TAUTOMERIC EQUILIBRIUM OF 1-β-D-RIBOFURANOSYL-2-KETO-4-(N-METHOXYAMINO)PYRIMIDINE

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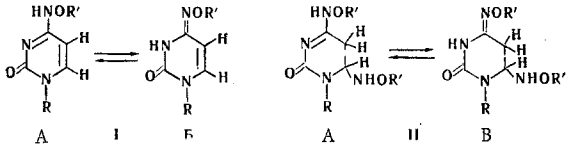
The 1-β-D-ribofuranosides of 2-keto-4-(N-methoxyamino)pyrimidine, 2-keto-4-(N-methyl-N-methoxyamino)pyrimidine, and 2-keto-3-methyl-4-(N-methoxyamino)pyrimidine were synthesized, and their pK_a values were determined by spectrophotometry. The pK_a values of the compounds are evidence that the tautomeric equilibrium between the oxime and hydroxyamine forms of 1-β-D-ribofuranosyl-2-keto-4-(N-methoxyamino)pyrimidine in aqueous solutions is shifted to favor the oxime form ($K_T \simeq 25$).

The widely distributed mutagens hydroxylamine and O-methylhydroxylamine lead to two types of genetic effects during their action on biological objects in weakly acid (pH 4-6) media – they induce transitions of the GC–AT type or lead to inactivation of the genetic material [1,2].

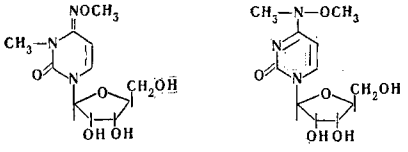
When hydroxylamine and O-methylhydroxylamine act on DNA, they primarily modify the cytosine ring to form compounds of the I and II types [2]:



Ia R=β-D-ribofuranosyl, R'=CH_3; Iб R=CH_3, R'=H.

We have previously assumed that the reason for the transitions during the action of hydroxylamine and O-methylhydroxylamine is the formation of compounds of the I type, while the reason for inactivation is the formation of compounds of the II type [3]. It has also been proposed that compound I is represented by imino form IB to a much greater degree than cytosine; the electronic structure of IB is closer to the uracil ring than to the cytosine ring.

Data on the analogous tautomeric equilibrium of 1-methylcytosine derivatives that attest to the predominance of the imino form were recently published [4]. However, a more adequate model of the nucleoside link of I is 1-β-D-ribofuranosyl-2-keto-4-(N-methoxyamino)pyrimidine (Ia), in connection with which we have studied the tautomeric equilibrium of this compound.



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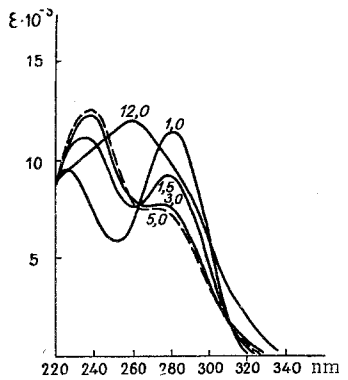

Fig. 1. Spectra of 1-β-D-ribofuranosyl-2-keto-4-(N-methoxyamino)pyrimidine in aqueous solutions at various pH values (0.002 M McElvain buffer). (The numbers on the curves are the pH values.)

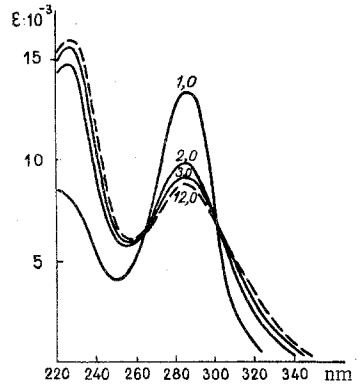

Fig. 2. Spectra of 1-β-D-ribofuranosyl-2-keto-3-methyl-4-(N-methoxyamino)-pyrimidine in aqueous solutions at various pH values (0.002 M McElvain buffer). (The numbers on the curves are the pH values.)

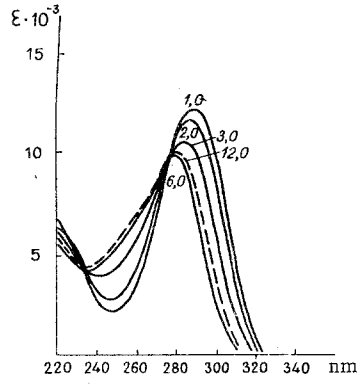

Fig. 3. Spectra of 1-β-D-ribofuranosyl-2-keto-4-(N-methyl-N-methoxyamino)-pyrimidine in aqueous solutions at various pH values (0.002 M McElvain buffer). (The numbers on the curves are the pH values.)

To determine the tautomeric equilibrium constants of Ia we used the well-known method of comparison of the ionization constants of the fixed tautomeric forms [5]. For this, we used 1-β-D-ribofuranosyl-2-keto-3-methyl-4-(N-methoxyamino)pyrimidine (III) and 1-β-D-ribofuranosyl-2-keto-4-(N-methyl-N-methoxyamino)pyrimidine (IV). The spectra of all three compounds are presented in Figs. 1-3. It is seen that the spectra of all of the products are analogous in the acid pH region (~1). Hence it can be concluded that their cations have the same electronic structure, and the method in [5] is applicable in this case.

Isosbestic points are observed at pH 1-5 for all three products, which apparently is evidence that the change in the spectra with changing pH is due to interaction of only two chromophores – the monoprotonated and neutral heterocyclic systems. This provides a possibility for determining the pK_a by the spectral method by construction of the dependence of the optical densities of solutions with the same concentration of the investigated compounds on their pH values. The pK_a values determined from the optical densities for Ia, III, and IV were 1.18, 1.53, and 3.05, respectively (determined from 280 nm for Ia, from 285 nm for III, and from 290 nm for IV). From the pK_a values of the latter two compounds, one can estimate the tautomeric equilibrium constant in Ia as $K_T = K_a^{IV}/K_a^{III} = 25$. The predominance of the oxime form over the methoxyamino form in the tautomeric equilibrium of Ia follows also from a comparison of the spectra of the above three compounds in the neutral form. It is easy to see (Figs. 1-3) that there is a close analogy in the spectra of Ia and III at pH 5-6, while the spectrum of IV differs substantially from the spectra of the first two compounds.

It should be noted that the oxime form apparently also predominates in the tautomeric equilibrium of I when R' = H, since the spectrum of its neutral form is practically identical to the spectrum of the neutral form of Ia [6].

The equilibrium constant is in agreement in order of magnitude with the tautomeric equilibrium constant (K_T ~ 10) obtained by Brown [4] for 1-methyl-2-keto-4-(N-hydroxyamino)pyrimidine (Ib). The larger numerical value of the constant obtained in this paper is apparently associated with the electronic effect of the substituents.

EXPERIMENTAL

O-Methylhydroxylamine hydrochloride was prepared by the method in [7] and had mp 146-148°. O,N-Dimethyldihydroxylamine hydrochloride was prepared by method in [8] and had mp 113-115°. The cytidine was obtained from the Reanal company and was used without further purification. 3-Methylcytidine was obtained from cytidine by methylation with dimethyl sulfate by the method in [9]. 4-Thiouridine was obtained by the method in [10] and was purified by chromatography on FN-12 paper with an isoamyl alcohol–acetone–water (3:2:1) system (system 1).

1-β-D-Ribofuranosyl-2-keto-4-(N-methoxyamino)pyrimidine (Ia). A solution of 50 mg (0.2 mmole) of cytidine in 0.75 ml of a 3.75 M solution of O-methylhydroxylamine hydrochloride (pH 5.0) was thermostatted at 35° for 72 h, after which the pH of the reaction mixture was brought up to zero with concentrated hydrochloric acid, and the mixture was thermostatted at 35° for 24 h. The solution was neutralized with 4 N KOH, and the excess O-methylhydroxylamine was removed by adding 0.5 ml of cyclohexanone and extracting the resulting cyclohexanone O-methyloxime with ten 1-ml portions of ether. The reaction product was isolated by paper chromatography with an isopropyl alcohol–ammonia–water (7:2:1) system (system 2); R_f 0.84. The R_f values of cytidine and uridine in this system were 0.55 and 0.56, respectively.

1-β-D-Ribofuranosyl-2-keto-3-methyl-4-(N-methoximino)pyrimidine (III). A solution of 70 mg (0.27 mmole) of 3-methylcytidine in 3 ml of 1.5 M solution of O-methylhydroxylamine hydrochloride (pH 5.0) was thermostatted at 40° for 88 h, after which the pH of the reaction mixture was brought to zero with concentrated hydrochloric acid, and the mixture was thermostatted at 40° for 24 h. The solution was neutralized with 4 N KOH, and the reaction product was separated from the salts by gel filtration on Sephadex G-10. The product was purified by paper chromatography in system 2; R_f 0.82. The R_f value in an ethanol – 0.5 M ammonium acetate (pH 3.0) (5:2) system (system 3) was 0.87.

1-β-D-Ribofuranosyl-2-keto-4-(N-methyl-4-N-methoximino)pyridimidine (IV). A solution of 19.32 mg (0.075 mmole) of 4-thiouridine in 3 ml of 1 M O,N-dimethylhydroxylamine hydrochloride (pH 5.0) was thermostatted at 50° for 96 h. The precipitate was removed by filtration, 8 ml of absolute alcohol was added to the supernatant liquid, and the precipitated O,N-dimethylhydroxylamine was removed by filtration. The reaction product was isolated by chromatography in system 2 (R_f 0.76), and the final purification was carried out by chromatography in the same system. The R_f values in system 3 and system 1 were 0.85 and 0.75, respectively. The extinctions of the products were determined by the relative percentage of ribose in the solutions of the investigated compounds after bromination by the method in [11].

The pH was determined with an LPU-0.1 potentiometer; the pK_a values were determined from the dependence of the optical density on the pH in a series of buffer solutions (McElvain buffer, 0.002 mole/liter) containing the same concentrations of the compound. The solutions had pH values ranging from 0 to 5.

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