HYDROXYETHYLATION OF URACIL, ADENINE, AND CYTOSINE WITH ETHYLENE CARBONATE

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It was established that both 1-(2-hydroxyethyl)uracil and 3-(2-hydroxyethyl)-uracil, which are converted to 1,3-bis(2-hydroxyethyl)uracil, are formed in the first step of the reaction of uracil with ethylene carbonate. The simultaneous formation of 9-(2-hydroxyethyl)adenine and 3-(2-hydroxyethyl)adenine occurs in the reaction of adenine with ethylene carbonate. The only product of hydroxyethylation of cytosine was 1-(2-hydroxyethyl)cytosine. Methods for the analytical separation of the hydroxyethylation products and the preparative isolation of the 1- and 9-hydroxyethyl derivatives are proposed.

The 1- and 9-hydroxyethyl derivatives of pyrimidine and purine bases of nucleic acids are of interest as the simplest models of nucleosides that do not contain a ribosyl residue and as intermediates in the synthesis of analogs of polynucleotides [1]. Of the methods for their preparation, the direct condensation of the bases with ethylene carbonate (I) is of great interest. In particular, prior protection of the exo-amino group of adenine (II) and cytosine (III) is not required in this case. However, as we demonstrated in a preliminary communication [2], both 9-(2-hydroxyethyl)adenine (IV) and 3-(2-hydroxyethyl)adenine (V) are formed in the condensation of I and II, whereas the yield of 3-(2-hydroxyethyl)uracil (VII), which is formed along with 1-(2-hydroxyethyl)uracil (VIII) and 1,3-bis(2-hydroxyethyl)-uracil (IX) in the condensation of I with uracil (VI) may reach 16%. The only product of hydroxyethylation of base III was 1-(2-hydroxyethyl)cytosine (X). The aim of the present research was to study the dynamics of this reaction, to develop methods for the analytical separation of the hydroxyethylation products, and to preparatively isolate chromatographically pure 1- and 9-(2-hydroxyethyl) derivatives.

The condensation of the heterocyclic bases with I was carried out with dry dimethylformamide (DMF) containing traces of NaOH. The compositions of the reaction mixtures and the characteristics of the reaction products are presented in Table 1. The analytical separations were accomplished by chromatography and electrophoresis on paper in various solvent systems. The R_f values and electrophoretic mobilities of the hydroxyethylation products are presented in Table 2. Compounds VII, VIII, and IX were detected in the reaction mixture in addition to VI in the reaction of I and VI. System A does not ensure the separation of VII and IX, and the spots of VI and VII are frequently overlapped; VII therefore was not detected in earlier studies [3] when this solvent system was used for analytical and preparative separations. System B and electrophoresis on paper in a lithium carbonate buffer at pH

TABLE 1. Compositions of Reaction Mixtures in the Hydroxyethylation of Uracil, Adenine, and Cytosine with Ethylene Carbonate

Heterocyclic base	Composition of the reaction mixture (mole %)	Isolated compound	Yield, %	mp, °C	Method of isolation
VI	VI (8); VII (4); VIII (47); IX (41)	VIII	40	137	Chromatography on Dowex-2 resin
II	IV (90); V (10)	IV	80	235	Recrystallization from alcohol
III	III (13); X (87)	X	70	229	The same

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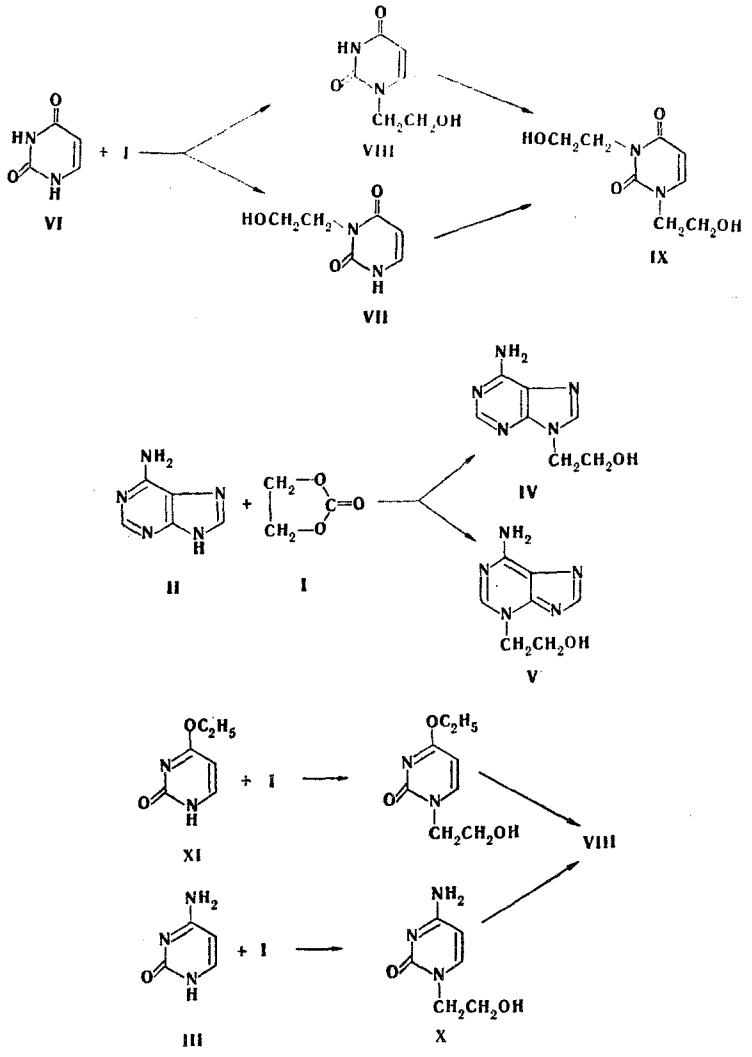
TABLE 2. Rf Values, Electrophoretic Mobilities, and Molar Extinction Coefficients of the Products of Hydroxyethylation of Uracil, Adenine, and Cytosine

Compound	R_f		Electrophoretic mobilities (pH)	UV spectrum	
	A	B		λ, nm	ε×10⁻³
VI	0,36	0,26	1 (11)	272	5,08
VIII	0,38	0,32	0,69 (11)	245	3,66
VII	0,49	0,44	0,44 (11)	268	5,73
IX	0,49	0,50	(11) at cathode	270	8,90
II	1,0	0,36	1 (3,8); (11) at anode		
IV	0,86	0,45	0,9 (3,8); (11) at cathode	261	13,10
V	0,71	0,57	1,7 (3,8); (11) at cathode	273	15,00
III			(12) at anode	280	10,40
X			(12) at cathode	280	15,50

11 make it possible to separate all four components in one step. The mixture of II, IV, and V was separated in both system B and system A; however, a considerably longer path is necessary for separation in system A, and the mobilities of isomers IV and V in this system were therefore assigned to the mobility of II. Electrophoresis carried out successively in alkaline and acidic media also makes it possible to separate these three components. The mixture of III and X is not separated by electrophoresis at pH 11, but the compounds migrate to different sides from the start at pH 12.

The attachment of the hydroxyethyl groups to the nitrogen atoms in the hydroxyethylation products was proved by spectrophotometry [2]. The λ_{max} bands of VI at pH 7.2 and 12 are found at 260 and 280 nm, respectively, the bands of the isobestic points are located at



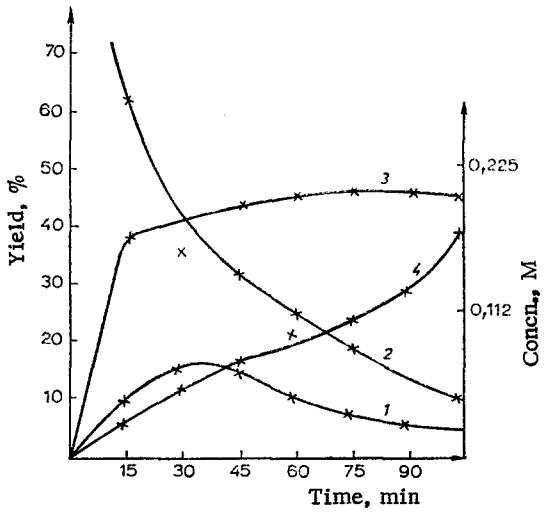


Fig. 1. Consumption of uracil during its hydroxyethylation with ethylene carbonate as a function of the time: 1) concentration and yield of 3-(2-hydroxyethyl)uracil (VII); 2) conversion of uracil VI; 3) concentration and yield of 1-(2-hydroxyethyl)-uracil (VIII); 4) concentration and yield of 1,3-bis(2-hydroxyethyl)uracil (IX).

235 and 268 nm. In the case of isomer VIII the λ_{max} bands at pH 7.2 and 12 are found at 265 nm, and the band of the isobestic point is located at 245 nm. A change in the pH does not affect the UV spectrum of uncharged IX. The λ_{min} band at pH 1 for V is found at 236 nm, and the band at pH 12 is located at 245 nm ($\Delta\lambda = -9$ nm). The λ_{min} band for isomeric IV is found at 235 nm at pH 1 and at 230 nm at pH 12 ($\Delta\lambda = +5$ nm). The structure of VIII was additionally confirmed by alternative synthesis through 4-ethoxy-2-pyrimidone (XI). Hydroxyethylation of pyrimidone XI excludes attachment of the hydroxyethyl group to 3-N. The presence of an exo-amino group in X was confirmed by its conversion to VIII by deamination with nitrous acid.

To study the dynamics of hydroxyethylation we periodically selected samples from the reaction mixtures and analyzed them by chromatography or electrophoresis on paper. The conversion of the heterocyclic base (α), the yields of the products (β), and the concentrations of the components were determined by the method in [4]. For base VI and its condensation products we can write

$$M_{VI}^{1} = M_{VI} + M_{VII} + M_{VIII} + M_{IX},$$

where M_{VI}^{1} is the number of moles of VI in the sample undergoing separation prior to the start of the reaction, and M_{VI-IX} is the number of moles of VI-IX in the spots of the separated sample. For the conversion of VI (α_{VI}) and VII (β_{VII})

$$\alpha_{VI} = \frac{M_{VII} + M_{VIII} + M_{IX}}{M_{VI} + M_{VII} + M_{VIII} + M_{IX}} \cdot 100\%,$$

$$\beta_{VII} = \frac{M_{VII}}{M_{VI} + M_{VII} + M_{VIII} + M_{IX}} \cdot 100\%.$$

The yields of the other compounds were similarly determined. The concentrations of the components were determined by multiplication of their fractions by the initial concentration of the base. The number of moles of analyzable substances in the spots were determined by spectrophotometry after their elution. The molar extinction coefficients are presented in Table 2. This method of calculation does not require allowance for the volume of the sample.

The consumption of base VI during its hydroxyethylation is presented in Fig. 1. It is apparent from Fig. 1 (curve 1) that hydroxyethylation in the 3-N position was accompanied by an increase in the concentration of isomer VII to 0.07 mole/liter (16% yield) in 30 min; the concentration of unchanged base VI in this case was 0.19 mole/liter (curve 2). After 105 min, the concentration of base VI decreased to 0.04 mole/liter, and the concentration of VII decreased to 0.0225 mole/liter. The hydroxyethylation of VI position proceeds at a higher rate in the 1-N than in the 3-N position (curve 3), and the ratio of the initial rates of formation of isomers VII and VIII was 1:3.6. The maximum concentration of isomer VIII (0.21 mole/liter) was attained after 75 min, and the concentration of the unchanged base VI was 0.085 mole/liter. It is interesting that the rate of formation of IX changed only slightly

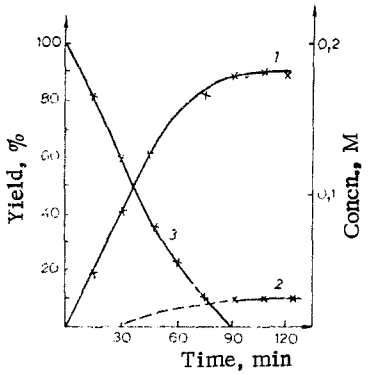


Fig. 2

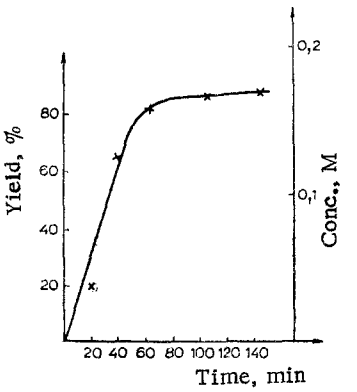


Fig. 3

Fig. 2. Consumption of adenine during its hydroxyethylation with ethylene carbonate as a function of the time: 1) concentration and yield of 9-(2-hydroxyethyl)adenine (IV); 2) concentration and yield of 3-(2-hydroxyethyl)adenine (V); 3) conversion of adenine.

Fig. 3. Accumulation of hydroxyethylcytosine in the hydroxyethylation of cytosine with ethylene carbonate.

during the reaction (curve 4), although the conversion of base VI reached 92%. This trend of the kinetic curves indicates that hydroxyethylation of both the 1-N and 3-N positions in base VI occurs in the first step of the reaction and that isomers VII and VIII are intermediates in the synthesis of bis(hydroxyethyl) compound IX.

The accumulation of IV and V proceeded in a different manner in the hydroxyethylation of base II (Fig. 2, curves 1 and 2). The concentrations of both isomers increased continuously and at the end of the reaction (curve 3) reached 0.18 and 0.02 mole/liter, respectively (90 and 10% yields). Simultaneous hydroxyethylation of the two tautomeric forms of base II evidently occurs in this case. The only hydroxyethylation product detected in the reaction of III with I was X (Fig. 3). Thus the exo-amino groups of II and III do not react with reagent I.

Chromatographically pure IV and X were isolated by recrystallization from alcohol (Tables 1 and 2). To isolate VIII we used chromatography on Dowex-2 anion-exchange resin with elution of the reaction products with 0.1 N acetic acid.

EXPERIMENTAL

The pH values were measured with an LPU-01 pH meter. The optical densities were measured with an SFD-2 spectrophotometer in a 1-cm thick quartz cuvette. The molar extinction coefficients of the analogs of the examined compounds [5, 6] were used (Table 2). Descending chromatography and electrophoresis on paper were used for the analytical separation of the reaction mixtures. Leningrad S paper from the Volodarsk Plant was used. The electrophoretic separations were carried out with a Labor horizontal electrophoretic apparatus (Hungary); the distance between the electrode cuvettes was 30 cm, the field strength was 30 V/cm, and the separation time was 50 min. The following solvent systems were used: A) butanol saturated with water; B) butanol—concentrated ammonium hydroxide—water (50:8:42). The mobilities relative to adenine are presented for IV and V in system A. The electrophoretic separation of mixtures of VI, VII, VIII, and IX and II, IV, and V was carried out at pH 11 in an 0.05 mole/liter lithium carbonate buffer, at pH 3.8 in an 0.1 mole/liter ammonium acetate buffer, and at pH 12 in 0.01 N NaOH. The mobilities of VII and VIII were assigned to the mobility of base II. The reaction mixture was applied to the chromatographic paper in 2 cm bands, and, after separation of the reaction mixture, the chromatograms were dried in a stream of cold air and developed with a UI-1 chromatoscope (254 nm). The reaction products were eluted with distilled water or 0.1 N HCl (6 ml).

9-(2-Hydroxyethyl)adenine (IV). A mixture of 1.67 g (12.4 mmole) of dried [at 110°C (2 mm)] base II and 1.09 g (12.4 mmole) of I in 62 ml of dry DMF containing traces of NaOH was heated at 160°C for 2 h, after which the DMF was removed by vacuum distillation, and the residue was recrystallized from alcohol to give chromatographically pure hydroxyethyladenine IV (Tables 1 and 2). 1-(2-Hydroxyethyl)cytosine (X) was similarly obtained and isolated.

The concentrations of VI and I were 0.45 and 0.5 mmole, respectively, during the study of the dynamics of the hydroxyethylation of uracil.

Isolation of 1-(2-Hydroxyethyl)uracil (VIII). A 25-g sample of the combined reaction mixtures obtained in different factor experiments was dissolved in 300 ml of water, and the insoluble VI was removed by centrifugation. A 10^6 OE (265 nm) sample of the supernatant liquid was made alkaline to pH 11 with LiOH and sorbed from a volume of 1 liter with a 4 × 30 cm column filled with Dowex 2 × 8 resin (50-100 mesh) in the OH^- form at a rate of 150 ml/h. Compound IX was not sorbed under these conditions. The column was washed with an LiOH solution (pH 11) until IX was removed, after which $0.41 \cdot 10^6$ OE of IX in 2.2 liters of eluate were obtained. The column was washed with water until the eluate was neutral, after which it was washed with 6 liters of 0.1 N acetic acid. Subsequent elution with 0.1 N acetic acid gave $0.28 \cdot 10^6$ OE of VIII (in the first 1.3 liters of eluate) and $0.18 \cdot 10^6$ OE of a mixture of VI, VII, and VIII (in the next 0.1 liter of eluate). The residual base VI was eluted with 1 N acetic acid. The mixture of VI-VIII was rechromatographed with a 2 × 25 cm column to give an additional $0.09 \cdot 10^6$ OE of isomer VIII. The fractions containing VIII were combined and evaporated to dryness, and the residue was recrystallized from alcohol (Tables 1 and 2).

Preparation of 1-(2-Hydroxyethyl)uracil (VIII) through 4-Ethoxy-2-pyrimidone (XI). Pyrimidone XI was obtained by the method in [7]; the absence of 2-ethoxy-4-pyrimidone in it was confirmed by paper chromatography in system B. A mixture of 1 g (7.1 mmole) of XI and 0.7 g (7.9 mmole) of reagent I was refluxed in 60 ml of dry DMF containing traces of NaOH. The mixture was cooled, and the resulting sediment was removed by filtration. The DMF was removed by vacuum distillation, and the syrupy residue was refluxed for 1 h in a mixture of concentrated HCl and water (1:3). The HCl was removed by codistillation with alcohol, and the residual syrup was crystallized by treatment with absolute alcohol. Recrystallization from alcohol gave a substance identical to VIII (Tables 1 and 2). The yield was 0.6 g (60%).

Deamination of 1-(2-Hydroxyethyl)cytosine (X). A solution of 0.1 g of $NaNO_2$ in concentrated HCl was added in the course of 4 days in 24 h to a cooled (to 0°C) solution of 0.1 g of hydroxyethylcytosine X in 2 ml of concentrated HCl. Samples were periodically removed and analyzed by electrophoresis on paper (with an 0.05 N ammonium acetate buffer at pH 3.8). By the fifth day X was virtually completely converted to VIII.

LITERATURE CITED

1. Z. A. Shomshtein and S. A. Giller, No. 1, 27 (1976).
2. G. E. Ustyuzhanin, V. V. Kolomeitseva, and N. S. Tikhomirova-Sidorova, Zh. Obshch. Khim., 43, 2093 (1973).
3. J. Pitha and P. Ts'o, J. Org. Chem., 33, 1341 (1968).
4. N. S. Tikhomirova-Sidorova, G. E. Ustyuzhanin, and É. M. Kogan, Biokhimiya, 32, 867 (1967).
5. J. J. Fox and D. Shugar, Biochim. Biophys. Acta, 9, 199 (1952).
6. N. Ueda, T. Kawabata, and K. Takemoto, J. Heterocycl. Chem., 8, 827 (1971).
7. G. E. Hilbert and E. F. Jones, J. Am. Chem. Soc., 57, 552 (1935).
8. A. R. Katritzky and A. J. Waring, J. Chem. Soc., No. 1, 1540 (1962).