STUDY OF THE HYDROLYTIC STABILITY OF 5-TRIMETHYLSILYL-2'-DEOXY-α-D-URIDINE, POSSESSING ANTIVIRAL ACTIVITY

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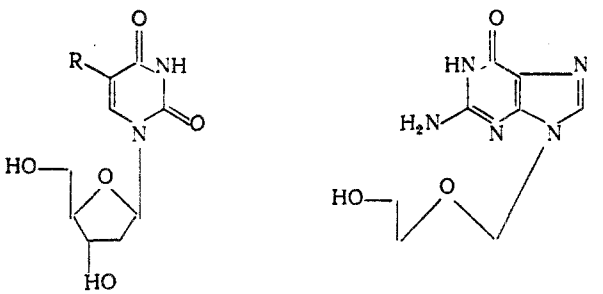
Among the analogs of natural nucleosides and deoxynucleosides, substances have been detected that selectively suppress the development of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) [6]. In this series we should mention first 5-iodo-2'-deoxyuridine (I), used for the treatment of herpetic eye lesions, (E)-5-(2-bromovinyl)-2'-deoxyuridine (II), which has been shown to be effective toward HSV-1, as well as acyclovir-9-(2-hydroxyethoxymethyl)guanine (III), which is being investigated widely in clinics throughout the world. Whereas I and II are active only toward HSV-1 virus, III also acts on HSV-2, which causes genital infections. The selectivity of the antiviral action of these preparations is due to the fact that the first stage of their bioactivation – o-phosphorylation – occurs under the action of thymidine kinase, encoded by the viral genome; these substances are not substrates for the thymidine kinase of mammalian cells and therefore are nontoxic for uninfected cells.



I—II III

I: R = I; II: R = —HC=CHBr- trans

The side study of a large group of 5-substituted 2'-deoxyuirdines led to the discovery of antiherpetic activity of 5-trimethylsilyl-2'-deoxy-α-uridine (IV) [4, 5]. The substance is effective when used systematically in experimental herpetic encephalitis of cotton rats, in local application in herpetic lesions of the skin and eyes of rabbits (induced by HSV-1 virus), as well as in experimental herpes of the genitals of guinea pigs, induced by HSV-2 virus.

An important structural peculiarity of the deoxynucleoside IV is the fact that it is a 2'-deoxy-α-nucleoside, which differs from the natural 2'-deoxynucleosides and their known biologically active analogs in the configuration of the glycoside bond. Up to the present time, no antiviral antimetabolites with an α-configuration of the glycoside bond had been known. It can be assumed that the mechanism of the bioactivation of IV in virus-infected cells differs from the mechanism of the bioactivation of preparations I-III. In such a case, IV may prove effective toward strains of herpes virus resistant to the action of preparations I-III.

To develop drug forms of IV and to predict their storage periods, its hydrolytic stability must be investigated. Hydrolytic decomposition in several directions is possible for IV: the C-Si bond may be cleaved and unsubstituted 2'-deoxy-α-uridine (V) may be formed from IV. The examples of the hydrolysis of C-trimethylsilyl-derivatives of heterocycles with the formation of trimethylsilanol and the corresponding C-unsubstituted heterocycle are known [8]. The possibility of cleavage of the glycoside bond and the formation of

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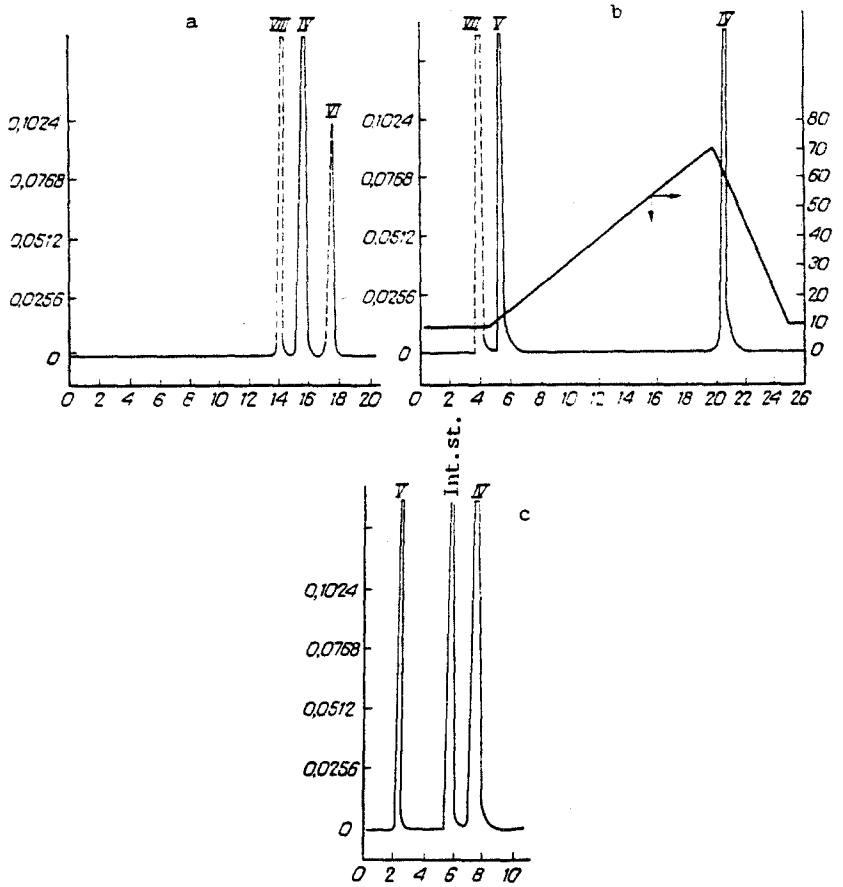
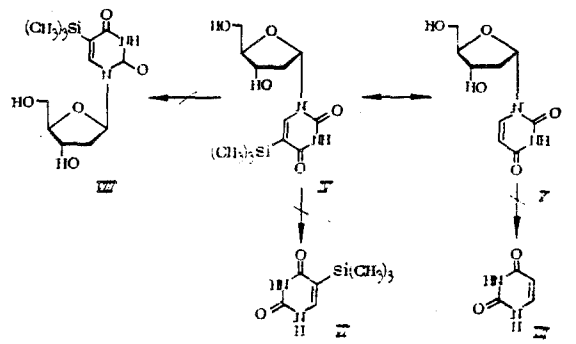


Fig. 1. Chromatogram of the separation of possible hydrolysis products of the nucleosides IV. The dotted lines show compounds not found in the hydrolysis of IV; a) isocratic system, 0.05 M $(NH_4)H_2PO_4$—methanol (60-40%); b) the same system, but with a gradient. The profile of the gradient is indicated; c) isocratic system, water—methanol (60-40%). Along x-axis: time (in min); along y-axis: optical density (in optical density units); in Fig. 1b, along the right-hand y-axis: methanol concentration (in %).

2-deoxy-D-ribose and 5-trimethylsilyluracil (VI) could not be excluded; VI might give the unsubstituted uracil (VII). As described in [9], during the revision of the article in the British Pharmacopoeia (BP, 1973) for 5'-iodo-2'-deoxyuridine, it was established by high-performance liquid chromatography that during its hydrolysis there is a cleavage of the glycoside bond with the formation of 2-deoxyribose and 5-iodouracil, which is then converted to uracil. Moreover, it was necessary to reckon with the possibility of anomerization of IV and the formation of 5-trimethylsilyl-2'-deoxy-β-uridine (VIII).



In a study of the products of hydrolysis of the deoxynucleoside IV we used the method of HPLC in a reversed-phase variant. The conditions of analysis under which complete separa-

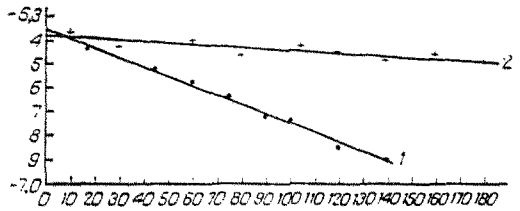


Fig. 2

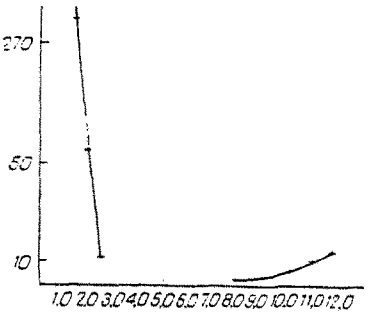


Fig. 3

Fig. 2. Semilogarithmic plots of the kinetic curve of hydrolysis of IV. 1) pH 1.5, T 333 K; 2) pH 12.0, T 348 K. Along x-axis: time (in min); along y-axis: log C (C is the concentration of IV).

Fig. 3. Dependence of the rate constant of hydrolysis (k) on the pH of the medium. Along x-axis: pH; along y-axis: values of $k \cdot 10^6$ (in sec^{-1}).

TABLE 1. First-Order Rate Constants k (M ± m) and Half-Life ($t_{1/2}$) of the Hydrolysis of Nucleosides IV and VIII

pH	T, K	IV		VIII	
		$k \cdot 10^6, sec^{-1}$	$t_{1/2}$, h	$k \cdot 10^6, sec^{-1}$	$t_{1/2}$, h
1,5	333	64,2±2,5	3,0	23,7±2,9	7,2
1,5	338	108,2±1,8	1,78	40,2±4,1	4,8
1,5	343	189,0±2,8	1,02	68,4±6,8	2,8
1,5	348	276,6±3,1	0,68	115,0±11,5	1,7
2,0	348	55,4±2,1	3,4	—	—
2,5	348	10,8±1,0	17,7	—	—
8,0	348	2,1±0,4	90,9	—	—
10,3	348	5,1±0,3	38,5	—	—
11,2	348	9,2±0,7	20,9	—	—
12,0	343	6,7±0,5	28,7	—	—
12,0	348	10,7±1,0	17,9	—	—
12,0	353	15,5±0,7	12,4	—	—

tion of IV and possible products of its hydrolysis – V, VI, VII, and VIII – was achieved were developed. The separation of IV, VIII, and VI could be achieved in an isocratic system (Fig. 1a). The more complex problem of separating IV, V, and VII was solved by the method of gradient elution. The results obtained and the profile of the gradient are shown in Fig. 1b. Subsequently these methods were used in the analysis of the reaction mass of hydrolyzed IV.

For a more complete disclosure of the possible pathways of hydrolysis of IV we studied rigorous conditions of hydrolysis: temperature 348 K and pH 1.5. In analysis by the HPLC method it was found that the reaction mass contains only the initial IV and V. This provided us with a basis for believing that the hydrolysis of IV occurs exclusively to 2'-deoxyuridine with breaking of the C–Si$(CH_3)_3$ bonds. In this case evidently trimethylsilanol is also formed and is rapidly converted to hexamethyldisiloxane. From this it follows that the rate of decomposition of IV can be monitored by observing the change in the concentration of IV or V. For greater convenience, we monitored the content of IV.

The concentration of IV in the reaction mass was determined by an internal standard method [2]; a derivative of 2-deoxyuridine resistant to hydrolysis – 5-(1-hydroxyhexafluoroisopropyl)-2'-deoxyuridine [3] – was selected as the internal standard.

A chromatogram of the separation of the internal standard, IV, and its only hydrolysis product, V, is presented in Fig. 1c.

We established experimentally that the hydrolysis of IV is described by a first-order kinetic equation. As an example, Fig. 2 presents semilogarithmic plots of the kinetic curve

TABLE 2. Activation Parameters of the Hydrolysis of Nucleosides IV and VIII

Compound	pH	ΔE, kJ/mole	ΔH*, kJ/mole	ΔS*, kJ/(mole·deg)
IV	1,5	94,3	91,4	—76,8
	12,0	84,9	81,9	—83,4
VIII	1,5	95,9	92,9	—78,6

*The values of ΔH and ΔS were determined at the temperature 348 K.

in the hydrolysis of IV in acid and alkaline media. The pseudocontacts of the rate of hydrolysis, cited in Table 1, were calculated by the method of least squares [1].

To select the optimum pH value for the development and storage of the drug form we studied the hydrolytic stability of IV in the range of pH 1.5-12.0. The data obtained are presented in Fig. 3. With increasing pH value in acid medium, the rate of hydrolysis of IV decreases sharply, while in alkaline medium it gradually rises. In the range of pH 6.5-7.1, even at a temperature of 348 K for 24 h, no hydrolysis products were observed.

Extremely important for the prediction of the stability of the drug form, as well as a further elucidation of the mechanism of the hydrolysis of IV is a study of the activation parameters of the hydrolysis process. We investigated the hydrolysis of IV both in acid and in alkaline media, in the temperature range 333-353 K. The activation energy (ΔE) was calculated by the method of least squares [1], the enthalpy (ΔH) and entropy (ΔS) according to the recommendations of [1]. The data obtained are presented in Table 2. We should mention the closeness of the values of the activation parameters in the hydrolysis of IV in acid and alkaline media, despite a substantial difference in the reaction rate.

It was also of interest to study the hydrolysis of the β-anomer VIII to compare its reactivity with the α-anomer to determine the possibility of anomerization of IV during hydrolysis. The hydrolysis of IV and VIII was investigated by the method of competitive reactions [7]. The reaction mass was analyzed by HPLC in an isocratic system. The calculated rate constants of hydrolysis and the half-lives are presented in Table 1, while the activation parameters of the reaction are presented in Table 2. It was established that the breakdown of the β-anomer VIII is somewhat slower than that of the α-anomer IV. Consequently, the possibility of the formation of the anomerization product (VIII) in the hydrolysis of IV and its subsequent rapid hydrolysis at the glycoside bonds can be excluded.

Thus, it was established by HPLC that the main products of the hydrolysis of IV and VIII are the corresponding 2'-deoxyuridines, i.e., cleavage occurs only at the C—Si$(CH_3)_3$ bond and there is no breakdown at the glycoside bond. Nor was anomerization of the α-nucleoside IV noted. A study of the hydrolytic stability of IV in a wide range of pH values at the temperatures selected permitted a determination of the region of pH values, namely, from 5.0 to 7.1, at which aqueous solutions of IV are most stable, which is of practical significance in the creation of its drug forms.

EXPERIMENTAL

A solution of IV in distilled water [10 ml, concentration of IV $(1.6\text{-}3.4)\cdot10^{-3}$ M] was introduced into a four-necked round-bottomed 50-ml flask, equipped with a mixer, reflux condenser, and thermometer, and kept at a constant set temperature with an accuracy ±0.1 K for 10-15 min with mixing; the buffer with the requisite pH was thermostatically controlled separately. The solution was adjusted to pH 1.5 by adding hydrochloric acid; the remaining buffer solutions were prepared on the basis of 0.2 M KH_2PO_4, adding solutions of H_3PO_4 or KOH. A 10-ml portion of the thermostatically controlled buffer was introduced into the flask, and samples were collected at definite time intervals, cooled rapidly, and stored at 273 K.

High-performance liquid chromatography was performed on a 1084-B liquid chromatograph from Hewlett-Packard (USA), using a 250 × 4 mm column, packed with the adsorbent Silasorb

C18 (Czechoslovakia) with particle size 10 μ; column temperature 313 K. The rate of flow of the mobile phase was 1 ml/min, volume of the sample 10 μl.

The concentration of IV in the reaction mass was determined by the internal standard method in an isocratic system. The mobile phase used was 40% aqueous methanol. The standards and the hydrolysate of IV were analyzed on 250 × 4.6 mm columns from Du Pont (USA), packed with the adsorbent Zorbax C8 from Du Pont (USA) with particle size 7 μ. The following system was used as the mobile phase: 0.05 M $(NH_4)H_2PO_4$–methanol. In the analysis of IV, VIII, and VI in the reaction mass, a system containing 30% methanol was used. V and VII were analyzed using a gradient system. The profile of the gradient is indicated in Fig. 1b.

LITERATURE CITED

1. A. J. Gordon and R. Ford, The Chemist's Companion [Russian translation from the English], Moscow (1976).
2. L. A. Kogan, Quantitative Gas Chromatography [in Russian], Moscow (1975), p. 127.
3. S. Ya. Mel'nik, A. A. Bakhmedova, T. P. Nedorezova, et al., Bioorg. Khim., 7, No. 7, 1047-1053 (1981).
4. S. Ya. Mal'nik, A. A. Bakhmedova, T. D. Miniker, et al., Bioorg. Khim., 10, No. 12, 1645-1654 (1984).
5. M. N. Preobrazhenskaya, S. Ya. Mel'nik, A. A. Bakhmedova, et al., USSR Patent No. 671287; Otkrytiya, No. 32 (1983).
6. M. N. Preobrazhenskaya, and S. Ya. Mel'nik, Results of Science and Technology. Bioorganic Chemistry Series [in Russian], Vol. 1, Moscow (1984), p. 109.
7. N. M. Émanuél' and D. G. Knorre, Course in Chemical Kinetics [in Russian], Moscow (1983).
8. R. S. Brown, H. Slebocka-Tilk, I. M. Bushchek, and J. G. Ulan, J. Am. Chem. Soc., 106, 5979-5984 (1984).
9. G. P. R. Carr, J. Chromatogr., 157, 171-184 (1978).