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Photophysics of 1-methyllumichrome

Ewa Sikorska [a], Igor V. Khemlinskii [b], David R. Worrall [c], Siân L. Williams [c],
Rafael Gonzalez-Moreno [d], Jose L. Bourdelande [d], Jacek Koput [e], Marek Sikorski [e,*]

[a] *Faculty of Commodity Science, Poznapń University of Economics, al. Niepodległości 10, 60-967 Poznań, Poland*
[b] *FCT, Universidade do Algarve, Campus de Gambelas, Faro 8000-117, Portugal*
[c] *Department of Chemistry, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK*
[d] *Unitat de Quimica Organica, Universitat Autonoma de Barcelona, Bellaterra, Barcelona 08193, Spain*
[e] *Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*

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Abstract

Singlet and triplet excited states properties of 1-methyllumichrome have been studied in a series of non-polar, polar aprotic, and polar protic solvents, and adsorbed to a cellulose matrix. These observations are discussed in terms of the possible solvent-solute interactions. The absorption and emission spectra and the fluorescence lifetimes and quantum yields of 1-methyllumichrome have been measured, along with the transient absorption spectra. The excited state decays are all single-exponential, suggesting a single emitting species present in all cases. The spectroscopic data show that the singlet excited state properties of 1-methyllumichrome depend on the solute-solvent hydrogen interaction. Significant changes in fluorescence quantum yields and fluorescence lifetimes were recorded and explained by variations of the non-radiative decay rate constant. Placing the 1-methyllumichrome in a restricted environment caused pronounced changes in its behaviour under laser flash photolysis. Transient absorption measurements of 1-methyllumichrome in $H_2O + \beta$-CD and in a cellulose matrix, provided the spectra of the radical anion and of the triplet excited state of the 1-methyllumichrome anion, respectively. Singlet oxygen is shown to be photosensitised in high yield, and this observation provides an insight into possible photodegradation pathways mediated by this molecule. In addition to the experiments, the nature of the electronic structure of 1-methyllumichrome has been studied by means of the time-dependent density functional theory.

1. Introduction

1-Methyllumichrome (1,7,8-trimethylalloxazine = 1,7,8-trimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) is an alloxazine, a member of a class of nitrogen heterocycles related to lumazine and flavins. Alloxazines have active centres at N(10), N(5), N(3) and N(1), and at both carbonyl oxygens at C(4) and C(2). The structure of the 1-methyllumichrome discussed here is presented in Fig. 1. Substituted alloxazines, mainly lumichromes, are present in many foods and are formed in the normal metabolic handling of ingested riboflavin. The early interest in the photophysical and photochemical properties of lumichrome and its derivatives was mainly driven by comparison to flavins. It has been important to assess the toxicity of lumichromes as they are products formed by photochemical reactions of riboflavin.

It has been shown that lumichrome, like riboflavin, is in fact non-mutagenic, non-genotoxic and non-clastogenic [1].

Most of the early work on the photochemistry of alloxazines has been performed in aqueous solutions [2–9]. Recent studies have shown that lumichrome may act as an efficient photoinitiator of free-radical polymerisation of 2-hydroxyethyl methacrylate in the presence of triethanolamine [10]. Another interesting application is an optical transistor device with a thin film of lumichrome on conductive SnO_2 glass [11]. The alloxazine nucleosides are potentially of interest as fluorescent probes and have been predicted to exhibit hydrogen-bonding characteristics similar to thymidine [12]. From the point of view of possible biological roles and for the elucidation of the mechanism of excited-state proton-transfer reactions it is especially interesting to study lumichromes with and without methyl substituents in the N(1) and/or N(3) positions. However, the available information about the photochemistry of lumichrome and its derivatives is still rather limited. Recently,

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* Corresponding author. Tel.: +48-61-8291309; fax: +48-61-8658008.
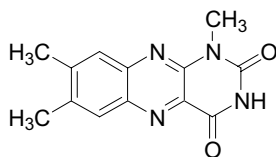*E-mail address:* sikorski@amu.edu.pl (M. Sikorski).

Fig. 1. Structure of 1-methyllumichrome.

the interest in their photochemistry has become more intense, but still there is a lack of information about the effect of the environment on the lifetimes of singlet and triplet excited states. The previous studies of 1-methyllumichrome are scattered and usually performed in protic solvents, e.g. water, alcohols and acetic acid [5–8]. The previous papers concerning 1-methyllumichrome are focused mostly on its steady-state emission and absorption spectroscopy and properties of 1-methyllumichrome in its triplet states. This paper describes a steady-state and time-resolved study of the singlet and triplet states of 1-methyllumichrome in six different solvents and also adsorbed onto cellulose. The present investigation was carried out with the aim of giving a more systematic insight into the photophysics of 1-methyllumichrome as a function of environment.

2. Experimental

β-Cyclodextrin and the solvents acetonitrile, 1,2-dichloroethane, 1,4-dioxane, ethanol and methanol, all from Aldrich, were used as received. Purified, distilled, deionised unbuffered water was used in all experiments and the pH of all of the aqueous solutions prepared was ca. 6. Acetonitrile was dried by refluxing over calcium hydride just before use. The purity of the solvent was confirmed by the absence of fluorescence at the maximum sensitivity of the spectrofluorometer. The 1-methyllumichrome was available from previous work [13,14].

Transient absorption measurements were performed using two different nanosecond laser flash photolysis systems available in Barcelona and Loughborough, both with right-angle geometry. In Barcelona the LKS50 instrument from Applied Photophysics was used: the third harmonic (355 nm) of a Q-switched Nd:YAG laser (Spectron Laser Systems, UK; pulse width ca. 9 ns) was used for laser flash excitation. The measurements in Loughborough were performed using a nanosecond laser flash photolysis system as described previously elsewhere [14]. Fluorescence decay curves of all samples were obtained using 340 nm excitation. Time-resolved fluorescence was recorded using a time-correlated single-photon counting system which has been described in detail in [15].

Singlet oxygen luminescence experiments were carried out by excitation of the sensitiser with the third harmonic of a Lumonics hyper YAG HY200 Nd:YAG laser (355 nm, 8 mJ per pulse, 8 ns FWHM). The excitation energy was attenuated using solutions of sodium nitrite in water. De-tection was using an EO-980P liquid nitrogen cooled germanium photodiode detector (North Coast Scientific), with a 1270 nm interference filter (Melles Griot) interposed between sample and detector to reduce detection of laser scatter and sensitiser emission, and to isolate the singlet oxygen phosphorescence. Data capture was with a 250MS/s digitising oscilloscope (Tektronix 2432A) and data analysis was using Microcal Origin. Perinaphthenone (Aldrich) was used as a reference standard, $\phi_\Delta = 0.95 \pm 0.05$, independent of solvent [16].

Steady-state fluorescence spectra were obtained with a Jobin Yvon–Spex Fluoromax 3-11 spectrofluorometer. UV-Vis absorption spectra were recorded on a Varian Cary 5E spectrophotometer.

Unless otherwise indicated, the samples were purged with nitrogen. All measurements were performed at room temperature.

3. Results and discussion

3.1. Spectroscopic and photophysical properties of 1-methyllumichrome in their ground and excited singlet states

1-Methyllumichrome exhibits absorption spectra with several major bands in the UV-Vis region, see Fig. 2, typical for lumichromes. The absorption spectrum of 1-methyllumichrome in the near-UV region shows two well-resolved maxima at approximately 330 nm (ca. 30 300 cm^{-1}) and 380 nm (ca. 26 300 cm^{-1}), depending on the solvent. The molar absorption coefficients and positions of the two lowest-energy bands for the six solvents examined and cellulose are listed in Table 1. In aprotic solvents, the longer-wavelength absorption band does not show significant dependence on the solvent polarity e.g. when dioxane is replaced by acetonitrile. The absorption and the corrected fluorescence excitation spectra agree well with each other in all solvents examined. The fluorescence emission spectrum of 1-methyllumichrome excited at 355 nm is presented in Fig. 2. The fluorescence emission spectra of 1-methyllumichrome in all six solvents show a single band, the exact position of the maximum depending on the environment and varying from 437 nm in dioxane and acetonitrile to 475 nm in aqueous solution. As can be seen from the data in Table 1, the fluorescence emission maximum shows no systematic dependence on the solvent polarity. The electronic structure of 1-methyllumichrome has been studied by means of the time-dependent density functional theory (TD-DFT) [17]. Recently, similar TD-DFT calculations have been made for the singlet and triplet absorption spectra of lumiflavin [18]. Both the previous results for lumiflavin and our results for 1-methyllumichrome demonstrate some very encouraging improvements as compared to previous semi-empirical and ab-initio calculations [19,20], in that they succeeded in reproducing the correct order of
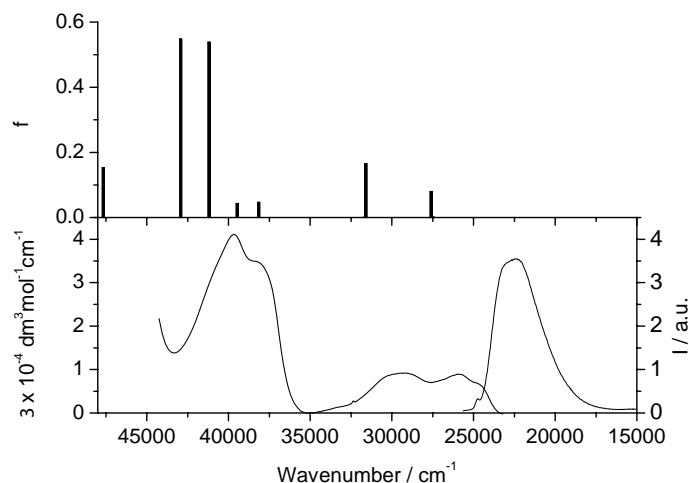
Fig. 2. Calculated lowest-energy singlet–singlet transitions of 1-methyllumichrome. The experimental ground-state absorption spectrum together with the fluorescence spectrum refers to 1-methyllumichrome in 1,2-dichloroethane.

the observed singlet excited states and oscillator strengths of the respective transitions. Moreover, to our best knowledge, there are no published theoretical predictions of the electronic spectra for 1-methyllumichrome, while the available theoretical predictions for similar compounds were obtained only using the semiempirical methods [19,21]. In this work, the TD-DFT calculations were performed using the hybrid method B3LYP [22] in conjunction with the modest split-valence polarized basis set 6-31G* [23]. The excitation energies and transition intensities were calculated for the optimized ground-state geometry of the 1-methyllumichrome molecule. Oscillator strengths were calculated in the dipole length representation. The calculations were performed using the Gaussian 98 package of ab initio programs [24]. The results are presented in Table 2 and Fig. 2. The two calculated lowest-energy transitions in the Fig. 2 are of the π–π^* character at approximately 316.5 nm (31 593 cm^{-1}) and 362.4 nm (ca. 27 590 cm^{-1}), and are accompanied by two closely located n–π^* transitions at 315.0 nm (27 462 cm^{-1}) and 364.1 nm (31 742 cm^{-1}) of low oscillator strengths.

The difference between the predicted and observed transitions energies in 1,2-dichloroethane is about 1500 cm^{-1}. As is the case for many aza-aromatics, 1-methyllumichrome possess close-neighbouring n, π^* and π, π^* (calculated $\Delta E = 128$ cm^{-1}) singlet excited states, having a lowest excited singlet state of n, π^* character in non-polar solvents, but π, π^* in alcohols and other hydrogen bond donors. This so-called level inversion arises from the sensitivity of the energy of n $\rightarrow \pi^*$ transitions to the hydrogen bond donor ability of the solvent. In contrast, the energies of n $\rightarrow \pi^*$ transitions are not strongly influenced by changes in the hydrogen bond donor properties of solvents. Thus, the first excited singlet state of 1-methyllumichrome is expected of n, π^* character in non-polar solvents but π, π^* in protic media. It is very difficult to locate the n $\rightarrow \pi^*$ absorption bands in alloxazines, because they tend to be submerged in the much more intense $\pi \rightarrow \pi^*$ bands. Theoretical calculations and polarised luminescence data in ethanol indicate that all the UV-Vis absorption and emission bands of alloxazines are attributable to the electric-dipole allowed $\pi \rightarrow$

Table 1

Spectroscopic and photophysical data for the singlet states of 1-methyllumichrome in different solvents

Solvent	λ_2 (nm)	λ_1 (nm)	λ_F (nm)	$\Delta\nu_F$ (cm^{-1})	ϕ_F	τ_F (ns)	k_r ($\times 10^8$ s^{-1})	$\sum k_{nr}$ ($\times 10^8$ s^{-1})	k_{ic} ($\times 10^9$ s^{-1})	k_{isc} ($\times 10^9$ s^{-1})	ε
Dioxane	328	381 (8100)	437	3263	0.028	0.51	0.51	19.0			2.21
1,2-Dichloroethane	334	382 (7600)	438	3377	0.021	0.61	0.34	16			10.37
Acetonitrile	334	379 (7600)	437	3304	0.027	0.63	0.43	15	0.51	1.0	35.94
Ethanol	337	385 (7400)	459	3341	0.032	0.94	0.34	10			24.55
Methanol	340	385 (7500)	453	3434	0.037	0.94	0.39	10			32.66
Water[a]	354	386 (7200)	475	3736	0.079	2.2	0.35	4.2	0.19	0.23	80.20
Cellulose[b]	354	392	462			1.0					6.7

λ_1, λ_2 are the positions of the two lowest–energy bands in the absorption spectra, molar absorption coefficients in parentheses, λ_F the fluorescence emission maximum, $\Delta\nu_F$ width of fluorescence band, ϕ_F the fluorescence quantum yield, τ_F the fluorescence lifetime, k_r the radiative rate constant and $\sum k_{nr}$ the sum of nonradiative rate constants, ε dielectric constant. The rate constants for internal conversion is k_{ic} and intersystem crossing is k_{isc}.

[a] From [9].
[b] From [36].

Table 2

Excitation energies out of the S_0 ground state of 1-methyllumichrome (in cm^{-1}; some of the triplet states are omitted), calculated triplet excitation energies starting from the first excited triplet state, T_1, of 1-methyllumichrome and corresponding oscillator strengths, f

$S_0 \rightarrow S_i$	TD-DFT (B3LYP)/6-31G*	f	$S_0 \rightarrow T_i$	TD-DFT (B3LYP)/6-31G*	f	$T_1 \rightarrow T_i$	UTD–DFT UB3LYP/6-31G*	f
$^1(n, \pi^*)$	27500	0.0015	$^3(\pi, \pi^*)$	21400	0	$\rightarrow T_2$	6650	0.0102
$^1(\pi, \pi^*)$	27600	0.0799	$^3(\pi, \pi^*)$	26300	0	$\rightarrow T_3$	6850	0
$^1(\pi, \pi^*)$	31600	0.1657	$^3(n, \pi^*)$	31000	0	$\rightarrow T_4$	11700	0
$^1(n, \pi^*)$	31700	0.0004				$\rightarrow T_5$	14000	0.0052
$^1(\pi, \pi^*)$	38200	0.0468				$\rightarrow T_6$	16400	0.0239
$^1(n, \pi^*)$	38800	0				$\rightarrow T_7$	16800	0.0032
$^1(\pi, \pi^*)$	39500	0.0434				$\rightarrow T_8$	18000	0.0001
$^1(\pi, \pi^*)$	39500	0				$\rightarrow T_9$	19700	0.091
$^1(n, \pi^*)$	40900	0.0001				$\rightarrow T_{10}$	20200	0
$^1(\pi, \pi^*)$	41200	0.5385				$\rightarrow T_{11}$	24700	0.0748
$^1(\pi, \pi^*)$	42900	0.5486				$\rightarrow T_{12}$	26900	0.0266
$^1(n, \pi^*)$	44400	0.0001				$\rightarrow T_{13}$	27500	0.0007
$^1(\pi, \pi^*)$	47700	0.1534				$\rightarrow T_{14}$	30500	0
						$\rightarrow T_{15}$	30700	0.1176
						$\rightarrow T_{16}$	31400	0.2894

π^* transitions [21]. It is well known, that as the hydrogen bond-donating ability of the solvent increases, the energy of a π, π^* excited singlet state decreases to a greater extent than that of the ground state. For 1-methyllumichrome, the lowest $\pi \rightarrow \pi^*$ absorption band shifts to longer wavelength by only 7 nm, but the $\pi^* \rightarrow \pi$ fluorescence transitions are more solvent-sensitive, and the fluorescence spectrum shifts by 38 nm (from 437 to 475 nm) from acetonitrile to water [9,14].

In all solvents the fluorescence decays are modelled well by single-exponential functions, as shown by the usual statistical "goodness-of-fit" criteria. The results of fluorescence lifetime measurements are collected in Table 1. 1-Methyllumichrome exhibits relatively short fluorescence decay times, typical for lumichromes in solution [25,26]. 1-Methyllumichrome has very similar lifetimes of 0.61 ns and 0.63 ns in 1,2-dichloroethane and acetonitrile, respectively, and a shorter fluorescence lifetime of 0.51 ns in dioxane, similar to previously reported fluorescence lifetimes of alloxazines [14,26]. The recorded fluorescence lifetimes and quantum yields in polar acetonitrile, and non-polar solvents 1,2-dichloroethane and dioxane, indicate that polarity is not an important factor influencing these properties. In alcohols and water, both polar protic solvents, the absorption and emission bands of 1-methyllumichrome undergo red shifts, the fluorescence quantum yields becoming higher and the lifetimes significantly longer, as compared to 1-methyllumichrome in aprotic solvents. It is reasonable to expect that for 1-methyllumichrome in protic solvents a range of hydrogen bonds can be formed between the solute and the solvent, involving N(3), N(5), and N(10) and both carbonyl oxygens, C(2) and C(4). Our recent results for lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives in acetonitrile and in methanol show that the hydrogen-bonding interactions between methanol and the N(1) and N(3) positions of lumichrome are unimportant [27]. Therefore, the hydrogen-bond inter-

actions of 1-methyllumichrome involving N(10), and N(5) and both carbonyl oxygens, C(2) and C(4), and protic solvent molecules should be important. In fact, MINDO/3 calculations suggest that both oxygen atoms are more electronegative than any of the nitrogen atoms in the lumichrome structure [28], and should therefore be of importance. Particularly interesting seems the simple hypothesis that the hydrogen bonding interaction between a protic solvent and 1-methyllumichrome at the N(10) position, and also hydrogen-bond interactions between solvent and 1-methyllumichrome at the N(5) and both carbonyl oxygens, C(2) and C(4) influences the conjugation in such a way that there is a rearrangement of the entire electronic structure to yield a more flavin-like structure.

The photophysics and photochemistry of 1-methyllumichrome has been of special interest for comparison to alloxazines, which are capable of undergoing excited state proton-transfer reactions. In contrast to alloxazines unsubstituted at the N(1) position, which undergo excited state proton transfer from N(1) to N(10) to form the corresponding isoalloxazine in aqueous solutions [29], such a process of excited-state proton transfer is impossible in 1-methyllumichrome. The observation of a single-exponential fluorescence decay of 1-methyllumichrome in aqueous solution is consistent with the presence of a single emitting species. This suggestion is also supported by the corrected fluorescence excitation spectra being identical to the absorption spectra throughout the near UV-Vis range, the emission spectra being independent on the excitation wavelength, and the excitation spectra—independent on the emission wavelength. Lasser and Feitelson [30] have reported a 2.4 ns fluorescence lifetime for neutral lumichrome, which is very similar to the value of 2.2 ns obtained by us for 1-methyllumichrome at pH $\cong$ 6. At pH 6, both lumichrome and 1-methyllumichrome exist in the neutral form; at higher pH values, however, the neutral

and monoanionic forms of lumichromes coexist in the $6 < pH \leq 10$ range [5,29,30]. As shown previously from the fluorescence intensity observed from the three forms, the neutral form is the only emitting component at pH $\cong$ 6 (see Fig. 6 in [30]). For example, the deprotonation of 1-methyllumichrome could take place at the N(3) nitrogen. The reported value of pK_a for 1-methlumichrome is 8.65 [29], interestingly, the p$K_a^* = 7.83$ value for deprotonation of 1-methyllumichrome at N(3) in the excited singlet state is only slightly lower than the pK_a value in the ground state [29]. The experimental apparent pK_a values of deprotonation in the ground state were determined by Koziolowa [29]. The pK_a values of deprotonation in the ground state were estimated spectrophotometrically in buffers of constant ionic strength of 0.05 within the 4.5–12.7 pH range and in NaOH solutions of suitable concentration for higher pH values. The dissociation at the N(3) nitrogen gives a monoanion with the typical *alloxazine-like* structure and the fluorescence lifetime of about 1.2 ns [30]. Thus, the 2.2 ns emitter corresponds to the lowest excited singlet state of the neutral form of 1-methyllumichrome. However, the singlet lifetime in water is relatively short, in this time is very difficult to reach the deprotonation–protonation equilibrium in the excited state. Hence, the observation of an increase of the fluorescence quantum yield and of a significantly longer fluorescence lifetime, if compared to 1-methyllumichrome in aprotic solvents, may point to an important role of the hydrogen-bond interaction between water molecules and 1-methyllumichrome at N(10), N(5), N(3) and both carbonyl oxygens, C(2) and C(4), resulting in a charge redistribution yielding a more flavin-like structure.

The radiative and non-radiative decay constants for the lowest excited singlet state can be calculated from $k_r = \phi_F/\tau_F$, and $\sum k_{nr} = (1 - \phi_F)/\tau_F$. The values of k_r and $\sum k_{nr}$ are also tabulated in Table 1. The data show that the decay of the singlet state is dominated by the rates of the non-radiative

processes, these being more than an order of magnitude larger than those of the radiative processes. It is interesting to note some differences for 1-methyllumichrome in aqueous solution, where the largest effect is on the non-radiative components, if compared to other solvents. However, the rate of the radiative process is similar to those in the other solvents examined. These differences between the rates of non-radiative processes of 1-methyllumichrome in water as compared to other solvents can be explained by a shift to the flavin-like structure. It is well known that flavins exhibit longer fluorescence lifetimes and a similar order of magnitude for the rates of both radiative and non-radiative processes, which are similar to those of radiative processes for alloxazines. For example, the fluorescence lifetime of lumiflavin has been determined as 7.6 ns, due mainly to a remarkable reduction in the rate of non-radiative processes relative to 1-methyllumichrome, by more than an order of magnitude. Hence the observation of the decrease of non-radiative decay rate for 1-methyllumichrome in aqueous solutions may point to hydrogen bonding, resulting in charge redistribution yielding a more flavin-like structure.

3.2. Alloxazines in their lower triplet states

The 1-methyllumichrome in 1,2-dichloroethane, acetonitrile and aqueous solutions produces upon laser excitation at 355 nm a transient species that decays on a microsecond timescale. Transient difference spectra of 1-methyllumichrome in the three solvents at different time delays are shown in Fig. 3. The spectra exhibit a sharp maximum at about 370 nm, a broader absorption maximum near 450 nm and a broad absorption centred at about 530 nm. The negative absorbance change near 400 nm is attributed to ground-state depletion. The spectra are similar to those previously reported for lumichromes and alloxazines and are assigned to the triplet–triplet absorption of the triplet excited state
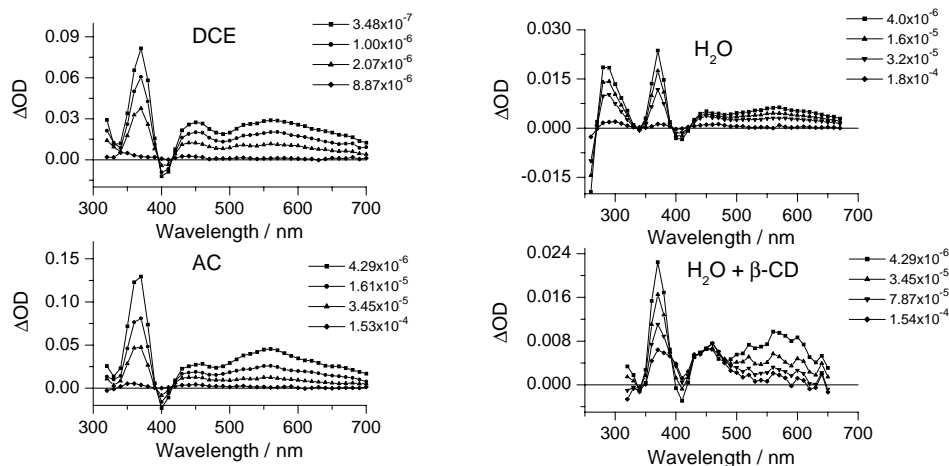

Fig. 3. Transient absorption spectra of 1-methyllumichrome in deoxygenated 1,2-dichloroethane, DCE, acetonitrile, AC, in deoxygenated aqueous solutions, H$_2$O, and in deoxygenated aqueous solutions with β-cyclodextrin, H$_2$O + β-CD. The numbers refer to the time in seconds after laser excitation at 355 nm, 1 cm cell pathlength.

of the neutral molecule [4,6,8,9,14]. The decay kinetics of the triplet state has been measured at the longer-wavelength maximum. Although the transient absorption spectra in all three solvents are similar, there are some apparent differences in the decay kinetics. The triplet lifetime recorded in non-polar 1,2-dichloroethane is the shortest, being longer in polar acetonitrile, and the longest in aqueous solutions.

For all three solvents the decay kinetics at 450 nm are complicated by a second, weaker-absorbing species decaying with a much longer lifetime tentatively assigned as the radical anion. It has been shown that lumichrome, and some alloxazines form inclusion complexes with β-cyclodextrin in water [31,32]. The equilibrium constant for the formation of this inclusion complex is determined by fluorimetry as 966 mol/dm³ and by solubility as 491 mol/dm³ [31]. The changes in the triplet lifetime and transient spectra observed in water with and without β-cyclodextrin clearly demonstrate that the presence of β-cyclodextrin affects the photophysics and photochemistry of 1-methyllumichrome in its triplet state. The 1-methyllumichrome produces a slightly different transient absorption spectrum, and a different time profile is observed in the region of 420–480 nm in aqueous solutions in the presence of β-cyclodextrin upon laser excitation at 355 nm. These changes are shown in Fig. 3. The spectrum of the long-lived species corresponds fairly well to the spectrum of the lumichrome anion radical [5,33].

The triplet–triplet absorption spectra (Fig. 4) were calculated by correcting the transient spectra for the ground state depletion, using the previously measured values of the triplet extinction coefficient at 370 nm [14,34] and the steady-state absorption spectra taken in the respective solvents. In the

calculations, we assumed the existence of only two absorbing species after the first singlet excited state has decayed, namely ground-state and first triplet excited state molecules. The spectra presented correspond to the respective shortest time delays of Fig. 3. Note that the triplet extinction coefficients measured by the triplet–triplet energy transfer from benzophenone have relative uncertainties of 10%; also, the unknown triplet extinction value in H_2O + β-CD was assumed to be equal to that in water, for the purpose of these calculations. This latter assumption may be justified by the weak dependence of the triplet extinction coefficient on the solvent polarity [6,8,9,14] and by the overall uncertainties of the values measured for other solvents.

To the best of our knowledge, the spectrum reported in Fig. 5 is the first theoretical T–T spectrum available in the literature, describing excitation from the lowest triplet state of 1-methyllumichrome, and of alloxazines in general. It was calculated using the unrestricted formalism (UB3LYP/6-31G*). The T–T excitation energies and transition intensities were determined for the optimized geometry of the lowest triplet state (T_1), the results are shown in Table 2 and Fig. 5. These results are particularly important when its necessary to distinguish the true tripleT–Triplet absorption spectrum from other possible species, readily formed during the photochemistry of alloxazines in different environments. The detectable transitions shown in the Fig. 5, at ca. 19 700, 24 700, 26 900, 30 700 and 31 400 cm⁻¹ are similar to the available experimental results. We notice that there are several lower-lying transitions (see Table 2), but because of limitations of the flash photolysis setup these transitions are not visible in the experimental spectrum. Fig. 5 refers to experimentally measured T–T spectrum of
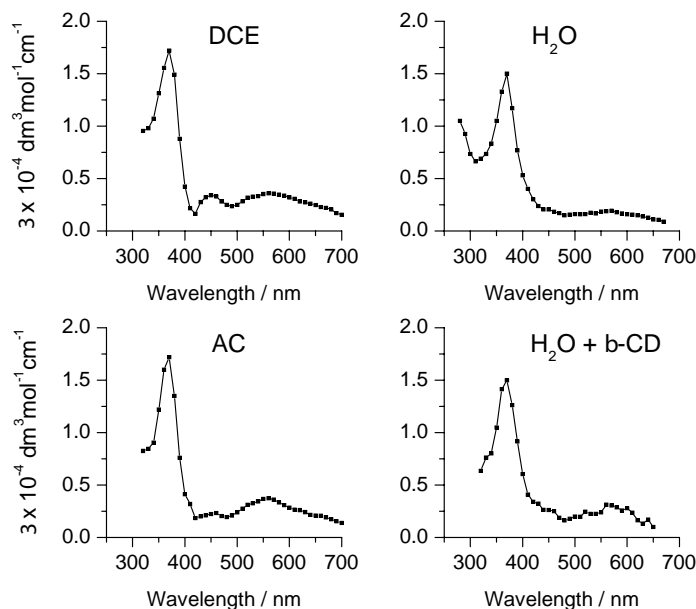

Fig. 4. The absolute absorption spectra of 1-methyllumichrome triplet in: 1,2-dichloroethane, DCE, acetonitrile, AC, in aqueous solution at pH 6, H_2O, and in aqueous solution with β-cyclodextrin, H_2O + β-CD. The spectra presented correspond to the respective shortest time delays of Fig. 3.
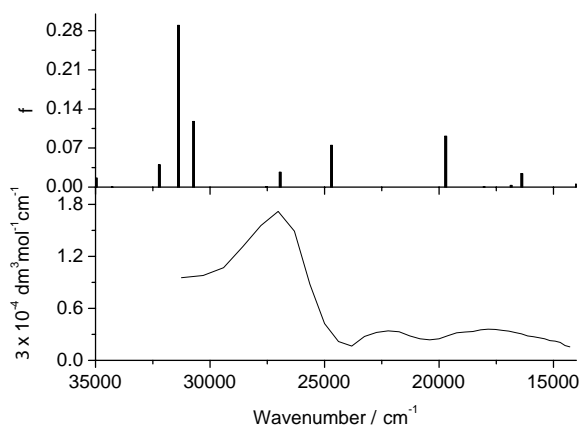
Fig. 5. Calculated T–T transitions of 1-methyllumichrome obtained with DFT method. For comparison, the experimental absolute absorption spectrum of 1-methyllumichrome triplet in 1,2-dichloroethane is given. The spectrum presented correspond to the shortest time delay of Fig. 3. Excitation is at 355 nm.
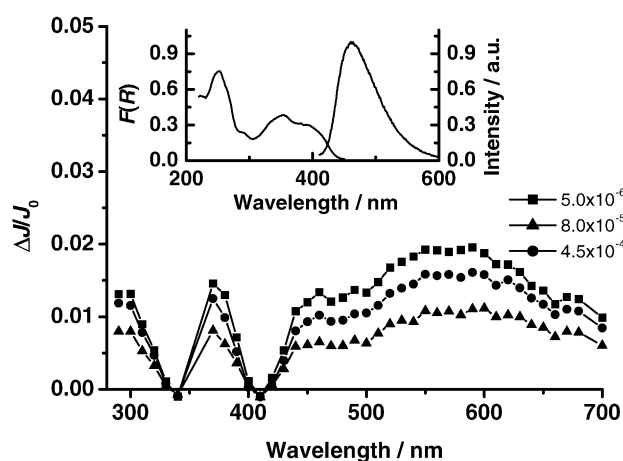

Fig. 6. The time-resolved diffuse reflectance laser flash photolysis spectra of 1-methyllumichrome, 1 MLch, at a loading of 1 mg/g on cellulose at room temperatures in deaerated samples. Excitation is at 355 nm. The delay times in seconds are indicated on the panel. Insert: ground-state diffuse reflectance absorption spectrum and normalised fluorescence emission of 1-methyllumichrome on cellulose.

1-methyllumichrome in non-polar 1,2-dichloroethane. For the theoretical spectrum, we succeeded in reproducing position of experimental T–T transitions and the respective oscillator strengths. The difference between the predicted and observed transitions energies in 1,2-dichloroethane are higher then those for singlet states and are about 2000 cm^{-1} for the lowest T–T transitions with a tendency to increase for higher-energy T–T transitions. The energy gap between the ground singlet state and first excited triplet (T$_1$) was predicted to be 19,700 cm^{-1} (508 nm).

Further evidence for the photochemistry of 1-methyllumichrome can be derived from our recent studies on cellulose [35,36]. Laser excitation at 355 nm of 1-methyllumichrome on cellulose leads to transients whose spectra and decay rates can be followed by transient diffuse reflectance methods. We demonstrated that in diffuse reflectance spectra of 1-methyllumichrome deposited onto cellulose at least two species are present. The short-lived species has a lifetime of the order of microseconds and is quenched by oxygen, and has been identified as the lowest triplet of the 1-methyllumichrome. The identification of the long-lived species was more complicated. The spectrum of the long-lived species is to some extent similar to those recorded for 1-methyllumichrome in solution, although the kinetics and intensity ratio of the maxima observed are substantially different. Comparing the transient spectrum of the long-lived species on cellulose (see Fig. 6) to the spectra of 1-methyllumichrome in solution and in H$_2$O + β-CD we conclude that the spectrum on cellulose does not belong either to the triplet or the anion radical. The comparison of the spectrum of lumichrome recorded in methanol in the presence of NaOH (see Fig. 5 in [33]), to the spectrum of 1-methyllumichrome on cellulose leads us to the conclusion that the recorded spectrum of the long-lived species on cellulose corresponds to the anionic triplet of 1-methyllumichrome.

3.3. Singlet oxygen measurements

For general interest we measured the emission at 1270 nm, which is highly specific to the O$_2$($^1\Delta_g$) → O$_2$($^3\Sigma_g^-$) transition, under laser excitation at 355 nm of the 1-methyllumichrome in air-equilibrated solutions. The emission intensity at 1270 nm increased in samples with higher oxygen concentrations and was extinguished by bubbling N$_2$ through the solution for a few min. The emission lifetime values recorded at 1270 nm in air-equilibrated solutions and presented in Table 3 are typical for singlet oxygen in all examined solutions [37]. All these observations confirm that 1-methyllumichrome used in this study acted as a photosensitiser for singlet oxygen, and that O$_2$($^1\Delta_g$) is responsible for the emission at 1270 nm.

Generally, the quantum yield for formation of singlet oxygen by sensitisation, ϕ_Δ, is given by the sum of the contributions due to oxygen quenching of the lowest excited

Table 3
The lifetime of triplet state, τ_T, the quantum yields of photosensitized production of singlet oxygen, ϕ_Δ, and the singlet oxygen lifetimes in solutions, τ_Δ

Solvent	τ_T (µs)	ϕ_Δ	τ_Δ (µs)
Dioxane	–	0.50	24[a]
1,2-Dichloroethane	2.4	0.84	57
Acetonitrile	6.9	0.90, 0.67[b]	78
Ethanol	–	0.91	14
Methanol	–	0.87	10
H$_2$O	18	0.67	4.5
D$_2$O	–	0.4[c]	60

[a] See the text.
[b] From [14].
[c] From [9].

singlet state (S_1) and the lowest excited triplet state (T_1) of the oxygen sensitizer:

$$\phi_\Delta = \phi_\Delta(S_1) + \phi_\Delta(T_1)$$

However, the fluorescence lifetimes of 1-methyllumichrome are short in the solvents used (Table 1) and thus oxygen should not significantly quench the first singlet excited state in aerated solvents by any mechanism; assuming a collisional mechanism only some 2% of the excited singlet states should be quenched by oxygen in acetonitrile, note from our calculations $\Delta E(S_1-T_1) = 7916\,cm^{-1}$. Considering the data on singlet oxygen yields presented in Table 3, and having in mind that the ϕ_Δ values should be interpreted as the lower limits of the respective triplet yields, it could be suggested that the previously determined values of ϕ_T have been underestimated. This explanation of the results presented in Table 3 seems reasonable in the light of the uncertainties in the triplet yields resulting from their determination by energy transfer in the alloxazine-benzophenone system. Thus, the results on the singlet oxygen yields suggest high values of ϕ_T for 1-methyllumichrome in all solvents applied. However, the results in water and heavy water are significantly lower, but still relatively high. The observed yield of singlet oxygen depends on the triplet quantum yield ϕ_T, the fraction of triplet states quenched by oxygen $f_T^{O_2}$ and the fraction of triplet states quenched which give singlet oxygen f_Δ^T. If both of these latter parameters are unity then the singlet oxygen quantum yield is equal to the triplet yield and internal conversion is a contributing pathway in the overall relaxation, although a relatively small one in most solvents. The dioxane result is especially interesting since there are clearly quenching photolysis products produced, as the rate constant for the singlet oxygen decay increases as the laser energy is increased for this sample, but not in the other solvents. The lower value of ϕ_Δ in this solvent may therefore be tentatively attributed at least in part to a lower value of f_Δ^T, since oxygen quenching of the triplet to produce singlet oxygen competes with reaction. The photophysics of 1-methyllumichrome with its relatively high absorption in the spectral range convenient for laser excitation at 355 nm makes this molecule interesting for many reasons. The results presented in this paper indicate the need for future studies of the possible applications of 1-methyllumichrome, and alloxazines in general, in such areas as: the photodegradation of polymers in aqueous solutions, the photooxidation of substituted phenols in water, photooxidation and photodegradation of food products, and the role of alloxazines, present as stable photoproduct of flavins, in the biologically active structures.

Acknowledgements

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