

PHOTOPHYSICAL PROPERTIES OF SOME PYRICHROMINE DERIVATIVES

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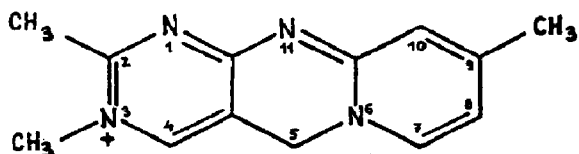
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Summary

The absorption-emission characteristics, photophysical properties and acid-base equilibria of trimethylated pyrichrominium ions in the ground and lowest excited singlet and triplet states were studied. Analysis of the lifetime quantum yield data indicated that at 77 K fluorescence and phosphorescence are the main processes of deactivation of the excited states for the A^+ and HA^{2+} forms. The strong temperature dependence of the fluorescence of the protonated form HA^{2+} is discussed on the basis of a phototautomerization reaction. The acidities in the excited singlet and triplet states are several pK_a units higher than those in the ground state. The trimethylpyrichrominium ions in water undergo a slow photochemical reaction in the presence of oxygen.

1. Introduction

Non-fluorescent natural products can sometimes be converted into highly fluorescent derivatives which can be detected in very low concentrations and used in quantitative analysis [1]. Facile syntheses of highly fluorescent heterocycles from *N*-methylated vitamin B_1 have recently been described [2 - 4]. Heating a methanolic mixture of 1'-methylthiaminium ion with 2-aminopyridines or substituted 2-aminopyridines gives pyrichromine derivatives with a high fluorescence efficiency. To gain a clearer understanding of the photophysical and photochemical properties of these compounds we decided to study the trimethylated pyrichrominium ion



more extensively. As described earlier [2], it is easy to synthesize trimethylated pyrichrominium perchlorate ($A^+ClO_4^-$) and its conjugate acid form $HA^{2+}(ClO_4^-)_2$.

2. Experimental details

2.1. Materials

2,3,9-Trimethyl-5*H*-pyrido[1,2-*a*]pyrimido[4,5-*d*]pyrimidinium perchlorate and its conjugate acid form were synthesized as described in ref. 2. Water was double distilled from a quartz apparatus. Fluorescence grade 95% ethanol (Merck) and concentrated analytical grade H₂SO₄ (POCh) were used without further purification. *n*-Propanol was purified by fractional distillation. Acetate and phosphate buffers and HCl were used for the p*K*_a(S₀) determination. The pH was checked before and after each experiment.

All solvents were carefully checked for luminescence prior to use. Merck silica gel F₂₅₄ or Kieselgel 60 were used for the thin-layer chromatography (TLC) analyses. The solvent systems were 10:1 and 5:1 trichloromethane-methanol mixtures.

2.2. Techniques

UV-visible absorption spectra were recorded on Zeiss (Jena, G.D.R.) VSU-2P or Specord UV-VIS spectrophotometers. The fluorescence and phosphorescence spectra and the phosphorescence decay times were measured using an MPF-3 (Perkin-Elmer-Hitachi) spectrofluorometer. All the fluorescence spectra were corrected for spectral response of the emission monochromator and photomultiplier, whereas the phosphorescence spectra were uncorrected. Fluorescence quantum yields were obtained using quinine bisulphate in aqueous 0.5 M H₂SO₄ ($\Phi_f = 0.55$ [5, 6]) and 9,10-diphenylanthracene in ethanol ($\Phi_f = 0.95$ [7]) as standards for measurements at room temperature and 77 K respectively. The measurements were made in deoxygenated solutions with optical densities below 0.05. Φ_{ph}/Φ_f was measured as described in ref. 8. The samples for the quantum yield measurements were deoxygenated by bubbling oxygen-free nitrogen through them for 15 min and they were then stoppered.

The irradiations were carried out on an optical bench using a high pressure HBO-200 mercury lamp (Narva) with a BC-7 glass filter (Mashpriborintog, U.S.S.R.) for $\lambda > 360$ nm and a Zeiss interference filter for $\lambda = 366$ nm. A solution of benzophenone-benzhydrol in benzene was used as the actinometer ($\Phi = 0.68$ [9]). Oxygen-free nitrogen or oxygen was bubbled through the solutions in a 1 cm cell for 15 min prior to irradiation.

3. Results and discussion

3.1. Absorption and luminescence spectra

The absorption, fluorescence and phosphorescence spectra are shown in Fig. 1. Table 1 gives the calculated and measured photophysical properties of the trimethylpyrchrominium ions. A⁺ and HA²⁺ showed fluorescence and phosphorescence. The excitation spectra for fluorescence and phosphorescence were identical with the absorption spectra. The fluorescence of A⁺

showed little dependence on temperature and exhibited vibrational structure even at room temperature, whereas the fluorescence of HA^{2+} was strongly temperature dependent and showed vibrational structure only at 77 K (see Fig. 1(b) and Table 1).

The fluorescence and phosphorescence quantum yields of A^+ and HA^{2+} were independent of the excitation wavelength. The high values of the fluorescence quantum yields showed that radiative processes are dominant in the lowest excited singlet state. The quantum yield of non-radiative processes in the S_1 state of A^+ can be calculated as $1 - \Phi_f = 0.2$ at room temperature, and the non-radiative rate constant k_{nr} is given by

$$k_{nr} = (1 - \Phi_f)\tau_s^{-1} = 3.6 \times 10^7 \text{ s}^{-1}$$

Comparison of the Φ_f and Φ_{ph} values from Table 1 showed that the quantum yield of the intersystem crossing $\text{S}_1 \rightarrow \text{T}_1$ at 77 K can be estimated from the relation

$$\Phi_{ISC} = 1 - \Phi_f \quad (1)$$

Hence the internal conversion $\text{S}_1 \rightarrow \text{S}_0$ can be neglected at 77 K. Because of the high values of the fluorescence quantum yields it was impossible to calculate accurate values of the intersystem crossing yields. We could only estimate the values of Φ_{ISC} as about 0.04 and about 0.03 for A^+ and HA^{2+} respectively.

By using the values of the singlet lifetimes τ_s from Table 1 the rate constants of the intersystem crossing $\text{S}_1 \rightarrow \text{T}_1$ were estimated as about $7 \times 10^6 \text{ s}^{-1}$ and about $6 \times 10^6 \text{ s}^{-1}$ for A^+ and HA^{2+} respectively. The fluorescence rate constants $k_f = 1/\tau_s^\circ$ for A^+ and HA^{2+} were $1.5 \times 10^8 \text{ s}^{-1}$ and $1.8 \times 10^8 \text{ s}^{-1}$ respectively (see Table 1).

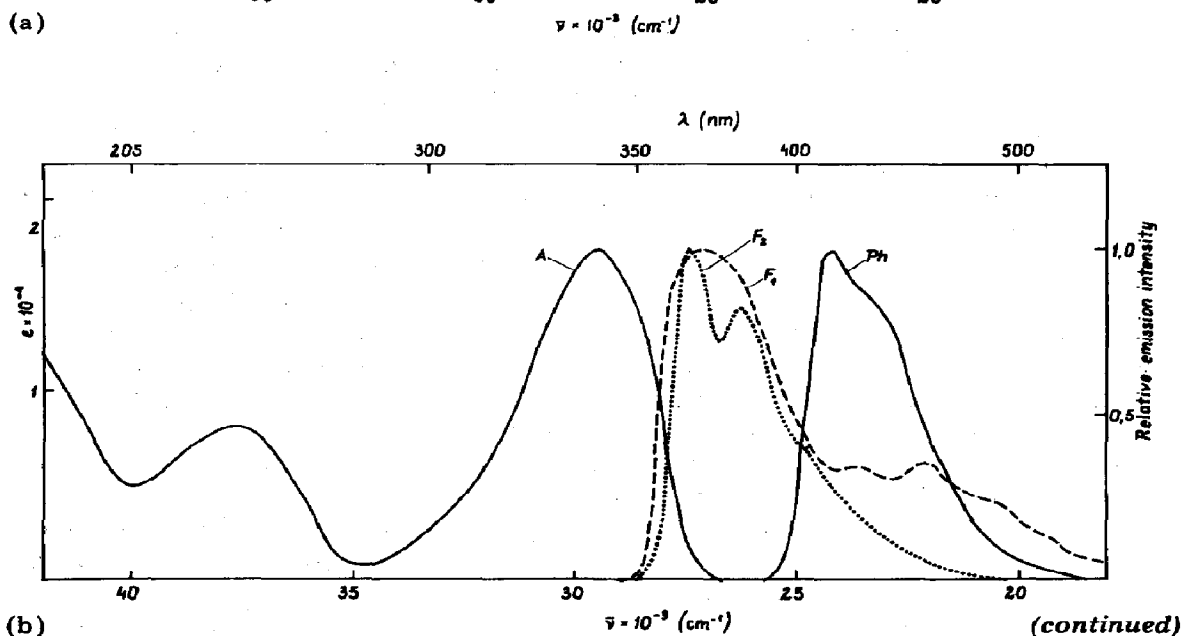
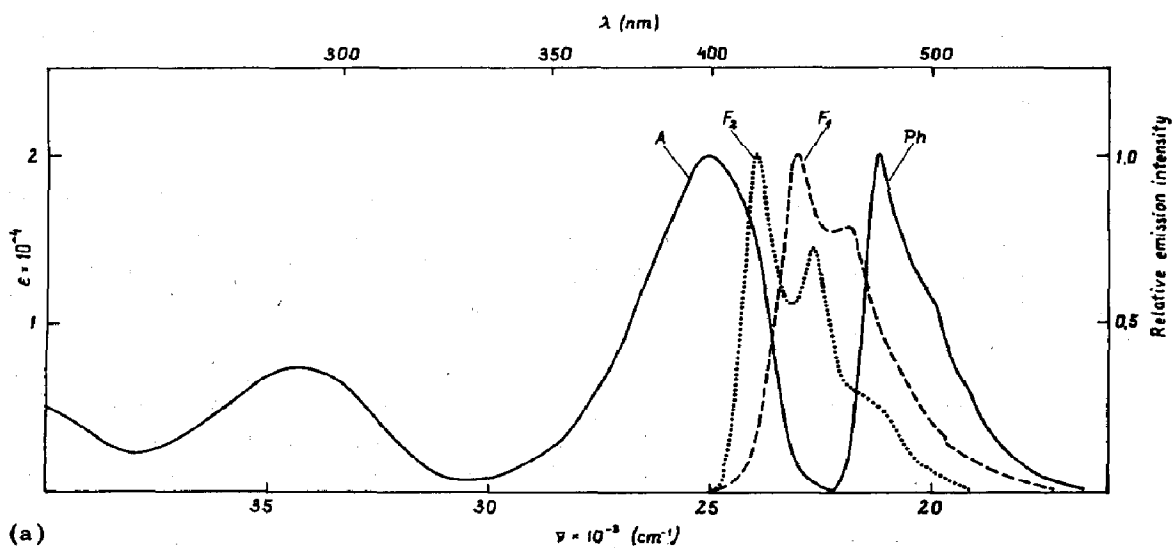
Comparison of Φ_{ISC} and Φ_{ph} (Table 1) showed that phosphorescence is the main deactivation path of the lowest excited triplet state. From the results obtained we concluded that the radiationless processes from the S_1 and T_1 states are generally much weaker than the radiative processes in the pyrichromine ions A^+ and HA^{2+} at 77 K.

A comparison of the radiative lifetimes τ_{cal}° calculated from the absorption spectra with the experimental results $\tau_{exp}^\circ = \Phi_f^{-1}\tau_s$ was made for A^+ in water at 293 K. Under these conditions the lifetime of the S_1 state was measured directly as 5.6 ns [2]. The value of 7.1 ns for τ_{exp}° is in good agreement with the calculated value of 6.6 ns. Radiative lifetime anomalies such as those reported by Birks [11] are not observed in this case. Therefore the singlet state lifetime τ_s calculated from the formula $\tau_s = \Phi_f\tau_{cal}^\circ$ (Table 1) should also give correct values in other solvents.

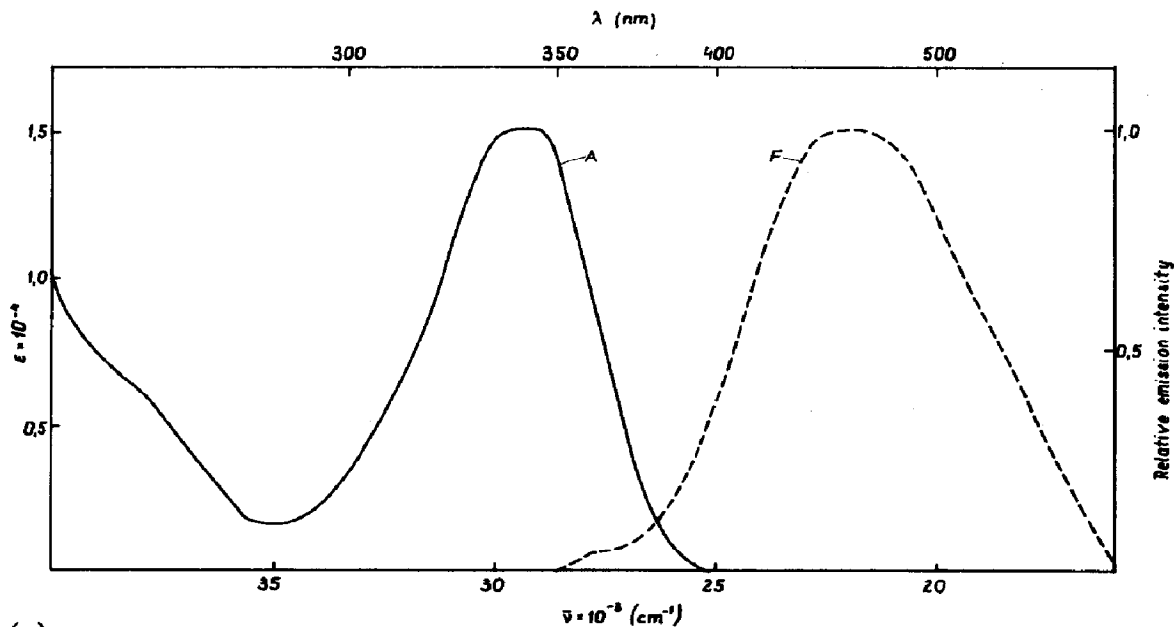
The absorption spectra of pyrichrominium ions in H_2SO_4 were essentially unchanged when the acid concentrations were varied from 0.025 M to 12 M and $\text{pK}_a(\text{S}_0)$ was 4.00. However, some changes occurred in the absorption spectrum in concentrated H_2SO_4 (18 M) (Fig. 1(c)): the low energy absorption band was slightly shifted to a longer wavelength ($\lambda_{max} = 343 \text{ nm}$) compared with its position in dilute H_2SO_4 ($\lambda_{max} = 340 \text{ nm}$), and the spectra

in the region below 280 nm were significantly different. As shown below this can be attributed to the formation of the H_2A^{3+} species. The fluorescence spectrum in concentrated H_2SO_4 changed drastically. The fluorescence quantum yield Φ_f in concentrated H_2SO_4 was 0.05 ± 0.01 at room temperature. Phosphorescence of pyrichrominium ions in concentrated H_2SO_4 was not detected.

We also observed that the trimethylated pyrichrominium ions A^+ were photochemically active. Therefore we carried out some preliminary experiments. The trimethylpyrichromine derivative A^+ was irradiated at $\lambda > 360$



(continued)



(c)

Fig. 1. Absorption, fluorescence and phosphorescence spectra: (a) A^+ in ethanol (curve A, absorption at 293 K; curve F_1 , fluorescence at 293 K; curve F_2 , fluorescence at 77 K; curve Ph, phosphorescence at 77 K); (b) HA^{2+} (curve A, absorption in 10 M H_2SO_4 at 293 K; curve F_1 , fluorescence in 10 M H_2SO_4 at 293 K; curve F_2 , fluorescence in 1:5:2 H_2SO_4 - n - C_3H_7OH - C_2H_5OH at 77 K; curve Ph, phosphorescence in 1:5:2 H_2SO_4 - n - C_3H_7OH - C_2H_5OH at 77 K); (c) in concentrated H_2SO_4 (probably H_2A^{3+}) (curve A, absorption at 293 K; curve F, fluorescence at 293 K).

nm under nitrogen and in oxygen-saturated aqueous solutions (pH 7.0). The reaction was monitored by UV and TLC analysis. A relatively slow photo-oxidation reaction was observed in the presence of oxygen. Deoxygenation inhibited this photoreaction.

The measured quantum yield Φ of the disappearance of A^+ in oxygen-saturated solutions was 0.01, whereas in oxygen-free solutions it was 5×10^{-4} . Both quantum yields were determined for irradiation with monochromatic light of wavelength 366 nm ($I_0 = 0.0029$ einsteins $l^{-1} \text{ min}^{-1}$) and were extrapolated to zero per cent of A^+ conversion.

3.2. Acid-base equilibria

The first acid-base equilibrium step can be expressed as shown below by making the reasonable assumption that N11 undergoes protonation:

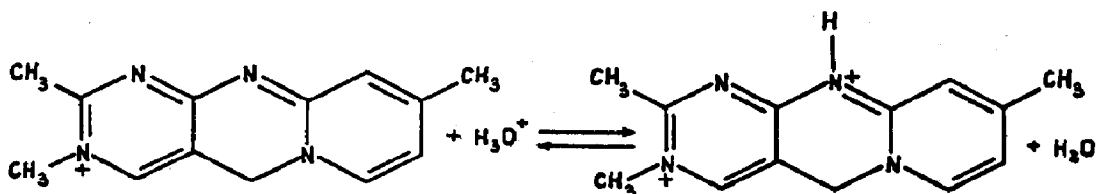


TABLE I

Photophysical properties of A^+ and HA^{2+}

Species	T (K)	Solvent	$\lambda_{00}(S_1)^a$ (nm)	τ_{cal}^b (ns)	Φ_f	τ_S (ns)	Φ_{Ph}/Φ_f	Φ_{Ph}	τ_T (s)	$\lambda_{00}(T_1)^c$ (nm)
A^+	293	H_2O (pH 7.0)	416	6.6	0.79	5.6 ^d	--	--	--	--
					± 0.03					
	293	C_2H_5OH	424	6.7	0.81	5.4 ^e	--	--	--	--
	77	C_2H_5OH	417	--	0.96	6.4 ^e	0.034	0.03	3.0	471
					± 0.05				± 0.5	
HA^{2+}	293	10 M H_2SO_4	357	5.6	≈ 0.03	$\approx 0.2^e$	--	--	--	--
	77	H_2SO_4 - <i>n</i> - C_3H_7OH - C_2H_5OH	365	--	0.97	5.4 ^e	0.046	0.04	2.0	413
					± 0.05				± 0.5	

^aCalculated from the intersection point of the mutually normalized absorption of the fluorescence spectra.^bThe Strickler-Berg formula [10] was used to calculate τ_{cal}^b .^cCalculated from the 0-0 phosphorescence band.^dMeasured [2].^eCalculated from $\tau_S = \tau_{cal}^b \Phi_f$.



The ground state $pK_a(S_0)$ value was determined by spectrophotometric methods based on changes of the absorption (for $\lambda = 392$ nm) and fluorescence (for $\lambda_{em} = 427$ nm and $\lambda_{exc} = 392$ nm) of A^+ with pH. $pK_a(S_0)$ is 4.00 ± 0.03 .

The pK_a values of the acid-base equilibria for the first excited singlet and triplet states were calculated using the following expression for the Förster cycle [12]:

$$pK_a = pK_a(S_0) - \frac{0.625}{T} (\bar{\nu}_{00}^{HA} - \bar{\nu}_{00}^A) \quad (3)$$

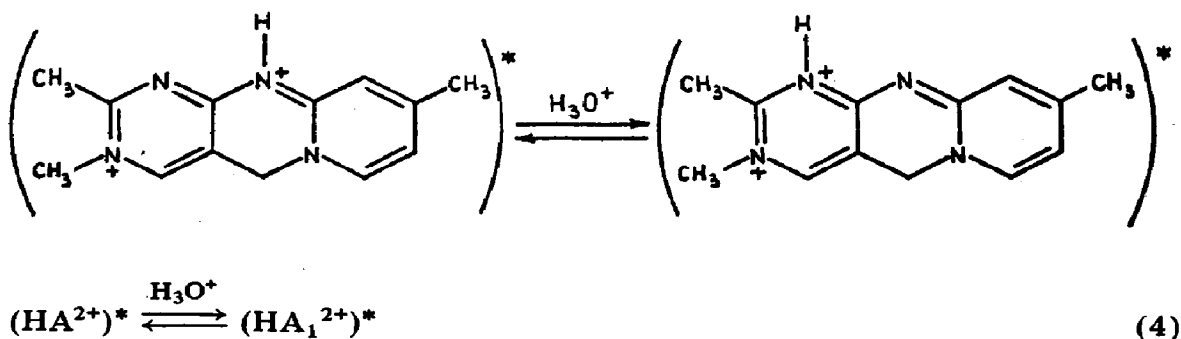
where $\bar{\nu}_{00}^{HA}$ and $\bar{\nu}_{00}^A$ are the wavenumbers of the pure electronic transitions in the conjugated acid and the base respectively. The values of $\bar{\nu}_{00}^{HA}$ and $\bar{\nu}_{00}^A$ were calculated from the intersection point of the mutually normalized absorption and fluorescence spectra for the singlet state S_1 and from the 0-0 phosphorescence band for the triplet state T_1 . Substituting the values of λ_{00} from Table 1 together with the ground state value of $pK_a(S_0)$ gives

$$pK_a(S_1) = -4.3 \pm 0.3 \quad \Delta pK_a(S_1) = -8.3$$

$$pK_a(T_1) = -2.2 \pm 0.3 \quad \Delta pK_a(T_1) = -6.2$$

The acidities of the excited singlet and triplet states are several pK_a units higher than those of the ground state.

Two fluorescence spectra were observed in more concentrated H_2SO_4 (8 M - 14 M) at room temperature: fluorescence of HA^{2+} ($\lambda_{max} = 370$ nm) increasing with H_2SO_4 concentration and fluorescence associated with an equilibrated structure ($\lambda \approx 450$ nm) (see Fig. 1(b)). This latter emission cannot be explained as fluorescence of the A^+ species. For example A^+ fluorescence should not be observed in 14 M H_2SO_4 ($H_0 \approx -7.5$), and the excitation spectra of both the short- and long-wavelength fluorescences are in good agreement with the absorption spectrum. Additionally, we observed only fluorescence from HA^{2+} at 77 K. The experimental results can be explained by the following tautomerization reaction in the excited S_1 state:



Increasing viscosity inhibits proton site exchange in the excited singlet state [13]. Intramolecular proton transfer (via the solvent) from N11 to N1 (reaction (4)) is inhibited in the rigid solution at 77 K. Then only the HA^{2+} fluorescence is observed and Φ_f increases from about 0.03 at room temperature to 0.97 in the rigid solution at 77 K (see Table 1). Therefore we suggest that the long-wavelength fluorescence ($\lambda \approx 450$ nm) in more concentrated H_2SO_4 solutions can be attributed to emission from the HA_1^{2+} species. The results of semi-empirical intermediate neglect of differential overlap calculations [14] are consistent with this proposal.

As shown in Fig. 1(c) the absorption and fluorescence spectra obtained in concentrated H_2SO_4 (18 M) are shifted to longer wavelengths. This absorption and emission is assumed to occur from the doubly protonated species; the second acid-base equilibrium can be expressed as



If it is assumed that absorption and emission processes in concentrated H_2SO_4 occur from the H_2A^{3+} species and the Förster cycle method is used, the value of $\Delta\text{p}K_a^{\text{II}}(\text{S}_1)$ can be estimated:

$$\Delta\text{p}K_a^{\text{II}}(\text{S}_1) = \text{p}K_a^{\text{II}}(\text{S}_1) - \text{p}K_a^{\text{II}}(\text{S}_0) \approx +3$$

Hence, on the basis of the above assumption, for the second protonation, the acidity in the excited singlet state is about a factor of 1000 lower than the value of $\text{p}K_a^{\text{II}}(\text{S}_0)$ in the ground state.

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