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Potentiometric investigation of acid-base equilibria of two new pyrimidine derivatives in various methanol–water media

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Abstract: The acid-base properties of 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one ($\mathbf{L}^1$) and 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-thione ($\mathbf{L}^2$) were investigated potentiometrically at an ionic strength of 0.10 M (LiCl) in 19.8, 33.6 and 55.9 % (v/v) methanol–water mixtures at 25.0 ± 0.1 ºC. The apparent dissociation constants (p_sK_a) were calculated for the di-protonated form ($\mathbf{L}^1H_2^{+2}$ and $\mathbf{L}^2H_2^{+2}$) of pyrimidine bases, using a software package TITFIT, which were then extrapolated to pure water to derive the dissociation constants in aqueous solution (pK_a). The aqueous pK_a constants were found to be: $\mathbf{L}^1$, $pK_{a1} = 3.76$ and $pK_{a2} = 6.95$; $\mathbf{L}^2$, $pK_{a1} = 3.57$ and $pK_{a2} = 6.90$. At pH ≤ 2.00, the dominant species in solution were the protonated form of the amino group substituted at the 1-position, while at a pH around 5.00, they were the protonated form of the pyrimidine ring nitrogen at the 3-position. An effect of intramolecular hydrogen bonding on the p_sK_a values was observed with $\mathbf{L}^1$ but not $\mathbf{L}^2$. The effects of molecular structure and solvent medium on the p_sK_a values are also discussed.

Keywords: dissociation constant, pK_a, potentiometry, pyrimidine derivatives, protonated amines, nitrogen-protonated pyrimidine bases, intramolecular hydrogen bonding.

INTRODUCTION

Dissociation constants (pK_a values) due to the protonation or deprotonation of a reactant during chemical treatment are useful physico-chemical measurements describing the extent of dissociation of functional groups with respect to pH. These parameters are important for choosing appropriate acidic or basic reagents in drug discovery and development, as knowledge of the dissociation state of a particular functional group is critical for understanding the pharmacokinetic and pharmacodynamic properties of new drug substances.[1]

Since pyrimidine bases are minor constituents of nucleic acids, the chemistry of these compounds has been the subject of extensive research because of their ap-

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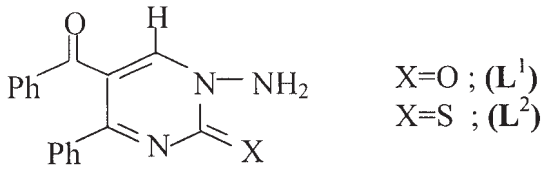
plications in molecular biology and medicine.[2] These compounds display many activities, including antibacterial, antifungal, antiviral, insecticidal and miticidal effects,[3,4] and colony inhibition.[5]

Traditionally, pH-metric titration was employed to determine the pK_a ,of dissociating groups in aqueous solution. However, the success of this approach is sometimes hampered by poor aqueous solubility (<10^{-4} M). If the compound is sufficiently soluble in a water-miscible organic solvent, it is possible to determine the apparent acidity constant, p$_s K_a$, pH-metrically, in a mixed solvent medium. The aqueous pK_a values can then be determined by extrapolation of the p$_s K_a$ values to zero organic solvent content.[6]

In this study, recently synthesized 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one (**L**^{1}) and 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-thione (**L**^{2}) were investigated using the potentiometric titration method in order to i) elucidate their chemical behavior, ii) determine the aqueous dissociation constants and the species present at different pH values, iii) determine the effect of molecular structure and solvent medium on the p$_s K_a$ values. The chemical structures of the studied compounds are given in Scheme 1. The measurements were made in methanol–water media of three different compositions. The apparent p$_s K_a$ values obtained in these mixtures were extrapolated to zero organic solvent content to determine the aqueous pK_a values.



Scheme 1.

EXPERIMENTAL

Materials and solutions

All chemicals were obtained from Fluka, as reagent grade materials. Methanol was used after distilling. Triple-distilled water was prepared in an all-glass apparatus by first redistilling single-distilled water from alkaline permanganate solution, the middle fractions being collected. Then, the double-distilled water was redistilled over an acidic sodium chromate solution, the middle fractions again being collected.[7] The so-obtained triple-distilled water was used for the preparation of the aqueous solutions.

The compounds, 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one (**L**^{1}) and 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-thione (**L**^{2}), the syntheses of which were described elsewhere,[3,4] were obtained from Erciyes University. Their purity was tested by thin-layer chromatography using mixtures of methanol–chloroform (1:9, v/v) and methanol–benzene (1:9, v/v) as solvents and also verified by IR data.

Stock solutions of the compounds (for compound **L**^{1}, *c*: 4.11×10^{-4} M, for compound **L**^{2}, *c*: 3.286×10^{-3} M) were prepared in methanol. The working solutions were prepared from these stock solutions daily by appropriate dilutions with triple distilled water to give 16.3, 33.3 and 50 % (w/w) methanol contents. These compositions were used in this study to prepare the standard buffer solutions for the calibration of the electrode, as described elsewhere.[8] Initially, the methanol–water mixtures were prepared by

w/w dilutions as 16.3, 33.3 and 50 %, which were then expressed as 19.8, 38.6 and 55.9 % (v/v), respectively. All future preparations were made by v/v dilutions to avoid the long w/w preparation using a balance. The stock solutions of the compounds were kept in the dark to avoid decomposition.

Sodium hydroxide solution (0.1 M) was prepared for each solvent system from pellets with carbon dioxide-free triple distilled water and stored in a glass bottle to avoid atmospheric exposure. The solution was standardized by using the linear least-squares fit of a Gran plot for the end-point determination against potassium hydrogenphthalate solution.[9] The electrode assembly consisted of an aqueous saturated calomel electrode as the reference electrode and a Metrohm 6.0202.100 glass electrode filled with a solution of 3M KCl as the indicator electrode.

Hydrochloric acid solution was prepared from a stock solution of known concentration in water and was standardized against standard sodium hydroxide solution using the alkalimetric titration method. All solutions were stored at room temperature.

Apparatus

Titrations were carried out at 25.0 ± 0.1 ºC by circulating thermostated water through a double-walled glass vessel of 50-ml capacity. A constant stream of purified nitrogen gas was passed through the test solutions to ensure an environment free of oxygen and carbon dioxide, and to assist in stirring the solutions. The nitrogen gas was purified as described for argon in a previous study.[10]

The cell containing the electrode, a nitrogen inlet, a thermometer and a capillary tip from a micro-pipette was closed with a Teflon stopper. All pH measurements were made using a Metrohm pH meter (model 744) with an accuracy of ± 0.01 units. The meter was fitted with a Metrohm combined pH electrode (model 6.0202.100) filled with a solution of 3M KCl. The electrode was stored in water and was soaked for 10 min in the methanol–water mixture to be studied before the potentiometric measurements. An Eppendorf micro-pipette was used for the addition of the titrant (*e.g.*, NaOH) solution. A Sartorius A120 S analytical balance (sensitivity of ± 0.0001 g) was used for measuring the masses of the compounds ($\mathbf{L}^1$, $\mathbf{L}^2$) and the chemicals.

pH determinations in different water–methanol media

To avoid inaccuracies from the calibration of the meter, the glass electrode used to measure the pH* values was calibrated using appropriate standard buffer solutions of acetate (pH* = 4.94 and 5.24) and succinate (pH* = 5.07) of known activity, which were prepared in the appropriate water–methanol composition of 16.3, 33.3 and 50.0 % (w/w) methanol. The reference values of the pH* of these buffer solutions and their preparations in different methanol–water mixtures has been reported previously.[8] The electrode was recalibrated when the solvent system was changed. The glass electrode is known to be responsive[11] under these experimental conditions. pH measurements in methanol–water mixtures can be performed similarly to those in water taking into account the pH values assigned to standard buffer solutions in methanol–water mixtures.[12,13] The pH meter readings (pH*) were converted into hydrogen ion concentration by using the activity coefficients (γ) obtained for each mixed solvent medium as explained below and used in the calculations. The desired pH of a solution was adjusted by dotting with a relatively concentrated HCl or NaOH solution on a thin glass rod.

Potentiometric measurements

The pH-potentiometric titrations were performed in a methanol–water mixture that contained either 19.8, 38.6 or 55.9 % (v/v) methanol. The common anion in all solutions was chloride (LiCl, HCl). For the working medium, the ionic strength (I) was kept constant at 0.10 M by using LiCl as the supporting electrolyte. Titrations in each methanol–water mixture were repeated twice and the values were reported as an average of two replicates.

Procedure

The working solutions, *i.e.*, blank and test solutions for each solvent medium (**1**: 19.8, **2**: 38.6 and **3**: 55.9 % v/v methanol), were prepared by adding a known volume of stock compound solution to the vessel containing a known quantity of HCl (to obtain a pH of approximately 2.0) and the re-

quired quantity of LiCl. The contents were diluted to a total volume of 20.0 ml with triple distilled water. After degassing for 10 min, potentiometric titration was performed with a standardized NaOH solution prepared in the solvent medium being studied, whereby the pH-titration curve was obtained. The first reading was taken without addition of the base solution. After each titration, the volume of the test solution was remeasured to calculate the degree of dilution. The pH was measured for each addition of NaOH solution in 0.03 ml increments.

The procedure for the preparation of the tests solutions of the compounds is summarized as follows with compound $\mathbf{L}^1$ for the each solvent medium:

Blank Solutions: HCl (1 ml, 0.094M), LiCl (solid 0.085 g), methanol (3.96 ml for solvent **1**; 7.72 ml for solvent **2**; and 11.18 ml for solvent **3**) and diluted to 20 ml with triple distilled water.

Test Solutions: For solvent **1**; HCl (1 ml, 0.094M), LiCl (solid 0.085 g), 3.96 ml of 4.119×10^{-4} M compound solution (prepared in methanol) and diluted to 20 ml with triple distilled water.

For solvent **2**; HCl (1 ml, 0.094M), LiCl (solid 0.085 g), 3.96 ml of 4.119×10^{-4} M compound solution + 3.76 ml methanol (in order to keep 38.8 % (v/v)) and diluted to 20 ml with triple distilled water.

For solvent **3**; HCl (1 ml, 0.094M), LiCl (solid 0.085 g), 3.96 ml of 4.119×10^{-4} M compound solution + 7.22 ml methanol (in order to keep 55.9 % (v/v)) and diluted to 20 ml with triple distilled water.

Calculation of the activity coefficients and operational autoprotolysis constants, $p\mathrm{K}_{ap}$ values

An aqueous-organic solvent SH, such as water–methanol, can undergo autoprotolysis according to the general process[14]:

$$2\mathrm{SH} \rightleftarrows \mathrm{SH}_2^+ + \mathrm{S}^- \tag{1}$$

The general equilibrium constant expression for (1) is called the autoprotolysis constant, K_{ap}, and is defined by:

$$\mathrm{K_{ap}} = a_{\mathrm{SH}_2^+} \times a_{\mathrm{S}^-} / a_{\mathrm{SH}}^2 \tag{2}$$

where $a_{\mathrm{SH_2}^+}$ and a_{S^-} are the ionic acitivites of the lyonium and lyate ions, and a_{SH} for the undissociated solvent SH is unity.

For each investigated system methanol–water, the activity coefficient (γ) for H^+ and the value of the operational autoprotolysis constant (pK_{ap}) were determined from titrations of the blank solutions mentioned above under the same experimental conditions as for the titrations of the test solutions with the standardized sodium hydroxide solution prepared in the same solvent system. The values for $[H^+]$ were obtained from the pH meter readings. However, $[OH^-]$ values were determined from the amount of base added to the solution. Blank solution titrations were repeated at least three times and the equivalence volumes were determined using second derivative method. The ionic strength (I) of the medium was kept constant at 0.10 M by using LiCl as the supporting electrolyte. The results are given in Table I.

Calculation of apparent dissociation constants ($p_s\mathrm{K}_a$) from potentiometric data

The concentrations of the compounds varied from 8.156×10^{-5} to 7.543×10^{-5} M for $\mathbf{L}^1$ and from 6.507×10^{-5} to 5.915×10^{-4} M for $\mathbf{L}^2$ during the potentiometric titrations. Under such conditions, the ionic strength deviation did not exceed 0.01 M over the entire pH range studied. The number of experimental points was 70–80 (ml-pH) in the pH range 2.0–12.0, for each titration curve. The values of the apparent dissociation constants, p_sK_a were calculated as concentration constants using the activity coefficients for H^+. The operational autoprotolysis constants, pK_{ap}s, given in Table I, were obtained by fitting the potentiometric data obtained from the titration of the di-protonated form of the compounds using a software package, TITFIT.[15] The obtained p_sK_a values were extrapolated to pure water to derive the dissociation constants in aqueous solution (pK_a).

The TITFIT program uses the Newton–Gauss–Marquardt technique supplemented by the use of analytical derivatives ($\partial ml_{\mathrm{calc}}/\partial \log p_i$). For a model which consists of a set of species $M_mL_lH_h$, the basis equation related to the equilibrium constants, β_{mlh}, (charges are omitted for simplicity) is:

TABLE I. Dissociation constants of di-protonated pyrimidine bases ($\mathbf{L}^1$, $\mathbf{L}^2$) and some characteristics in solutions containing different volume ratios of methanol and water at 25 ºC (I = 0.10 M LiCl).

Compound name	Methanol % (w/w)	Methanol % (v/v)	Activity coefficient of H^+	[b]Operational autoprotolysis constant of medium pK_{ap}/M^2	p_sK_{a1}	p_sK_{a2}	[c]Average standard deviation
$\mathbf{L}^1$	16.3	19.8	0.94	13.81	3.76	6.95	3.03×10^{-2}
	33.3	38.6	0.80	13.83	3.70	7.05	9.74×10^{-3}
	50.0	55.9	0.68	13.86	3.69	7.15	7.64×10^{-2}
	0	0	1.08[a] ($\pm7\times10^{-3}$) R = –0.99	13.78[a] ($\pm1\times10^{-3}$) R = 0.99	pK_{a1} = 3.79[a] ($\pm2\times10^{-2}$) R = –0.93	pK_{a2} = 6.84[a] ($\pm5\times10^{-3}$) R = 0.99	
$\mathbf{L}^2$	16.3	19.8	0.94	13.81	3.57	6.90	7.10×10^{-3}
	33.3	38.6	0.80	13.83	3.53	7.00	1.19×10^{-2}
	50.0	55.9	0.68	13.86	3.50	7.10	1.67×10^{-2}
	0	0	1.08[a] ($\pm7\times10^{-3}$) R = – 0.99	13.79[a] ($\pm1\times10^{-3}$) R = 0.99	pK_{a1} = 3.61[a] ($\pm2\times10^{-2}$) R = – 0.99	pK_{a2} = 6.79[a] ($\pm5\times10^{-3}$) R = 0.99	

$\mathbf{L}^1$: 1-Amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one; $\mathbf{L}^2$: 1-Amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-thione. Values in parenthesis are standard deviation for at least three replicates and were calculated by the linear regression method. [a]Values obtained by linear extrapolation to pure water. See text for details. [b]$pK_{ap} = pH_{meas.} - \log [OH^-]$; *i.e.*, the negative logarithm of the product resulting from the concentration of OH^- and the value measured for H^+. [c]Average standard deviation values by means of TITFIT. R = Correlation coefficient.

$$\beta_{mlh} = [M_m L_l H_h] / [M]^m [L]^l a^h_H \quad (3)$$

where m, l and h are the number of the metal (in this study, its value is 0), the compound being investigated and proton, respectively, and a_H is the measured proton activity.

TITFIT calculates the values of the cumulative protonation or dissociation constants that minimizes the sum of the error squares QS between the experimental and the calculated milliliter values, according to the equation given below:

$$QS = \sum_{po\ int\ s} (ml_{exp} - ml_{calc})^2 \quad (4)$$

RESULTS AND DISCUSSION

The measurements were made at low concentrations of the compounds ($\mathbf{L}^1$ = 8.156×10^{-5} M; $\mathbf{L}^2$ = 6.507×10^{-5} M) and at the slowest speed of titrant addition. The low compound concentrations were chosen to avoid complications arising from the formation of polynuclear species. Under these experimental conditions, stable pH values were obtained.

Activity coefficients and operational autoprotolysis constants, pK_{ap}s values

Differences were observed in the values of the activity coefficients which decreased as the dielectric constant of the solvent composition decreased. This is because the activity coefficient of the proton decreased with increasing percentage of methanol in the medium. This agrees well with the fact that the values of the activity coefficient vary with solvent composition and, in general, decrease as the dielectric constant of the solvent decreases.

On the other hand, the operational autoprotolysis constant values, pK_{ap}s, increased slightly with increasing percentage of methanol in the solvent mixture, from pure water up to 55.9 % (v/v) of methanol (Table I). This can be interpreted by the fact that the basicity of the water molecules increased linearly thereby resulting in a proportional increase in the operational autoprotolysis constants (pK_{ap}) with increasing amount of methanol.

When the value of the operational pK_{ap} were plotted against the mole fraction of methanol, χ, a linear relationship was obtained from pure water to methanol–water (55.9 + 44.1, v/v). The equation of the regression line for the operational pK_{ap} = $0.00133\chi + 13.81179$, $R^2 = 0.9991$, defines the operational autoprotolysis constant, pK_{ap}, scale in methanol–water for any solvent composition up to 55.9 % methanol.

Some general considerations and definitions

The compounds have some similarities and some differences in their structures. Each has one amino group, $-NH_2$, substituted at position 1, and one pyrimidine ring nitrogen, >N–, at position 3, which are able to be protonated depending on the pH of the medium. It is well known that amino groups can be protonated by acid or water molecules in acidic solutions. In a previous study, it was found that

both compounds partially exist in their di-protonated form, *i.e.*, in their cationic form, in acidic solutions.[10] However, they exist in their molecular form (Scheme 1) in basic solutions, hence the compounds under investigation are basic in character. Thus, compounds $\mathbf{L}^1$ and $\mathbf{L}^2$ were considered to exist in the $\mathbf{L}^1H_2^{+2}$ and $\mathbf{L}^2H_2^{+2}$ forms, respectively, in acidic solution.

For a constant ionic strength, the dissociation equilibria of the di-protonated compounds, $\mathbf{L}^1H_2^{+2}$ and $\mathbf{L}^2H_2^{+2}$, can be characterized by the related apparent dissociation constant, p_sK_a:

$$\mathbf{L}^1H_2^{+2} \rightleftarrows \mathbf{L}^1H^+ + H^+ \quad (5)$$
$$p_sK_{a1} = [\mathbf{L}^1H^+][H^+] / [\mathbf{L}^1H_2^{+2}]$$

$$\mathbf{L}^1H^+ \rightleftarrows \mathbf{L}^1 + H^+ \quad (6)$$
$$p_sK_{a2} = [\mathbf{L}^1][H^+] / [\mathbf{L}^1H^+]$$

Determination of the aparent dissociation constants (p_sK_a) of the di-protonated compounds

Identical dissociation constants were obtained for both compounds. Both compounds were found to undergo protonation at low pH values and the fully protonated form of each compound can liberate two protons; *i.e.,* for $\mathbf{L}^1$ p_sK_{a1} = 3.76 and p_sK_{a2} = 6.95, within the pH range 2.0–12.0. The similarity between the structures of the compounds (Scheme 1) indicated that the p_sK_{a1} and p_sK_{a2} values obtained for both compounds were consistent with the acidity of the protonated

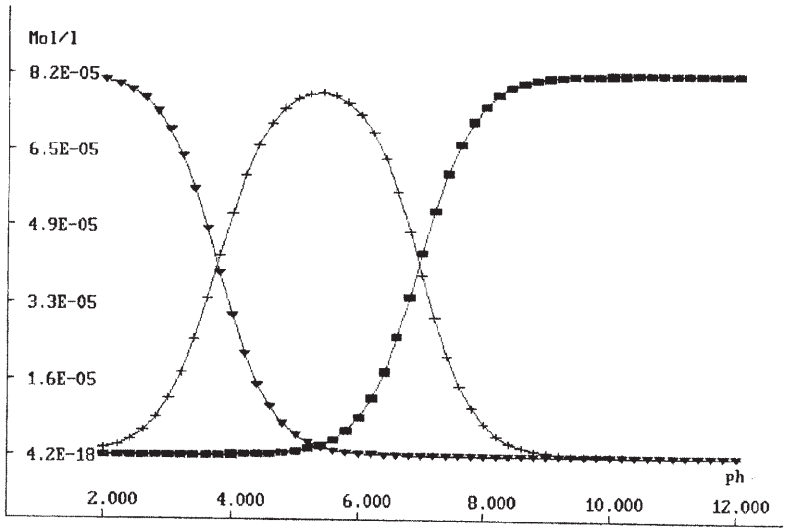


Fig. 1. A typical species distribution diagram as a function of pH for the compound $\mathbf{L}^1$. ■: original compound, ▼: protonated form of the amino group substituted at position 1, +: protonated pyrimidine nitrogen at position 3. Methanol 19.8 % (v/v). [$\mathbf{L}^1$] = 8.156×10^{-5} M, [NaOH] = 0.093 M.

forms of the amino group, $–NH_3^+$ substituted at position 1, and the protonated pyrimidine ring nitrogen, $>N^+H–$ at position 3, for which the apparent dissociation constants were easily recognised by comparison of the p_sK_a values. The apparent dissociation constants calculated for both compounds for each solvent system at 25.0 ± 0.1 ºC and at I = 0.10 M LiCl are given in Table I.

The relative importance of the various species in each pH range is shown in Fig. 1 as a concentration distribution diagram for the compound $\mathbf{L}^1$ system. The best fit of the potentiometric data for the studied solvent systems was obtained when the species reported in Fig. 1 were used. For both compound systems, the concentration distribution of species showed an identical dependence on pH.

The plot given by symbol (■) in Fig. 1 shows the change in the concentration of the original form of compound $\mathbf{L}^1$ (Scheme 1) with pH. However, the symbols (▼) and (+) show the pH dependence of the concentrations of the species protonated at the amino group (*i.e.*, ammonium group, $–NH_3^+$) substituted at position 1 and the species protonated at the pyrimidine ring nitrogen at position 3, respectively, which undergo dissociation as the pH increases from pH 2.0 to 12.0.

On examination of the concentration distributions of the species as a function of pH (Fig. 1), it can be seen that the original form of the compound practically did not exist in the medium at pH values below 6.00. On the other hand, the ammonium group, represented by the symbol (▼), is the only predominant species at pH values of 2.00 and below. Its concentration decreased at pH values above 2.00, and it disappeared completely at a pH between 5.00 and 6.00, exhibiting an apparent dissociation constant, p_sK_{a1}, at pH 3.76. However, the concentration of the protonated form of the pyrimidine ring nitrogen, represented by the symbol (+), started to increase when the pH of the medium was increased from the pH value of 2.00, reaching a maximum concentration at a pH between 5.00 and 6.00. Increasing the pH above 6.00 led to a concentration decrease, the species practically disappearing at pH values around 8.00, exhibiting the second apparent dissociation constant, p_sK_{a2}, at pH 6.95.

The obtained p_sK_{a1} values (Table I) are similar to these of Albert *et al.*,[6] who reported a pK_a value of 5.71 for the protonation of the amino group of 4-aminopyrimidine in water and Bacarella *et al.*[16] who found a pK_a value of 4.51 for the anilinium ion in 20 % (v/v) methanol.

The p_sK_{a2} values (Table I) are also similar to the values reported by Rived *et al.*[17] for some nitrogen-protonated heterocyclic bases, *i.e.,* 5.44, 5.16, 6.99 and 7.26 for the protonation of the N atom of pyridine, quinoline, papaverine and narcotine, respectively, in methanol. Similarly, Bruni *et al.*[18] and Tissier *et al.*[19] reported pK_a values of 5.43 and 6.05 for the pyridine nitrogen and the 4-methylpyridine nitrogen, respectively.

Based on the obtained p_sK_{a1} and p_sK_{a2} values (Table I), it is possible to suggest that the following equilibrium reactions 7 and 8 occur for p_sK_{a1} and p_sK_{a2}, respectively.

$$–NH_3^+ + OH^- \rightleftarrows –NH_2 + H_2O \quad (7)$$

$$>N^+H– + OH^- \rightleftarrows >N– + H_2O \quad (8)$$

Effect of molecular structure on p_sK_a

The values of p_sK_{a1} and p_sK_{a2} for $\mathbf{L}^1$ and $\mathbf{L}^2$ are very similar ($\mathbf{L}^1$, p_sK_{a1} = 3.76 and p_sK_{a2} = 6.95; $\mathbf{L}^2$, p_sK_{a1} = 3.57 and p_sK_{a2} = 6.90). A comparison of the obtained p_sK_a values (Table I) shows that the amino group substituted at position 1 undergoes protonation or dissociation first with respect to the nitrogen atom at position 3. This result is in agreement with the theoretical result.[20] This demonstrates that the amino group, $–NH_2$, of both compounds are considerably weaker bases than the pyrimidine ring nitrogen at position 3. A comparison of the p_sK_{a1} values for both compounds shows that the amino group of $\mathbf{L}^1$ is a slightly stronger base the amino group of $\mathbf{L}^2$.

Effect of solvent medium on the apparent dissociation constants, p_sK_a

A linear relationship was found between the obtained dissociation constants, p_sK_a, and the percentage of methanol. The linear equations and the related correlation coefficients for both compounds are given in Table I. Inspection of the p_sK_a values in Table I shows that both p_sK_{a1} and p_sK_{a2} values of both compounds are dependent on the amount of methanol.

The p_sK_{a1} values were found to decrease slightly with increasing percentage of methanol in the medium. This observation agrees well with the report of Bacarella[16] who observed a similar decrease in the dissociation constant of the anilinium ion with increasing percentage of methanol in the medium. The observed decrease can be interpreted by the fact that the basicity of the water molecules increases linearly and, accordingly, the operational autoprotolysis constants in the solvent mixture, pK_{ap}, increase with increasing amount of methanol.

The decrease observed in the p_sK_{a1} values of the compounds with increasing percentage of the organic solvent in the medium can also be attributed to the large stabilization of protons by their interaction with water molecules only.[21] Consequently, the activity coefficient of the proton decreased with increasing percentage of methanol in the medium. This would result in an increase in the apparent dissociation constant (low p_sK_{a1}) of these compounds. This demonstrates that the amino groups of both compounds are weaker bases in the methanol–water mixture of 55.9 % (v/v) than the mixture of 19.8 % (v/v).

On the other hand, as the methanol fraction in the mixture was increased, the p_sK_{a2} values of both compounds were found to increase slightly. This behavior suggests that the solvating ability of the mixture decreased as the percentage of methanol increased. Since ε_{H_2O} = 78.30 (25 ºC) and ε_{MeOH} = 32.63 (25 ºC), the energy required for the separation of charges increases and thus the extent of the apparent dissociation constant was lowered.[11] For higher methanol contents, the protonated base is mostly solvated by the less basic methanol and the p_sK_{a2} values

increase with the methanol contents up to values slightly higher than the p_sK_{a2} value obtained in solvents with a lower methanol–water ratio.

Intramolecular hydrogen bonding

Inspection of the p_sK_a values given in Table I obtained in different methanol–water systems for both compounds shows that the values of p_sK_{a2} are higher than those for p_sK_{a1}. This could be explained by the formation of internal hydrogen-bonds, as were reported in a previous study to be formed in compound **L**[1], between the $-NH_3^+$ group at position 1 and the oxygen of the >C=O group at position 2, but not in compound **L**[2].[10] When the amino group at position 1 is in the $-NH_3^+$ form, it withdraws electrons from the oxygen of the >C=O group at position 2 through hydrogen bonding. In this case, the O atom compensates the electron deficiency either by withdrawing electrons from the C atom at position 2 or from the N atom at position 3, which makes the protonation of the N atom at position 3 more difficult (*i.e.*, a higher p_sK_a) compared with the substituted $-NH_2$ at position 1. This will result in an increase in the stability of the protonated nitrogen at position 3 of **L**[1], and its dissociation becomes more difficult (*i.e.*, a higher p_sK_{a2} value).

In the case of **L**[2], hydrogen bonding does not occur between the sulphur atom of the thiocarbonyl group at position 2 and the hydrogens of the substituted amino group at position 1. Thus, such interactions will be weak and the N atom at position 3 will be more easily protonated than that in **L**[1] (*i.e.*, a lower p_sK_{a2} value).

The low values for p_sK_{a1} when compared with those for p_sK_{a2} can also be explained by the fact that the amino group attached to the N atom at position 1 is fed mesomerically by the electrons of this N atom, which consequently favors the protonation of the amino groups in comparison to the N atom at position 3 (lower p_sK_{a1}).

Determination of the aqueous dissociation constants, pK_a

The values of apparent dissociation constants, p_sK_{a1} and p_sK_{a2} obtained in methanol–water mixtures were plotted against the alcohol content (expressed as volume %) of the solution.[22] The plots yielded straight lines, as can be seen in Figs. 2 and 3 which also include the corresponding equations for the relationship be-

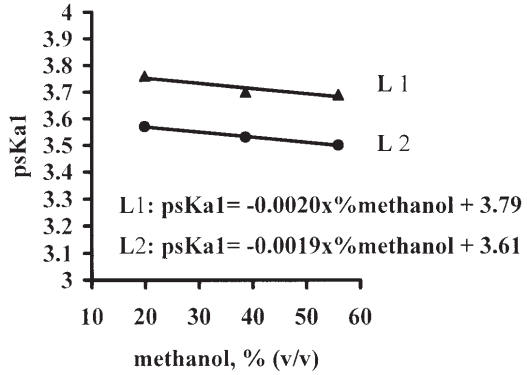


Fig. 2. Variation of the p_sK_{a1} values of 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one (**L**[1]) and 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-thione (**L**[2]) against the percentage of methanol in the medium: (▲), **L**[1]; (●), **L**[2].

tween the p_sK_a values and the percentage of methanol. For each compound, the obtained straight lines were extrapolated to zero methanol content to obtain the aqueous pK_a values. The p_sK_a values given in Table I were used for the extrapolation of the aqueous pK_a. The calculated pK_a values, together with the corresponding correlation coefficient, *R*, are given in Table I.

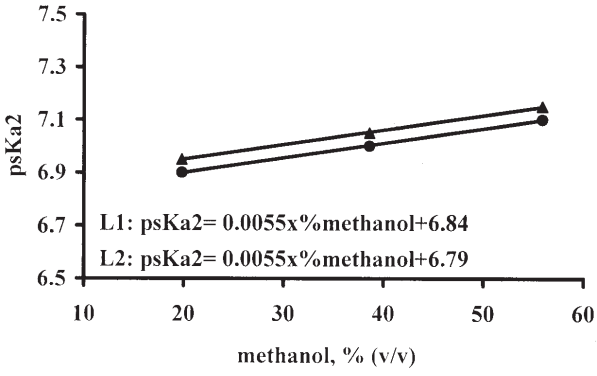


Fig. 3. Variation of the p_sK_{a2} values of 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-one (**L**[1]) and 1-amino-5-benzoyl-4-phenyl-1*H*-pyrimidine-2-thione (**L**[2]) aganinst the percentage ofmethanol in the medium: (▲), **L**[1]; (●), **L**[2].

CONCLUSION

No p_sK_a value was obtained for the sulphur atom of the thiocarbonyl group substituted at position 2 of **L**[2]. One reason for this could be the presence of water in the medium that inhibited the occurrence of possible thione-thiol tautomerization. Thus, repeating the experiments in completely non-aqueous medium is expected to be useful.

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ИЗВОД

ПОТЕНЦИОМЕТРИЈСКО ИСПИТИВАЊЕ КИСЕЛИНСКО-БАЗНЕ РАВНОТЕЖЕ ДВА НОВА ДЕРИВАТА ПИРИМИДИНА У РАЗЛИЧИТИМ СМЕШАМА МЕТАНОЛ–ВОДА

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Киселинско-базна својства 1-амино-5-бензоил-4-фенил-1*H*-пиримидин-2-она (L^1) и 1-амино-5-бензоил-4-фенил-1*H*-пиримидин-2-тиона (L^2) испитивана су потенциометријски при јонској јачини 0,10 M (LiCl) у 19,8, 33,6 и 55,9 % (v/v) смешама метанола и воде на температури 25,0 ± 0,1 °C. Коришћењем софтверског пакета TITFIT израчунате су привидне константе дисоцијације (p_sK_a) за ди-протоновани облик пиримидинских база ($L^1H_2^{+2}$ и $L^2H_2^{+2}$), које су екстраполисане да би се добиле константе дисоцијације у воденом раствору (pK_a). Нађено је да су pK_a вредности: L^1, pK_{a1} = 3,76 и pK_{a2} = 6,95; L^2, pK_{a1} = 3,57 и pK_{a2} = 6,90. При pH ≤ 2,00 у раствору доминира протонован облик амино групе супституисане у положају 1, док је при pH око 5,00 доминантан облик пирими-

динског прстена са протонованим азотом у положају 3. Код L^1 је примећен утицај међумолекулске водоничне везе на p_sK_a вредности, што није случај са L^2. Дискутовани су и утицаји молекулске структуре и растварача на p_sK_a вредности.

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REFERENCES

1. L. Z. Benet, J. E. Goyan, *J. Pharm. Sci.* **56** (1967) 665
2. D. T. Hurst, *The Chemistry and Biochemistry of Pyrimidines, Purines and Pteridines*, Wiley, New York, 1980
3. Y. Akçamur, B. Altural, E. Saripinar, G. Kollenz, O. Kappe, *J. Heterocycl. Chem.* **25** (1988) 1419
4. B. Altural, Y. Akçamur, E. Saripinar, I. Yildirim, G. Kollenz, *Monatsh.Chem.* **120** (1989) 1015
5. M. S. Motawia, A. E. S. Adbelmegied, E. B. Pederson, C. M. Nielsen, P. Ebbesen, *Acta Chem. Scand.* **46** (1992) 77
6. A. Albert, E. P. Serjeant, *The Determination of Ionization Constants, A Laboratory Manual*, 3rd Ed., Chapman and Hall, London, 1984
7. I. M. Kolthoff, E. B. Sandell, E. J. Meehan, S. Bruckenstein, *Quantitative Chemical Analysis*, 4th Ed., The Macmillan Company, New York, 1969
8. D. D. Perrin, B. Dempsey, *Buffers for pH and Metal Ion Control*, Chapman and Hall, London, 1974
9. F. J. C. Rossotti, H. Rossotti, *J. Chem. Educ.* **42** (1965) 375
10. H. Kilic, M. L. Berkem, *J. Serb. Chem. Soc.* **69** (2004) 689
11. R. G. Bates, *Determination of pH*, 2nd Ed., Wiley, New York, 1973
12. J. Barbosa, V. Sanz-Nebot, *Fresenius*, *J. Anal. Chem.* **353** (1995) 148
13. P. R. Mussini, T. Mussini, S. Rondinini, *Pure Appl. Chem.* **69** (1997) 1007
14. S. Rondinini, P. Longhi, P. R. Mussini, T. Mussini, *Pure Appl. Chem.* **59** (1987) 1693
15. A. D. Zuberbühler, T. A. Kaden, *Talanta* **29** (1982) 201
16. A. L. Bacarella, E. Grunwald, H. P. Marshall, E. L. Purlee, *J. Org. Chem.* **20** (1955) 747
17. F. Rived, M. Rosés, E. Bosch, *Anal. Chim. Acta* **374** (1998) 309
18. S. Bruni, F. Cariati, P. G. Daniele, E. P. Prenesti, *Spectrochim. Acta Part A* **56** (2000) 815
19. C. Tissier, M. Tissier, *J. Chem. Phys.* **73** (1976) 149
20. J. A. Joule, G. F. Smith, *Heterocyclic Chemistry*, Van Nostrand Reinhold Company, London, 1976
21. R. G. Bates, J. F. Coetzee, C. D. Ritchie, *Solute-Solvent Interactions*, Marcel Dekker, New York, 1969
22. N. F. Hall, M. R. Sprinkle, *J. Am. Chem. Soc.* **54** (1932) 3469.