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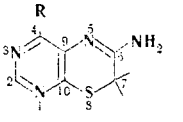
STRUCTURE OF THE CATIONIC FORMS OF PYRIMIDO[4,5-b][1,4]THIAZINES — A NEW TYPE OF FOLIC ACID ANTAGONIST

G. G. Dvoryantseva, T. N. Ul'yanova, T. Ya. Filipenko, M. P. Nemeryuk, T. S. Safonova, Yu. N. Sheinker, P. V. Petrovskii, and É. I. Fedin

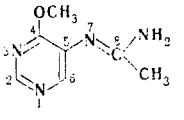
UDC 547.853.7'854.1'859'869:543.422.25

The protonation of 4-methoxy-, 4-amino-, and 4-dimethylamino-6-aminopyrimido[4,5-b][1,4]thiazines and N-(4-methoxy-5-pyrimidinyl)acetamidine was studied by ^1H and ^{13}C NMR spectroscopy. It is shown that the addition of a proton in the first three compounds takes place at the N_5 atom of the thiazine ring, whereas in the case of N-(4-methoxy-5-pyrimidinyl)acetamidine primary protonation is observed at the nitrogen atom of the amidine group, and the second proton adds to the N_1 atom of the pyrimidine ring.

Previous research on pyrimido[4,5-b][1,4]thiazine derivatives has led to the detection of a new type of folic acid antagonist that has antitumorigenic activity [1]. 4-Methoxy-6-aminopyrimido[4,5-b][1,4]thiazine hydrochloride (tomizin) has been selected from this group of substances for clinical study [2]. The participation of an acid–base reaction in the mechanism of inhibition of dihydrofolatereductase (DFR) has been established for a number of previously known folic acid antagonists [3]. In this connection, it seemed of interest to study the structure of the protonated forms of pyrimido[4,5-b][1,4]thiazines and to establish the possible centers of bonding of compounds of this type with the acidic functions of the active centers of DFR. With this end in mind, we investigated the protonation of I–IV by



I–III



IV

I $R=OCH_3$; II $R=NH_2$; III $R=N(CH_3)_2$

S. Ordzhonikidze All-Union Scientific-Research Pharmaceutical-Chemistry Institute, Moscow 119815. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 1, pp. 49-54, January, 1979. Original article submitted December 28, 1977.

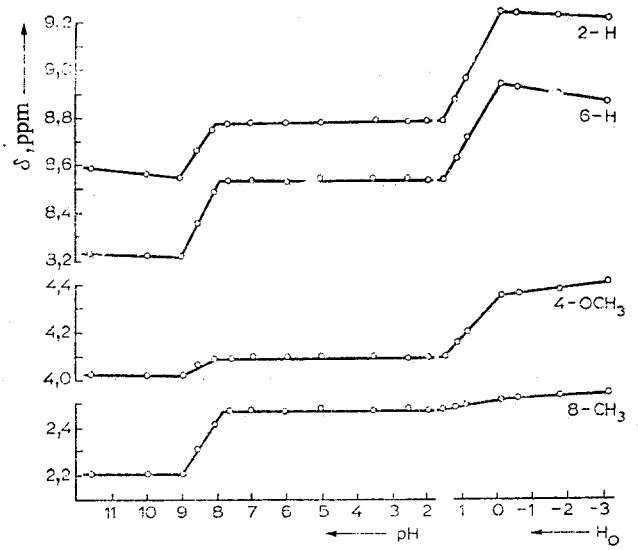


Fig. 1. Dependence of the chemical shifts of the protons in the PMR spectra of N-(4-methoxy-5-pyrimidinyl)acetamidine (IV) on the pH values and acidity function H_0.

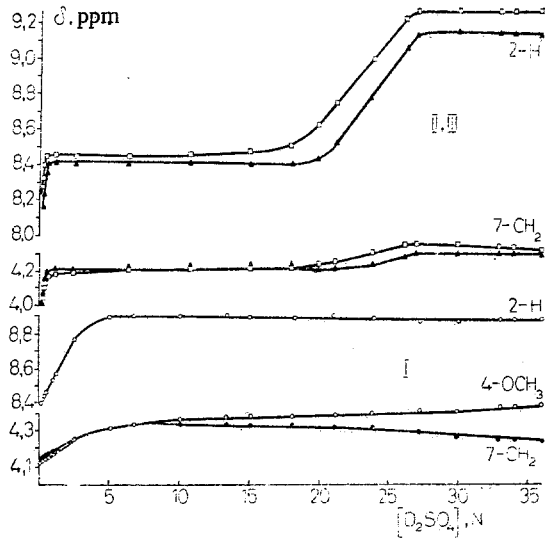


Fig. 2. Dependence of the chemical shifts of the protons in the PMR spectra of pyrimido[4,5-b][1,4]-thiazines (I-III) on the D_2SO_4 concentration in D_2O: for I (○), II (□), and III (▲).

^1H and ^{13}C NMR methods and measured the UV spectra at various pH values and the ionization constant of I in water.

Pyrimidothiazines I-III contain three potential cationoid centers, viz., the nitrogen atoms in the 1, 3, and 5 positions. Protonation of a model compounds — N-(4-methoxy-5-pyrimidinyl)acetamidine (IV) — should be directed primarily at the nitrogen atom of the amidine group (N_7). In order to establish the degree of ionization of I-IV at various media acidities and to determine the parameters of the spectra of the mono- and dications we measured the dependence of the chemical shifts of the protons on the D_2SO_4 concentration in D_2O for I-III and the pH values of aqueous buffer solutions for IV. It follows from the results of the measurements, which are presented graphically in Figs. 1 and 2, that in aqueous solutions of sulfuric acid 4-methoxy-6-aminopyrimidothiazine (the base of tomizin) I gives exclusively the monoprotonated form. The successive formation of a mono- and dication is observed in the case of II-IV. A comparison of the PMR spectra of the compounds in CF_3COOH and D_2O/D_2SO_4 showed that in trifluoroacetic acid the protolytic equilibrium is shifted to favor the monocations of I-III.

The changes in the spectral parameters in the step involving primary protonation of model compound IV (Fig. 1 and Table 1) are in agreement with monocation structure IV^+, which

TABLE 1. Chemical Shifts in the PMR Spectra of Neutral Molecules and Cations

Compound	Medium	Charge	δ, ppm			
			2	4*	5	7
I	DMSO	0	8,12	3,89	—	3.44
	D_2O†	0	8.40	4.12	—	4.14
	10,07 N D_2SO_4	1+	8.89	4.36	—	4.33
	36 N D_2SO_4	1+	8,87	4.44	—	4.25
	CF_3COOH	1+	8.96	4,47	—	4.41
II	DMF	0	7.86	—	—	3,57
	D_2O†	0	8.20	—	—	4.00
	CF_3COOH	1+	8.63	—	—	4.25
	8,0 N D_2SO_4	1+	8.46	—	—	4.21
	30,0 N D_2SO_4	2+	9,28	—	—	4,34
III	DMF	0	7.93	3.17	—	3.48
	D_2O†	0	8.07	3.15	—	3.95
	CF_3COOH	1+	8,40	3.52	—	4.27
	8.0 N D_2SO_4	1+	8.41	3.40	—	4.20
	30,0 N D_2SO_4	2+	9.17	3.66	—	4,31
IV	$CDCl_3$	0	8.45	4.00	8.01	($J_{2,6}$<0,3 Hz)
	H_2O, pH 10	0	8.57	4.04	8.22	The same
	H_2O, pH 6.05	1+	8.78	4.09	8.54	„ „
	3.2 N H_2SO_4	2+	9.26	4.36	8.95	($J_{2,6}$=1.0Hz)
	36 N D_2SO_4	2+	9,12	4.46	8.71	The same

*The chemical shifts of the protons of the OCH_3 (I and IV) and $N(CH_3)_2$ (III) groups.
†The δ values were found by extrapolation to zero D_2SO_4 concentration in D_2O.

corresponds to the addition of a proton to N_7. Thus the signal of the methyl protons of the acetamidine group experiences an appreciable weak-field shift ($\Delta\delta$ = 0.27 ppm), while the change in the chemical shift of the protons of the OCH_3 group in the 4 position of the pyrimidine ring does not exceed 0.1 ppm. A different pattern is observed in the step involving secondary protonation of the IV molecule: the 4-OCH_3 signal is shifted ~0.3 ppm to weak field, and the position of the signal of the protons of the methyl group changes only slightly. The position of the center of secondary protonation of IV was established from the change in the magnitude of the meta constant of spin–spin coupling (SSC) of the 2-H and 6-H protons of the pyrimidine ring. In the spectrum of the neutral IV molecule the $J_{2,6}$ constant is close to zero, and the corresponding signals are observed in the form of singlets. The first protonation does not affect the character of the multiplicity of these signals. On passing from the monocation to the dication the $J_{2,6}$ constant increases to 1 Hz, and the 2-H and 6-H signals are converted to doublets. A similar effect of protonation of the nitrogen atom of the heteroaromatic ring on the magnitude of the meta constant of SSC of the protons adjacent to the cation center has been previously observed in the case of a number of pyrazine derivatives and their 1-oxides [4] and condensed systems that include a pyrimidine fragment [5]. On the basis of these data a structure corresponding to the addition of a second proton to N_1 should be assigned to the IV^{2+} dication. Consequently, the relative basicities of the cationoid centers in IV decrease in the order $N_7 > N_1 > N_3$. The lower proton-acceptor capacity of N_3 as compared with N_1 is evidently explained by the electron-acceptor inductive effect of the OCH_3 group in the ortho position relative to N_3.

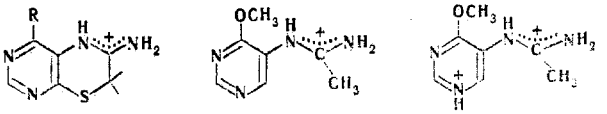
The data on the structures of the mono- and dication of IV make it possible to assume that the most likely protonation center of 4-methoxy-6-aminopyrimidothiazine I is the nitrogen atom of the cyclic amidine group (N_5). Replacement of the OCH_3 group in the 4 position of the two-ring system by an amino group should lead to an increase in the relative basicity of N_3 in II and III as compared with I. However, a comparison of the PMR spectra recorded under similar conditions (aqueous solutions of sulfuric acid) showed that there is an analogy in the relative changes in the 2-H chemical shift in the steps involving primary and secondary protonation of II and III and model compound IV. The addition of a second proton to the nitrogen atom of the pyrimidine ring in IV leads to considerably greater deshielding of 2-H ($\Delta\delta_2$ = 0.47 ppm) as compared with the effect observed in the case of primary protonation of IV at the nitrogen atom of the amidine group ($\Delta\delta_2$ = 0.21 ppm). Such ratios in the changes in the 2-H chemical shift in the primary (0.25–0.35 ppm) and secondary (0.72–0.76 ppm) protonation of II and III indicate that protonation in these compounds also is directed primarily at N_5.

TABLE 2. ^{13}C Chemical Shifts and $^{13}C^{1}H$ Spin–Spin Coupling Constants*

Compound	Positions	Neutral molecule		Monocation	
		δ ^{13}C, ppm	$^{1}J^{13}C^{1}H$, Hz	δ ^{13}C, ppm	$^{1}J^{13}C^{1}H$, Hz
I	2	148,00	204,4	151,33	219,1
	4	160,28		162,96	
	6	150,08		148,15	
	7	24,29	145,6	27,49	150,5
	9	127,08		120,27	
	10	153,72		160,49	
	4-OCH_3	53,20	147,1	59,78	152,9
III	2	148,52	198,5	146,55	216,2
	4	150,08		154,60	
	6	156,54		148,30	
	7	24,55	145,6	27,12	151,5
	9	125,59		113,34	
	10	150,54		158,04	
	4-$N(CH_3)_2$	40,08	136,8	41,03	142,6

*The spectra of the neutral I and III molecules were recorded from solutions in DMSO, the spectra of the I^+ monocations were recorded from solutions in CF_3COOH, and the spectra of the III^+ monocations were recorded from solutions in 8.0 N D_2SO_4; the chemical shifts relative to tetramethylsilane are presented.

The structures of the monocations of the investigated pyrimidothiazines were confirmed by measurement of the ^{13}C NMR spectra of the neutral and monoprotonated forms of I and III.



I^+–III^+ IV^+ IV^{+2}

The assignment of the ^{13}C signals (Table 2) was made on the basis of the character of the multiplicity of the spectral lines in the absence of suppression of the ^{13}C—^{4}H SSC and a comparison with the spectra of quinazoline [6] and pteridines [7, 8]. A $C_{(2)}$ doublet with $^{1}J^{13}C^{1}H$ = 198–220 Hz, a $C_{(9)}$ signal at substantially stronger field (δ = 110–130 ppm) relative to the signals of the remaining sp^2 carbon atoms of the two-ring system (140–170 ppm), and a triplet of the ring 7-CH_2 group (δ_7 = 24–28 ppm; $^{1}J^{13}C^{1}H$ = 145–152 Hz) are readily identified in the spectra of the neutral molecules and cations of the pyrimidothiazines. The $C_{(6)}$ signal is observed in the form of a triplet with geminal constant $J_{6,7}$ = 7–8 Hz. The $C_{(4)}$ signal is a doublet ($J_{4,2}$ = 9–10 Hz) that is additionally split due to SSC with the protons of the methyl groups of the substituents attached to $C_{(4)}$.

It has been previously shown [9, 10] that the addition of a proton to the nitrogen atom of a heteroaromatic ring is accompanied by a characteristic shift in the signals of the α-

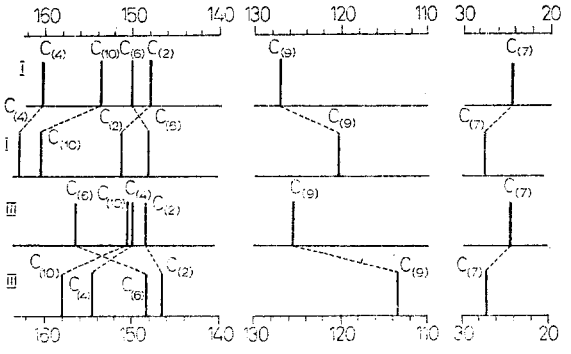


Fig. 3. Graphical representation of the effect of protonation of I and III on the ^{13}C chemical shifts.

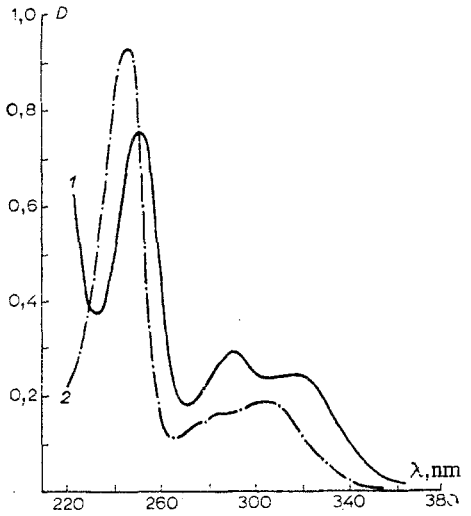


Fig. 4. UV spectra of the neutral 4-methoxy-6-aminopyrimido[4,5-b]-[1,4]thiazine (I) molecules (1) and its monocation (2).

carbon atoms to strong field, whereas the signals of the C atoms in the β and γ positions relative to the cation center generally experience weak-field shifts. The $C_{(6)}$ and $C_{(9)}$ signals are shifted to strong field and the $C_{(7)}$ and $C_{(10)}$ signals are shifted to weak field on passing from neutral I and III molecules to the corresponding monocations. These results are in agreement with protonation of the investigated compounds at N_5 (Table 2 and Fig. 3).

Measurements of the UV spectra of aqueous solutions of 4-methoxy-6-aminopyrimidothiazine (I) at various pH values showed that the shift in the protolytic equilibrium to favor the monocation is accompanied by a hypsochromic shift of the electron transitions observed above 220 nm and by a decrease in the intensities of the long-wave absorption bands (Fig. 4). This sort of effect of protonation on the low-energy transitions is also observed in other heteroaromatic systems that form ions of the amidinium type [11] and is evidently due to delocalization of the positive charge from the cation center on the exocyclic amino group.

Since I, in contrast to IV, does not form a dication over the investigated range of media acidities, it may be concluded that the presence of a sulfur atom in the two-ring system leads to a significant decrease in the basicity of N_1. Replacement of the OCH_3 group in the 4 position by an NH_2 or $N(CH_3)_2$ group, which have strong +C and weak —I effects, should lead to a change in the relative proton-acceptor capacity of the nitrogen atoms of the pyrimidine ring. With allowance for the effect of the sulfur atom, this provides a basis for the assumption that the N_3 atom is the preferred center of secondary protonation of II and III.

It follows from the magnitude of the ionization constant of 4-methoxy-6-aminopyrimidothiazine (pK_a 5.23) measured spectrophotometrically in water that at pH values above six tomizin exists primarily in the form of the neutral I molecule and can form H complexes primarily of the molecular type with DFR. On the basis of the available data on the dependence of the effect of inhibition of DFR at various pH values on the pK_a of the substrate [3] it may be assumed that the maximum effect of inhibition of DFR for tomizin should be observed near the physiological pH (7.2).

EXPERIMENTAL

The PMR spectra of 0.2 M solutions of the investigated compounds were recorded with a JNM-4H-100 spectrometer. Tetramethylsilane (TMS) was used as the internal standard in nonaqueous media, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) and $N(CH_3)_4Cl$, the chemical shifts of which relative to DSS were found from a calibration graph constructed for various acid concentrations, were used as the internal standards in aqueous buffer solutions in D_2O/D_2SO_4 mixtures. The pH values of the solutions were measured with a Radiometer PHM-26 pH meter at 25 ± 0.05°C. The values of the H_0 acidity function for 0.02-6.8 M H_2SO_4 solutions were found from tables [12, 13].

The ^{13}C NMR spectra of the neutral I and III molecules were measured in 15% solutions of the compounds in DMSO, while CF_3COOH and D_2SO_4 were used as the solvents in the case of the I^+ and III^+ monocations, respectively. The position of the protolytic equilibrium in the indicated media was monitored from the titration curves measured by PMR spectroscopy. The measurements of the ^{13}C NMR spectra were made with an HX-90 spectrometer with an operating frequency of 22.635 MHz under conditions of Fourier transformation and $^{13}C-\{^1H\}$ double

NMR with noise modulation of the 90-MHz frequency, as well as without suppression of the $^{13}C-\{^1H\}$ coupling. The external standard was $CHCl_3$, the ^{13}C chemical shift of which relative to TMS was taken as 77.17 ppm. The sample temperature was 30-40°C under the conditions of noise suppression and ~20°C in the absence of suppression. The error in the measurement of the ^{13}C chemical shifts was ≤ 0.1 ppm, and the error in the measurement of the $^{13}C^1H$ constants was ≤ 1.0 Hz.

The UV spectra of the neutral molecule and cation of I were measured with an Hitachi EPS-3 spectrophotometer at solution concentrations of $3\text{-}9 \cdot 10^{-5}$ M. The weighed samples were dissolved in 96% ethanol, and the solutions were then diluted with buffer solutions with known pH values. The ethanol concentration in the test solution did not exceed 1%.

UV spectra of I [λ_{max} ($\varepsilon \cdot 10^{-3}$)]: neutral molecule (pH 7.12), 317 (6.7), 290 (7.56), and 253 (22.0); monocation (pH 3.02), 305 (5.2), 285 (4.7), 275 (4.0), and 247 nm (28.5). The ionization constant of I was measured spectrophotometrically in water: λ_{anal} = 290 nm, $c = 9 \cdot 10^{-5}$ M, t = 25°C, and $pK_a = 5.23 \pm 0.03$.

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