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Phototautomerism of 5-deazalumichrome (in the presence of acetic acid)

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Abstract

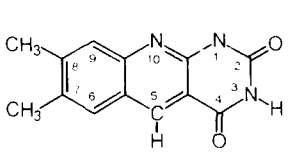
The phototautomerism of 5-deazalumichrome was studied in 1,2-dichloroethane in the presence of acetic acid. In comparison with 3-methyllumichrome, 5-deazalumichrome undergoes excited state proton transfer with higher efficiency, manifested by its fluorescence spectrum and lifetimes. The replacement of N-5 by carbon in the pyrazine ring of alloxazine restricts the electron density redistribution involved in the phototautomerism to the region of N-1:C-10a:N-10, and eliminates the competitive contribution of the rest of the conjugated double bond system. Semiempirical calculations confirm the experimental observations.

Keywords: Phototautomerism; 5-Deazalumichrome; Alloxazine; Excited state proton transfer; Lumichrome

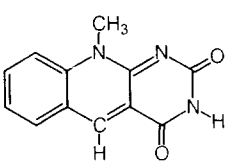
1. Introduction

A flavin moiety with the nitrogen atom at position 5 replaced by methenyl has been synthesized [1]. It has a low redox potential involving two e^- steps [2]. The discovery of the natural occurrence of 5-deazaflavins in coenzymes [3] has stimulated extensive studies on the chemical, photochemical and biochemical properties of these molecules [2,4].

As in the case of lumichrome and flavins, the isomeric decomposition product of 5-deazalumichrome (5-deaza-7,8-dimethylalloxazine, 5DL), with the 5-deaza-alloxazine structure, has not been studied in detail. Differently substituted alloxazines, mainly lumichromes (the decomposition products of flavins), have been found in many organisms [5], and it can safely be assumed that 5-deazalumichromes, the products of the metabolic and photochemical decomposition of 5-deazaflavins (as a model compound, 5-deaza-10-methylisoalloxazine (5DIA) was used in this study), will be found in organisms containing these compounds [6].



5DL



5DIA

The synthesis of 5-deazariboflavin and its derivatives has been reported [4,7], and the basic properties of 5DL have been discussed [7]. A preliminary study on the physicochemical properties of 5DL in comparison with lumichrome [8] has revealed similarities (ground and excited state pK_a values, solvatochromism) and differences (lack of reversible pyrimidine ring opening in aqueous alkaline solutions [9]). More recently, it was found that these two molecules are similarly solvated (hydrogen bonded to solvent molecules) and have similar small differences between the dipole moments of the ground and excited states [10]. In has also been noted [8,10] that, despite the differences in the absorption and fluorescence emission maxima, 5DL exhibits phototautomerism in the presence of the same molecules as lumichrome, e.g. acetic acid and pyridine [8,11]. In this paper, we present the results of a detailed study on the phototautomerism of 5DL in the presence of acetic acid, including the fluorescence lifetimes of the tautomers with the alloxazine and isoalloxazine structures.

2. Materials and methods

2.1. Compounds

5DL and 5DIA were generous gifts from Professor P. Hemmerich and Professor S. Ghisla, University of Konstanz, Germany. For spectral measurements, methanol (MeOH), 1,2-dichloroethane (DCE) and acetic acid of spectral purity were used. Other chemicals used were of p.a. grade.

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2.2. Spectroscopy

Absorption spectra were recorded on a Cary-14 (computerized by On-Line Instr. Systems, Inc.) spectrophotometer; corrected emission and excitation spectra were measured using an SLM DMX-1000 (SLM Aminco, Urbana, IL) spectrofluorometer. Fluorescence decay and lifetime measurements were performed using the equipment described previously [12]. Slit widths were kept constant at 2.5 nm throughout the study. The emission spectra were recorded for excitation wavelengths selected according to the excitation spectra. For lifetime measurements, excitation was at 345 nm for 5DL and 5DIA.

2.3. Methods

The fluorescence spectra were recorded at different wavelengths and averaged (three or more) before analysis on a personal computer. Fluorescence decay measurements and the associated data analysis, using the maximum entropy method, were performed as described previously [12]. Throughout the study, the concentration of the sample compounds was kept constant at 16.6 μM. Quartz cells (1 cm^3) were used; measurements were carried out at 20 °C. The purity of all the compounds studied and the lack of possible decomposition were checked using thin layer chromatography (TLC) on silica gel (Bakerflex 1B2) in solvent systems consisting of chloroform, methanol, 2-butanone and acetic acid (14:2:1:1, v/v) and without acetic acid [13].

3. Results and discussion

3.1. Phototautomerism

To avoid possible complications, which have been encountered when methanol is used as bulk solvent [14], DCE was chosen for this study. This should allow better resolution of the tautomeric forms and the identification of hydrogen bonding, as has recently been shown for 3-methyllumichrome [15].

The spectra of 5DL and 5DIA, in comparison with regular ("aza") isomers [8,11,15], generally show a blue shift in the apparent near-UV absorption and fluorescence emission maxima (Table 1).

On addition of acetic acid, starting from 0.002 M and increasing to 0.8 M, small changes in the absorption spectrum of 5DL are observed, i.e. the disappearance of vibronic structure (shoulder at 370 nm), a bathochromic shift of the first maximum (longest wavelengths) from 352 to 356 nm with a slight increase in intensity and a bathochromic shift of the second maximum from 320 to 322 nm (not shown).

In the emission spectra, the changes are more dramatic and start from the lowest acetic acid concentration applied (0.002 M) (Fig. 1). Broadening of the emission maximum is observed at this concentration, which splits into three peaks at about 400, 415 and 435 nm (the peak at 413 nm is the result of the overlapping of bands of comparable intensity). The long- wavelength emission can be ascribed to the product of the excited state proton transfer with an isoalloxazine structure [8,11,14,15]. At this acetic acid concentration, excitation at 390 nm (the red edge of the absorption spectrum) results in an emission spectrum with a maximum at about 455 nm, close to the value expected for the tautomer with an isoalloxazine structure and similar to that of 5DIA (Fig. 1). This indicates that, in the ground state, there are at least two differently solvated 5DL molecules in solution, and this difference is due to the interaction with acetic acid. This is further confirmed by the apparent difference in the excitation spectra recorded by monitoring the fluorescence at 400 and 490 nm (also for 0.008 M, 0.016 M and 0.064 M acetic acid). The spectrum obtained by monitoring at 490 nm is bathochromically shifted by about 5 nm (not due to isoalloxazine) (Fig. 2) and reflects the presence of a form which contributes about 20% of the 5DL present in solution (estimated from the absorbance and fluorescence intensity, assuming that at 490 nm there is only negligible fluorescence of the species with an emission maximum at about 400 nm). As for 3-methyllumichrome [15], the sum of the two excitation spectra gives curves practically identical to the absorption spectra at the same acetic acid concentration.

These results closely resemble the interaction observed between 3-methyllumichrome and acetic acid in DCE, which has been interpreted as the formation of complexes of different structure in the ground state, only one of which can undergo phototautomerism [15]. The main difference is the higher sensitivity of 5DL to low acetic acid concentrations; in such conditions, in the case of 3-methyllumichrome, no conclusive observations were possible based on the excitation

Table 1
Absorption and emission maxima (nm) and fluorescence lifetimes (ns) of 5DL and 5DIA

Compound	Solvent	Absorption	Emission	Fluorescence lifetime [b]
5DL	DCE	320, 352, 370 [a]	413, 402 [a]	3.3
	MeOH	315, 360, 375 [a]	422	5.9
5DIA	DCE	330, 380 [a], 405, 430 [a]	453	4.0
	MeOH	330, 400, 420 [a]	458	4.4

[a] Shoulder.
[b] Emission wavelength at 454.9 nm.

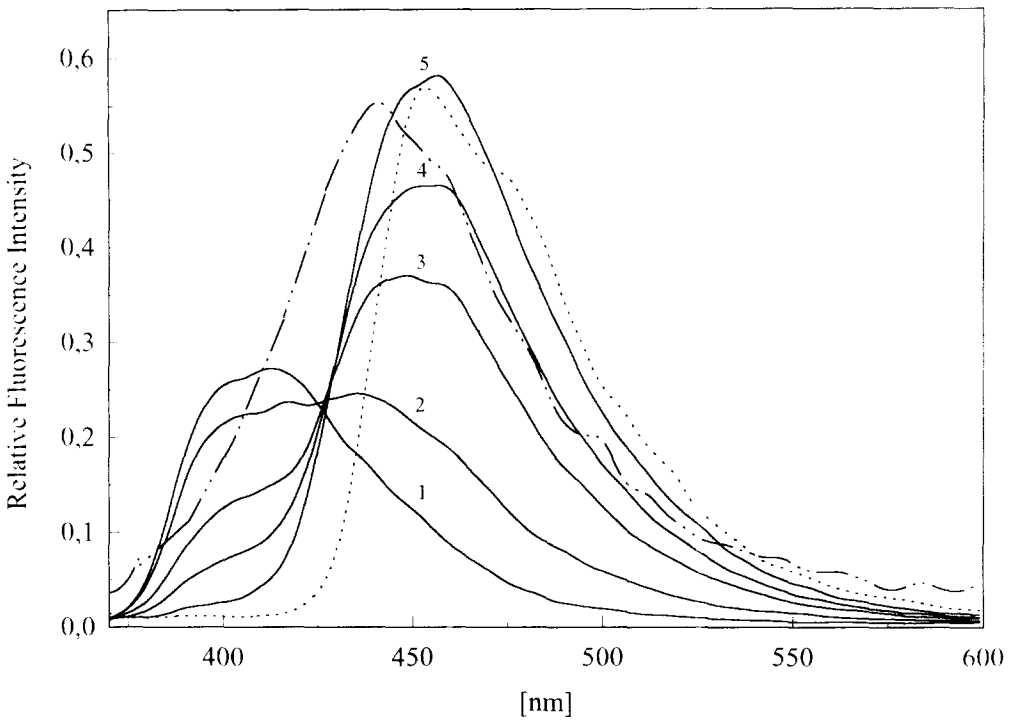


Fig. 1. Changes in the fluorescence emission spectrum of 5DL in DCE on addition of acetic acid (excited at 350 nm). Concentration of acetic acid: 1, no acetic acid; 2, 0.002 M; 3, 0.016 M; 4, 0.064 M; 5, 0.8 M. Curve – · · –, fluorescence emission spectrum of 5DL in the presence of 0.002 M acetic acid (excited at 390 nm, enlarged four times); curve · · · · ·, fluorescence emission spectrum of 5DIA in DCE (excited at 400 nm).

spectra [15]. Isoalloxazine (also 5-deazaisoalloxazine) does not exist in the ground state in measurable concentrations because, according to AM1 calculations, the alloxazine structure is more stable by 43.6 kJ mol^{-1} [14] (a value of 234.6 kJ mol^{-1} has been found by Palmer et al. [16] by ab initio calculations). The difference in stability between the 5-deazaisoalloxazine and 5-deazaalloxazine structures may be slightly smaller considering the differences in the electron

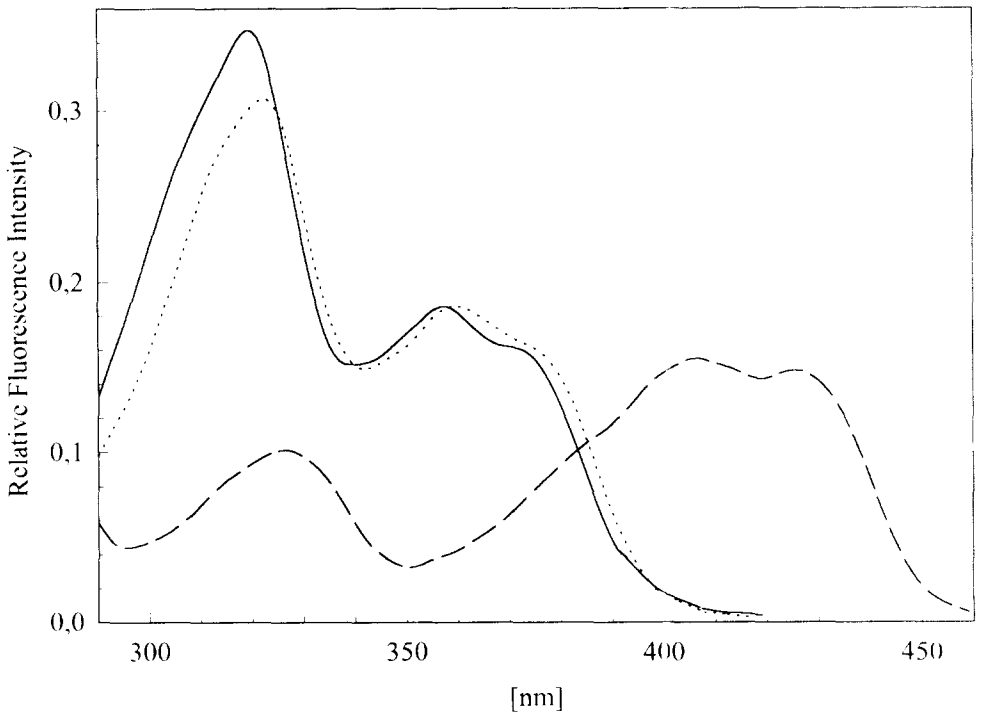


Fig. 2. Fluorescence excitation spectra of 5DL in DCE in the presence of 0.016 M acetic acid: full curve, monitored at 400 nm; dotted curve, monitored at 490 nm. Broken curve, fluorescence excitation spectrum of 5DIA in DCE monitored at 460 nm.

Table 2
Computed electron density differences (Δq) on N-10 and N-1 atoms in the ground and lowest singlet states and the dipole moments in both states

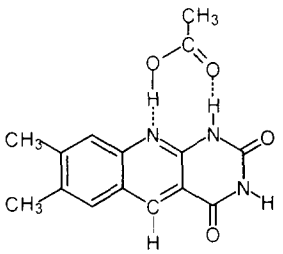
Compound	S_0		S_1	
	Δq	μ	Δq	μ
5-Deazaalloxazine	0.212	5.0	0.261	6.5
5-Deazalumichrome	0.213	6.1	0.267	8.0
Alloxazine	0.165	5.1	0.308	7.7
Lumichrome	0.167	6.3	0.315	9.2

density distribution calculated using the INDO/S approach (Table 2). The iso structures exist only when a substituent in the N-10 position is introduced (e.g. alkyl).

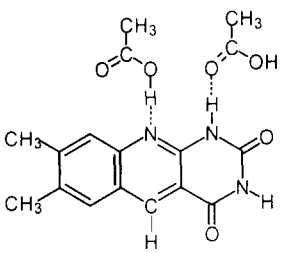
A further increase in the acetic acid concentration in the system causes a sharp decrease in the emission intensity with a maximum at about 415 nm, together with an increase in the emission intensity of the band with a longer wavelength maximum; this shifts bathochromically up to 457 nm for 5DL in the presence of 0.8 M acetic acid. At this acetic acid concentration, the band with a maximum at shorter wavelengths practically disappears (Fig. 1), constituting less than 5% of the initial spectrum (without acetic acid), and our attempts to record an excitation spectrum by monitoring the emission at 400 nm failed.

The set of emission spectra of 5DL in DCE with increasing concentrations of acetic acid (Fig. 1) shows a clear-cut isoemissive point at about 425 nm, similar to that observed for 3-methyllumichrome [15]. The shifts in the maxima may be due, in part, to the effect of "pulling" caused by overlapping bands of different intensity, so that the exact maxima positions cannot be given without a thorough band resolution analysis. This, in turn, will be very difficult (lack of vibrational fine structure, maxima too close).

It is apparent that the most remarkable difference between 5DL and 3-methyllumichrome is the almost complete disappearance of the species emitting fluorescence characteristic of the alloxazine structure at 0.8 M acetic acid concentration for 5DL, whereas in the case of "regular" lumichromes this was not achieved even at concentrations one order of magnitude higher [15,17]. Taking into account the presence of 5DL molecules differently solvated in the ground state, the alloxazine fluorescence should be ascribed exclusively to those solvated only by DCE molecules (a weak hydrogen bonding agent [18]) or to those solvated by acetic acid molecules, but not at sites contributing to the formation of the phototautomerically active complex. The almost complete shift of the equilibrium towards molecules forming complexes with acetic acid in such a way as to promote phototautomerism seems to favour the first assumption. It may also mean that the replacement of N-5 by carbon in the pyrazine ring (to give a pyridine ring) of alloxazine restricts the electron density redistribution involved in the excited state proton transfer to the region of N-1:C-10a:N-10, and eliminates the competitive contribution of the rest of the conjugated double bond system due to the lack of the hydrogen bonding active acceptor, i.e. lone pair of electrons on N-5. The real structure of the 5DL–acetic acid complex, towards which the equilibrium shifts in the ground state, can only be surmised; it is probably one of the most favourable combinations proposed previously for 3-methyllumichrome [15]. The complex structures favouring phototautomerism at low (1) and high (2) acetic acid concentration are shown below. The complex with DCE and acetic acid (3) is unable to phototautomerize.



(1)



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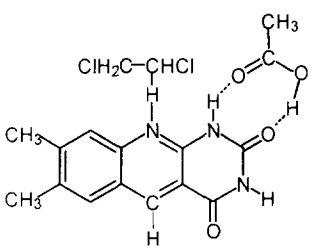

(3)

The long-wavelength part of the 5DL emission spectrum with a maximum at about 460 nm is most probably due to the phototautomeric species with the isoalloxazine structure, created by double proton transfer in the excited state as proposed previously [8]; this is analogous to the case of lumichrome and other derivatives of alloxazine [11,15,17]. The emission spectrum of 5DIA (used as a model compound), with an isoalloxazine structure (lack of two methyl groups in the benzene ring does not influence the spectral properties of isoalloxazines [19]), is practically (within experimental error) identical with the phototautomeric fluorescence emission of 5DL in the presence of 0.8 M acetic acid (Fig. 1).

Truly dramatic differences are observed in the relative intensities of fluorescence (and lifetimes, see below), which for 5DL in MeOH (and also in 1,2-propanediol [10]) are roughly twofold higher than in DCE. Practically no difference in the relative fluorescence intensities could be found for 5DIA in DCE and MeOH. Moreover, in the correlation between the solvent polarity (in ϵ or other experimental units) and the relative fluorescence intensity (closely related to the quantum yield; excited at practically constant absorbance), 5DL in DCE shows a strong deviation from linearity, whereas only a slight deviation is observed for 3-methyllumichrome. For the latter compound, the relative fluorescence intensity in DCE is only about 20% lower than that observed in MeOH. Such linearity is a general and characteristic feature of all the alloxazine derivatives studied so far (a linear cor-

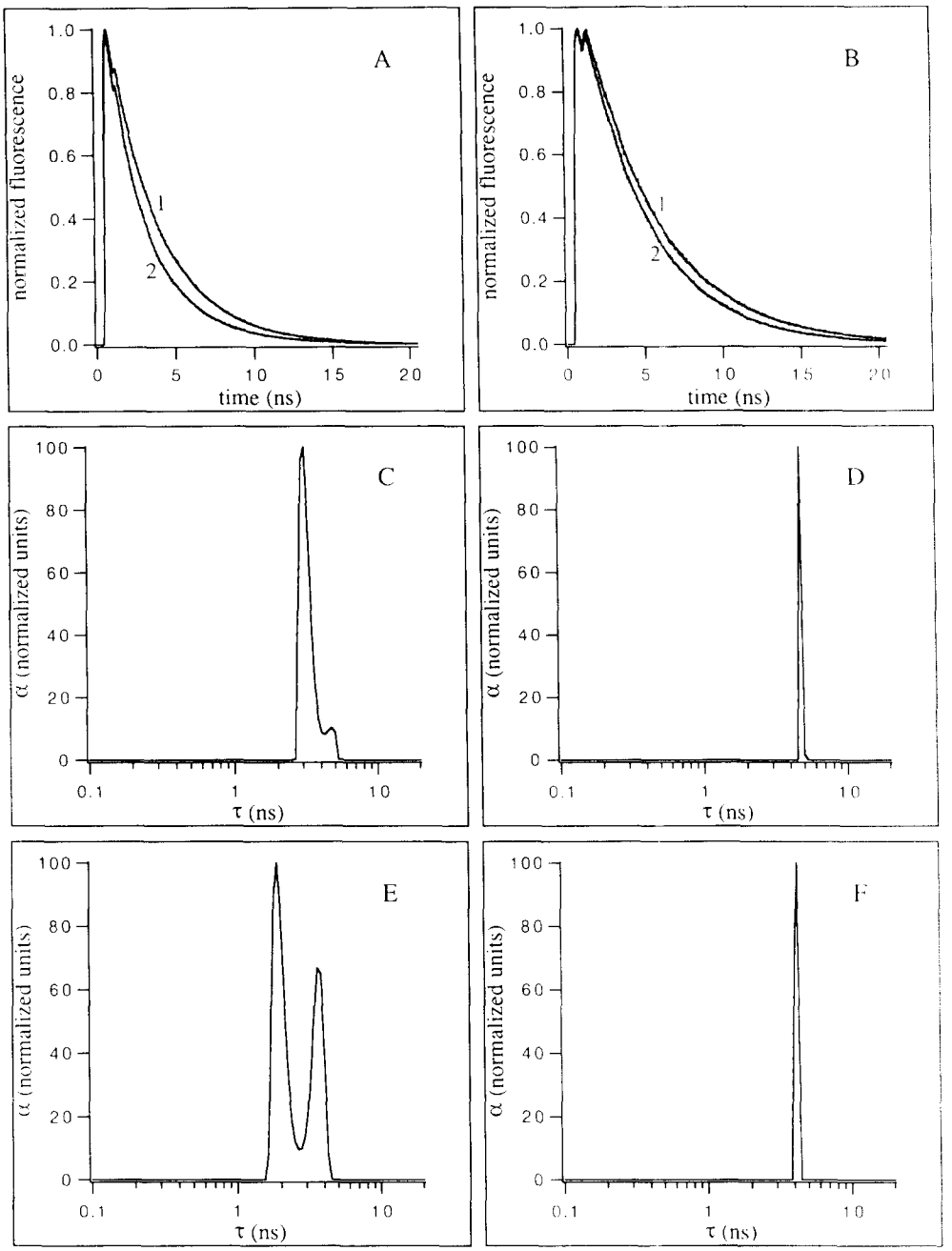

Fig. 3. Fluorescence decay analysis. Experimental fluorescence decay curves measured at 410.3 nm (A) and 468.9 nm (B) of 5DL in DCE in the presence of 0.016 M acetic acid (1) and 0.064 M acetic acid (2). Decay analysis of lifetime distribution (using maximum entropy method) of 5DL in DCE in the presence of 0.016 M acetic acid (C, D) and 0.064 M acetic acid (E, F) measured at 410.3 nm (C, E) and 468.9 nm (D, F).

relation is not observed for solvents which catalyse phototautomerism, i.e. acetic acid, pyridine and water) [11,14,15,20,21].

The most plausible explanation of the influence of DCE (unable to catalyse the phototautomerism of alloxazines) on the fluorescence intensities of 5DL and 3-methyllumichrome is the heavy atom effect of chlorine atoms in the DCE molecule. This is supported by the fact that in 1,1,2,2-tetrachloroethane the fluorescence intensity of 5DL is strongly decreased (by 60% compared with that in DCE) and disappears in 1,2-dibromoethane (not shown). The reason why this effect manifests itself in a much stronger way in the case of 5DL must be associated with the lack of nitrogen at position 5. Taking into account that, in the case of alloxazines, position N-10 is the site of the highest probability of hydrogen bonding [15], we can suggest that DCE solvates molecules of 5DL predominantly at N-10. This is in agreement with the previous assumption that, in the presence of acetic acid, an equilibrium is established between 5DL molecules solvated by DCE or acetic acid molecules, the latter being able to form stronger and more stable structures (see above). The above explanation, based on the assumed role of N-10 in the 5DL molecule,

Table 3
Distribution of lifetimes, using the maximum entropy method, of 5DL in the presence of acetic acid (in ns, contribution given in parentheses)

Acetic acid (M)	Emission wavelength (nm)		
	410.3		468.9
	τ_1	τ_2	τ
0	3.3 [a]		
0.016	3.1 (0.93)	4.6 (0.07)	4.7
0.064	1.9 (0.59)	3.5 (0.41)	4.2

[a] Emission wavelength, 454.9 nm.

is further supported by the lack of a quenching effect of DCE for the isoalloxazine structure (5DIA).

3.2. Fluorescence lifetimes

The total fluorescence decays of standard solutions of 5DL and 5DIA in DCE were measured and the expected difference in the lifetimes was obtained after analysis by the maximum entropy method [12]. The monoexponential decay curves in both cases give well-defined lifetimes of 3.3 ns for 5DL and 4.0 ns for 5DIA (Table 1). The rather long lifetime for 5DL seems to indicate that its structure is more rigid than that of 3-methyllumichrome (with a lifetime of 0.99 ns [15]).

Measurements performed for both compounds dissolved in methanol result in slightly longer lifetimes of 4.4 ns for 5DIA and 5.9 ns for 5DL. A similar result of 6.9 ns was obtained recently for 5DL in 1,2-propanediol [10] (more polar than DCE). We assume that the result is justified because of the high viscosity of 1,2-propanediol (the absorption and fluorescence spectra of 5DL in MeOH and 1,2-propanediol match each other closely). Although both 5DL and 5DIA show some sensitivity to the solvent polarity, and comparable red shifts in the maxima on going from DCE to MeOH, such a dramatic change (almost doubling) in the lifetime of 5DL requires an explanation. The most plausible comment we can propose in discussing this phenomenon is that (as already suggested above) the chlorine atoms of DCE exert a heavy atom quenching effect on the fluorescence of 5DL molecules which enter into close contacts with the solvent molecules.

To analyse the phototautomerization process of 5DL in terms of the appearance of the isomeric species with an isoalloxazine structure and the different fluorescence lifetimes, solutions containing acetic acid at 0.016 M and 0.064 M were chosen (Fig. 3) because, according to results of spectral measurements (see above), at these acetic acid concentrations, both species (alloxazine and isoalloxazine) are present in sufficiently high concentrations. To facilitate the recognition of both forms, one set of measurements was performed using detection at 410.3 nm, i.e. in the region close to the fluorescence emission maximum of the alloxazine form and also within the blue edge of the band ascribed to the isoalloxazine (phototautomeric) form. As expected, for both acetic acid concentrations, the fluorescence decay curves were multiexponential and gave, after analysis of the distribution of the lifetimes using the maximum entropy method, an indication of the presence of both species (Table 3).

There is no doubt that τ_1 should be ascribed to the alloxazine form and τ_2 to the isoalloxazine form, provided that the rate of phototautomerism is much larger than the decay rates of both forms.

The second set of measurements was performed using detection at 468.9 nm, i.e. on the red side of the emission band at longer wavelengths and ascribed to the isoalloxazine (phototautomeric) form. At this wavelength, the emission of the decreasing concentration of the alloxazine form was assumed to be negligible. The results obtained confirm this assumption giving, in the presence of acetic acid at 0.016 M and 0.064 M, monoexponential decays and fluorescence lifetimes of 4.7 ns and 4.2 ns respectively (Table 3). The reason for the discrepancy in the fluorescence lifetime of the isoalloxazine species in the presence of 0.064 M acetic acid measured at different wavelengths is unclear and requires further study.

4. Conclusions

Distinct differences in the phototautomeric process of 5DL relative to 3-methyllumichrome [15] were observed in the presence of acetic acid in DCE as bulk solvent, in particular in the fluorescence intensity. The fluorescence of the alloxazine form of 5DL is quenched by DCE (heavy atom effect) in comparison with more polar solvents containing no heavy atoms in their structure. This is also manifested in the fluorescence lifetime. These effects are ascribed to the replacement of N-5 by carbon and the subsequent changes in the electron density distribution.

Acknowledgements

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