CONVERSIONS OF THE ANTINEOPLASTIC PREPARATION PROSPIDIN IN AQUEOUS MEDIA

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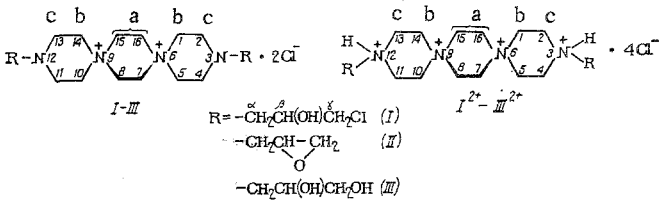
Prospidin — 3,12-bis(2'-hydroxy-3'-chloropropyl)-3,12-diaza-6,9-diazoniadispiro[5.2.5.2]hexadecanedichloride (I) — is an antineoplastic drug used in the treatment of a number of forms of malignant neoplasms [4, 6]. For a study of the mechanism of the antineoplastic action of I and possible pathways of its metabolism, data on the structure of products of its chemical conversions, associated with functional changes in the terminal cytotoxic groups, are of great importance.

It is known that the γ-chloro-β-hydroxypropyl group possesses substantial mobility. In alkaline media (pH > 8.0), this group in compound I is converted at an appreciable rate to the β,γ-epoxypropyl group of compound II [5]. The epoxy form (II) is aqueous solution in turn undergoes hydrolysis, forming a β,γ-dihydroxy derivative III [2, 3]. Analogously, I is hydrolyzed in water to a protonated form of the diol III^{2+} [2]. The kinetics and mechanism of these reactions were not investigated previously, whereas such data are necessary for the modeling of processes of biotransformation of I.

The present work is devoted to a study of the mechanism of the conversions of I and II in aqueous media.



We used ^{13}C NMR spectroscopy as the main method of investigation. The assignment of the signals was based on the nature of their multiplicity in entirely correlated spectra, and the values of the chemical shifts (CS) and spin—spin coupling constants (SSCC) $^1J(^{13}C^1H)$, as well as on a comparison with the spectra of analogs of I described previously [1, 2]. A comparison of the spectra of protonated and deprotonated forms of compounds I-III shows that a change in the structure of the side chains has virtually no effect on the spectral parameters of the spiroheterocycle (Table 1). At the same time each of the compounds considered is unambiguously identified according to the values of the CS δ (^{13}C) and the SSCC $^1J(^{13}C^1H)$ of carbon atoms in the β- and γ-positions of the terminal groups. Thus, for example, as we go from I to III a substantial weak field shift it observed for C_γ ($\Delta\delta = -16.2$ ppm), which permits monitoring of the conversion of I to III during hydrolysis.

The hydrolysis of prospidin was studied in water, as well as aqueous solutions of HCl and NaX (X = Cl, Br, I) in the temperature interval from 25 to 85°C.

Our investigations showed that the process of conversion of I to aqueous media includes two main successive steps: the step of intramolecular cyclization according to an S_N1-type mechanism, with the formation of an intermediate compound IV and terminal spiran azetidinium groups and its hydrolysis forming an equilibrium mixture of protonated and deprotonated diol III. The structure of IV was demonstrated on the basis of an investigation of the ^{13}C NMR spectra of a model compound — 2-hydroxy-4-azoniaspiro-[3.5]nonane (V) — and calculation

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TABLE 1. Chemical Shifts ($\delta^{13}C$, ppm) and Spin–Spin Coupling Constants $^1J(^{13}C^1H)$ (Hz) in the Spectra of Compounds I–III*

Compound	Measured, ppm	δ (^{13}C)						^1J ($^{13}C^1H$)					
		C_a	C_b	C_c	C_α	C_β	C_γ	C_a	C_b	C_c	C_α	C_β	C_γ
I	D_2O	51,7	59,2	45,9	59,4	67,9	47,9	149	147	138	133	144	151
I^{2+}	DCl/D_2O	53,0	56,5	46,2	59,4	65,3	46,8	150	150	147	146	143	154
II	D_2O	51,7	59,1	45,8	58,7	50,3	45,6	149	147	136	134	180	177
II^{2+}	DCl/D_2O	52,6	56,2	45,6	59,0	45,8	46,2	151	150	149	146	179	180
III	D_2O	51,7	59,3	45,9	58,8	68,5	64,1	149	147	136	133	144	143
III^{2+}	DCl/D_2O	52,5	56,6	45,8	58,8	66,1	63,4	151	150	150	144	141	142

*The notation of the groups of magnetically equivalent carbon nuclei is cited on the structural formulas (see text).

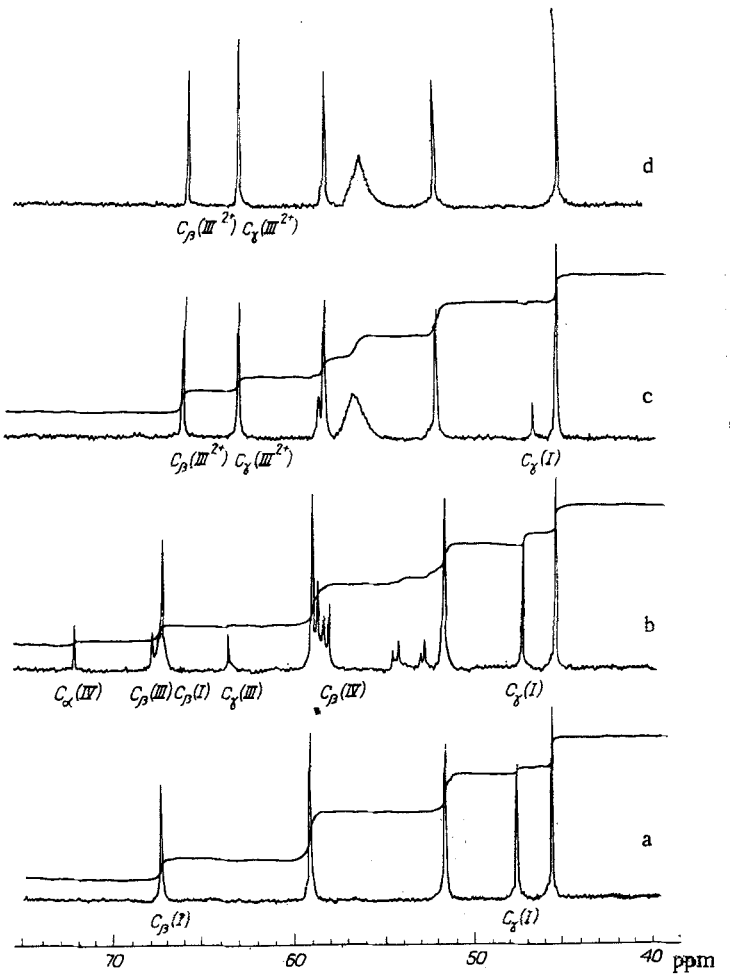
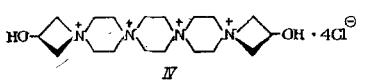
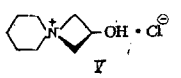


Fig. 1. ^{13}C NMR spectra of a solution of I in D_2O, initial (a), after 3 h of heating at 70°C (b), after 40 h (c), and a 0.4 M solution of III in 0.8 DCl in D_2O (d).

of the parameters of the spectrum of azetidinium groups in IV according to an additive scheme using the literature data on the effects of substituents [11] and the spectral parameters of azetidine derivatives [10] (Table 2).



IV



V

TABLE 2. Observed and Calculated Chemical Shifts of ^{13}C (ppm) in the Spectra of Compounds IV-VII

Compound	Measured, ppm			Calculated, ppm		
	C_α	C_β	C_γ	C_α	C_β	C_γ
IV	72,4	58,4	—	72,9	59,0	—
V	71,9	59,1	—	72,9	59,0	—
VI	59,4	67,2	37,4	58,4	67,9	36,9
VII	61,4	67,1	11,7	56,4	67,9	10,9

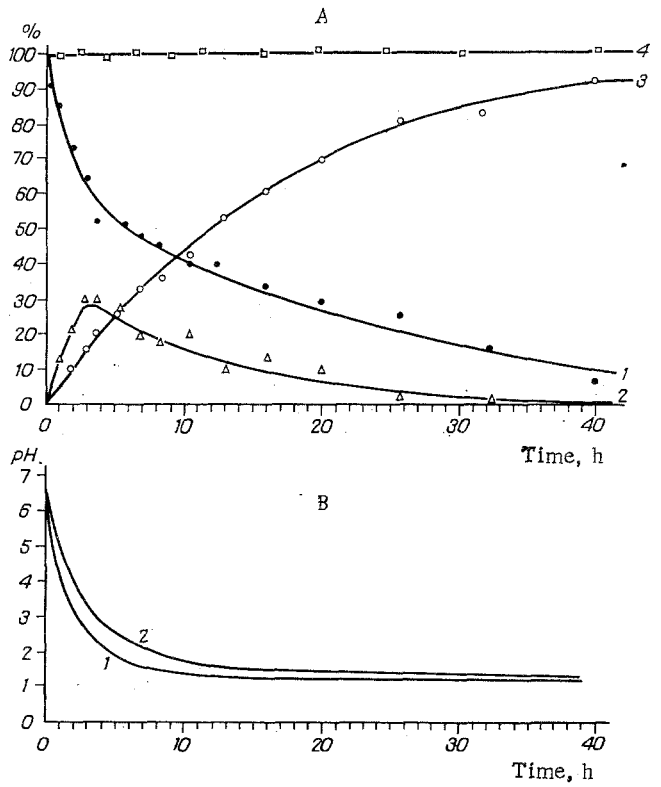


Fig. 2. Kinetics of the conversions of I in aqueous media. A) Kinetic curves: 1, 4) consumption of I; 2) accumulation of IV; 3) accumulation of III during hydrolysis of a 0.4 M solution of I in D_2O (1-3) and 0.8 M DCl (4) at 70°C; B: 1) pH change of a 0.2 M solution of I in H_2O; 2) in 1 M NaCl at 70°C.

The most characteristic signal in the spectrum of compound IV is a triplet at 72.4 ppm with constant $^1J(^{13}C^1H)$ = 155.0 Hz, belonging to the carbon atom of the azetidinium ring in the α-position to the quaternary nitrogen. We should mention the close correspondence of these parameters to the values of $\delta(^{13}C)$ = 71.9 ppm and ^1J = 152.7 Hz for the analogous atoms in IV.

A measurement of the spectra of aqueous solutions of I in the kinetic system of recording makes it possible to follow the process of conversion of I to IV and III: The intensity of the signals of I gradually decreases, while that of III increases in time. In addition, the appearance of signals of an intermediate compound IV is observed, the intensity of which initially increases but then decreases until they disappear entirely (Fig. 1).

The rate of the summary reaction, primarily the step of hydrolysis IV → III, decreases sharply with increasing temperature. Thus, 5 h after the beginning of the reaction, the decrease in the concentration of the compound I at the temperatures 85, 70, and 25°C is 85, 50, and less than 3%, respectively.

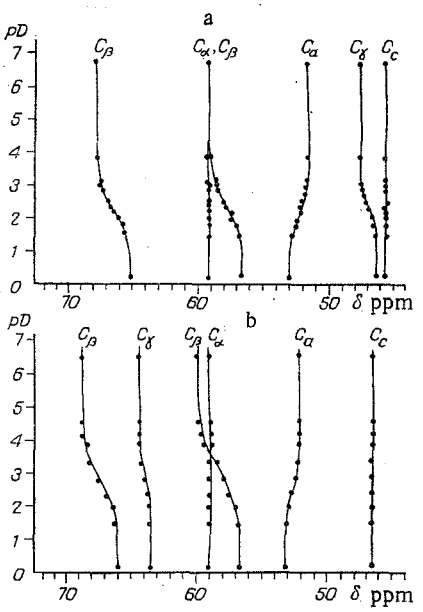
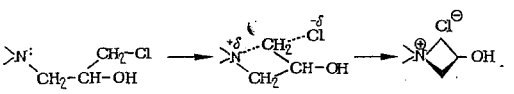


Fig. 3. Dependence of the chemical shifts of ^{13}C of 0.1 M solutions of I (a) and III (b) and D_2O on pD at 25°C.

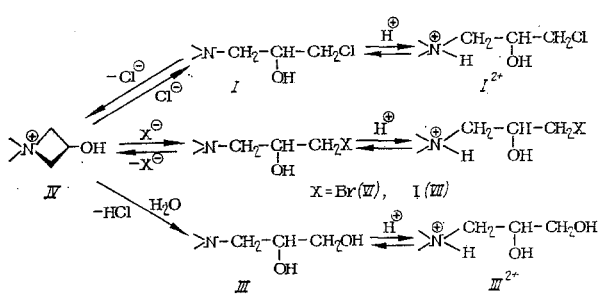
At the temperature 70°C the summary reaction and both of its steps proceed at a kinetically controlled rate. From an analysis of the kinetic curves constructed according to the integral intensities of the signals in the spectra recorded at this temperature (see Figs. 1 and 2A), it follows that the two steps of the process studied are successive.

The key step is the reaction of intramolecular nucleophilic substitution of the chlorine atom, occurring during attack of the γ-carbon atom of the unshared pair of nitrogen



The binding of the unshared pair of the tertiary nitrogen atoms in the case of protonation of I excludes the possibility of an intramolecular alkylation reaction. The spectrum of an aqueous solution of I in the presence of an equinormal element of DCl under the same reaction conditions does not undergo any changes (see Fig. 2A, curve 4). From these data follows the practically important conclusion of a significant increase in the stability of aqueous solutions of the dihydrochloride I^{2+}.

The step of intramolecular cyclization is reversible. This was confirmed by an investigation of the reaction in the presence of alkaline metal halides. In 5 M solutions of NaX (X = Cl, Br, I) at 70°C, exchange of a halide in the γ-halo derivatives under these conditions occurs as a result of nucleophilic attack by halide ions on the α-carbon atoms of the azetidinium ring, accompanied by its opening according to the following scheme:

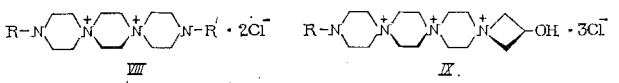


This unambiguously indicates reversibility of the step of intramolecular alkylation. The structure of compounds VI and VII was confirmed by a comparison of the values of the chemical shift observed in the spectra fo these compounds with those calculated according to an additive scheme using the increments for substituted alkanes [11] (see Table 2).

The increase in the relative concentration of the intermediate compound IV is explained by the salt effect. It is known that an increase in the ionic strength of the solution leads to an increase in the rates of the reactions characterized by greater polarity of the transition state in comparison with the initial state [7]. Consequently, the primary salt effect leads to the fact that the rate of the intramolecular cyclization reaction increases with increasing ionic strength of the solution, since the polarity of its transition is higher than the polarity of the initial state I and, conversely, the rate of its reverse reaction decreases. This leads to a shift of the equilibrium I → IV to the right.

The second step in the hydrolysis reaction is opening of the azetidinium rings of IV by water, forming the diol III. The acid liberated in this case protonates both the final product and the starting material of the reaction. An increase in the relative content of the protonated form I^{2+} leads to a gradual slowing of the summary reaction I → III. The process of conversion of I in aqueous media is accompanied by a decrease in the pH to a definite limit (Fig. 2B). A comparison of the curves of the pH change during hydrolysis, measured for solutions of I in water and 1 M NaCl, permits us to conclude that the increase in ionic strength and increasing Cl^- ion concentration lead to a slowing of the second step of the reaction.

The mechanism considered for the conversion of I in water and aqueous solutions of NaX suggests the possibility of the formation not only of VI but also of asymmetric dispirotripiperazinium derivatives of the type of VIII and IX as intermediate products.



VIII

R,R′: $-CH_2CH(OH)CH_2X$.

IX

X=Cl, Br, I, OH

The absence of any appreciable mutual influence of the terminal groups associated with the tricyclic structure on the corresponding spectral parameters does not permit the identification of compounds of the type VIII and IX in the reaction system containing analogous symmetrical derivatives (I, III, IV, VI, VII).

As a result of the existence of a system of proteolytic equilibria in the solution for the study of the conversion of prospidin in aqueous media, a characterization of the relative basicity of the initial and final reaction products is important. For this purpose we measured the ionization constants of I and III in water by the ^{13}C NMR method. The use of other methods of determining pK_a is limited by the fact that prospidin (I) is not titrated in aqueous media and does not exhibit absorption in the UV spectrum in the region $\lambda > 210$ nm.

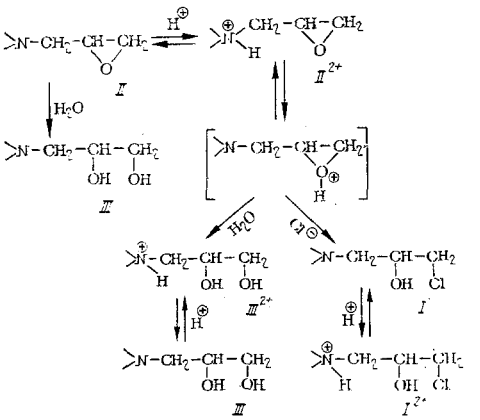
Compounds I and III each contain two cationic sites in their molecules — tertiary nitrogen atoms in the positions 3 and 12 of the spiroheterocycle. However, the dependence of the chemical shifts on pD respond to the presence of one proteolytic equilibrium in solution in both cases, which corresponds to close values of the first and second ionization constants. The absence of any appreciable mutual influence of the two cationic sites in the molecule is due to their separation by an extensive spiran system, containing two quaternary nitrogen atoms. Thus, the values of the ionization constant pK_a (I) = 1.90, pK_a (III) = 2.45, found from the dependence of the chemical shifts $\delta(^{13}C)$ on pD (Fig. 3), pertains the process of diprotonation of I and III and correspond to the greater basicity of III in comparison with I. Such a difference in basicity, evidently due to a substantial degree to solvation effects, leads to the fact that the system of proteolytic equilibria, observed under conditions of the hydrolysis reaction, always corresponds to a larger concentration of the dihydrochloride III^{2+} in comparison with I^{2+}. This determines the possibility of virtually complete conversion of I to III^{2+} in aqueous medium.

As we go to alkaline media, the conversion of I to III may occur through the formation of the epoxy derivative II. As was noted earlier [5], reaction I → II proceeds rather rapidly at pH > 8.0. The epoxy form II in aqueous medium readily undergoes hydrolysis, forming the diol III. Under similar conditions the rate of the reaction II → III significantly

exceeds the rate of hydrolysis of I in water. Thus, at 55°C the time of half-conversion II → III is about 2.5 h.

In aqueous solutions of HCl, opening of the epoxide ring with the formation of the γ-chloro-β-hydroxypropyl group is observed. At a mole ratio of acid and base $C_{HCl}/C_{II} > 4$ under mild conditions (t = 25°C), II is quantitatively converted to I^{2+}. The spectrum, measured 30 min after the beginning of the reaction, corresponds to the presence only of this compound in solution.

A lowering of the acid concentration to an equinormal ratio with II leads to a substantial slowing of the reaction under the same conditions. In this case the first step of the process — formation of the protonated form II^{2+} — can be distinguished (see Table 1). Further changes in the spectra with time correspond to the process of opening of the epoxide rings of the cation II^{2+} under nucleophilic attack of chloride ions according to the following scheme:

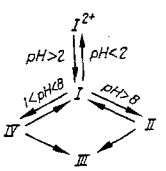


It can be assumed that the reaction proceeds through the formation of a tautomeric hydroxonium form of the cation II^{2+} as an active intermediate. This hypothesis is supported by the fact that the reaction system contains not only the main reaction product I and I^{2+} but also an equilibrium mixture of protonated and deprotonated forms of the diol III and III^{2+}, evidently formed as the result of the hydrolysis of the cation II^{2+}, despite the significantly lower nucleophilicity of the H_2O molecules in comparison with chloride ions. An increase in the concentration of Cl^- ions as we go toward a more acid medium $C_{HCl}/C_{II} > 4$ leads to a substantial increase in the rate of the main reaction, as a result of which the formation of hydrolysis products is not observed under these conditions.

Thus, the aggregate of results described earlier [1-3, 5] and those obtained in this work permits us to conclude that the conversions of prospidin in aqueous media are due to changes in the structure of the terminal groups. Under the investigated conditions, no reactions associated with elimination of side chains and structural changes in the tricyclic transport fragment are observed.

The most characteristic of I are reactions of conversion to the epoxy form II in alkaline media (pH > 8) and intramolecular alkylation with the formation of compounds with terminal spiran azetidinium groups in neutral and weakly acid media (1.0 < pH < 8.0). Both types of compounds formed undergo hydrolysis, forming the biologically inactive [3] diol III. The azetidinium fragment is hydrolyzed under similar conditions significantly more slowly than the epoxy ring of compound II.

The ability of the epoxy group of II to be converted to a γ-chloro-β-hydroxypropyl group of compound I evidently explains the high biological activity of II [5]:



In acid media there is a protonation of I with the formation of the dihydrochloride I^{2+}, stable to hydrolysis.

The results obtained suggest that the formation of a structure with azetidinium groups, observed for I under conditions close to the physiological, may play an important role in the mechanism of the biological action of prospidin.

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EXPERIMENTAL

Compounds I-III and V were synthesized as described earlier [3, 5, 8]. The ^{13}C NMR spectra were measured on an XL-100-12/A instrument from Varian (Switzerland) with working frequency 25.2 MHz. Conditions of recording of the spectra: scanning width 2500 Hz, duration of pulse 18 μsec (40°C), time of collection of data 0.82 sec, number of passes during accumulation of the spectrum from 512 to 2048, depending on the concentration of the compounds measured in solution. In the measurement of entirely correlated spectra of ^{13}C, to improve the signal-to-noise ratio the method of saturation of ^{1}H nuclei during the time lag between sample collections, usually established equal to 2 sec, was utilized. The time of collection of the data in this case was 1.6 sec.

The pH of the solutions was measured on a pH-Meter-27 instrument from Radiometer (Denmark) with a thermostatically controlled measuring cell. The scale was calibrated with standard buffer solutions. The values of pD in the case of measurement in D_2O was converted to the corresponding values of the pH by means of the ratio pH = pD − 0.4 [9].

The values of pK_a of compounds of I and III were calculated by treating the dependence of the chemical shifts of ^{13}C on the pH of the measurable solutions according to the method of least squares.

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