Absorption and Luminescence Studies of Some Highly Fluorescent Derivatives of Vitamin B_1; Solvent and pH Effects

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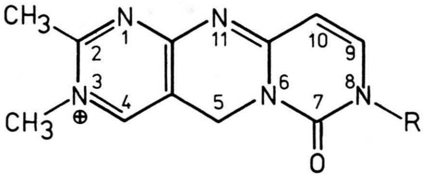
The influence of solvent on the UV-visible absorption and luminescence spectra of some highly fluorescent vitamin B_1 derivatives, the products of the reaction of N-methylated vitamin B_1 with cytidine (I), adenosine (II) and 2-amino-4-methylpyridine (III) is studied. Spectroscopic manifestations of protonation of I and II are also investigated using a semiempirical INDO/S CI method. Singlet and triplet energy levels of the free ion and several protonated species are calculated, and transition energies and oscillator strengths are compared with the experimental spectra. Calculated charge densities on heteroatoms in the ground and excited singlet and triplet states are correlated with changes of the experimental pK_a values with excitation. The results for I and II are compared with those for the trimethylated pyrichrominium ion (III) previously studied.

Introduction

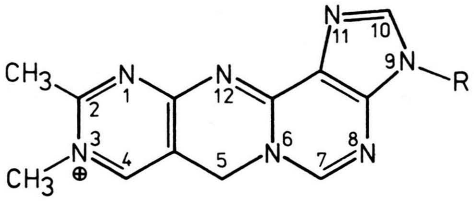
Non-fluorescent natural products can sometimes be converted into highly fluorescent derivatives which can be detected in a very low concentration using emission techniques. It has been recently found that N-methylated vitamin B_1 can be converted into highly fluorescent tricyclic products in nucleophilic substitution by 2-aminopyridines, cytidine, or other nucleophiles [1–3]. The derivatives of cytidine (I), adenosine (II) and 2-amino-4-methylpyridine (III) are studied.

A possible application of these compounds in quantitative determinations of vitamin B_1 (thiamine) by fluorescence techniques [4], and as potential luminescence probes in nucleic acids was the reason for more extensive studies of their photophysical and photochemical properties [5–8]. In this paper an effect of solvent on UV-visible absorption and luminescence properties of compounds I–III will be presented. The spectroscopic manifestations of protonation of the compounds I and II will also be discussed and com-

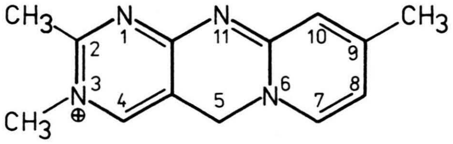


I

R= ribosyl



II



III

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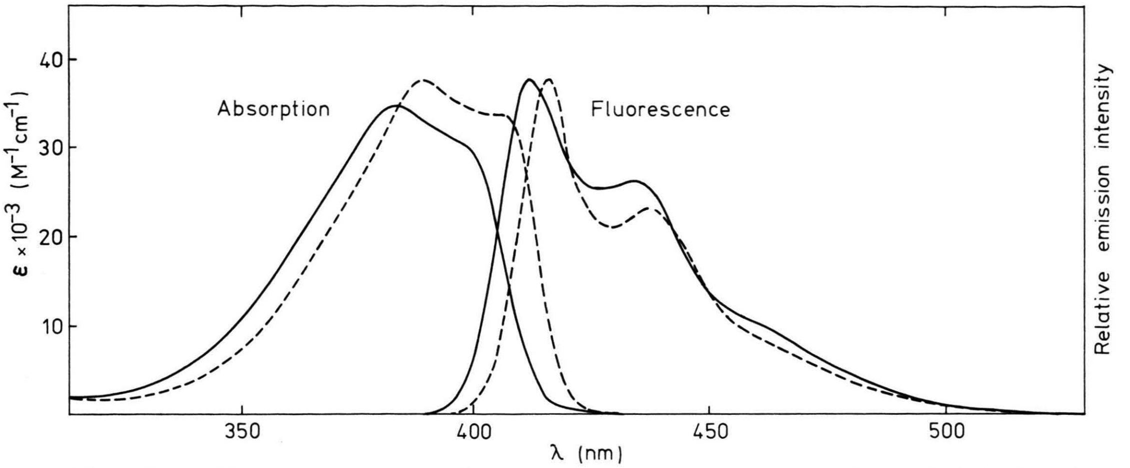

Fig. 1. Absorption and fluorescence spectra of the compound I in water (solid line), and acetonitrile (broken line) at room temperature.

pared to the protonation effects for the trimethylated pyrichrominium ion (III) previously studied [6].

Experimental

2,3-Dimethyl-7-oxo-8-ribosyl-7,8-dihydro-5H-dipyrimido[1,6-a:4′,5′-d]pyrimidinium perchlorate (I), 2,3-dimethyl-9-ribosyl-5H-pyrimidopurino[1,6-a:4′,5′-d]pirimidinium perchlorate (II) and 2,3,9-trimethyl-5H-pyrido[1,2-a]pyrimido[4,5-d]pyrimidinium perchlorate (III) were synthesized and purified by Zoltewicz et al. [1–3]. All solvents were spectrograde quality and were used without further purification.

UV-visible absorption spectra were recorded using a Specord M-40 spectrometer (Zeiss). The emission spectra were measured by means of MPF-3 and MPF-44B (Perkin-Elmer) spectrofluorimeters and were corrected for spectral response of the emission monochromator and photomultiplier. The fluorescence quantum yield measurements were carried out according to the procedure described previously [5].

Electronic properties were calculated using the spectroscopically parametrized semiempirical INDO/S CI method [9]. The calculations were performed for compounds I and II with the methyl group instead of the ribosyl substituent and for various protonated species, with a proton placed at the assumed site of protonation. The cristallographic geometries were used. Details of calculations were described in [6].

Results and Discussion

The photophysical properties and the acid-base equilibria in the ground and lowest excited singlet and triplet states of I, II, and III have been previously investigated [5, 6]. It has been shown that these compounds, subjected to sulfuric acid-water mixtures, undergo a proton transfer reaction characterized by a equilibrium constant K_a:

$$A^+ + H_3O^+ \rightleftarrows HA^{2+} + H_2O, \qquad (1)$$

where A^+ and HA^{2+} represent the free ion and the monoprotonated species, respectively.

For I, II, and III the absorption spectrum in water at pH ≈ 6.5 shows an intensive maximum at 385, 383 and 392 nm, respectively. The position and bandshape of this long-wavelength absorption band as well as the fluorescence spectrum of the A^+ form of the three compounds vary only slightly with solvent. This is illustrated in Fig. 1; numerical results are given in Table 1, showing solvent effects on the absorption and flourescence spectra. The small spectral shifts observed in the absorption and fluorescence spectra in different solvents are difficult to explain quantitatively. A possible specific solute-solvent interaction that can occur for some solvents and small changes in the dipole moments of the A^+ ion with excitation (see below) can be a reason for the observed solvent effects. A relatively large influence of the type of solvent on the fluorescence quantum yield is found only for compound I. This effect can be explained in terms of

Table 1. Absorption and fluorescence spectra of the compounds I, II, and III at room temperature.

Compound	Solvent	Absorption			Fluorescence		
		λ_{max} (nm)	$\Delta\bar{\nu}_{1/2}$ (cm^{-1})	$\varepsilon \times 10^{-3}$ ($M^{-1}\,cm^{-1}$)	λ_{max} (nm)	$\Delta\bar{\nu}_{1/2}$ (cm^{-1})	Φ_f [a]
I	Acetonitrile	390	3000	37.6	416	1900	0.99
	Acetone	391	3000	34.4	418	1900	0.94
	Ethanol	390	3050	31.6	417	2000	0.14
	Water	385	3100	33.8	413	2100	0.38
II	Acetonitrile	392	2600	41.3	415	2300	0.95
	Ethanol	389	2700	38.3	416	2400	0.99
	Water	383	2700	40.3	409	2250	0.95
III	Acetonitrile	403	3200	23.6	432	2200	0.85
	Ethanol	398	3200	22.6	433	2200	0.86
	Water	392	3300	22.7	427	2500	0.79 [b]

[a] accuracy ±(3–8)%; [b] from [5].

changes in radiationless rates with solvent and will be discussed elsewhere [10].

Upon acidification, in 1–18 M sulfuric acid-water solutions, the A^+ ion is protonated to form the HA^{2+} ion, and in all absorption spectra of vitamin B_1 derivatives studied, a "blue" shift of 40–50 nm of the long-wavelength band is observed. (For III, the double protonated species H_2A^{3+} was additionally observed in concentrated (18 M) H_2SO_4 solution [6].) Thus, protonation of the free ion A^+ leads to both a hypsochromic shift and a decrease in the intensity of the long-wavelength band in the absorption spectra of the three compounds. A similar behaviour is observed in the fluorescence spectra upon protonation; the HA^{2+} forms are characterized by "blue" shifted weak fluorescence [5, 8]. It is noteworthy that for the compound III in more concentrated sulfuric acid solution an additional fluorescence is observed, which is assigned to the emission from the monoprotonated tautomer [5–7]. This type of dual fluorescence is not observed for I and II. Both A^+ and HA^{2+} forms of I, II, and III exhibit weak phosphorescence in rigid solutions at 77 K; the HA^{2+} phosphorescence is "blue" shifted in comparison to the phosphorescence of the A^+ ion.

The experimental pH effect on the absorption and luminescence properties of I and II can be compared with calculated electronic properties of the free ions and their protonated forms with the proton placed at the assumed site of protonation. By using the semi-empirical INDO/S CI method we have calculated the transition energies and oscillator strengths for the free ions A^+ and different monoprotonated forms HA^{2+}. The calculations were performed for the following species: the free ions A^+ of I and II, the HA^{2+} ion of I protonated at the N-11, N-1 and O atoms, and the HA^{2+} ion of II protonated at the N-12, N-1, N-8, and N-11 atoms. The observed and calculated spectra are summarized in Tables 2–6.

The observed and calculated absorption spectra of the free ions A^+ of I and II are in good qualitative agreement. In the case of monoprotonated ions HA^{2+}, the best correlation between the observed and calculated spectra is found for the compound I proto-

Table 2. Observed and calculated transition energies, molar absorption coefficients (ε) and oscillator strengths for compound I.

Observed		Calculated		
Energy (cm^{-1})	ε ($M^{-1}\,cm^{-1}$)	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states [a]				
26 000	33 500	A′	28 200	0.752
		A″	29 600	0.000
		A″	33 800	0.000
34 200	3 150	A′	35 600	0.069
		A″	36 300	0.000
		A″	36 900	0.016
39 500	4 800	A′	38 300	0.037
		A″	42 100	0.003
42 500	10 800	A′	43 300	0.246
44 900	13 300	A′	44 700	0.251
		A″	45 000	0.001
		A′	46 100	0.220
Triplet states [b]				
21 300	–	A′	24 000	–
		A′	27 800	–

[a] From absorption spectra in water at room temperature.
[b] From phosphorescence spectra in ethanol at 77 K.

Table 3. Observed and calculated transition energies, molar absorption coefficients (ε) and oscillator strengths for the N-11 protonated compound I (transitions with the oscillator strengths greater than 0.050 are only quoted).

Observed		Calculated		
Energy (cm^{-1})	ε (M^{-1} cm^{-1})	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states [a]				
28 200	29 300	A′	30 000	0.520
		A′	33 800	0.050
39 000	2 700	A′	40 600	0.094
		A′	43 300	0.098
		A′	45 600	0.053
45 000	22 600	A′	48 500	0.407
		A′	48 900	0.683
Triplet states [b]				
23 800	–	A′	24 300	–
		A′	29 700	–

[a] From absorption spectra in 2 M H_2SO_4 at room temperature.
[b] From phosphorescence spectra in H_2SO_4:n-C_3H_7OH:C_2H_5OH (2:5:1) at 77 K.

Table 4. Calculated transition energies and oscillator strengths for the N-1 and O-monoprotonated ion of compound I (transitions with the oscillator strengths greater than 0.050 are only quoted).

N-1 monoprotonated ion			O-monoprotonated ion		
Symmetry	Energy (cm^{-1})	Oscillator strength	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states					
A′	27 100	0.752	A″	28 000	0.001
A′	30 600	0.095	A′	28 700	0.585
A′	39 300	0.083	A′	29 800	0.249
A′	40 900	0.050	A′	38 500	0.100
A′	45 200	0.102	A′	41 400	0.073
A′	47 900	0.341	A′	42 300	0.266
			A′	44 200	0.167
			A′	47 000	0.318
Triplet states					
A′	21 600	–	A′	22 500	–
A′	25 100	–	A″	28 200	–

Table 5. Observed and calculated transition energies, molar absorption coefficients (ε) and oscillator strengths for compound II.

Observed		Calculated		
Energy (cm^{-1})	ε (M^{-1} cm^{-1})	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states [a]				
26 100	40 300	A′	26 300	0.738
		A″	30 100	0.000
34 000	3 900	A′	32 700	0.316
		A′	35 800	0.047
		A″	36 100	0.007
		A″	38 100	0.009
		A′	38 300	0.028
		A″	39 200	0.001
		A′	40 700	0.033
		A″	40 900	0.016
		A″	42 500	0.002
41 000	12 700	A′	42 900	0.137
		A′	43 800	0.030
		A′	45 200	0.143
		A″	45 700	0.011
		A′	45 800	0.328
		A″	46 300	0.003
48 000	29 400	A′	47 500	0.384
Triplet states [b]				
21 150	–	A′	23 300	–
		A′	26 500	–

[a] From absorption spectra in water at room temperature.
[b] From phosphorescence spectra in ethanol at 77 K.

Table 6. Observed and calculated transition energies, molar absorption coefficients (ε) and oscillator strengths for the N-12 protonated compound II (transitions with the oscillator strengths greater than 0.050 are only quoted).

Observed		Calculated		
Energy (cm^{-1})	ε (M^{-1} cm^{-1})	Symmetry	Energy (cm^{-1})	Oscillator strength
Singlet states [a]				
29 900	24 200	A′	29 700	0.553
		A′	35 100	0.050
~37 000	6 900	A′	37 000	0.161
41 800	16 300	A′	40 800	0.319
		A′	41 700	0.073
		A′	45 500	0.300
47 000	24 600	A′	46 400	0.569
Triplet states [b]				
23 900	–	A′	26 600	–

[a] From absorption spectra in 1.5 M H_2SO_4 at room temperature.
[b] From phosphorescence spectra in H_2SO_4:n-C_3H_7OH:C_2H_5OH (2: 5:1) at 77 K.

nated at the N-11 atom, in spite of the large negative charge on the oxygen atom (see Fig. 2), and for the compound II protonated at the N-12 atom. However, comparing the spectra one must bear in mind that the theoretical spectra refer to ions in vacuo, while the experimental spectra were taken in highly polar solvents. Despite of this limitation there is good agreement between the observed and calculated changes

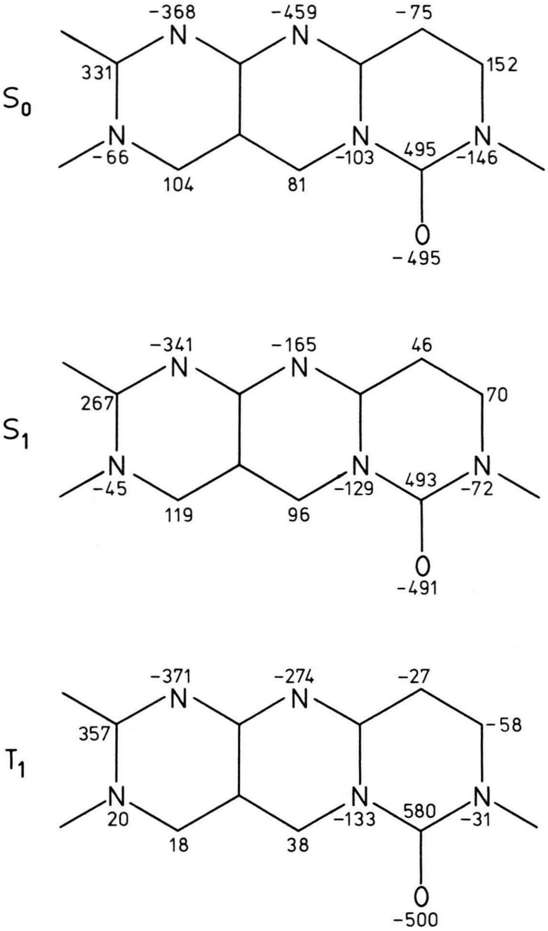

Fig. 2. Net atomic charges in the ground and excited singlet and triplet states of compound I (in 0.001 e units).

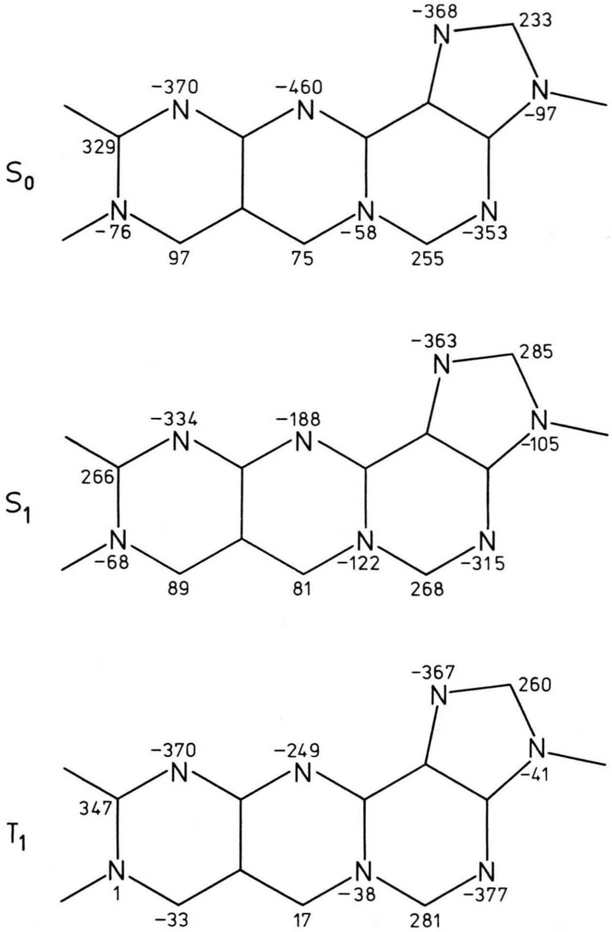

Fig. 3. Net atomic charges in the ground and excited singlet and triplet states of compound II (in 0.001 e units).

in the transition energy and oscillator strength of the long-wavelength absorption band upon protonation. It was recently been shown [6] that the N-11 atom is the site of protonation for the trimethylated pyrichrominium ion (III).

The lowest excited singlet state of the A^+ and HA^{2+} ions for I, II, and III are calculated to be of the π, π^* type. This is consistent with such experimental results as large values of the molar absorption coefficient of the low-energy band, $\varepsilon \approx 10^4\ M^{-1}\ cm^{-1}$, and efficient fluorescence with a lifetime in the nanosecond range. The fluorescence quantum yields for the A^+ and HA^{2+} forms are determined to be nearly 1 in rigid solution at 77 K [5, 8] (the intermolecular proton transfer reaction (1) is inhibited.) It is noteworthy that the fluorescence bands show near mirror images of the low energy absorption bands and, therefore, can be assigned to the primary excited S_1 states of the free ions and monoprotonated forms of I, II, and III.

The triplet state energies of the A^+ and HA^{2+} ions are also well reproduced in the calculations (see Tables 1, 2, 4, and 5 for I and II, and data for III in [6]). As for the S_1 states, the energies of the lowest triplet states for the three compounds increase upon protonation. The lowest triplet state of the A^+ and HA^{2+} ions are calculated to be of the π, π^* type; this is consistent with the spectral characteristic of these states: weak phosphorescence with lifetime in the second range.

Since the absorption, fluorescence and phosphorescence spectra of the free ions A^+ and their conjugated acid species are known for I, II, and III, the pK_a values

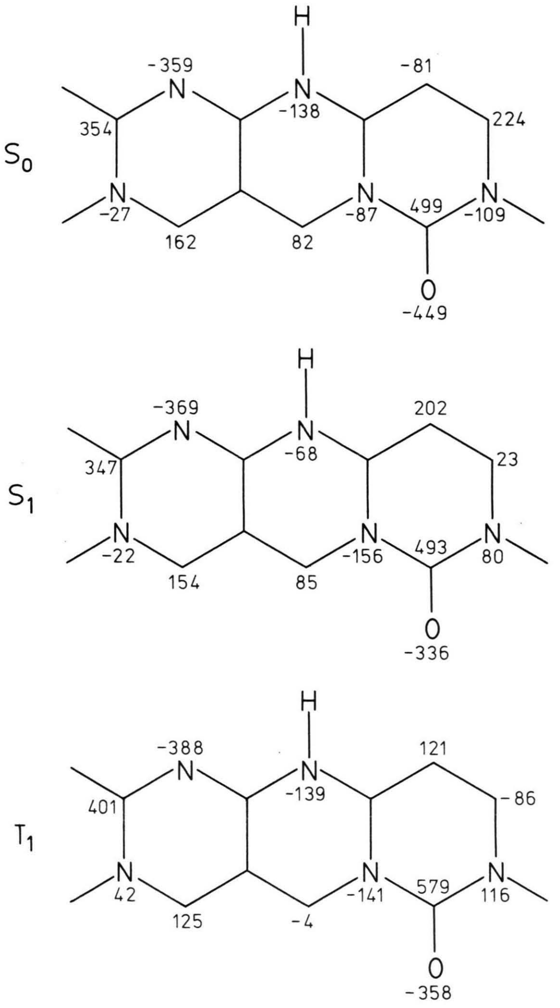

Fig. 4. Net atomic charges in the ground and excited singlet and triplet states of compound I protonated at the N-11 atom (in 0.001 e units).

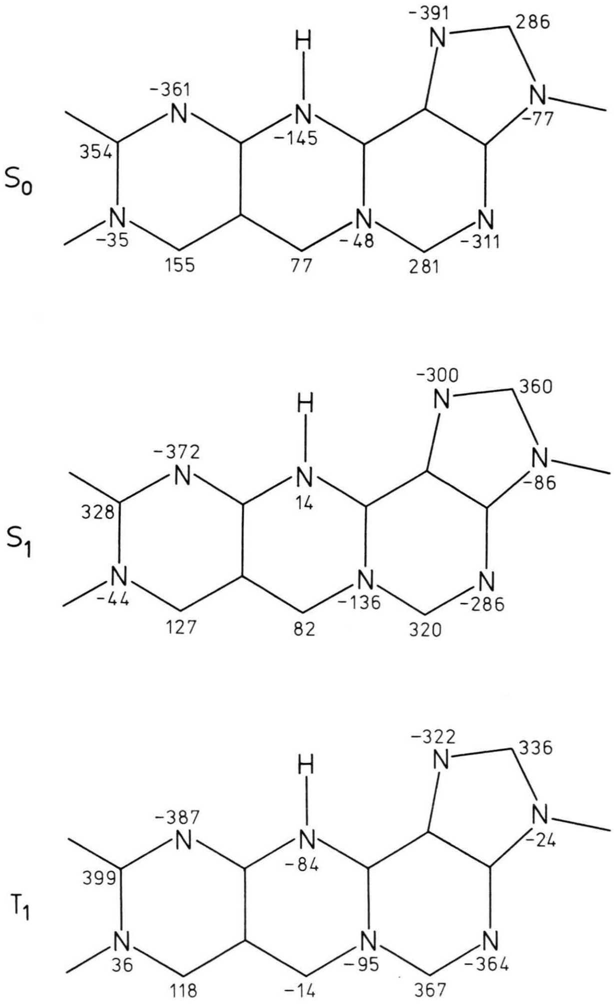

Fig. 5. Net atomic charges in the ground and excited singlet and triplet states of compound II protonated at the N-12 atom (in 0.001 e units).

of acid-base equilibrium (1) for the ground state could be determined spectrophotometrically, and for the excited singlet and triplet states by using the Förster cycle [5, 8]. As for compound III [6], to distinguish between different protonation sites in the free ion A^+ of I and II, we have calculated net atomic charges in the ground and lowest excited singlet and triplet states. The results are summarized in Figs. 2 and 3. It is apparent that among the seven heteroatoms of the free ion II only four: N-1, N-8, N-11, and N-12 atoms, can be considered as the most probable site of protonation. The N-12 atom is predicted to be the most basic heteroatom, and it is therefore the most probable site of protonation in the ground state. The values of net atomic charges along with changes in the absorption spectrum upon protonation and values of the pK_a constants for the ground and excited states [8] strongly indicate that the N-12 atom is the site of protonation in the ground state. This is consistent with the results for the trimethylated pyrichrominium ion (III) [6] having very similar molecular structure. Moreover, as for the compound III, changes in net atomic charge on the N-12 atom with excitation are in qualitative agreement with changes of the pK_a values determined experimentally [8]: $\Delta pK_a(S_1) = -7.0$ and $\Delta pK_a(T_1) \approx -5.8$.

For the compound I, the net atomic charges (Fig. 2) suggest that the N-11 and O atoms are the most probable sites of protonation in the ground state. The changes in the absorption spectra upon protonation as well as changes in the pK_a values with excitation indicate protonation on the N-11 atom. Similarly to compounds II and III, the changes in net atomic charge on the N-11 atom with excitation correlate with the increase of acidity of I in the lowest excited singlet and triplet states: $\Delta pK_a(S_1) = -5.8$ and $\Delta pK_a(T_1) = -5.3$ [8]. (The values of $\Delta pK_a(S_1)$ was calculated from the Förster cycle taking 0–0 fluorescence bands measured at 77 K as pure electronic transitions in conjugated acid and bases.) Changes in net atomic charges with excitation for the monoprotonated molecules are illustrated in Figs. 4 and 5, and these are consistent with the observed increase of acidity in the lowest excited singlet and triplet states.

The determined net atomic charges for the free ion A^+ in the ground and excited states (Figs. 2 and 3) allow one to calculate dipole moments. It was found that, in spite of changes in the electron density distribution, the calculated dipole moments were essentially constant with excitation. Therefore, the solvent shifts due to dipole-dipole interactions [11] can be expected to be very small. This is consistent with small solvent shifts in the absorption and fluorescence spectra (see Table 1).

A comparison of the experimental and theoretical results for the three compounds leads to the following conclusions:

- the N-11 atom for I and III and N-12 for II are the protonation sites for the free ion A^+,
- the lowest excited singlet and triplet states for the free ion A^+ and monoprotonated species HA^{2+} are of the π, π^* type,
- calculated changes in charge densities on the nitrogen atom (N-11 for I and III, and N-12 for II) in the ground and excited and singlet and triplet states are consistent with changes of the pK_a values with excitation,
- the observed small shift of low energy absorption and fluorescence bands in different solvents are consistent with small changes in calculated dipole moments of the A^+ form upon excitation.

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