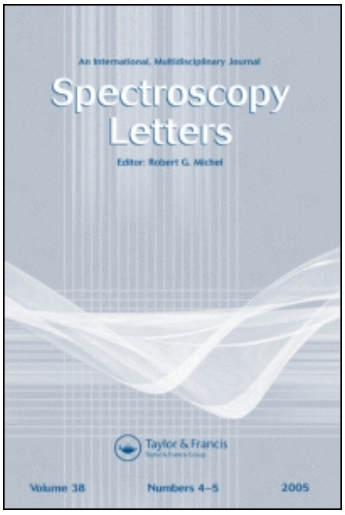

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Fluorescence Titration of Some Purines Determination of Lowest Excited-State Ionization Constants

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FLUORESCENCE TITRATION OF SOME PURINES DETERMINATION OF LOWEST EXCITED-STATE IONIZATION CONSTANTS

Key words: 6-methyl purine, xanthine

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ABSTRACT

Excited-state ionization equilibria of 6-methyl purine and xanthine were investigated in a wide range of pH.

6-Methyl purine is known to have two ionization equilibria in its ground-state. It was found that it had one more ionization equilibrium in strongly acidic region. In the excited state, four ionization equilibria were observed. The pK^* values of three of them were determined by means of fluorescence titration.

In the case of xanthine, there exist three ground-state equilibria. In the excited-state, four equilibria were observed. The pK^* values of two of them were determined by means of fluorescence titration.

Application of nucleic acides in biochemistry and clinical chemistry has increased the interest in the photochemical processes of these compounds and the excited-state properties of purine derivatives have been the object of a number of investigations. There have been few studies upon the excited-state ionization equilibria of purine derivatives, and they have been undertaken within limited pH range[1-9]. In this study, the first excited-state ionization constants of 6-methyl purine and xanthine have been investigated in a wide range of pH.

TABLE 1

ion or molecule	H_3MePu^{++}	H_2MePu^{+}	HMePu	$MePu^{-}$	$MePu^{=}$
λ_{max}(nm) for absoption	263	265[a]	261[a]	271[a]	b
λ_{max}(nm) for fluorescence	440	390	380	364	361

a) The values exist also in literature[2].
b) This anion doesn't occur in ground-state in the pH range investigated.

EXPERIMENTAL

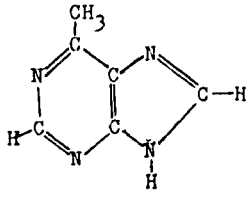
Chemicals: 6-methyl purine was from Fluka(purum), xanthine was from Merck(pure). Bidistilled water used as solvent. Buffer solutions (pH range 2-12) were prepared by adding the necessary amounts of NaOH solution into H_3PO_4-CH_3CO_2H-H_3BO_3 mixture. H_2SO_4 and NaOH were used to prepare strongly acidic and basic solutions.

Instrumentation: Absorption spectra were obtained using an Unicam SP 800A spectrophotometer. Fluorescence spectra were measured by a Fica 55 spectrofluorometer. Radiometer 26 pH meter was used for measuring the pH values of buffer solutions. All measurements were made at room temperature. Optimum concentrations of 6-methyl purine and xanthine were determined as 2.10^{-5} M and 4.10^{-5} M respectively from the relative fluorescence intensity versus concentration plots.

RESULTS

A) 6-Methyl purine

Absorption and fluorescence spectra of 2.10^{-5} M 6-methyl purine solutions at different pH values were obtained (pH differences were approximately 0.5 unit). Absoption spectra showed that four different protolytic species exist in gound-state in the pH range investigated. Fluorescence spectra, on the other hand, showed five different protolytic species in excited-state. Absorption and fluorescence band maxima are summarized in table 1 (6-methyl purine is shown as HMePu).



HMePu

6-methyl purine

6-Methyl purine is known to have two ionization equilibria in its ground-state. They can be written as follows:

(I) $H_2MePu^+ + H_2O \rightleftharpoons HMePu + H_3O^+$ pK = 2.6

(II) $HMePu + H_2O \rightleftharpoons MePu^- + H_3O^+$ pK = 9.02

In this work, one more equilibrium which occurs in 2.5 M H_2SO_4 solution was found. This equilibrium can be shown as follows:

(III) $H_3MePu^{++} + H_2O \rightleftharpoons H_2MePu^+ + H_3O^+$ pK = -1.8

The pK value was determined using following equation[13]

$$H_o = pK + \log \frac{[H_2MePu^+]}{[H_3MePu^{++}]}$$

H_o is the Hammett acidity function.

In the excited-state, one more equilibrium which doesn't occur in ground-state was observed in the solution at pH=12.6 .

(IV) $(MePu^-)^* + H_2O \rightleftharpoons (MePu^=)^* + H_3O^+$

In order to determine the excited-state pK^* (excited-state ionization constant) values, relative fluorescence intensity versus pH curves were obtained.

From FIG.1, the value of pK^* = 3.6 was found for the equilibrium (I).

From FIG.2, the value of pK^*= 9.0 was found for the equilibrium (II). This value must correspond to the ground-state pK= 9.02 . pK^*= 7.4 had been estimated from fluorescence band shifts with pH[12] (If fluorescence emission is faster than the establishment of the excited-state equilibrium fluorescence titration gives the ground-state pK value[10].

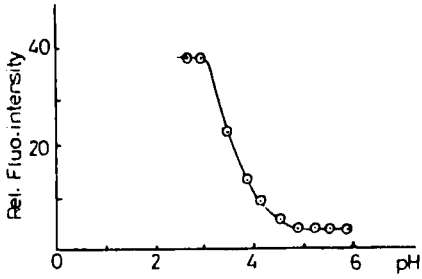


FIG.1. Fluorescence titration curve for 6-Methyl purine at 370nm.

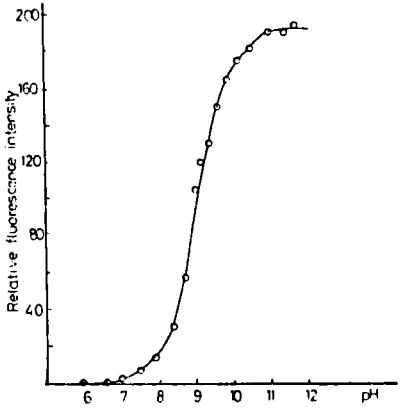


FIG. 2. Fluorescence titration curve for 6-methyl purine at 345nm

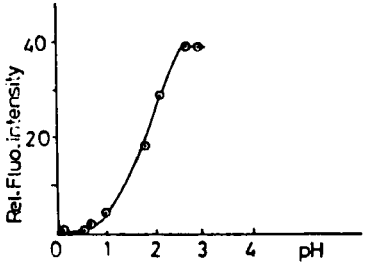


FIG. 3. Fluorescence titration curve for 6-methyl purine at 370nm.

From FIG.3, the value of pK^{*}=1.8 was found for the equilibrium (III).

Equilibrium (IV) couldn't be investigated by this method because of the overlapping of the fluorescence bands of the relevant species. The pK^{*} value had been estimated from the fluorescence band shifts with pH as approximately 12.6 (12).

B) Xanthine

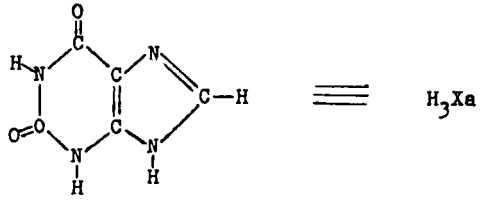
Fluorescence spectra of 4.10^{-5} M xanthine solutions at different pH values were obtained (pH differences were approximately 0.5 unit). Four different protolytic species of xanthine exist in its ground-state. Only three of them emitted.

λ_{max} values of absorption and fluorescence bands are summarized below.

TABLE 2

ion or molecule	H_4Xa^+	H_3Xa	H_2Xa^-	HXa^{*}	Xa^{3-}
λ_{max}(nm) Absorption(1,4,5)	260	267	240;277	283	a
λ_{max}(nm) Fluorescence	b	440	450	440	b

a) This anion doesn't occur in ground-state in the pH range investigated.
b) Fluorescence emission is weak.



Xanthine

Xanthine is known to have three ionization equilibria in its ground-state. They can be written as follows (neutral xanthine is abbreviated as H_3Xa).

(I) $H_4Xa^+ + H_2O \rightleftharpoons H_3Xa + H_3O^+$ pK=0.8

(II) $H_3Xa + H_2O \rightleftharpoons H_2Xa^- + H_3O^+$ pK=7.44

(III) $H_2Xa^- + H_2O \rightleftharpoons HXa + H_3O^+$ pK=11.12

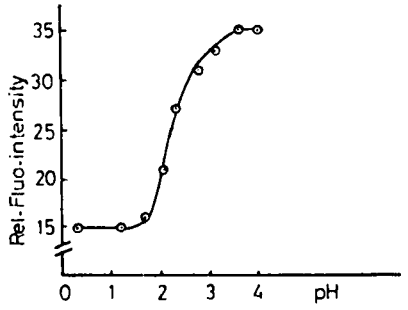


FIG.4. Fluorescence titration curve for xanthine at 440nm.

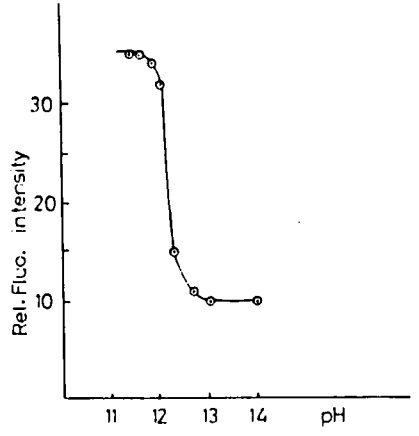


FIG.5. Fluorescence titration curve for xanthine at 440nm.

In the excited-state one more equilibrium which doesn't occur in ground-state was observed in the solutions at pH=12.6 . This equilibrium can be shown as follows.

(IV) $$(HXa^{=})^{*} + H_2O \rightleftharpoons (Xa^{3-})^{*} + H_3O^{+}$$

In order to determine the excited-state pK^{*} values, relative fluorescence intensity versus pH curves are obtained.

pK^* values for the equilibria (I) and (IV) were found as 2.2 and 12.15 respectively from FIG. 4 and FIG.5. The pK^* values of the equilibria (II) and (III) couldn't be investigated by this method due to overlapping of the fluorescence bands of relevant species. These values had been estimated as 4.2 and 9.4 from fluorescence band shifts with pH [12].

DISCUSSION

It has been found that in the purine derivatives studied, the ionization equilibria in the excited-state shift to the acidic region for the proton dissociation from molecules. However, for the proton binding to the molecules, the reverse has been observed. That is, the excited-state equilibria shift to the basic region.

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