PROTONATION OF 4-HYDROXYPYRAZOLO[3,4-d]PYRIMIDINE AND ITS METHYL DERIVATIVES

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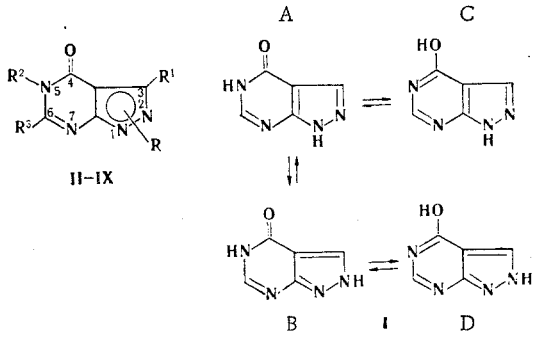
The ability of 4-hydroxypyrazolo[3,4-d]pyrimidine and its methyl derivatives to form mono- and dications was shown by UV and PMR spectroscopy; an assumption regarding the structures is expressed on the basis of the data obtained.

In a continuation of our research on a number of purine analogs — pyrazolo[3,4-d]pyrimidines [1, 2] — we made a detailed study of the protonation of 4-hydroxypyrazolo[3,4-d]-pyrimidine (I) — the well-known medicinal preparation "allopurinol" [3] — and its methyl derivatives (II-V). These compounds are extremely complex subjects for spectral investigation: they are only slightly soluble in water and aprotic solvents and are also capable of existing in different tautomeric forms, for example, A-D for I.*



II R=1-CH_3, $R^1=R^2=R^3=H$; III R=2-CH_3, $R^1=R^2=R^3=H$; IV R=1-CH_3, $R^1=R^3=H$, $R^2=CH_3$; V R=2-CH_3, $R^1=R^3=H$, $R^2=CH_3$; VI $R=R^2=R^3=H$, $R^1=CH_3$; VII $R=R^1=R^2=H$, $R^3=CH_3$; VIII R=1-CH_3, $R^1=R^2=CH_3$; $R^3=H$; IX R=1-CH_3, $R^1=R^2=H$, $R^3=CH_3$

The acid and base properties of a number of pyrazolo[3,4-d]pyrimidine derivatives were investigated in [4], but Zynch and Robins were unable to evaluate the ability of I and its 1-methyl derivatives (II) to undergo protonation by a spectrophotometric method, inasmuch as the spectra of the netural molecules and cations of these compounds proved to be practically identical.

A detailed study of the UV spectra of 4-hydroxypyrazolo[3,4-d]pyrimidine derivatives made it possible to establish that, in addition to the known absorption maximum at 255-285 nm, their spectra also contain another short-wave high-intensity maximum at 210-215 nm (Fig. 1). It was found that, in contrast to the long-wave absorption, which was used in [4] to determine the pK_α value, the intensity of the short-wave maximum of the investigated compounds decreases substantially on passing from neutral media to acidic media. This made

*In principle, the nitrogen atom in the 7 position may also participate in the tautomeric transformations.

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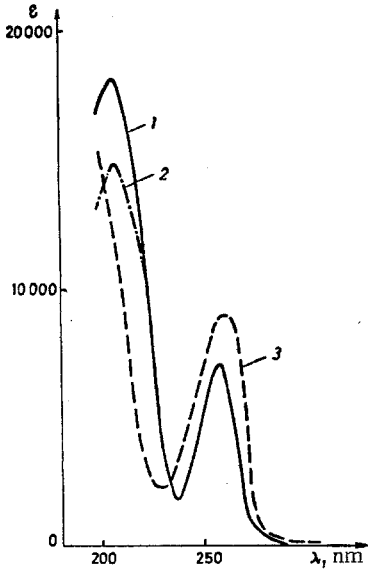

Fig. 1. UV spectra of 4-hydroxypyrazolo[3,4-d]pyrimidine I: 1) neutral molecule; 2) monocation; 3) dication.

it possible to determine the first protonation constant (K_a^I) of the indicated compounds within the limits of pH 5 to −1.

In order to ascertain the formation of dications we studied the UV spectra of I-VI in strongly acidic solutions from H_0 = −2 to concentrated sulfuric acid. We were unable to follow the change in the intensity of the short-wave band under these conditions, but in the H_0 interval from −4 to −8 we did observe an increase in the intensity of the long-wave absorption, which had a small bathochromic shift. Assuming that a proton added to the monocation in this case, we determined the pK_a^{II} values for the investigated compounds.

The pK_a values were calculated from formulas (1) and (2):

$$pK_a^I = pH + \frac{D - D_m}{D_n - D} \quad (1)$$

$$pK_a^{II} = H_0 + \frac{D_d - D}{D - D_m} \quad (2)$$

where D, D_n, D_m, and D_d are, respectively, the instantaneous optical density and the optical densities of the neutral molecule, the monocation, and the dication. The characteristics of the UV spectra and the pK_a values of the investigated compounds are presented in Table 1.

The confirmation of the successive formation of mono- and dications on passing from weakly acidic to strongly acidic media and the determination of the site of primary and secondary protonation were obtained by means of the PMR spectra.

Singlets of H_3 and H_6 protons and of protons of the methyl groups are observed in the spectra of solutions of I and its methyl derivatives in aqueous deuterotrifluoroacetic (D-TFA) and deuterosulfuric acids. The assignment of the signals to the H_3 and H_6 protons for I, III, and V was made by comparison with the spectra of model 3-methyl- (VI) and 6-methyl-4-hydroxypyrazolo[3,4-d]pyrimidines (VII) and for 1-methyl derivatives II and IV by comparison with the spectra of the additionally specially synthesized 1,3,5-trimethyl- and 1,6-dimethyl-substituted compounds (VIII, IX) (Table 2, Figs. 2 and 3).

It was observed that all of the signals of the investigated compounds are shifted to weak field as the D_2SO_4 concentration increases. The most pronounced change in the chemical shift occurs in D_2SO_4 solutions at pH 3 to −2. A further increase in the H_0 acidity to −3 leads to minor changes in the chemical shifts. When $H_0 < -3$, the chemical shifts of the protons again begin to increase. A comparison of these results with data from the UV spectra graphically confirms the assumption regarding the successive occurrence of primary and secondary protonation of the molecules of the investigated compounds.

Shifts of all of the PMR signals to stronger field are observed at high D-TFA and D_2SO_4 concentrations, and this effect is more appreciable for D-TFA (see Figs. 2-5). Complexes of the base/acid type are apparently formed over this range of acid concentrations. This specific interaction is similar to that observed in azaindoles [5].

It follows from the results obtained that 4-hydroxypyrazolo[3-4-d]pyrimidine (pK_a^I = 0.47) proved to be a considerably weaker base than its purine analog — hypoxanthine (pK_a = 1.98).

An analysis of the data obtained makes it possible to draw some conclusions and make some assumptions relative to the structures of the cations formed.

The closeness of the pK_a^I values and of the chemical shifts of the signals of the H_3 and H_6 protons and the N_1—CH_3 and N_2—CH_3 protons of the monocations of the dimethyl derivatives to the values of the corresponding monomethyl derivatives (compare II and IV and III and V in Tables 1 and 2 and Figs. 2-5) makes it possible to suppose that the cations of the investigated compounds have amide structures of the A and B type. On the basis of a comparison of the pK_a^I value with allowance for the indications in [6, 7] regarding the increase in the basicities of nitrogen-containing heterocycles by 0.3-1 pK_a units when a CH_3 group is introduced and also with allowance for the regularities of the change in the chemical

TABLE 1. Data from the UV Spectra and pK_a Values

Compound	Neutral molecule (alcohol)		Monocation		Dication		$pK_a^{I}\pm0,05$	$pK_a^{II}\pm0,15$
	λ_{max}, nm	lg ε	λ_{max}, nm	lg ε	λ_{max}, nm	lg ε		
I	206 255	4,31 3,83	210	4,23	265	3,94	0,47	−5,55
II	213 258	4,34 3,86	212,5	4,25	265	3,99	−0,65	−5,15
III	210 263	4,42 3,89	210	4,38	265	3,94	1,06	−5,50
IV	215 258	4,25 3,89	215	4,19	268	4,03	−0,6	−5,00
V	210 267	4,36 3,96	215	4,32	268	4,01	0,8	−5,50

TABLE 2. Data from the PMR Spectra

Compound	Solvent	Chemical shifts, δ, ppm					
		H_3	H_6	C_3-CH_3 C_6-CH_3	N_1-CH_3	N_2-CH_3	N_5-CH_3
I	3N D-TFA Conc. D-TFA	8,79 8,35	9,05 8,87				
II	D_2O 3N D-TFA Conc. D-TFA	8,40 8,20 8,14	8,47 8,24 8,22		4,26 4,02 3,79		
III	D_2O 3N D-TFA Conc. D-TFA	8,64 8,70 8,20	8,39 9,20 8,87			4,35 3,83	
IV	D_2O 3N D-TFA Conc. D-TFA	8,35 8,34 8,17	8,58 8,50 8,28		4,26 4,16 3,77		3,89 3,79 3,36
V	D_2O 3N D-TFA Conc. D-TFA	8,64 8,70 8,10	8,50 9,18 8,86			4,37 4,15 3,79	3,83 3,88 3,29
VI	D_2O 3N D-TFA Conc. D-TFA		8,40 9,16 8,80	2,90 2,88 2,42			
VII	D_2O 3N D-TFA Conc. D-TFA	8,53 8,77 8,29		2,81 3,00 2,55			
VIII	D_2O Conc. D-TFA		8,55 8,20	2,77 2,43	4,12 3,75		3,85 3,31
IX	D_2O Conc. D-TFA	8,25 8,12		2,54 2,05	4,00 3,67		

shifts of the proton signals, it can be concluded that I and its 2-methyl derivatives (III and V) are protonated in a different manner than their analogs (II and IV) with a methyl group in the 1 position and that structure B is the foundation of the monocation of 4-hydroxypyrazolo[3,4-d]pyrimidine (I). The substantial difference in the basicities of the 1-methyl and 2-methyl derivatives (approximately one order of magnitude in the pK_a^{I} values) undoubtedly attests to a difference in the structures of the monocations formed. These facts can be explained by the fact that protonation is directed to the pyrimidine ring for one series of derivatives and to the pyrazole ring for the other.

On the basis of the fact that the changes in the chemical shifts of the signals of the H_6 protons in weakly acidic solutions of I and its 2-methyl derivatives are much greater than for the signals of the H_3 protons, it can be assumed with a sufficient degree of probability that the addition of the first proton to these compounds takes place in the pyrimidine ring. This confirms the relative change in the chemical shifts of the signals of the methyl protons during primary protonation of VI and VII (Fig. 4).

For the 1-methyl derivative, primary protonation is complete at considerably lower H_0 values (Fig. 2). Moreover, the signal from the H_6 proton of the monocation of this compound

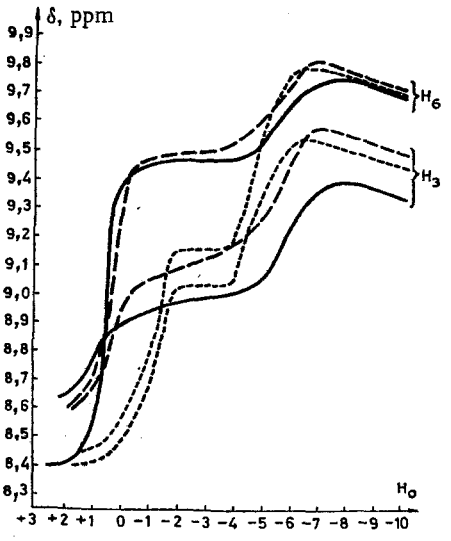

Fig. 2

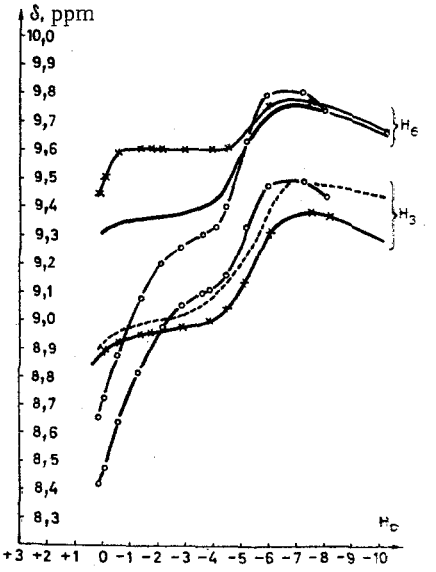

Fig. 3

Fig. 2. Relationship between the chemical shifts of the H_3 and H_6 proton signals and the acidity of the medium for I (---), II (...), and III (——).

Fig. 3. Relationship between the chemical shifts of the H_3 and H_6 proton signals and the acidity of the medium for IV (-·-), V (-×-), VI (——), and VII (---).

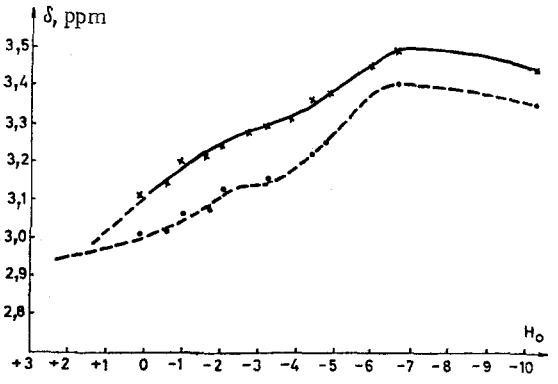

Fig. 4

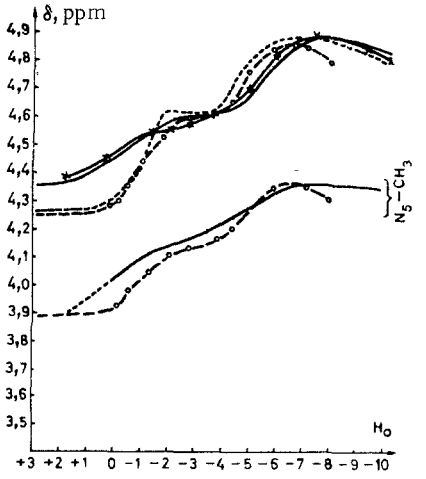

Fig. 5

Fig. 4. Relationship between the chemical shifts of the signals of the methyl group protons and the acidity of the medium for C_3—CH_3 in VI (---) and C_6—CH_3 in VII (×-×-×).

Fig. 5. Relationship between the chemical shifts of the signals of the methyl group protons on the acidity of the medium for N_1—CH_3 in II (---), N_2—CH_3 in III (-×-), N_1—CH_3 and N_5—CH_3 in IV (-○-) and N_2—CH_3 and N_5—CH_3 in V (——).

is shifted to strong field as compared with the signals of the H_6 proton of I, III, and V and is close to the signal of the H_3 proton. This provides a basis for the assumption that the primary protonation of II and the other 1-methyl derivatives proceeds in the pyrazole ring rather than in the pyrimidine ring. The fact that, in contrast to V, for which large changes in the positions of the signals of the N_5—CH_3 protons are observed on passing from D_2O to D_2SO_4 with H_o = 0.15 ($\Delta\delta$ = 0.18 ppm), these changes are practically

absent ($\Delta\delta$ = 0.02 ppm) (Fig. 5) for IV may serve as a confirmation of this. It is possible that the corresponding redistribution of electrons during protonation of the pyrazole ring is delocalized over the entire system to a greater degree than when the first proton adds to the pyrimidine ring. This is in agreement with the conclusion in [8] that endocyclic perturbation effects are transmitted more easily from the six-membered ring to the five-membered ring than in the opposite direction.

The secondary protonation constants (pK_a^{II}) are identical for I, III, and V, whereas they are somewhat higher for II and IV. This also may serve as a confirmation that addition of the second proton proceeds differently for these two series of compounds. The monocations of unsubstituted 4-hydroxypyrazolo[3,4-d]pyrimidine and its 2-methyl derivatives are apparently protonated in the pyrazole ring, whereas the 1-methyl derivatives are protonated in the pyrimidine ring.

EXPERIMENTAL METHOD

The PMR spectra were obtained with a Varian/HA-100 spectrometer with hexamethyldisiloxane as the internal standard. The UV spectra of solutions of the compounds (c ~ $1 \cdot 10^{-4}$ M) in H_2SO_4 with a known acidity function (H_0) [9] in thermostatted cuvettes with l = 1 cm at 27° were recorded with a shimadzu MPS-50L spectrophotometer. The methyl derivatives of 4-hydroxypyrazolo[3,4-d]pyrimidine were synthesized by the methods in [10-13].

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