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Photophysical properties of some highly fluorescent derivatives of vitamin B_1. Solvent effect

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Abstract

The influence of the solvent on the photophysical properties 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)) was studied using absorption and fluorescence techniques (both steady state and time resolved). For II and III, the photophysical properties do not change significantly in various solvents. For I, a large decrease in the fluorescence quantum yield and lifetime of the singlet state is observed on going from acetonitrile to alcohol. These large changes in the photophysical parameters of I occur in spite of the small changes in the absorption spectrum and the shape of the fluorescence spectrum. No evidence for a specific solute–solvent interaction, i.e. a complex of I with alcohols, is found in pure (including deuterated solvents) or mixed solvents. The measured values of the intersystem crossing (ISC) quantum yield of I in acetonitrile and methanol (in the region of 10^{-2}) indicate that the ISC process is not responsible for the observed solvent effect. Possible explanations for the influence of the solvent on the photophysical properties of I in its first excited singlet state are discussed.

Keywords: Vitamin B_1 derivatives; Photophysical properties; Fluorescence; Solvent effect

1. Introduction

Highly fluorescent vitamin B_1 derivatives are of particular interest for application as fluorescent probes in nucleic acids. In addition, the simple conversion of vitamin B_1 (thiamine) into highly fluorescent tricyclic products via nucleophilic substitution by 2-aminopyridines, cytidine or other nucleophiles [1–3] may have potential analytical applications (detection of very low concentrations of vitamin B_1 using emission techniques).

In previous papers [4–8], we have described the photophysical and photochemical behavior and acid–base equilibria of three fluorescent vitamin B_1 derivatives (products of the reaction of N-methylated vitamin B_1 with cytidine (**I**), adenosine (**II**) and 2-amino-4-methylpyridine (**III**)). The experimental results were discussed on the basis of theoretical semi-empirical calculations. In Ref. [7], it was shown that, in spite of the small spectral shifts with the solvent in the absorption and fluorescence spectra of all three compounds, the solvent had a large influence on the fluorescence quantum yield of compound **I**. To explain this unusual solvent effect, we have extended our study to a larger number of solvents (including deuterated solvents) and to the determination of additional experimental parameters, such as the singlet state lifetimes, triplet quantum yields and rate constants of radiationless processes, in several solvents.

2. Experimental details

2.1. Materials

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]-pyrimidinium 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] (see Scheme 1 for structural formulas). The peracetylated derivative **Ia** was obtained by treatment of **I** with acetic anhydride in pyridine at room temperature, and was purified by semi-preparative high

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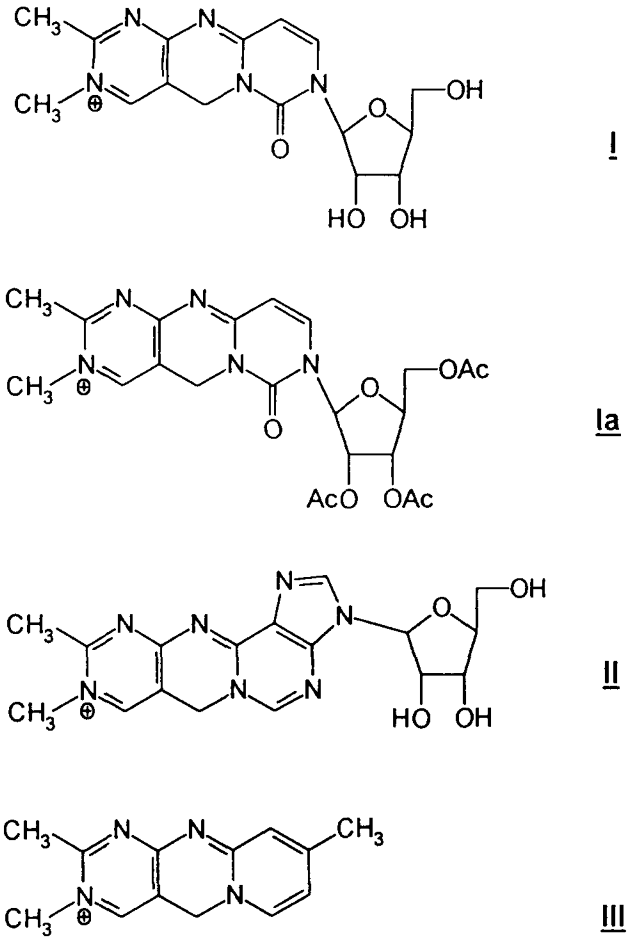

Scheme 1.

performance liquid chromatography (HPLC) (Waters, DELTA PAK C4 column). All solvents were of spectrograde quality and were used as supplied. Water was purified using a Millipore Milli-Q system.

2.2. Methods

Steady state UV–visible absorption and fluorescence spectra were recorded in 1 cm quartz cells, using a Specord M-40 (Zeiss) spectrometer and an MPF-66 (Perkin–Elmer) spectrofluorometer respectively. The emission spectra were corrected for instrumental distortion. The concentrations of the solutions for the fluorescence measurements were in the region of 10^{-6} M, so that the absorbance at the excitation wavelength did not exceed 0.1. The fluorescence quantum yields were determined according to the procedure described previously [4], using a solution of quinine sulfate in 0.1 N sulfuric acid as a standard [9].

Fluorescence lifetimes were measured using a time-correlated, single-photon-counting system with a nanosecond flash lamp filled with hydrogen (IBH 5000 system). Excitation was performed at 385 nm, and the fluorescence was monitored at 435 nm with bandwidths of about 8 nm. Fluorescence lifetimes in the picosecond range (for **I** in alcohols) were determined by Dr. Jan Konijnenberg (in Dr. C.A.G.O. Varma's laboratory at the University of Leyden) using a streak camera after excitation with a 265 nm laser pulse (pulse width, 25 ps) (experimental details are given in Refs. [8] and [10]).

Measurements of the triplet–triplet transient absorption spectra were performed using a nanosecond laser flash photolysis system at the Radiation Laboratory, University of Notre Dame; details of this apparatus are given in Ref. [11]. Laser excitation at 355 nm (from a Quanta Ray CDR-1 Nd:YAG laser system operated at 0.7–1.5 mJ; pulse width, approximately 6 ns) was employed in right-angle or front-face geometries. Rectangular quartz cells (0.5 cm × 1.0 cm) with an optical path length of 0.5 cm for the monitoring beam were used for right-angle geometry; 0.2 cm × 1.0 cm cells were used for front-face geometry. The transient absorptions at preselected wavelengths were monitored by a detection system consisting of a double monochromator, a photomultiplier tube and a pulsed xenon lamp (1 kW) as the monitoring source. The signal from the photomultiplier was processed by a 7912 AD Tetronix transient digitizer controlled by an LSI 11/2 microprocessor. Cut-off filters were used to avoid spurious responses due to second-order scattering from the monochromator gratings.

Triplet yields of **I** in acetonitrile and methanol were determined on the basis of the relative actinometry method [12] using benzophenone in acetonitrile as reference ($\epsilon_R(530\ \text{nm}) = 6500\ \text{M}^{-1}\ \text{cm}^{-1}$ [13], $\Phi_T(\text{R}) = 1.0$ [14]). If the same number of excitation photons are absorbed by both sample and reference (employing optically matched solutions at 355 nm), the triplet yield $\Phi_T(\text{T})$ is given by [12]

$$\Phi_T(\text{T}) = \frac{\Delta A_T(\lambda_1)\epsilon_R(\lambda_2)}{\Delta A_R(\lambda_2)\epsilon_T(\lambda_1)}\Phi_T(\text{R}) \qquad (1)$$

The molar absorption coefficients for the triplet–triplet absorption of **I** were estimated in acetonitrile using an energy transfer method [12] with benzophenone as sensitizer and in methanol using a singlet depletion method [12].

All experiments were performed at room temperature (22 ± 1 °C); the solutions were deoxygenated by bubbling with high-purity argon (except for fluorescence lifetime measurements in the picosecond range).

3. Results and discussion

The absorption and fluorescence spectra of compounds **I–III**, presented in Ref. [7], indicate that the position and shape of the fluorescence and long-wavelength absorption bands do not change significantly with the solvent, such as acetonitrile, alcohols and water. However, the fluorescence quantum yield is strongly affected by the type of solvent for compound I. In this work, we have extended the measurements to a larger number of solvents. The data are summarized in Table 1. Because compounds I–III are insoluble in non-polar solvents, solvents such as hydrocarbons could not be used. For all solvents, except water, the positions of the absorption maximum (ν_{max}) and the full width at half-maximum ($\delta\nu_{1/2}$) are constant in the range ±150 cm^{-1} (for water solutions, the values of ν_{max} are "blue" shifted by about 400 cm^{-1}). A similar behavior is observed in the fluorescence spectra measured in various solvents. The maximum and shape of the fluorescence bands do not change significantly with solvent (±100 cm^{-1}), except for water where a hypsochromic shift (approximately 200 cm^{-1}) is observed.

The lowest excited singlet state for compounds I–III is probably of π,π^* type, based on the following evidence: (1) the nearly mirror-image symmetry of the fluorescence spectrum and the low-energy absorption band; (2) the large values of the molar absorption coefficient; (3) the large fluorescence quantum yield; (4) the similarity between the radiative rate constants for all the solvents (Table 1); (5) the theoretical INDO/S calculations [5,7]. The energy level diagram obtained in the semi-empirical INDO/S calculations for compound I [7] is presented, as an example, in Fig. 1. In contrast with II and III [5,7], the $S_2(n,\pi^*)$–$S_1(\pi,\pi^*)$ energy gap for I is small (approximately 1200 cm^{-1}).

The small values of the solvent shifts in the absorption and fluorescence spectra for compounds I–III can be interpreted in terms of the small difference between the dipole moments of the S_0 and S_1 states. For example, the calculated (INDO/S) change in the dipole moment between the ground and first excited singlet states for compound I is equal to $\Delta\mu = 0.2$ D. Thus the dipole–dipole interaction with the solvent molecules is expected to be small and leads to a small hypsochromic shift of the S_0–S_1 electronic transition with increasing solvent polarity. Since the lowest excited singlet state is of the π,π^* type (energy level diagram in Fig. 1), hydrogen bond formation with the solvent is not expected to affect significantly the energy of the S_0–S_1 transition. Ground state complexation by alcohols and water is not observed in the absorption spectra of compounds I–III in pure or mixed solvents (on addition of alcohol or water to solutions of I–III in acetonitrile, no additional absorption in the low-energy region of the spectrum was observed).

For compounds II and III, the photophysical properties do not change significantly in different solvents (Table 1). On the other hand, for compound I, in spite

Table 1
Absorption and fluorescence data for compounds I–III at room temperature

Compound	Solvent	Absorption		Fluorescence				$k_n \times 10^{-9}$ [e] (s^{-1})
		λ_{max} (nm)	$\epsilon \times 10^{-3}$ (M^{-1} cm^{-1})	λ_{max} (nm)	Φ_f [a]	τ_S [c] (ns)	$k_f \times 10^{-9}$ [d] (s^{-1})	
I	CH_3CN	390	37.6	416	0.99	3.61	0.27	0.003
	$(CH_3)_2CO$	391	34.4	418	0.94	3.12	0.30	0.002
	CH_3OH	390	36.5	417	0.14	0.55[f]	0.26	1.56
	CH_3OD	390	–	417	0.15	0.58[f]	0.26	1.47
	C_2H_5OH	390	31.6	417	0.14	0.47[f]	0.30	1.83
	C_2H_5OD	390	–	417	0.14	0.50[f]	0.28	1.72
	n-C_3H_7OH	390	–	417	0.11	0.39[f]	0.28	2.28
	n-C_4H_9OH	390	–	417	0.12	–	–	–
	H_2O	384	33.8	414	0.38	1.58	0.24	0.39
	D_2O	384	30.6	414	0.42	1.86	0.23	0.31
Ia	CH_3CN	386	–	416	0.89	3.92	0.23	0.028
	C_2H_5OH	388	–	418	0.05	< 0.60	> 0.10	> 1.90
	H_2O	382	–	414	0.20	1.04	0.19	0.77
II	CH_3CN	392	41.3	415	0.95	3.50	0.27	0.014
	C_2H_5OH	389	38.3	416	0.99	3.24	0.31	0.003
	H_2O	383	40.3	409	0.95	3.38	0.27	0.014
III	CH_3CN	403	23.6	432	0.85	5.45	0.15	0.027
	C_2H_5OH	398	22.6	433	0.86	5.31	0.16	0.026
	H_2O	392	22.7	426	0.79 [b]	5.23	0.15	0.040

[a] Accuracy, ±(3–10)%.
[b] From Ref. [1].
[c] Accuracy, ±(1–5)%.
[d] Calculated from $k_f = \Phi_f \tau_S^{-1}$.
[e] Calculated from $k_n = (1 - \Phi_f)\tau_S^{-1}$.
[f] Measured in Dr. Varma's laboratory (see Section 2).

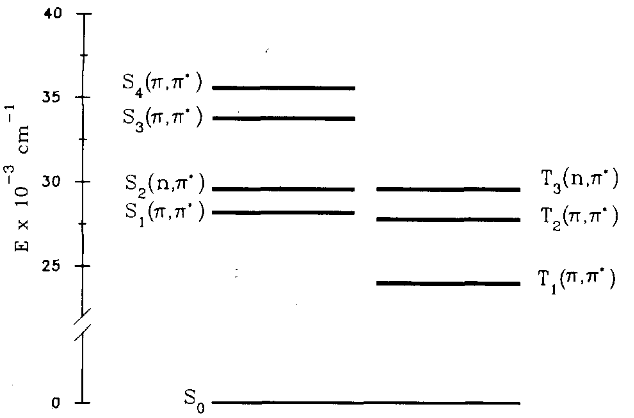

Fig. 1. Energy level diagram for compound **I** obtained from INDO/S CI calculations (from Ref. [7]).

of the small solvent shifts in the absorption and fluorescence spectra, there are large differences in the fluorescence quantum yields and lifetimes measured in different solvents. On going from acetonitrile to methanol, there is about a sevenfold decrease in both Φ_f and τ_S (Table 1). About the same degree of fluorescence quenching is observed for the other alcohols, including their deuterated forms. In all solvents, single-exponential emission decays are observed.

The results of UV–visible absorption measurements, including those in mixed acetonitrile–methanol solvents (see above), and the similar changes in the Φ_f and τ_S values with the solvent (Table 1), indicate no alcohol–solute ground state complex formation and no static quenching. Additional data, i.e. the negligibly small (except for water) solvent deuterium isotope effects on the radiationless rate constants k_n (Table 1) and the lack of correlation of k_n with variations in the acidity of the alcohols [10] or viscosity of the solvents [14], indicate that these solvent parameters do not control the observed effects.

The rate constant k_n in Table 1 represents the sum of the radiationless processes leading to deactivation of the S_1 state, i.e. internal conversion (IC) and intersystem crossing S_1–T_1 (ISC) processes. To distinguish between these processes, the quantum yields of ISC (Φ_T) were determined for **I** in acetonitrile and methanol. Since the triplet–triplet absorption spectrum of **I** is not known, it was characterized using direct excitation and sensitization techniques (with the benzophenone–biphenyl system as sensitizer). Compound **I** shows a triplet–triplet absorption with a maximum at 460 nm ($\epsilon \approx 13\,000$ M^{-1} cm^{-1}) in acetonitrile and at 480 nm ($\epsilon \approx 12\,000$ M^{-1} cm^{-1}) in methanol. Both absorptions decay with lifetimes in the range 20–30 μs and are quenched in air-saturated solutions. Applying the relative actinometry method [12] (Eq. (1)), the ISC quantum yields are estimated as $\Phi_T = 0.01$ and $\Phi_T = 0.02$ in acetonitrile and methanol respectively. These results clearly indicate that the ISC process is not responsible for the observed decrease in k_n on going from acetonitrile to methanol.

Specific solute–solvent interactions in the ground and excited states were studied by performing experiments with mixed solvents (see, for example, Refs. [10] and [15]). On addition of methanol to solutions of **I** in acetonitrile, no changes in the absorption spectra are found due to ground state complex formation with the alcohol (no additional absorption band is observed). A decrease in the fluorescence lifetime and relative quantum yield of **I** is observed with increasing methanol concentration to high values (Fig. 2 and Table 2). The identical, within experimental error, dependence of Φ_f and τ_S on the molar fraction of methanol (which implies no change in the radiative lifetime) and the results of the absorption spectra suggest an involvement of the

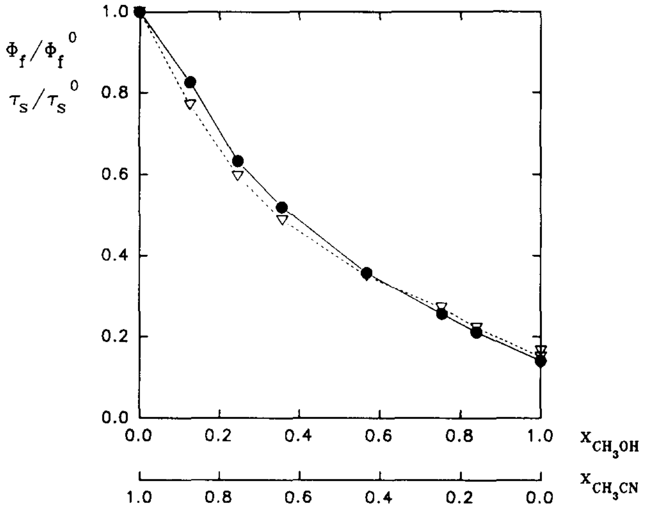

Fig. 2. Fluorescence quantum yields (circles) and singlet lifetimes (triangles) of **I** in binary mixtures of acetonitrile and methanol (x = molar fraction).

Table 2

Variation of the singlet state lifetime τ_S, relative lifetime τ_S/τ_S^0 and relative fluorescence quantum yield Φ_f/Φ_f^0 for **I** in mixed binary acetonitrile–methanol solvent (τ_S^0 and Φ_f^0 are the lifetime and the fluorescence quantum yield in acetonitrile respectively)

Molar fraction x_{CH3OH}	τ_S (ns)	τ_S/τ_S^0	Φ_f/Φ_f^0
0	3.61	1.00	1.00
0.13	2.78	0.77	0.82
0.25	2.15	0.60	0.63
0.36	1.76	0.49	0.52
0.57	1.26	0.35	0.36
0.75	0.98	0.27	0.26
0.84	0.80	0.22	0.21
1.00	0.60	0.15	0.14
1.00	0.55 [a]	0.17	–

[a] Measured in Dr. Varma's laboratory (see Section 2).

excited singlet state (no ground state complexation) in the observed "quenching" by alcohols. The large concentration of alcohol required for a significant decrease in Φ_f and τ_S may suggest weak interaction of the excited singlet state of **I** with the alcohol. Operationally, this may be treated as a quenching process with a small rate constant. Based on the results presented in Table 2, the formal rate constant for quenching of the singlet state of **I** by methanol in acetonitrile is estimated to be 4×10^7 M^{-1} s^{-1}; this is much lower than the diffusion rate constant in acetonitrile at room temperature ($k_{diff}=1.9\times10^{10}$ M^{-1} s^{-1} calculated from the Debye equation [14]). Thus the results obtained in mixed solvents show the quenching of the excited singlet state of **I** by the alcohol, but do not provide evidence for specific solute–alcohol interaction.

To exclude any possible influence of intramolecular hydrogen bond formation within molecule **I** (hydrogen bonds between the OH groups of the ribosyl and carbonyl groups) on its photophysical properties (including the mechanism of quenching by alcohols), we have determined the absorption and fluorescence properties of the peracetylated derivative **Ia**. In this molecule, the intramolecular hydrogen bond cannot be formed. Similar results obtained for **I** and **Ia** in various solvents (Table 1) clearly indicate that intramolecular hydrogen bond formation within molecule **I** is not responsible for the observed effects. The expected competition between intramolecular hydrogen bond formation and intermolecular hydrogen bond formation between the carbonyl group of **I** and the alcohol cannot be responsible for the solvent effects.

Possible explanations of the influence of the solvent on the photophysical properties of **I** are summarized below.

(1) An enhancement of the IC rate constant on going from acetonitrile to alcohols. This mechanism can be ruled out in our case on the basis of the expected effect of alcohols on the energies of the n,π* and π,π* states of **I**. Even though a small $S_2(n,\pi^*)$–$S_1(\pi,\pi^*)$ energy gap for **I** was obtained in the semi-empirical calculations [7] (Fig. 1), the interaction with an alcohol should lead to an increase in the $S_2(n,\pi^*)$–$S_1(\pi,\pi^*)$ gap [16]. As a consequence, the IC rate constant should decrease on adding alcohol, not increase. This counterargument assumes that the mixing of $S_2(n,\pi^*)$ and $S_1(\pi,\pi^*)$ states enhances IC via vibronic interactions [17].

(2) An increase in the radiationless depopulation rate constant of the excited singlet state via intermolecular hydrogen bonding with the solvent. We believe that this effect (well documented in the literature [18–20]) is not responsible for the quenching of the S_1 state of **I** by alcohols observed in this work. We observed no deuterium isotope effects from the solvents on the non-radiative rate constants k_n (Table 1) (except for a small effect for water), and no expected correlation of k_n with the proton donating ability of the alcohol used [10,21].

(3) The formation of non-emitting exciplexes with alcohols (charge transfer character of the exciplex). A larger singlet quenching in alcohols than in water (Table 1) can be taken as an argument in favor of charge transfer quenching. Since the rate constant for quenching by alcohols is very small (and probably located on the Arrhenius portion of the Rehm–Weller curve [22]), we expect a strong temperature dependence of the quenching. This expectation is consistent with the large value of Φ_f (i.e. a small contribution of charge transfer quenching) determined for **I** in rigid alcohol solution at 77 K ($\Phi_f=0.99$ [6]).

The unusual solvent effect on the photophysical properties of **I** is probably due to an intermolecular quenching (dynamic) process of the first excited singlet state of molecule **I** with the solvent molecules. The nature of the quenching is still unclear, but we suggest the formation of a non-emitting exciplex with the solvent. The lack of solubility of **I** in less polar solvents, such as hydrocarbons and ethers, precludes a more detailed study of the solvent effect.

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