Photophysical and Photochemical Properties of Some Fluorescent Derivatives of Vitamin B_1

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Z. Naturforsch. **42a,** 485–489 (1987); received December 31, 1986

Absorption and emission spectra, depopulation kinetics of the lowest excited singlet and triplet states and acid-base equilibria of two fluorescent vitamin B_1 derivatives, the products I and II of the reaction of N-methylated vitamine B_1 with cytidine and adenosine, respectively, were investigated. Analysis of the lifetime and quantum yield data indicate that at 77 K emissions are the main processes of deactivation of the S_1 and T_1 states for the free ion and protonated forms. The pKa values indicate a much higher acidity in the excited singlet and triplet states than in the ground state. I and II undergo very slow photochemical reactions in solution in the presence of oxygen ($\Phi \sim 10^{-4}$).

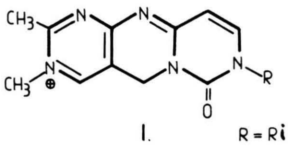
Introduction

It has been found recently that the nucleophilic substitution of N-methylated vitamin B_1 by 2-aminopyridines, cytidine, cytosine or other nucleophiles leads to the formation of highly fluorescent tricyclic products [1–3]. This reaction has been already proved to be important with regards to quantitative conversion of non-fluorescent natural products into highly fluorescent derivatives, which can be detected in very low concentration and quantitative determined by fluorescence measurements. Additionally, because of the possible application of these compounds as luminescence probes in nucleic acids they have become the subject of intense photophysical and photochemical studies.

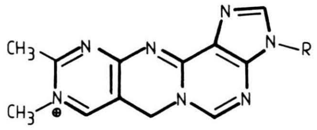
The photophysical properties of the trimethylated pyrichrominium ion have recently been described [4, 5]. In this paper, preliminary results of similar studies for highly fluorescent derivatives of cytidine and adenosine are discussed.

Experimental

2,3-Dimethyl-7-oxo-8-ribosyl-7,8-dihydro-5H-dipyrimido-[1,6-a: 4′,5′-d] pyrimidinium perchlorate (I) and 2,3-dimethyl-9-ribosyl-5H-pyrimidopurino-[1,6-a: 4′,5′-d] pyrimidinium perchlorate (II) have been synthesized and purified by Zoltewicz et al. [3]. The purification of the solvents and the preparation of the samples have been performed as in [4]. Merck Silicagel F_{254} or Silicagel 50 without fluorescence indicator and the solvent systems 1-butanol: H_2O and trichloromethane: methanol were used for the thin-layer chromatography analysis (TLC).



I.



R = Ribosyl

II.

UV-visible absorption spectra were recorded using a Specord M-40 spectrophotometer (Zeiss, GDR). The luminescence and excitation spectra were measured by means of MPF-3 and MPF-44B (Perkin-Elmer-Hitachi) spectrofluorimeters. The fluorescence spectra at room temperature were corrected for spectral response of the emission monochromator and photomultiplier, whereas the low temperature fluorescence and phosphorescence spectra are uncorrected.

Fluorescence decay times were determined by means of a Spectra-Physics synchronously pumped, cavity – dumped, frequency – doubled laser system [6]. Phosphorescence lifetimes were measured using a spectrophosphorimeter constructed by J. Najbar and his coworkers (Jagiellonian University, Cracow).

The luminescence quantum yield measurements and photochemical studies were carried out according to the procedure previously described [4].

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The radiative rate constants k_f were calculated on the basis of the absorption and fluorescence spectra from the Strickler-Berg formula [7] ($1/\tau^0_{cal}$), as well as from the measured fluorescence lifetimes (τ_s) and quantum yields (Φ_f) using the equation

$$k_f = \Phi_f \cdot \tau_s^{-1} . \qquad (1)$$

The nonradiative rate constants, k_n, were calculated from the equation

$$k_n = (1 - \Phi_f)\, \tau_s^{-1} . \qquad (2)$$

Results and Discussion

1. Absorption and Emission Properties

Low energy absorption, fluorescence and phosphorescence spectra are shown in Figs. 1 and 2. The absorption spectra of I and II are characterized by very strong $\pi \rightarrow \pi^*$ transitions in the 300–400 nm range depending on the nature of the solvent used. In water solutions ($pH = 6.5$) the spectra of the free A^+ ion revealed the maxima of absorption at 384 nm ($\varepsilon = 34100\ M^{-1}\,cm^{-1}$) and 383 nm ($\varepsilon = 37600\ M^{-1}\,cm^{-1}$) for I and II, respectively. In 1–18 M sulfuric acid solutions the spectra of the monoprotonated species (HA^{+2}) showed the maxima at 355 nm ($\varepsilon = 29300\ M^{-1}\,cm^{-1}$) and 335 ($\varepsilon = 24200\ M^{-1}\,cm^{-1}$) for I and II, respectively. As in the case of the trimethylated pyrichrominium ion [4, 5], the protonation of the free ion leads to both a hypsochromic shift and a decrease in the intensity of the long-wavelength band in the absorption spectra of I and II.

The fluorescence spectra of I and II at room temperature in aqueous solutions ($pH = 6.5$) show intensive bands at 413 nm and 409 nm, respectively. These emissions may be attributed to the primary excited singlet state S_1 of the monocation A^+. In $2\,M\,H_2SO_4$, the solute is protonated in the ground state and the absorption spectra show only bands from HA^{+2}, but nevertheless the fluorescence spectra remain unchanged relative to those at $pH = 6.5$. When the sulfuric acid concentration is increased, the absorption spectra remain unchanged but further new fluorescence bands appear at ~ 378 nm and ~ 370 nm for I and II, respectively. These emsissions may be attributed to the S_1 states of the monoprotonated species HA^{+2}.

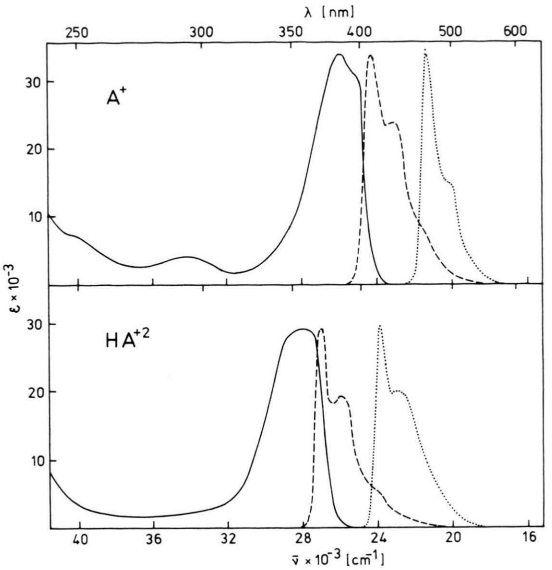

Fig. 1. Absorption-emission spectra of I in the free ion (A^+) and protonated (HA^{+2}) forms. A^+: solid and broken lines: absorption and fluorescence in H_2O at 293 K, respectively; dotted line: phosphorescence in C_2H_5OH at 77 K. HA^{+2}: solid line: absorption in $2\,M\,H_2SO_4$ at 293 K, broken and dotted lines: fluorescence and phosphorescence in H_2SO_4:n-C_3H_7OH:C_2H_5OH, (2:5:1) at 77 K, respectively.

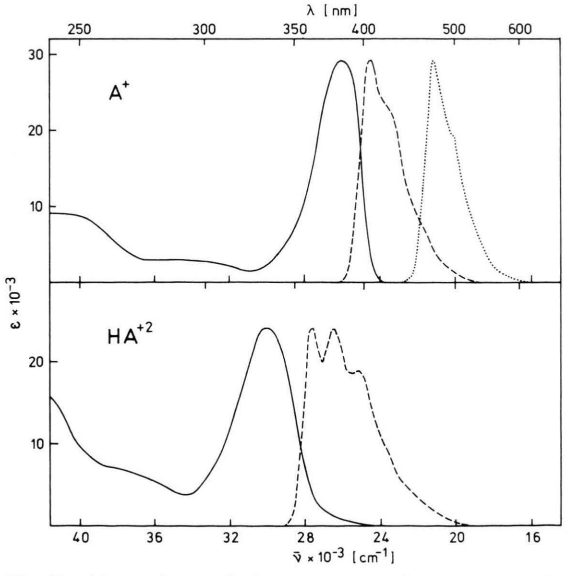

Fig. 2. Absorption-emission spectra of II in the free ion (A^+) and protonated (HA^{+2}) forms. A^+: solid and broken lines: absorption and fluorescence in H_2O at 293 K, respectively, dotted line: phosphorescence in C_2H_5OH at 77 K. HA^{+2}: solid line: absorption in $1.5\,M\,H_2SO_4$ at 293 K, broken line: fluorescence in H_2SO_4:n-C_3H_7OH:C_2H_5OH (2:5:1) at 77 K.

Table 1. Photophysical properties of I and II in solutions.

Compd.		Solvent	T K	$\lambda_{00}(S_1)$ [a] nm	Φ_f	τ_s ns	$1/\tau^0_{cal}$ [b] $s^{-1} \cdot 10^{-8}$	k_f [c] $s^{-1} \cdot 10^{-8}$	k_n [d] $s^{-1} \cdot 10^{-8}$	Φ_{ph}/Φ_f	Φ_{ph}	τ_T s	$\lambda_{00}(T_1)$ [e] nm
I	A^+	H_2O ($pH = 6.5$)	293	405	$\sim 0.38 \pm 0.03$	1.58	2.6	2.4	4.0	–	–	–	–
		C_2H_5OH	77	413	0.99 ± 0.05	3.9 [f]	2.6	–	$\leqq 0.15$	0.0079	0.008	2.0 ± 0.1	470
	HA^{+2}	18 M H_2SO_4	293	368	~ 0.06	~ 0.2 [f]	3.3	–	–	–	–	–	–
		H_2SO_4: n-C_3H_7OH: :C_2H_5OH	77	371	0.56 ± 0.07	–	–		–	0.080	0.045	1.7 ± 0.2	420
II	A^+	H_2O ($pH = 6.5$)	293	399	0.95 ± 0.03	3.48	2.7	2.7	0.14	–	–	–	–
		C_2H_5OH	77	411	1.00 ± 0.05	3.8 [f]	2.6	–	$\leqq 0.13$	0.0092	0.009	2.2 ± 0.1	473
	HA^{+2}	18 M H_2SO_4	293	352	~ 0.009	~ 0.03 [f]	~ 3	–	–	–	–	–	–
		H_2SO_4: n-C_3H_7OH: :C_2H_5OH	77	364	0.65 ± 0.07	–	–	–	–	< 0.003	< 0.002	–	~ 418

[a] Calculated from the intersection point of the mutually normalized absorption and fluorescence spectra at room temperature, or from the O–O fluorescence band at 77 K.

[b] Calculated from the Strickler-Berg formula [7].

[c] Calculated from (1). [d] Calculated from (2).

[e] Calculated from the O–O phosphorescence band.

[f] Estimated as $\tau_s = \tau^0_{cal}\,\Phi_f$.

The A^+ forms of I and II in solutions show intensive fluorescence at room temperature and weak phosphorescence at 77 K, while the protonated species HA^{+2} are characterized by weak fluorescence at room temperature, but strong fluorescence at 77 K, and weak phosphorescence at 77 K. The fluorescence of the A^+ form of I reveals a vibrational structure even at room temperature, whereas the HA^{+2} of I and both the free ion and protonated forms of II show a vibrational structure only at 77 K. The excitation spectra of fluorescence and phosphorescence are identical and have the same spectral shapes as the corresponding absorption spectra. All calculated and measured photophysical of I and II in solutions are summarized in Table 1.

The fluorescence and phosphorescence spectra and emission quantum yields are independent of the excitation wavelength. Similarly as for the trimethylpyrichrominium ion [4], the fluorescence at 77 K is the main deactivation path of the lowest excited singlet state of the A^+ form of I and II (Table 1). Because of the high fluorescence quantum yields it was not possible to calculate the accurate quantum yield and rate constant of the intersystem crossing process $S_1 \rightarrow T_1$. From (2) and the data given in Table 1 (including experimental errors), the rate constant of intersystem crossing could be estimated as $\leqq 0.15 \times 10^8\,s^{-1}$ and $\leqq 0.13 \times 10^8\,s^{-1}$ for I and II in ethanol at 77 K, respectively. The internal conversion $S_1 \rightarrow S_0$ could be neglected at 77 K, as it was shown before [4]. The low phosphorescence quantum yields together with low values of Φ_{ISC} suggest that phosphorescence is the main deactivation path of the lowest excited triplet state at 77 K. The phosphorescence decay lifetime of 2 s suggests that analogously as for the pyrichrominium ion [5], the T_1 states of I and II are of the (π, π^*) type.

It is important to note that, as it was previously described for the vitamin B_1 derivative [4], also in the case of I and II there is a good agreement between the radiative rate constant calculated ($1/\tau^0_{cal}$) and measured (k_f) in water solutions (Table 1). Therefore, the calculation of the singlet state lifetime, τ_s, according to $\tau_s = \tau^0_{cal}\,\Phi_f$ is expected to correct values also for the ethanol solutions (Table 1).

2. Acid-base Equilibria

The compounds I and II in an acid-water mixture undergo the proton transfer reaction

$$A^+ + H_3O^+ \rightleftharpoons HA^{+2} + H_2O\,, \tag{3}$$

which is characterized by the equilibrium constant pK_a. The typical pH dependent changes of the absorption spectra of the solutions are presented in Figure 3. The ground state equilibrium constants $pK_a\ (S_0)$ of I and II were determined on the basis of the changes of the long-wavelength absorption maxima with pH (Table 2). Similar values of $pK_a\ (S_0)$ were obtained from the fluorescence-titration curves (excitation at 384 nm or 383 nm, and emission at 413 nm or 409 nm for I and II, respectively).

The excited state pK_a's were estimated from the Förster cycle using (4) (discussed in detail in [8]) and from the data of Table 1.

$$pK_a^* = pK_a(S_0) - \frac{0.625}{T}\left(\bar{\nu}_{00}^{HA} - \bar{\nu}_{00}^{A}\right), \tag{4}$$

where: $\bar{\nu}_{00}^{HA}$ and $\bar{\nu}_{00}^{A}$ are the wavenumbers of the pure electronic transitions in the conjugated acid and base, respectively. The results obtained are shown in Table 2. The acidities of the excited singlet and triplet states are several pK_a units higher than in the ground state. The above cited results confirmed the assumption made by Zoltewicz et al. [3] that the higher acidity of the S_1 state, when compared to that of the ground state, explains some qualitative observations of the luminescence properties of I in 1 M perchloric acid. Under the experimental conditions ($pH \sim 0$), after light absorption by HA^{+2} ions ($pK_a(S_0) = 1.5$) and a very fast proton transfer in the excited singlet state the emission from the free ion A^+ was observed ($pK_a(S_1) = -3.7$). A detailed analysis of the spectroscopic characteristics of the protonation of I and II by semiempirical calculations as well as by a comparison with the pyrichrominium ions previously studied [5] will be published elsewhere [9].

Table 2. Ground and excited state pK_a's of I and II in water solutions.

Compd.	$pK_a(S_0)$	$pK_a(S_1)$	$pK_a(T_1)$
I	1.5 ± 0.1	-3.7 ± 0.4	-3.8 ± 0.4
II	1.3 ± 0.1	-5.7 ± 0.6	~ -4.5

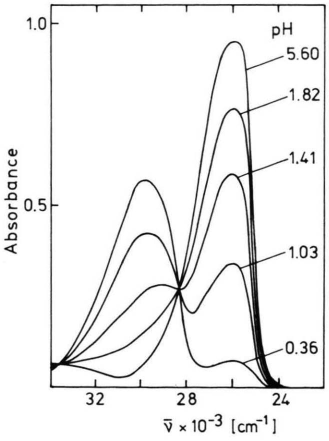

Fig. 3. Absorption spectra of II in buffer solutions ($c = 2.5 \times 10^{-5}$ M, $l = 1.0$ cm).

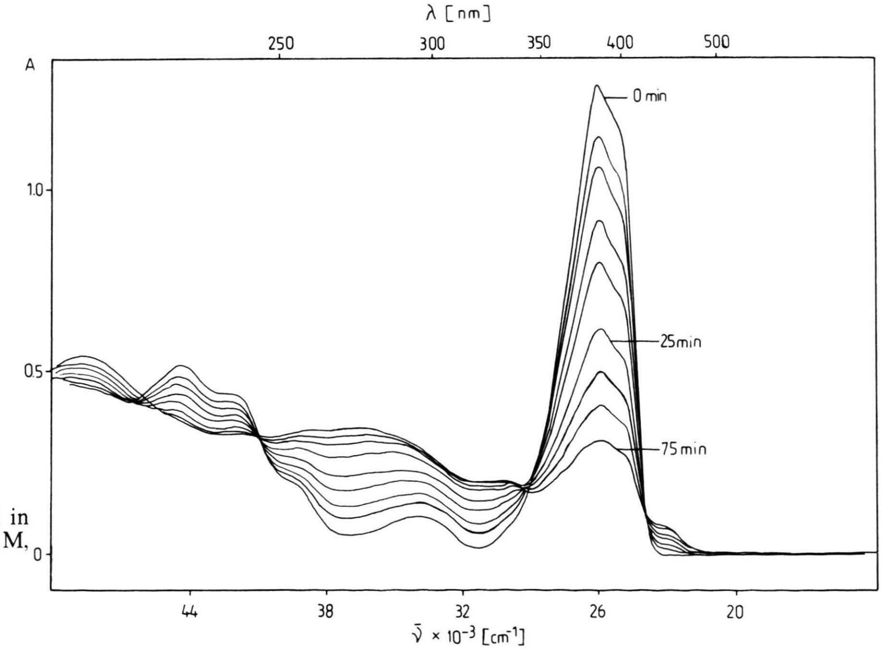

Fig. 4. Absorption spectra of I in oxygen saturated aqueous solution ($pH \sim 6$), during irradiation with monochromatic light of 366 nm ($c_0 = 3.8 \times 10^{-5}$ M, $l = 1.0$ cm).

In rigid solutions at 77 K the intermolecular proton transfer (3) is inhibited. Therefore, in the acid solutions at 77 K, the emission from the excited singlet states of the HA^{+2} form is observed with high fluorescence quantum yield. Unlike the previousy described pyrichrominium ions, where in more concentrated sulfuric acid solutions the monoprotonated tautomer was formed by a two step pseudo-intramolecular proton transfer reaction involving deprotonation and reprotonation in the first excited singlet state [4, 5, 10], that type of the phototautomerization has not been observed for I and II.

3. *Photochemical Properties*

I and II undergo photochemical reactions in aqueous solutions. Typical changes of their absorption spectra during irradiation are presented in Figure 4. However, the quantum yields of I and II disappearance are very small- in the range of 10^{-4}. The quantum yields determined for irradiation with monochromatic light of the wavelength of 366 nm ($I_0 = 0.0031$ einstein $dm^{-3} min^{-1}$) are $\leqq 2.0 \times 10^{-4}$ and $\leqq 6.0 \times 10^{-5}$ for deoxygenated solutions of I and II, respectively (after bubbling oxygen free nitrogen for 10 min). The saturation of the solutions with oxygen leads to an increase in the quantum yields to 5.0×10^{-4} and 2.3×10^{-4} for I and II, respectively, suggesting that under these conditions photooxidation occurs. The influence of oxygen, however, is much smaller in comparison with the pyrichromine derivatives previously studied [4]. From experiments in the presence of typical singlet oxygen sensitizers such as methylene blue and rose bengal, quenchers (sodium azide) and using the procedure previously described by Paszyc et al. [11] for the photooxidation of Y_t base in aqueous solution, photooxidation via singlet oxygen as a possible mechanism is excluded.

Preliminary irradiations showed a large number of relatively unstable photoproducts formed with low chemical yields (at least 7 products for I and 12 for II). Therefore, our attempts to separate and identify at least some of them were unsuccesful (TLC analysis, column chromatography).

To summarize the photochemical properties of I and II in aqueous solutions: In comparison with the very efficient photophysical processes of excited state deactivation, photochemical pathways can be neglected.

Acknowledgements

Acknowledgements are made to Professor S. Paszyc for his great interest in this work and helpful discussions. The author is indebted to Professor J. A. Zoltewicz for supplying him with samples of I and II, to Dr. B. Skalski for the fluorescence lifetime measurements and to Dr. J. Najbar for the permission to use his spectrophosphorimeter. – This work was supported by the Polish Academy of Sciences.

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