I) and (II) (Fig. 1, Ib, IIb). A quantitative determination of the sums of the free metabolites of these drugs showed that their amounts of biotransformation products were of the same order of magnitude, but DOP (I) was subjected to metabolic transformation to a far smaller degree than DOV (II) [2]. In view of this, the main material used for isolating the pure components and demonstrating their structures was the sum of the metabolites of DOV. As the result of a preliminary separation of the sum of the free metabolites of (II) on a column of alumina, in addition to the

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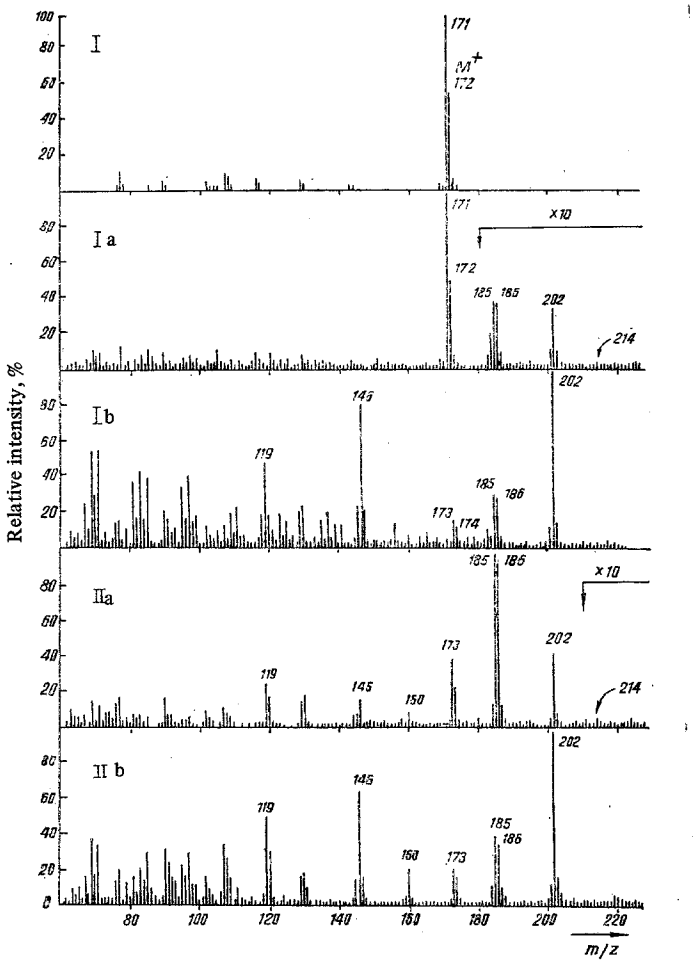


Fig. 1. Mass spectra: I) pure DOP; (Ia) sum of the free metabolites of DOP; (Ib) sum of the bound metabolites of DOP; (IIa) sum of the free metabolites of DOV; (IIb) sum of the bound metabolites of DOV.

initial compound (II), we isolated a metabolite with M^+ 202, the mass spectrum of which differed from that of vasicinone (III) [3] by an increased intensity of the peaks of fragmentary ions with m/z 174 and 173 (Fig. 2a). When the sum of the free metabolites of (I) was analyzed on a chromato-mass spectrometer, the spectrum of a metabolite (V) with M^+ 214 was obtained (Fig. 2b). When the sum of the bound metabolites of DOV, apart from the initial compound (II), were separated, vasicinone (III) and the metabolite (VI) with M^+ 246 were isolated (Fig. 2d).

STRUCTURE OF THE METABOLITE WITH M^+ 202

The PMR spectrum of a solution of the metabolite in $CDCl_3$ (0 — TMS) taken on a spectrometer working in the pulsed regime with subsequent Fourier transformation (Fig. 3) contains isolated signals of practically equal intensity — a triplet at 5.22 ppm with ^3J about 7.4 Hz and a quartet at 6.33 ppm with ^3J = 6.6 and 1.8 Hz — relating to protons located geminally to secondary OH groups. In the higher field, between 2.2 and 4.4 ppm, multiplets characteristic for nonequivalent methylene protons can be clearly seen. If we take as a basis the integral intensity of one of the isolated signals mentioned above, the group of resonance lines located in the 7.4-8.4 ppm region corresponds to aromatic protons. These facts permit the observation that the metabolite consists of a mixture of practically equimolar amounts of two related compounds.

The characteristics of the signals in the 7.4-8.4-ppm interval show the closeness of the values of the chemical shifts of similar pairs of aromatic protons (Table 1), which indicates an identity of the structures of the aromatic parts of the molecules of these two compounds.

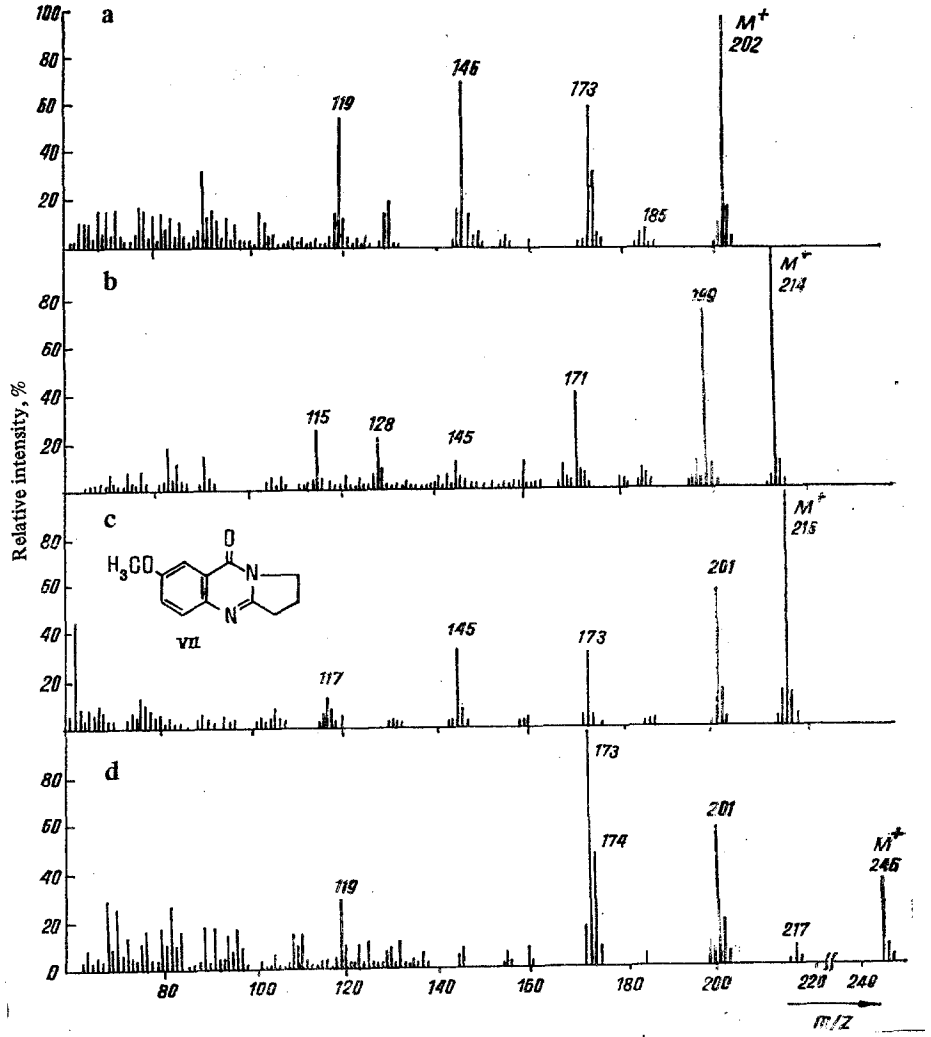


Fig. 2. Mass spectra: a) metabolite with M^+ 202; b) metabolite (V); c) 6-methoxy-DOV; d) metabolite (VI).

The use for the assignment of the signals of the method of multifrequency resonance permitted two groups of interacting multiplets to be revealed, which showed the presence of two similar $-CH(OH)-CH_2-CH_2-$ fragments in the molecules of both components of the metabolite with M^+ 202. To one of these fragments belong the signals located at 5.22 ppm [1 H, t, ^3J about 7.4 Hz, $>CH(OH)$]; 2.27 and 2.67 ppm (1 H each, m, $C-CH_2-C$); and 4.03 and 4.37 ppm (1 H each, m, $N-CH_2-C$). In the values of its chemical shifts and the nature of its multiplicities, this group of signals corresponds to that of the PMR spectrum of a sample of pure vasicinone (III).

Thus, the metabolite is a mixture of vasicinone and an isomeric hydroxyl-containing compound (IV) differing from it either by the position or by the orientation of the secondary OH group.

To the isomer (IV) belong signals at 6.33 ppm [1 H, q, ^3J = 1.8 and 6.6 Hz, $>CH(OH)$]; 2.27 and 2.52 (1 H each, m, $-C-CH_2-C-$); and 3.07 and 3.38 ppm (1 H each, m, $-C-CH_2-C=$). Multiplets at 2.27 and 2.52 ppm are assigned unambiguously to the methylene protons at C-10, which agrees well with the chemical shifts of the protons of the same time in vasicinone (see Table 1). It is known that in the PMR spectrum of deoxyvasicinone (II) a 2 H triplet at 4.15 ppm corresponds to the methylene proton located in the α position to the amide nitrogen (at C-11), while the protons of the α position to the double bond (at C-9) resonate in a considerably weaker field, at 3.13 ppm, i.e., the difference in the values of their chemical shifts amounts to 1.0 ppm [4]. It follows from this that the multiplets in the weaker field at 3.07 and 3.38 ppm observed in the spectrum of the metabolite must be assigned to nonequivalent methylene protons at C-9 of the (IV) molecule. Furthermore, in this case the resonance signal from the $>CH(OH)$ group undergoes a paramagnetic shift in relation to that of vasicinone (5.22 ppm, H-9)

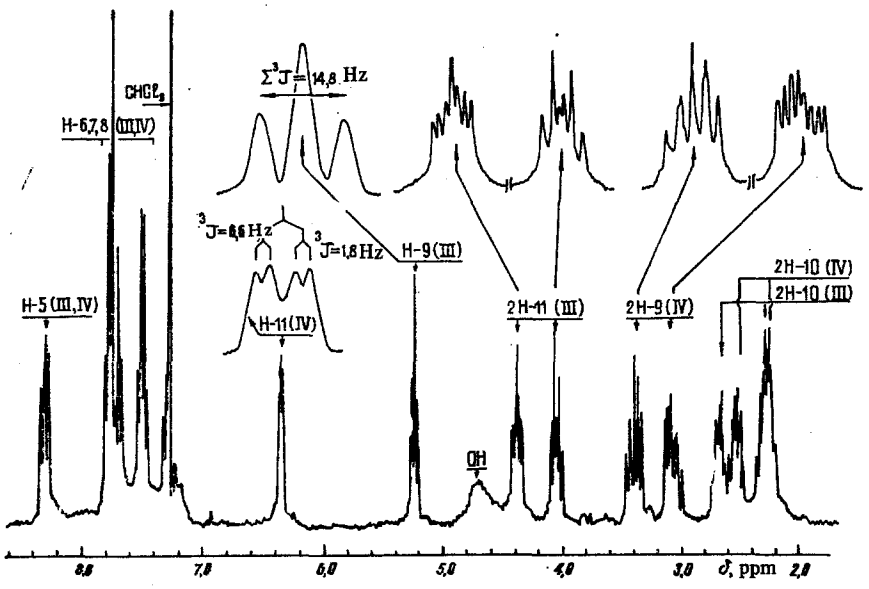


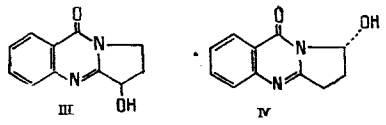
Fig. 3. PMR spectrum of the metabolite with M^+ 202 (solvent $CDCl_3$).

by 1.1 ppm, which correlates well with the above-mentioned difference in the chemical shifts of the protons at C-9 and C-11 of the deoxyvasicinone molecule. These facts in combination enable us to state that in the molecule of compound (IV) the secondary hydroxy group is located at C-11.

The crystal structure of the alkaloid peganine, which contains an OH group at C-9, has been studied by x-ray structural analysis. We also established that the pyrrolidine ring has the "envelope" conformation and the hydroxy group the β orientation [5]. No similar investigation has been carried out for vasicinone. However, in the PMR spectra of vasicinone and peganine the H-9 proton geminal to the hydroxy group appears in the form of a triplet with close values of the sum of the vicinal spin–spin coupling constants – Σ^3J = 14.9 and 16.0 Hz, respectively. This gives grounds for postulating the same β orientation of the OH functions of the molecules of these two bases.

A characteristic feature of the "envelope" conformation of the pyrrolidine rings of these bases is that the similarly oriented H-9 and H-11 protons form dihedral angles θ with the methylene protons at C-10 of identical size. Consequently, if in the isovasicinone (IV) molecule the OH group at the C-11 carbon atom had the β orientation, we would be justified in expecting in its spectrum a signal of triplet nature with $\Sigma^3J \approx 14$–16 Hz for the corresponding geminal proton. However, as mentioned above, in the spectrum of the metabolite (IV) the H-11 proton is represented by a quartet signal at 6.33 ppm with ^3J = 1.8 and 6.6 Hz, showing the α orientation of the secondary hydroxy group in the isovasicinone molecule.

Thus, isovasicinone (IV) is 11α-hydroxy-2,3-trimethylene-3,4-dihydro-4-quinazolone.



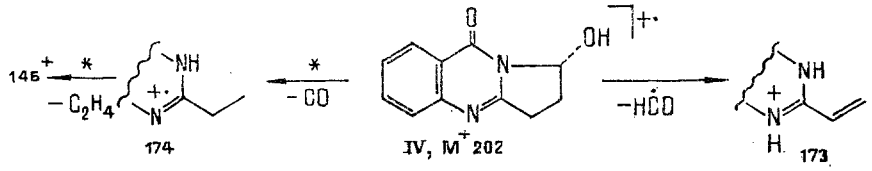
The structure of (IV) explains the above-mentioned increased intensity of the peaks of the ions with m/z 174 and 173 in the spectrum of the metabolite with M^+ 202 (Fig. 2a). Measurements of the accurate masses of these ions showed that the first consisted of the ions $(M - CO)^+$ and $(M - C_2H_4)^+$ in a ratio of 9:1, and the second of the $(M - HCO)^+$ ion. The $(M - C_2H_4)^+$ ions are most probably due to a contribution of vascisinone (III), since in the spectrum of a pure sample of (III) the fragments $(M - CO)^+$ and $(M - C_2H_4)^+$ were present in a ratio of 1:2, and the relative intensity of the peak with m/z 174 amounted to 8% [3]. The splitting out of the particles of CO and HCO from M^+ is characteristic for compounds con-

TABLE 1. Chemical Shifts of the Protons (δ, ppm) of Vasicinone (III), Isovasicinone (IV), Deoxyvasicinone (II), and the Metabolite (VI)

Proton	Vasicinone (III)	Isovasicinone (IV)	Deoxyvasicinone (II)	Metabolite (VI)
H-9	5,22 t ^3Jabout7,4 Hz	—	—	—
H-11	—	6,33 q ^3J=1,8 & 6,6 Hz	—	—
2H-9	—	3,07 m 3,38 m	3,13 t	2,94 m
2H-10	2 27 m 2,67 m	2,27 m 2,52 m	2 24 m	2,94 m
2H-11	4,03 m 4,37 m	—	4,15 t	—
H-5	8,28	8,32	8,23	8,31
H-6	7,48	7,51	7,43	7,49
H-7	7,77	7,69	7,67	7,78
H-8	7 75	7,75	7,70	7,71
O—CH_2 | CH_3	—	—	—	4,20 q 1,27 t

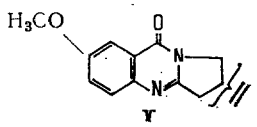
*t — triplet; q — quartet; m — multiplet.

taining the —N—CH—OH group [6], to which isovasicinone (IV) can be assigned. The fragmentation obviously takes place by the following route:



STRUCTURE OF THE METABOLITE WITH M^+ 214

In the sums (Ia) and (IIa), this metabolite amounted to thousands of a percentage part [2], and therefore it could not be isolated in the pure form. We give its most probable structure on the basis of a study of its mass spectrum (Fig. 2b). The M^+ peak in it has an intensity of 100%. The main direction of fragmentation consists in the successive elimination of 15 and 28 amu, which is characteristic for aromatic methoxy derivatives. The spectrum of (V) is very similar to that of 6-methoxydeoxyvasicinone (VII), which, as our investigations have shown [7], has a number of characteristic features (Fig. 2c) distinguishing it from isomers with CH_3O groups in positions 5, 7, and 8. The difference between the M^+ peaks of (V) and (VII) of 2 amu is most probably due to the presence of a double bond in ring C of the metabolite. Consequently, this compound may have the structure:



V

The elementary composition of the M^+ ion with m/z 214 ($C_{12}H_{10}N_2O_2$) confirms this hypothesis. We may note that the dehydrogenation of ring C has also been observed in the case of the metabolism of 2,3-isooxazole-4-quinazolone [8].

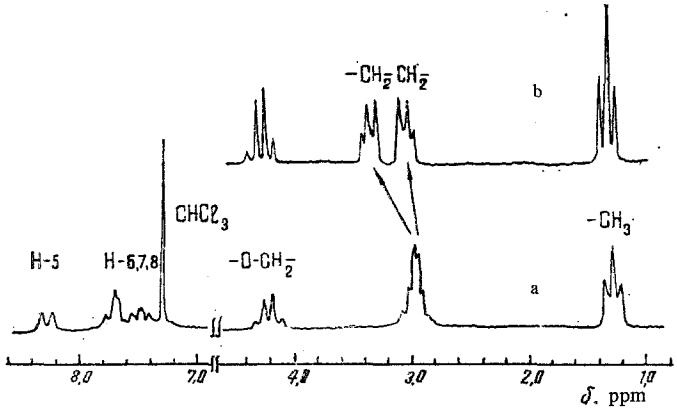


Fig. 4. PMR spectrum of the metabolite (VI): a) in $CDCl_3$; b) in $CDCl_3$ + CF_3COOH.

STRUCTURE OF THE METABOLITE (VI) WITH M^+ 246

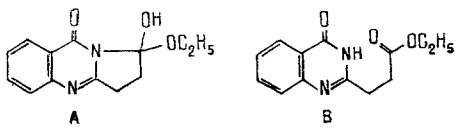
The PMR spectrum of metabolite (VI) ($CDCl_3$, 0 — TMS, Fig. 4a) lacks the signal corresponding to a proton geminal to a hydroxy group. At the same time, it contains signals at 1.27 ppm (3 H, t, CH_3—) and 4.20 ppm (2 H, q, —CH_2—), the multiplicities and values of the chemical shifts of which correspond to the protons of an O-ethyl group. The presence of this group is also confirmed by the ions $(M - C_2H_5)^+$ and $(M - OC_2H_5)^+$ with m/z 217 and 201 in the mass spectrum of the metabolite (VI) (Fig. 2d). The values of the chemical shifts of the four aromatic protons resonating at 7.49 (H-6), 7.71 (H-8), 7.78 (H-7) and 8.31 ppm (H-5) are close to those of the vasicinone and isovasicinone (see Table 1).

A complex multiplet of 4 H with its center at 2.94 ppm can be assigned to the protons of two methylene groups adjacent to one another. From a comparative consideration of the values of the chemical shifts of the methylene protons in the molecule of (VI) (see Table 1), it follows that the CH_2 groups at C-9 and C-10 are unsubstituted. Furthermore, the signals of the protons at C-10 undergo an additional downfield shift by 0.5 ppm as compared with the lines of this group in the spectrum of isovasicinone. One of the factors capable of causing such a change in the spectrum of compound (VI) may be the attachment of two oxygen functions to C-11, as is also indicated by the presence in its spectrum of a direct metastable transition $246^+ \rightarrow 173^+$, taking place with the loss of the particle $C_3H_5O_2$.

The following observations are in favor of this structural feature of the (VI) molecule. With the gradual addition of definite portions of dry fluoroacetic acid to a solution of deoxyvasicinone, a paramagnetic shift of the protons of all three methylene groups is observed in its PMR spectrum which is obviously due to the protonation of the pyridine nitrogen atom. When equilibrium has been reached in this exchange process, the chemical shifts of the protons practically cease to vary. At the same time, we find that the triplet from the two protons at C-9 has undergone a shift by 0.50 ppm, while the chemical shifts of the protons at C-10 and C-11 have changed by only 0.25 and 0.20 ppm, respectively. When an identical experiment was performed with vasicinone, we found that the paramagnetic changes in the chemical shifts of the protons at C-9, C-10, and C-11 amounted to 0.50, 0.20, and 0.18 ppm, respectively. It follows from this that when the pyridine nitrogen atom is protonated the greatest descreening effect is experienced by the C-9 protons regardless of the presence of a hydroxy group in the pyrrolidine ring.

An analogous effect is also observed in the case of the metabolite (VI). Under these conditions, the complex four-proton multiplet at 2.94 ppm appears in the form of intercoupled two-proton triplets at 3.33 and 3.04 ppm (Fig. 4b). Consequently, in this case, the contributions of protonation to the chemical shifts of the protons of the two methylene groups amount to 0.4 and 0.1 ppm. This means that the triplets at 3.33 and 3.04 ppm correspond to the protons at C-9 and C-10, respectively. Consequently, the ethoxy group and the second oxygen function in the molecule of the metabolite (VI) are present on the same carbon atom, C-11.

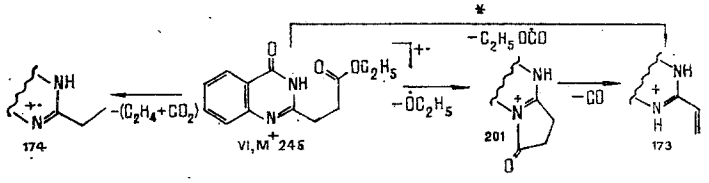
On the basis of all that has been said above, for metabolite (VI) it is possible to suggest the two alternative forms A and B:



A B

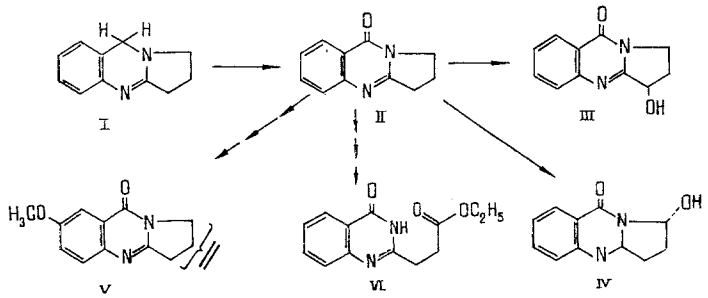
In view of the presence of two voluminous substituents at C-11 in the five-membered ring of structure A, one might expect the appearance in the PMR spectrum of metabolite (VI) of separate multiplet signals from four nonequivalent protons of two methylene groups at C-9 and C-10, as had been observed in the spectra of isovasicinone and vasicinone (Fig. 3), in the molecules of which the pyrrolidone ring is present in one preferred conformation. However, the presence of the two-proton triplets at 3.33 and 3.04 ppm shows the equivalence of the protons in each of the methylene groups as a consequence of their considerable mobility. It follows from this that in $CDCl_3$ solution structure B is more probable for metabolite (VI).

The fragmentation of metabolite (VI) is also in harmony with structure B:



The acyclic nature of the substituent at C-2 explains the lowered intensity of the M^+ peak with m/z 246 (Fig. 2d). Together with this, the possibility is not excluded that metabolite (VI) in the solid state exists in the form of the α-carbinolamine A, and transition to form B takes place under thermal conditions or under electron impact [9].

Summarizing the facts presented above, we suggest the following scheme for the metabolism of DOP (I) and DOV (II) in the rat organism:



It permits the conclusion that the anticholinesterase effect of DOP is due to the action of the alkaloid itself, since analogous activity of the main metabolites is 100 and more times lower than that of DOP. The toxicity of the main metabolites is also considerably smaller than that of the initial drug [10].

EXPERIMENTAL

The collection of the biological material and the extraction of the free metabolites (I) and (II) was carried out as described previously [1].

Hydrolysis. The residue after the extraction of the free metabolites was treated with 20% by volume of concentrated HCl and the mixture was heated on the water bath for 5 h; then the pH was brought to 10 with concentrated Na_2CO_3 solution and the total bound metabolites were extracted with chloroform.

The chromatographic fractionation of the total material was carried out on a column of Al_2O_3 (activity grade II), the material being eluted successively with mixtures of benzene and chloroform in ratios of 3:1, 2:1, 1:1, 1:2, 1:3, ..., 1:9, with chloroform, and with mixtures of chloroform and methanol in ratios of 99:1, 9:1, and 3:1.

PMR spectra were taken on XL-100-15 and XL-200 spectrometers (Varian) with $CDCl_3$ as the solvent, 0 — TMS, and mass spectra on a MKh-1303 instrument fitted with the system for the direct introduction of the sample into the ion source, the inlet temperature being 150°C, the collector current 50 μA, and the ionizing voltage 40 V. The accurate masses of the ions were measured on a MKh-1310 instrument with a SVP-5 sample inlet system using a collector current

of 60 μA, an ionizing voltage of 50 V, and a temperature of the ionization chamber of 150°C. The accuracy of the mass measurement was $5 \cdot 10^{-6}$. The conditions for chromato-mass spectrometric analysis have been published previously [1].

SUMMARY

The structures of the two main products of the biotransformation of deoxypeganine and deoxyvasicinone have been established by the PMR method as 11α-hydroxydeoxyvasicinone and 2-(β-ethoxycarbonyl)ethyl-4-quinazolone. The fragmentation of the compounds has been elucidated on the basis of the structure and measurements of the elementary compositions of the ions.

A minor component of the total metabolites has been isolated by chromato-mass spectrometry, and for this the structure of 6-methoxydidehydrodeoxyvasicinone is suggested.

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DNA-CELLULOSE — A NEW AFFINITY SORBENT FOR HUMAN LEUCOCYTIC INTERFERON

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UDC 576.858.095.383

The possibility has been studied of using affinity chromatography on DNA-cellulose for the purification of interferon. It has been shown that interferon at a low ionic strength binds to DNA-cellulose, and on this basis a new method is proposed for purifying interferon which permits its 200-fold purification with small losses.

The interferons are proteins of vertebrate cells (from fish to Man) which form wide controlling and regulating functions directed to preserving cell homeostasis. The most important of these functions are their antiviral, anticellular, immunomodeling, and radioprotective functions [1, 2]. In spite of the voluminous material on the study of interferon over more than 20 years, its use in medical practice has not previously been broad enough. This can be explained by the fact that there is no sufficiently effective and economic method of purifying interferon, since such demands are made on the preparation as the complete absence of toxicity and antigenicity in homologous organisms. The known methods are associated with great losses or envisage the use of difficultly accessible expensive materials [4-7]. In the present paper we give the results of a study of the possibility of using affinity chromatography on DNA-cellulose, preceded by ion-exchange chromatography, for purifying human leucocytic interferon.

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