Spectroscopic Study of the Basicity of 4,6-Dihydroxypyrimidine Derivatives

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ABSTRACT: The protonation of a number of 4,6-dihydroxypyrimidine derivatives is studied, and the features of the electronic spectra of free bases and protonated forms are considered. It is shown that the alkyl substituents in position 2 increase the basicity of the compound, and the nitro group in position 5 leads to its decrease. In an acid medium (0.1−99.5% H_2SO_4), 4,6-dihydroxypyrimidine, 6-hydroxy-2-methylpyrimidine-4(3H)-one, and 6-hydroxy-2-ethylpyrimidine-4(3H)-one have two protonation stages, barbituric acid is protonated in three stages, and 6-hydroxy-2-methyl-5-nitropyrimidine-4(3H)-one and 6-hydroxy-2-ethyl-5-nitropyrimidine-4(3H)-one form a monocation.
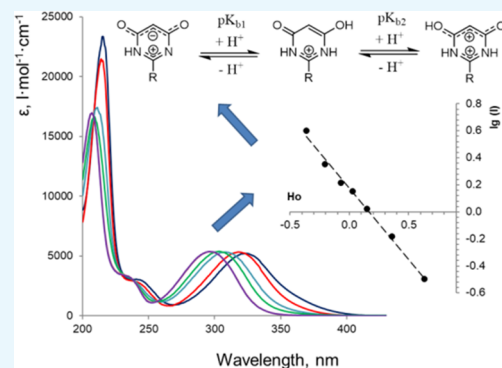
INTRODUCTION

An important feature of pyrimidinediones is the presence of amphoteric properties and prototropic tautomerism. In neutral and alkaline environments, many of them form mono- and dianions. In a strong acidic medium, protonation occurs, which, due to the presence of several centers, proceeds with the formation of mono-, di-, and tricharged cations. It is obvious that tautomerism and acid−base properties have a strong influence on the properties and reactivity of compounds. These issues have repeatedly been the subject of research.[1]

In particular, protonation has a strong influence on the nitration of heterocyclic compounds. The main works in this direction are the cycle of research by Katritzky, which considers, among other things, the nitration of pyrimidinones.[2] Using the same methods, we studied the effect of protonation on the nitration of 6-hydroxy-2-methylpyrimidine-4(3H)-one (1) and showed that the free base is involved in the process.[3]

Among the pyrimidine derivatives, the uracil analogue 4,6-dihydroxypyrimidine (2) can be distinguished. Its derivatives include the well-known barbituric acid (3), which is the parent compound for the same-name class of drugs. Recently, a number of 4,6-dihydroxypyrimidine derivatives have been found to have urease inhibition activity,[4] and it is also used in the synthesis of compounds that are immune-activated NO production inhibitors.[5] Nitration of 2 is proposed to obtain nitroform.[6] Industrial production of the insensitive explosive 1,1-diamino-2,2-dinitroethylene is based on the nitration of 1.[7]

Several studies are devoted to the study of the structure and tautomerism of 2 and its 5-alkyl derivatives. Short and Thompson[8] based on IR spectroscopy data suggested that in the solid state, it is in the lactam−lactim and dilactam forms (Figure 1, 2a, 2b, 2c, 2d). Based on nuclear magnetic

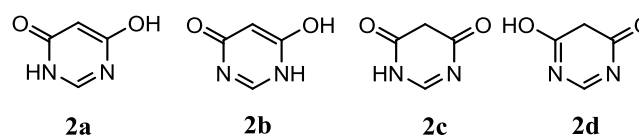**Figure 1.** Tautomeric forms of 4,6-dihydroxypyrimidine.

resonance (NMR) and UV spectroscopy data, a number of Russian and Australian researchers also tended to prefer the existence of form 2a in solutions in organic solvents.[9−11] Although this form dominates the solution, a small amount of dilactam (2c)[12,13] is allowed. Khromov−Borisov[9] based on NMR spectroscopy data and Katritzky[14] based on basicity measurements suggested that zwitterionic forms (Figures 2 and 3, 2e, 4a−d) are important in aqueous solutions.

Later, studies by Katrusiak and Katrusiak[15] using X-ray structure analysis showed that 4,6-dihydroxypyrimidine exists in two crystalline polymorphic forms—molecular and ionic. In the first of them, the molecules are in the form (2a), while in the ionic polymorph, the molecules are differentiated into positive and negative ions (Figure 2, 2f and 2g).
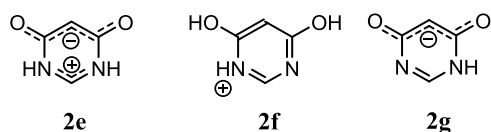
Figure 2. Ionic forms of 4,6-dihydroxypyrimidine.

An important argument for the existence of zwitterionic forms of 4,6-dihydroxypyrimidine derivatives is their asymmetric dimerization. Russian and Czech researchers[16,17] have shown the formation of such compounds **5a–d** in a series of N-methyl derivatives of 4,6-dihydroxypyrimidine (Figure 3). A crystal labile product of spontaneous dimerization of 4,6-dihydroxypyrimidine was obtained much later.[18] Its structure was determined as 2-(4,6-dioxo-5-pyrimidinyl)-4,6-dioxo-1,2,3,5,5-pentahydropyrimidine (**6**). It was shown that after dissolution in dimethyl sulfoxide (DMSO), the reverse transformation to **2** occurs.

Even more attention is paid to tautomerism and acid–base properties of barbituric acid. It is known that the most stable neutral form in solutions is keto form **3a**, which has been shown in numerous studies by both experimental and computational methods,[19,20] but the existence of a crystalline enol polymorph has been proved only recently.[21] The formation of mono- and dianions and their spectral properties are well studied.[22] Their protonation is less studied, and Zuccarello[23] considers the electronic spectra of barbituric acid and shows that in acidic media (up to 10 M HCl, H_o = 3.59), there is a cation that exists in two main equilibrium forms **3b** and **3c** with some percentage of **3d**. Olah,[24] in his work using the ^{1}H NMR spectroscopy method, shows the formation of the triprotonated form of **3e** in the medium trifluoromethane-sulfonic acid (H_o = 14.1), but data on pK_b are not given (Scheme 1).

A significant number of studies have been devoted to the peculiarities of protonation of thymine, uracil, 4,6-dihydroxypyrimidines. However, most of them focus on the issue of keto–enol tautomerism and forms of their existence in solutions and solid state, and not on the protonation site. For barbituric acid, there are no data of basicity constants for the second and third stages of protonation. Due to increased attention of researchers to 2-substituted 4,6-dihydroxypyrimidines as initial compounds for the synthesis of biologically active and energetic materials, major goals of this research were the investigation of the basicity of 6-hydroxy-2-methylpyrimidine-4(3H)-one (**1**), 4,6-dihydroxypyrimidine (**2**), barbituric acid (**3**), 6-hydroxy-2-ethylpyrimidine-4(3H)-one (**7**), 6-hydroxy-2-methyl-5-nitropyrimidine-4(3H)-one (**8**), and 6-hydroxy-2-ethyl-5-nitropyrimidine-4(3H)-one (**9**) (Figure 4) as well as the study of the influence of substituent in positions 2 and 5 on protonation direction and basicity constant determination.

It is well known that the chemical reactions of weak organic bases such as nitrosation, nitration, halogenation, etc., which take place in acidic media, could be strongly influenced by their acid–base properties. Basicity constants are a criterion for the quantitative assessment of acid–base properties and are used for the calculation of true rate constants of reactions. pK_b values allow us to choose a correct acidity of the reaction medium. There are no experimentally determined pK_b values for 2-substituted 4,6-dihydroxypyrimidines in the literature. Results of the present study could help us to obtain information about the structure and reactivity of 2-substituted 4,6-dihydroxypyrimidines and the mechanisms of reactions in acidic media. In addition, the basicity constants are reference values.

■ RESULTS

Protonation of 4,6-dihydroxypyrimidine and its derivatives was studied in a 0.1−100% sulfuric acid medium. The main method of research was spectroscopy in the ultraviolet region (UV spectroscopy). Liquid chromatography−mass spectrometry (LC−MS) and ^{1}H NMR were used to control possible chemical transformations.

There is no doubt that tautomeric transformations and dimerization of **2** and its derivatives can have a significant effect on protonation. Although according to IR, ^{1}H NMR, and LC−MS data, the **2** obtained by us does not contain **6** and in the solid state is in the lactam−lactim form **2a**, it was necessary to find the features of its behavior in solutions.

We studied its spontaneous dimerization in solutions in water, buffer solutions, and trifluoroacetic acid (TFA). In all cases, after dissolution **2**, dimer **6** begins to form (by LC−MS). The process is slow: at room temperature, in an aqueous solution, the equilibrium is reached in 2−3 days with a **6** content of 4%; in acidic buffer solutions with a pH of 4.8−3.6 and TFA, the equilibrium concentration is reached in 1−2 days with a content of ∼1%, while there is no noticeable change in the UV spectrum. Thus, the acidic medium suppresses dimerization and it does not affect the pK_b measurement. No dimeric forms were found in the study of **1**, **3**, and **7** solutions.

Protonation is a fast process, but if tautomeric transformations are slow and equilibrium is not achieved during the measurement, the result of pK_b determination will be distorted. Therefore, our study monitored changes in the UV spectra over time. The study showed that the spectra of **1**, **3**, and **7** in solutions in water and sulfuric acid of all concentrations do not change, the substances are chemically stable, and if tautomeric transformations occur during dissolution, then equilibrium is achieved during the preparation of solutions (about 5−15 min).

A different behavior was found for aqueous solutions of **2**: when registering the spectra 2−3 min after preparation, the
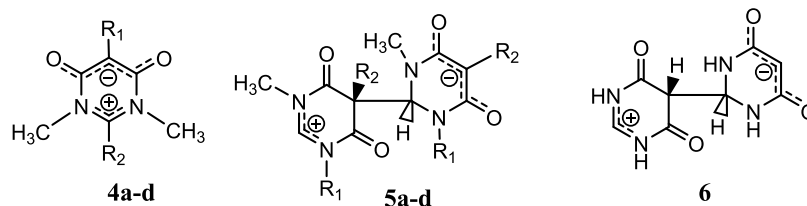

Figure 3. Zwitterionic and dimeric forms of the derivatives of 4,6-dihydroxypyrimidine: **4a**—$R_2 = R_1 = H$; **4b**—$R_1 = CH_3$, $R_2 = H$; **4c**—$R_1 = C_6H_5$, $R_2 = H$; **4d**—$R_1 = H$, $R_2 = CH_3$; **5a**—$R_1 = R_2 = H$; **5b**—$R_1 = H$, $R_2 = CH_3$; **5c**—$R_1 = CH_3$, $R_2 = H$; **5d**—$R_1 = R_2 = CH_3$.
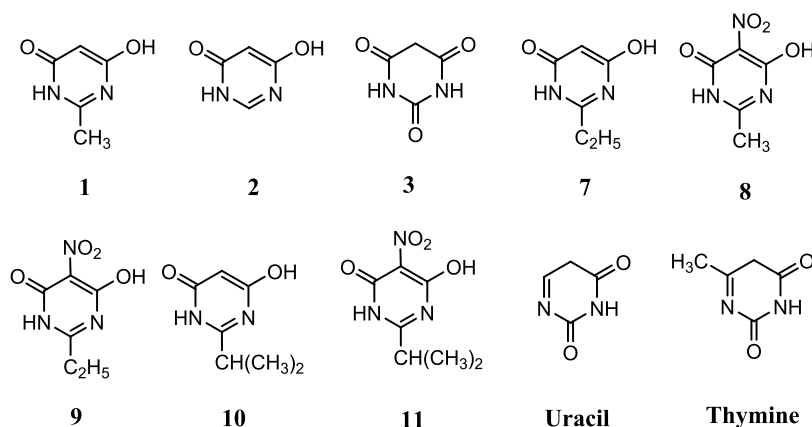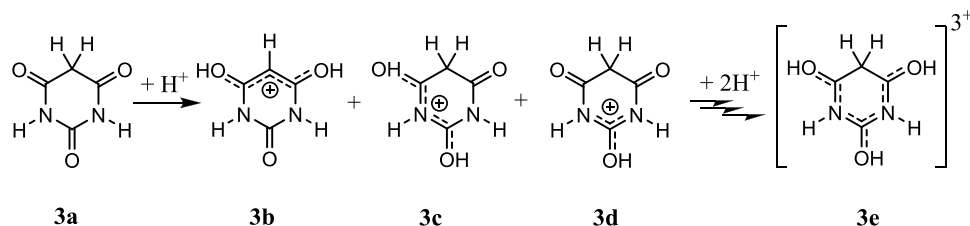
Scheme 1. Protonation of Barbituric Acid

Figure 4. Comparison of the structures of 4,6-dihydroxypyrimidine derivatives with uracil and thymine.

value of extinction coefficient (ε) at the maximum at 254 nm has a value of ~3000 L·mol^{-1}·cm^{-1}. Then, the optical density increases to the value of $\varepsilon \sim 8900$ L·mol^{-1}·cm^{-1}. The change in optical density is well described by the first-order kinetic equation of the reaction and takes about an hour at 20 °C. The position of the maximum and the profile of the spectrum do not change. These observations can be interpreted as the transformation of the lactam−lactim form **2a** in zwitterionic ion **2C**.[25] The ε value in a weak acid medium (0.5−1% H$_2$SO$_4$) is close to 9400 L·mol^{-1}·cm^{-1} and is reached immediately after the dissolution of **2** (<5 min). Repeated registration of the spectra after 1−4 h shows no changes, and therefore, tautomerism does not interfere with the measurement of pK_b.

In cases of **1**, **2**, and **7**, there is a two-jump transition of change in the maxima position, which corresponds to two protonation stages. For **8** and **9**, one protonation stage is observed, and for **3**, three protonation stages are observed, Figures 5 and 9.

The spectra of **1**, **2**, and **7** are close to each other, which indicates their existence in the same tautomers. They are characterized by intense absorption with maxima at 200−204 nm (lg $\varepsilon \approx 4.3$) and 252−254 nm (lg $\varepsilon \approx 4.0$). The maximum at 252−254 nm is the most convenient for measuring pK_b, since at these wavelengths, the influence of the solvent is minimal and the peak shift during the formation of cations does not lead to its going beyond the operating range of the spectrometer. Alkyl substituent at position 2 does not significantly affect the position of maxima, but there is a moderate increase in the extinction coefficients (Table 1).

The nitro group in 6-hydroxy-2-methyl-5-nitropyrimidin-4(3H)-one (**8**) and 6-hydroxy-2-ethyl-5-nitropyrimidin-4(3H)-one (**9**) does not significantly change a spectrum but causes a shift of maxima to the long-wavelength range.

During the transition from a weakly acidic to moderately acidic medium (H$_2$SO$_4$ with a concentration of 30−40%, $H_o \sim$
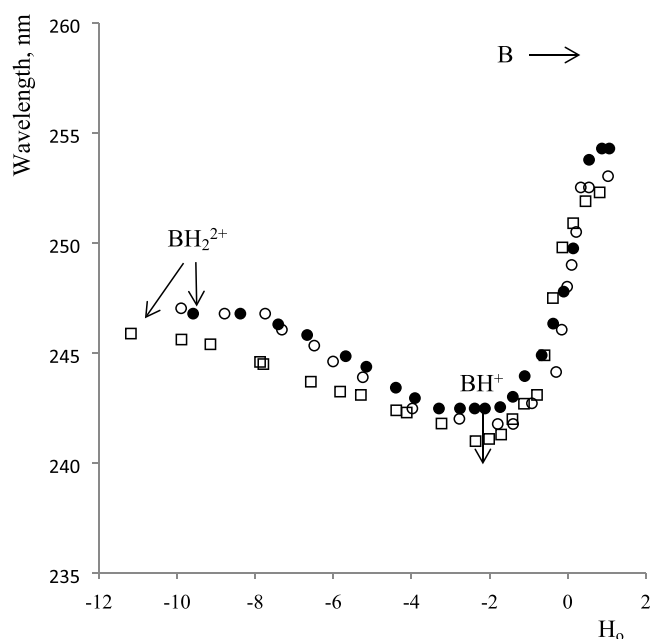

Figure 5. Dependence of the absorption maximum position on the acidity: ○—6-hydroxy-2-methylpyrimidine-4(3H)-one (**1**), □—4,6-dihydroxypyrimidine (**2**), ●—6-hydroxy-2-ethylpyrimidine-4(3H)-one (**7**).

1.7 to −2.5), changes in the **1**, **2**, and **7** spectra are the same, the main absorption peak shifts from 250−254 to 240−242 nm (Figures 6 and 7), and the extinction coefficient decreases.

An isobestic point is present on the group of spectra, which confirms the assumption that free and monoprotonated forms are present in this range of acidity (Figure 6). At $H_o \sim -2$ to −3, a region with a constant spectrum is observed, which makes it possible to accept the spectra of compounds in this region for the spectra of monoprotonated forms.

Table 1. Absorption Maxima and Extinction Coefficients of Free Base and Cations of 4,6-Dihydroxypyrimidine and Its Derivatives

| compounds | λ_{max}, nm/lg (ε), L·mol^{-1}·cm^{-1} | | | | | |
	1	2	3	7	8	9
B	252/4.07	252/3.97	254/3.00	253/4.08	324/3.72	325/3.74
BH$^+$	242/3.93	242/3.93	258/2.15	242/3.93	296/3.73	296/3.82
BH$_2^{2+}$	246/4.08	246/3.89	260/3.00	246/4.10		
BH$_3^{3+}$			258/4.50			
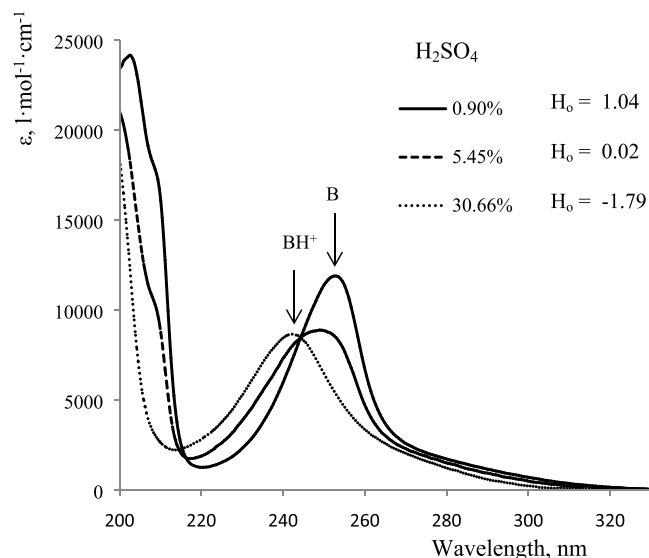
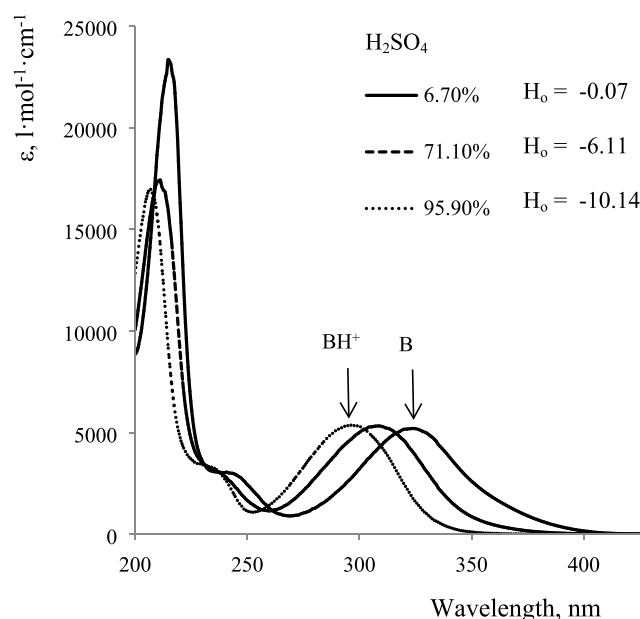
Figure 6. Changes in the UV spectra of 6-hydroxy-2-methylpyrimidin-4(3H)-one (**1**) upon its first protonation in solutions in 0.9−30.7% H$_2$SO$_4$.

With a further increase in H_o from −2 to −11, the absorption maximum shifts from 240 to 245 nm (Figure 7), accompanied by an increase in the extinction coefficient, and an isobestic point is also observed. In sulfuric acid with a
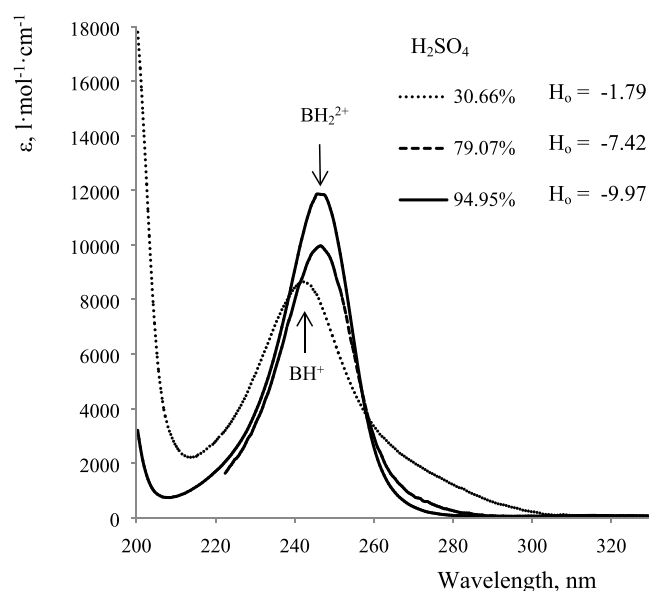
Figure 7. Changes in the UV spectra of 6-hydroxy-2-methylpyrimidin-4(3H)-one (**1**) upon its second protonation in solutions in 30−95.0% H$_2$SO$_4$.

concentration higher than 90% for **1** and **7**, the spectrum changes stop and the diprotonated form is present in the solution. In the case of **2**, the maximum shift continues up to 99.9% H$_2$SO$_4$.

In the case of nitro derivatives of **8** and **9**, with an increase in acidity, the absorption maximum shifts to the short-wavelength region (Figure 8). One protonation stage is observed.


Figure 8. UV spectra of 6-hydroxy-2-methyl-5-nitropyrimidine-4(3H)-one (**8**) in a solution of 6.7−95.9% H$_2$SO$_4$.

Changes in the spectrum of **3** with increasing acidity are more complex (Figure 9). Barbituric acid is an acid with p$K_a \approx$ 4, so it is largely in the form of an anion in water, which has an intense absorption at 258 nm.[22] When switching to solutions of sulfuric acid of 3−5%, ionization is suppressed and the neutral form prevails in the solution, which is characterized by an intense absorption below 220 nm.

With an increase in the concentration of sulfuric acid to 30%, a monotonous decrease in the optical density is observed at 210 nm and, especially, at 260 nm, which is replaced by an increase with a further increase in acidity. Thus, the optical density dependences at 210 and 260 nm (Figure 10) on H_o show three regions corresponding to three protonation stages: from 5 to 35%, monoprotonated form (BH$^+$); from 35 to 70%, diprotonated form (BH$_2^{2+}$); and from 70 to 100%, triprotonated form (BH$_3^{3+}$) are generated. The spectra of 30, 70, and 99.5% H$_2$SO$_4$ were assumed to be mono-, di-, and triprotonated forms of **3**, respectively, when calculating pK_b.
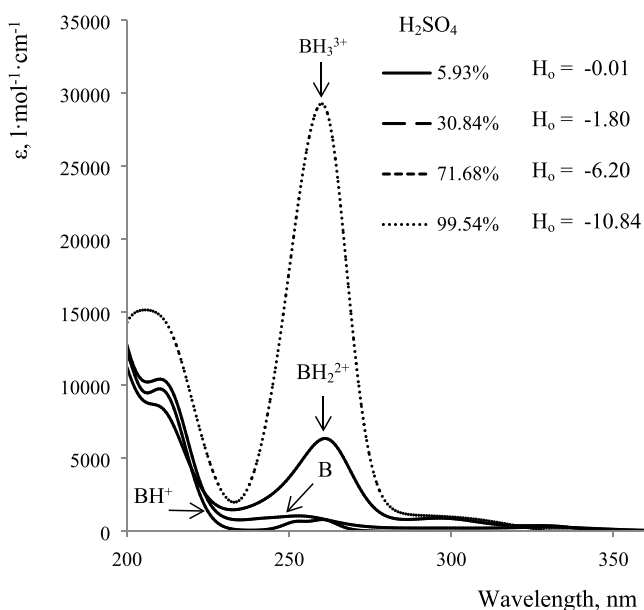
Figure 9. UV spectra of free base and protonated forms of barbituric acid (**3**).
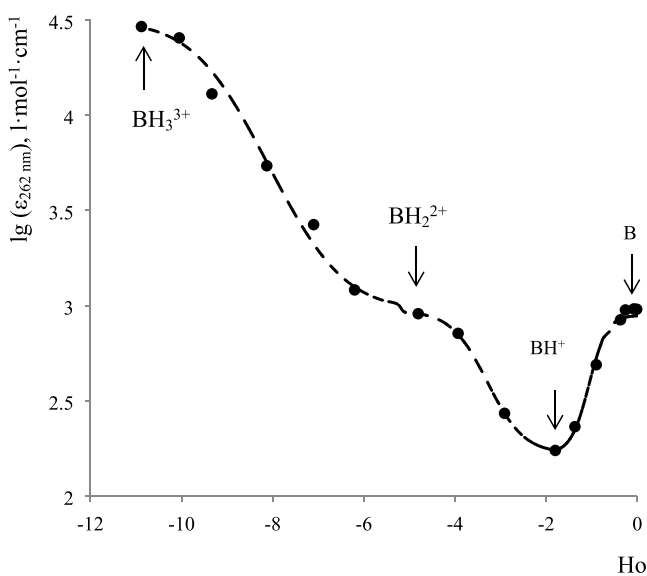
Figure 10. Change of extinction coefficient (at 262 nm) of barbituric acid (**3**) on acidity. Black circles—experiment, dotted line—calculation according to eq 4.

■ DISCUSSION

pK_b values were calculated using the Yates−McClelland method, which assumes that there is a proportional relationship between any values of the H_x acidity function[26]

$$\lg I = -mH_x + pK^*_b \tag{1}$$

$$I = \frac{[BH_n^{n+}]}{[BH_{n-1}^{(n-1)+}]}, \; n = 0, 1, 2, 3 \tag{2}$$

Thus, the dependence of the logarithms of the ionization ratios (I) on the H_x acidity function is linear and the criterion for the applicability of H_x to the study of protonation of a weak base is the angular coefficient $m = dH_x/dH_o$ close to 1. The function H_x indirectly indicates the type of protonation. The pK_b value determined by the H_o acidity scale and equal to the acidity of the medium at the half-protonation point ($\log I = 0$) was calculated by the equation

$$pK_{bn} = \frac{pK^*_{bn}}{m_n} \tag{3}$$

In this research, the acidity functions H_o,[27−30] H_a,[31,32] and H_I,[33] H_o'''[34] were used to analyze experimental data. The H_x acidity functions describe a type of protonation of very weak uncharged bases. When constructing them, the following reference reactions were used: H_o—protonation of nitroanilines; H_I—protonation of primary amides; H_a—protonation of indoles; H_o'''—protonation of tertiary aromatic amines.

In the cases of **1**, **2**, **7**, **8**, and **9**, the concentrations of free base (B), cation (BH$^+$), and dication (BH$_2^{2+}$) were calculated from UV spectroscopy data by Firordt's method by two wavelengths corresponding to their maxima (Table 1). The mixture is analyzed using Firordt's method by resolving the following system of equations

$$\begin{bmatrix} a_1 \times l \times C_X + b_1 \times l \times C_Y = A_1 \\ a_2 \times l \times C_X + b_2 \times l \times C_Y = A_2 \end{bmatrix} \tag{4}$$

where A_1 and A_2 denote adsorption at λ_1 and λ_2, respectively; a_1, a_2, b_1, and b_2 are the molar absorption coefficients of X and Y at λ_1 and λ_2, respectively; C_X and C_Y are the molar concentrations of X and Y in the mixture, respectively; and l is the thickness of the absorbing layer.[35]

For **3**, the maximum shift is small, which complicates the use of this method, but there is a significant change in the optical density at ∼260 nm, so ionization ratio was calculated by the equation

Table 2. Basicity of 4,6-Dihydroxypyrimidine Derivatives

| compound	pK_{b1}	pK_{b2}	pK_{b3}
1	0.21;[14] 0.23;[3] 0.17 ± 0.01	−6.10;[3] −6.24 ± 0.48	
2	−0.41 ± 0.03; −0.30[14]	−7.06 ± 0.37	
3	−0.90 ± 0.06	−3.62 ± 0.02	−9.27 ± 0.54
7	−0.03 ± 0.04	−5.81 ± 0.35	
8	−6.04 ± 0.32		
9	−5.92 ± 0.80		
uracil[36]	−2.38	−7.3 (H_a) ∼ (−12.1 H_o)[a]	
thymine[36]	−3.02	−6.8 (H_a) ∼ (−10.9 H_o)[a]	

[a]Acidity score at the half-protonation point.

Table 3. pK^*_b and Coefficient m Values for 4,6-Dihydroxypyrimidine Derivatives

compound	pK^*_{b1}	m	pK^*_{b2}	m	pK^*_{b3}	m
			H_o			
1	0.18 ± 0.01	1.06 ± 0.04	−2.84 ± 0.22	0.45 ± 0.04		
2	−0.32 ± 0.02	0.79 ± 0.02	−2.91 ± 0.15	0.41 ± 0.03		
3	−2.09 ± 0.08	2.31 ± 0.07	−4.14 ± 0.03	1.15 ± 0.01	−5.99 ± 0.35	0.64 ± 0.04
7	−0.026 ± 0.037	0.94 ± 0.05	−2.81 ± 0.17	0.48 ± 0.03		
8	−2.76 ± 0.11	0.46 ± 0.02				
9	−3.02 ± 0.41	0.51 ± 0.07				
			H_a			
1	0.00 ± 0.01	1.32 ± 0.05	−3.68 ± 0.21	0.94 ± 0.06		
2	−0.47 ± 0.04	1.02 ± 0.03	−3.67 ± 0.25	0.84 ± 0.07		
3	−2.49 ± 0.09	2.90 ± 0.09	−5.32 ± 0.21	2.022 ± 0.08	−6.10 ± 0.04	1.08 ± 0.08
7	−0.19 ± 0.04	1.18 ± 0.06	−3.75 ± 0.23	0.99 ± 0.06		
8	−3.72 ± 0.08	0.95 ± 0.02				
9	−3.41 ± 0.37	0.87 ± 0.10				
			H_I			
3	−1.85 ± 0.09	1.58 ± 0.06	−4.18 ± 0.10	0.84 ± 0.08	−6.64 ± 0.39	0.57 ± 0.04
			H_r			
3	−1.48 ± 0.11	1.16 ± 0.05	−3.70 ± 0.08	0.52 ± 0.01	−6.03 ± 0.61	0.33 ± 0.04

Scheme 2. Formation of Dimer from 4,6-Dihydroxypyrimidine (1) and 6-Hydroxy-2-Methylpyrimidine-4(3H)-One (2)
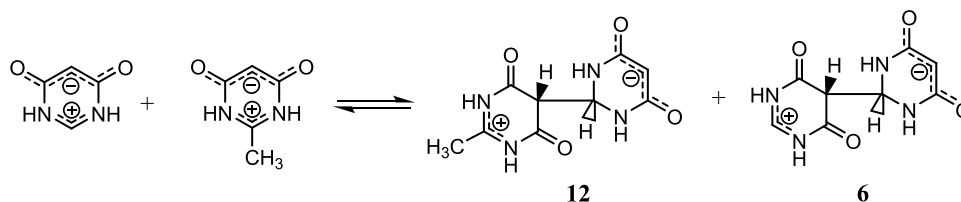
$$I = \frac{\varepsilon_{BH_{n-1}^{(n-1)+}} - \varepsilon}{\varepsilon - \varepsilon_{BH_n^{n+}}}, \quad n = 1, 2, 3 \tag{5}$$

The values of pK_{bn} obtained by us are shown in Table 2. As can be seen for **1** and **2**, they coincide with those given in ref 14. The introduction of a donor substituent in position 2 is expected to increase basicity. Replacing the methyl group with an ethyl group slightly reduces the basicity for the first protonation step, but increases it for the second.

The oxygen in the second position in barbituric acid (**3**) also acts in different directions. There is a strong decrease in basicity at the first stage of protonation, which correlates with the presence of pronounced acidic properties in **3** and is determined by its presence in the keto form. However, the second protonation of **3** is much easier than that of **1**, **2**, and **7**, and the same seems to apply to the third stage of protonation.

The presence of a nitro group with a strong electron-acceptor effect is expected to significantly reduce the basicity of compounds; only the first protonation is observed in the studied range of acidity.

As indicated, the type of acidity function that best describes protonation is indirectly related to the direction of protonation. Table 3 shows the values of coefficient m. At the first protonation of **1** and **7**, they are close to 1 on the H_o scale. The H_a scale is more suitable for **2**, but using H_o also gives a satisfactory value for the m coefficient. Their difference from 1 is not high. This fact does not allow us to make a clear choice in favor of one of these acidity scales.

The unified character of the spectra of protonated forms of **1**, **2**, and **7** indicates their existence in similar tautomeric forms and a single protonation mechanism. When considering a

possible proton attachment site for 4,6-dihydroxypyrimidine derivatives, their tautomerism must be taken into account. During the first protonation of **1**, **2**, and **7**, the value of the coefficient m is close to 1 when using the acidity function H_o. It is different from the first protonation stage of thymine and uracil, which is described by the H_a function. The site of protonation is oxygen.[36]

This suggests the addition of a proton to the "pyridine" nitrogen atom of lactam−lactim form with the formation of cation **13a** (Scheme 3). Although the protonation of the lactam−lactim form is possible, in our opinion, the presence of zwitterionic forms will be an important factor. In this case, it is preferable to attach a proton to the negative center of the zwitterionic form - the oxygen atom of carbonyl group. This process leads to the formation of a cation with localization of the charge on the "pyridine" nitrogen atom, the structure of which is the same as in the protonation of the lactam−lactim form. The final structure of the cation will be determined by the relative stability of its tautomeric forms. Protonation of the lactam−lactim form is possible at a low rate of its conversion to zwitterion and a low equilibrium concentration of the latter.

The existence of ionic forms of **1** and **7** was previously assumed but, unlike **2**, there is no direct evidence for this. However, the presence of a certain amount of them in solutions cannot be excluded. Dimerization could indicate their presence, but it is not observed in aqueous solutions, probably due to the steric effect of the substituent in position 2. To test this assumption, we used LC−MS to study the interaction of **1** and **2** in an aqueous solution (molar ratio 1:1). Along with **6**, the formation of a mixed dimer was achieved, presumably having the structure **12** (Scheme 2). Their equilibrium content, as in the case of **2**, was ∼4%. The ratio

Scheme 3. Protonation of 4,6-Dihydroxypyrimidine (2), 6-Hydroxy-2-Methylpyrimidine-4(3H)-One (1), and 6-Hydroxy-2-Ethylpyrimidine-4(3H)-One (7)

R=H, CH$_3$, C$_2$H$_5$, CH(CH$_3$)$_2$ 13a   13b   14a   14b
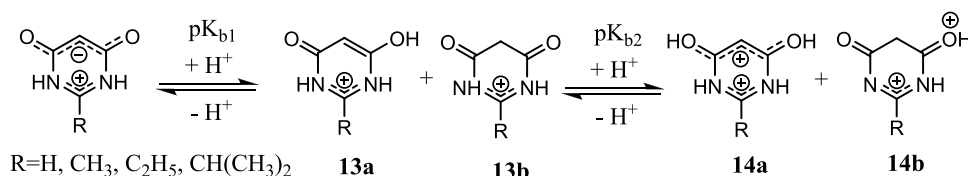
Scheme 4. Protonation of 6-Hydroxy-2-Methyl-5-Nitropyrimidine-4(3H)-One (8), 6-Hydroxy-2-Ethyl-5-Nitropyrimidine-4(3H)-One (9), and 6-Hydroxy-2-Isopropyl-5-Nitropyrimidine-4(3H)-One (11)

R = CH$_3$, C$_2$H$_5$, CH(CH$_3$)$_2$
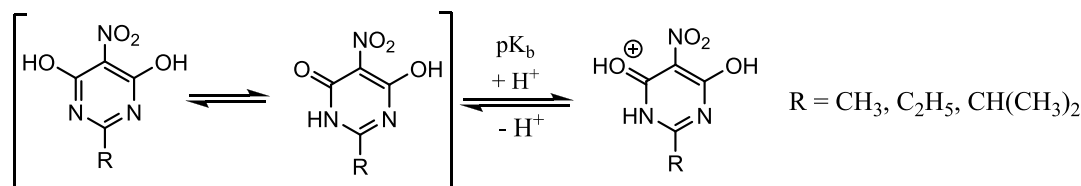
of **6** and **12** is approximately 3:2, which indicates close interaction rates and equilibrium concentrations of zwitterion forms of **1** and **2**.

The first protonation of **1**, **2**, and **7** is described by the H_o function constructed using anilines, so we can expect the addition of a proton to pyridine nitrogen atom (Scheme 3).

The comparison of the ^1H NMR spectra of **2** in DMSO-d_6 and acidic media shows that the base form of the cation can be the lactam–lactim forms, the ratio of proton integrals in 2 and 5 positions is 1:1. During monoprotonation in the TFA medium, a shift of proton signals at positions 2 and 5 to a weak field is observed ($\Delta\delta_H = +0.98$ and $+0.81$ ppm) and a larger shift at position 2 is in good agreement with the structure **13a** (R = H) (Scheme 3), with a predominant localization of charge on nitrogen atoms. In addition, solutions contain up to 10% dilactam forms **13b** (R = H). It is characterized by a methylene group signal: δ_H 2.24 ppm (d, 2H, J = 0.8 Hz). Its presence was also proved in ref 12 by the isotopic exchange of hydrogen at position 5 for deuterium in the D$_2$SO$_4$.

The protonation of substances **1** and **7** occurs in a similar way. When monocations are formed in TFA, the signals of protons in position 5 are shifted into a weak field: $\Delta\delta_H = +0.92$ and $+0.83$ ppm accordingly. The signal of the methyl group in monocation **13a** (R = CH$_3$) is also strongly shifted downfield: $\Delta\delta_H = +0.49$ ppm. The same effect is exerted by monoprotonation on the chemical shift of the protons of the ethyl group: $\Delta\delta_H = +0.32$ ppm for CH$_3$ and $\Delta\delta_H = +0.54$ ppm for CH$_2$. At the same time, no dilactam form is observed for **1**. For **7**, as well as for **2**, up to 5% of the form of dilactam **13b** (R = C$_2$H$_5$) is present.

The second protonation of **1**, **2**, and **7** is described by the H_a function constructed using amides, so we can expect the addition of a proton to oxygen (Scheme 3).

Other changes in the ^1H NMR spectrum are observed during the formation of dication **2** in 95% sulfuric acid. Compared to the monocation, the signal of the proton in position 5 is even more biased into the weak field ($\Delta\delta_H = +0.22$ ppm). On the contrary, the signal of the proton in position 2 is shifted into a strong field ($\Delta\delta_H = -0.32$ ppm). This fact is in good agreement with structure **14a** (R = H) (Scheme 3), and the second protonation occurs at carbonyl oxygen. The dilactam form **14b** (R = H) is also present. It is

characterized by the signal of the methylene group: $\delta_H = 2.15$ ppm (s, 2H).

Comparison of the ^1H NMR spectra of mono- and dications of **1** and **7** shows changes similar to those for compound **2**. A shift of the signals of protons in position 5 in a weak field is observed: $\Delta\delta_H = +0.24$ and $+0.23$ ppm accordingly. This indicates that the position of the second protonation site is close to position 5. On the contrary, the signals of the alkyl group are shifted to strong field: $\Delta\delta_H = -0.38$ ppm for CH$_3$ and $\Delta\delta_H = -0.63$ (CH$_2$) and $\Delta\delta_H = -0.46$ (CH$_3$) ppm for ethyl group. In the cases of **1**, **7**, and 6-hydroxy-2-isopropylpyrimidine-4(3H)-one (**10**), in contrast to **2**, no forms of dilactam were found.

The ^1H NMR spectra of the dication **14a** in concentrated H$_2$SO$_4$, compared to the solution of the free base in DMSO-d_6, show that in all cases, the proton signals in position 5 are strongly shifted to a weak field: R = CH$_3$, $\Delta\delta_H = +1.17$ ppm; R = C$_2$H$_5$, $\Delta\delta_H = +1.10$ ppm; R = CH(CH$_3$)$_2$, $\Delta\delta_H = +1.08$ ppm. It can be seen that an increase in the donor effect of the alkyl group increases the electron density at the pyrimidine core and correlates with a decrease in pK_{b2}.

The signal of the methyl group of dication **14a** (R = CH$_3$) is shifted to the weak field significantly less ($\Delta\delta_H = +0.17$ ppm) than in the case of thymine ($\Delta\delta_H = +0.74$ ppm), which is consistent with different protonation directions and may indicate a significant charge delocalization.

For dication **14a** (R = C$_2$H$_5$), a shift of the signals of the methylene group to the weak field by 0.08 ppm is also observed, and the signals of the methyl group, on the contrary, are shifted to the strong field $\Delta\delta_H = -0.34$ ppm, which is typical of an ethyl group connected to electron-acceptor radical. In the case of 6-hydroxy-2-isopropylpyrimidine-4(3H)-one, all signals of the alkyl substituent are shifted to a strong field: CH $\Delta\delta_H = -0.26$ ppm and CH$_3$ $\Delta\delta_H = -0.31$ ppm. In all cases, J does not change significantly.

All of this confirms the generality of the second protonation of **1**, **2**, and **7** and the location of the second protonation center on the oxygen atom of the carbonyl group.

For nitro derivatives, it is most likely to be found in the lactam–lactim and dilactim forms, but not in the dilactam form. ^1H NMR spectra of **8** and **9** in DMSO-d_6 and H$_2$SO$_4$ show the absence of a proton at position 5. The UV spectra of neutral and protonated forms of nitro derivatives do not differ,

Scheme 5. Protonation of Barbituric Acid (3)
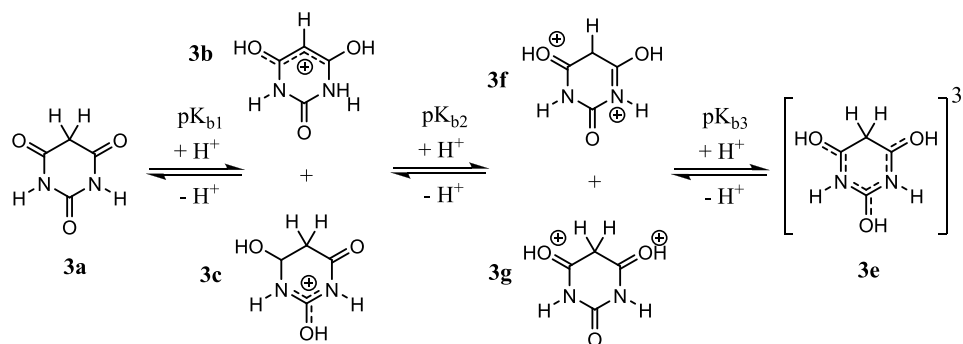
in principle, from the spectra of the initial compounds; a maximum shift is observed due to the presence of an acceptor substituent.

Protonation of **8** and **9** is described by the H_a function, which suggests the addition of a proton by oxygen of the carbonyl group. The observed shift of the substituent proton signals to a strong field (**8**: CH_3, $\Delta\delta_H = -0.14$ ppm; **9**: CH_2, $\Delta\delta_H = -0.08$ ppm, CH_3, $\Delta\delta_H = -0.35$ ppm; **12**: CH, $\Delta\delta_H = -0.26$ ppm, CH_3, $\Delta\delta_H = -0.44$ ppm) also indicates a different nature of protonation in contrast to the parent structures and is consistent with the addition of a proton to the carbonyl group.

The nitro group in position 5 reduces the basicity of nitrogen atoms and makes it difficult to delocalize the negative charge, preventing the formation of zwitterionic forms. Consequently, they do not make a significant contribution to protonation, which occurs on the oxygen of the carbonyl group (Scheme 4).

Analysis of the coefficients m during protonation of barbituric acid showed that for the first stage, it is satisfactorily described by the acidity function H_r; the second stage is satisfactorily described by the acidity functions H_o and H_I; the third stage is described by the acidity function H_a. As already mentioned, the change in UV spectra during **3** protonation is fundamentally different from that observed for other studied compounds. A significant change in both the position and intensity of the absorption maxima is consistent with the available data on the structure of free and protonated forms of **3**. Based on the structures of the monoprotonated and triprotonated forms, let us consider the possible structure of the diprotonated form. Note that the UV spectrum of BH_2^{2+} shows great similarity to the spectrum of BH_3^{3+}, but is significantly different from BH^+. This suggests a large contribution of the triketo form **3g** in equilibrium with the diketo form **3f** (Scheme 5).

CONCLUSIONS

4,6-Dihydroxypyrimidine and 6-hydroxy-2-methylpyrimidine-4(3H)-one are stronger bases compared to the isomeric thymine and uracil. The first protonation of 4,6-dihydroxypyrimidine, 6-hydroxy-2-methylpyrimidin-4(3H)-one, and 6-hydroxy-2-ethylpyrimidin-4(3H)-one easily occurs by the addition of a proton to the zwitterionic form. Thus, it is most likely that the primary protonation site is the pyridine nitrogen atom. The second protonation with the formation of a doubly charged cation occurs by the addition of a proton to the oxygen of the carbonyl group.

The presence of a nitro group at position 5 reduces the basicity of nitrogen atoms and prevents the formation of a

zwitterion structure, which leads to a sharp decrease in basicity and protonation of 6-hydroxy-2-methyl-5-nitropyrimidine-4(3H)-one and 6-hydroxy-2-ethyl-5-nitropyrimidine-4(3H)-one by carbonyl groups.

Protonation of barbituric acid takes place in three stages, while its basicity at the first protonation step is lower than 4,6-dihydroxypyrimirine, which is due to its presence in the keto form. The formation of dication occurs at a lower acidity than 4,6-dihydroxypyrimidine, 6-hydroxy-2-methylpyrimidine-4(3H)-one, and 6-hydroxy-2-ethylpyrimidine-4(3H)-one.

Based on the experimental data obtained for all studied compounds, basicity constants and values of the m coefficient were calculated using various acidity functions.

METHODS

The following substances for the study were synthesized using known methods: 4,6-dihydroxypyrimidine (**2**),[37] barbituric acid (**3**),[38] 6-hydroxy-2-methylpyrimidine-4(3H)-one (**1**),[39] 6-hydroxy-2-ethylpyrimidine-4(3H)-one (**7**),[40] 6-hydroxy-2-iso-propylpyrimidine-4(3H)-one (**10**),[41] 6-hydroxy-2-methyl-5-nitropyrimidine-4(3H)-one (**8**),[42] 6-hydroxy-2-ethyl-5-nitro-pyrimidine-4(3H)-one (**9**),[43] and 6-hydroxy-2-isopropyl-5-nitropyrimidine-4(3H)-one (**11**).[43] According to LC/MS and ^{1}H NMR spectroscopy data, their purity is higher than 98%.

Solutions of sulfuric acid were prepared by mixing sulfuric acid (95%), oleum (105%), and distilled water. The concentration was determined titrometrically and by density with an error of no more than 0.1%.

Absorption spectra in the ultraviolet and visible regions were recorded in a temperature-controlled cell on a Specord M-40 spectrophotometer at 25 ± 0.1 °C. To control the stability of the solutions, the measurements were repeated up to three to five times for 3−6 h. Data were averaged. The deconvolution of spectra was carried out using the "OMNIC" program and mixed Gauss−Lorentz functions.

Liquid chromatography−mass spectrometry (LC−MS) experiments were performed using a Thermo Finnigan Surveyor MSQ with electrospray ionization (ESI, positive and negative modes).

^{1}H NMR spectra were recorded on a Varian Mercury Plus instrument (300 MHz).

Infrared (IR) spectra were recorded on an Avatar 360 FTIR instrument in KBr.

ASSOCIATED CONTENT

ⓈⅠ **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00671.

Decomposition of UV spectra of the free base and protonated forms of 4,6-dihydroxypyrimidine derivatives; chemical shifts of protons in the free base and protonated form of 4,6-dihydroxypyrimidine derivatives; calculation of pK_b values of 4,6-dihydroxypyrimidine derivatives; mass and UV spectra of dimer **6** and **12**; and ^{1}H NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

LC−MS, liquid chromatography−mass spectrometry; IR, infrared; UV, ultraviolet; NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide

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