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FLUORESCENCE LIFETIMES OF FUROCOUMARINS. PSORALENS

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The fluorescence lifetimes of linear furocoumarin drugs and model compounds are reported in water, ethanol and water/ethanol mixtures. In most cases more than one lifetime is observed and this is discussed in terms of complex and excited-state complex formation between the solvent and furan moiety of the drugs.

1. Introduction

Psoralens (linear furocoumarins – FC) especially 8-methoxypsoralen (8-MOP) are used in the treatment of various skin diseases including psoriasis [1] and leukoderma [2]. Such treatment involves either topical or oral application of psoralens plus UVA light (320–400 nm) and is known as PUVA phototherapy [3]. There has been considerable work on the lowest excited triplet states of psoralens and parameters such as triplet lifetimes, extinction coefficients, and quantum yields (Φ_T) have been reported in several solvents [4–6]. However, there has been little reported work on the first excited singlet state (S_1) of these molecules, yet it has been suggested that the formation of the monoadduct between the thymine base of DNA and psoralens (probably the first photochemical event in PUVA phototherapy), occurs via the first excited singlet state [7]. This Letter presents the first measurements of the fluorescence lifetimes of 8-MOP, 5-methoxypsoralen (5-MOP), 5,8-dimethoxypsoralen (5,8-DiMOP), psoralen (Ps), 4′-aminomethyl-4,5′,8-trimethylpsoralen hydrochloride (AMT) as well as the model compounds 7-hydroxycoumarin (7-HC) and 4,5′-dimethyl-4′,5′-dihydroangelicin, a substituted coumarin (DMDHA), in the solvents water and ethanol together with some observations in benzene, and also reports the effect of thymine and tryptophan on the fluorescence lifetimes of AMT, Ps and 8-MOP in water. Fig. 1 gives the structures of the molecules studied in the present work.

2. Experimental

The 8-MOP, Ps, L-tryptophan (tryp) and thymine were obtained from Sigma, 5-MOP was a gift from Professor Rodighiero, AMT was kindly donated by Professor Hearst, 5,8-DiMOP was kindly donated by Dr. Poh-Agin. The 7-HC and DMDHA were synthesised by standard procedures. The solvents were all of spectroscopic grade and the water was distilled prior to use.

The absorption spectra were taken using a Perkin–Elmer 554 UV–VIS spectrophotometer with 2 nm slit and matched quartz cuvettes of 1 cm optical pathway. The emission spectra under excitation at 364 nm were

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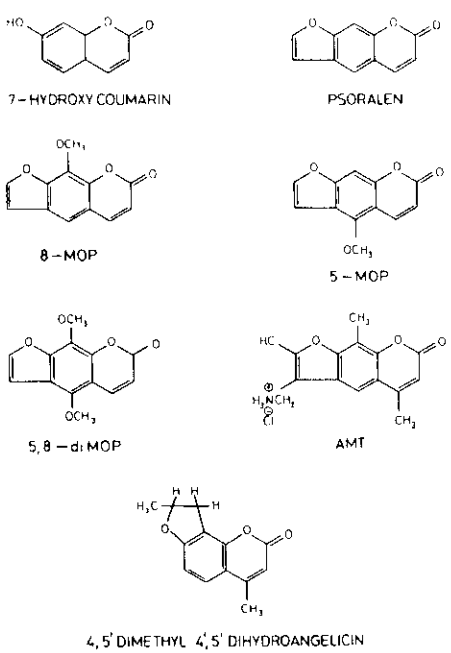

Fig. 1. Structures of the linear furocoumarins and model compounds investigated.

measured using a Perkin–Elmer 650-40 spectrofluorometer with 5 nm slits in both the excitation and the observation monochromators. The emission spectra were not corrected for the spectral response of either the monochromator nor the photomultiplier.

The pulsed excitation at 364 nm for the time-resolved fluorescence experiments was provided by a Coherent CR-18 mode-locked argon laser with an acousto-optic pulse picker on the output beam to reduce the laser pulse repetition rate to 800 kHz.

The solution to be measured was contained in a 1 cm pathway quartz cuvette and the fluorescence was detected at 90 degrees through a Kodak Wratten no. 4 cut-off filter using the single-photon timing apparatus described [8]. To increase the data acquisition rate, the excitation pulse and the fluorescence photons were made to provide the stop and the start signals, respectively, to the time-to-amplitude converter. The fwhm duration of the excitation pulse as detected by this apparatus was 240 ps at 364 nm.

The experimental fluorescence decay curves were then transferred to a Tektronix 4051 graphic system for processing and plotting. The experimental curves were fitted by the sum of up to three exponential decay components using a non-linear least-squares program on a PDP 11/23 computer. Typical data showing log plots of the experimental fluorescence decay, linear plots of the weighted residuals and auto-correlation of the residuals, are shown in figs. 2 and 3 for psoralen in water and ethanol, respectively.

Fluorescence emission and excitation spectra were measured on a Perkin–Elmer MPF/44A spectrofluorometer incorporating the low-temperature attachment.

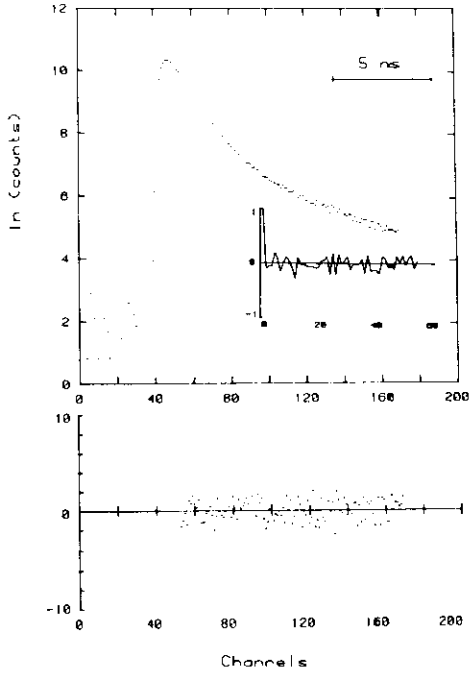

Fig. 2. Logarithmic plot of the fluorescence decay of psoralen in water versus time. The weighted residuals (bottom) and their autocorrelation function (inset) are also shown.

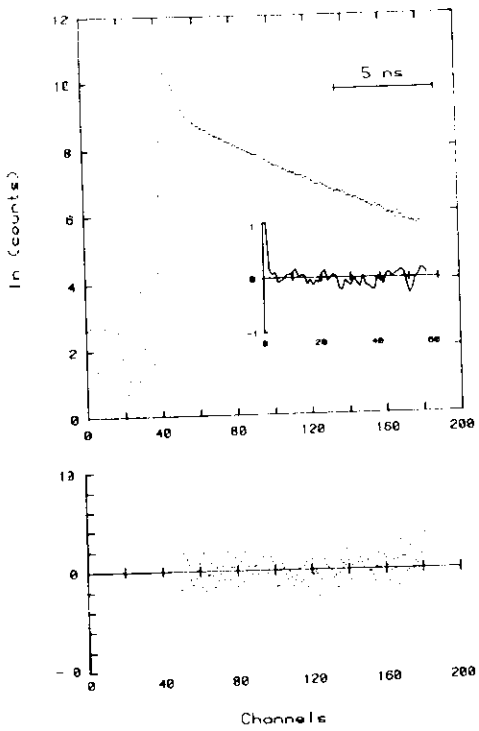

Fig. 3. Logarithmic plot of the fluorescence decay of psoralen in ethanol versus time. The weighted residuals (bottom) and their autocorrelation function are also shown.

3. Results

Photobiological work has often involved water [6], small percentages of ethanol in water [9] or phosphate buffer [10] as solvents and our preliminary work considered the effect of these "similar" environments. In all cases no effect on the fluorescence data was obtained by these minor solvent variations.

Tables 1 and 2 report the photophysical data for the FC and models studied in water and ethanol respectively. In addition table 3 reports such data for psoralen itself, in varying water/ethanol mixtures.

As can be seen Ps, 8-MOP and 5,8-DiMOP all have three lifetimes in ethanol and only two ($\tau_1 \approx 4.5 \pm 0.5$ ns and $\tau_2 \approx 0.9 \pm 0.1$ ns) in water where the very short-lived ($\tau_3 \approx 0.2$–0.3 ns) component is no longer evident even though it is the predominant species (≈70–90%) in ethanol. However, 5-MOP shows rather complex behaviour in that the three components in water with the short-lived species predominant become a single long-lived component (≈3–4 ns) in ethanol. AMT has two components (τ_1 and τ_2) both in ethanol and water with the τ_2 (≈1 ns) predominant. The two model compounds 7-HC and DMDHA show similar behaviour both having only the long-lived ($\tau_1 \approx 4.5$ ns) component in water. In ethanol the single fluorescence lifetime for 7-HC is quite similar to that of water. However for DMDHA in water the single τ is changed to two components with τ_2 (≈1.7 ns) being predominant.

We have not studied the FC molecules in detail in benzene but our preliminary results for these mole-

Table 1
Fluorescence decay time constants (τ_1, τ_2, τ_3) and relative initial amplitudes (P_1, P_2, P_3) of linear furocoumarins and models in water [a]

Molecule	τ_1 (ns)	P_1(%)	τ_2 (ns)	P_2(%)	τ_3(ns)	P_3(%)
7-HC	5.26	100	–	0	–	0
DMDHA	4.24	100	–	0	–	0
Ps	4.08	4.6	0.89	95.4	–	0
8-MOP	3.99	3.2	1.06	96.8	–	0
5-MOP	5.44	14.8	1.32	9.2	0.43	76.1
5/8-DiMOP	4.91	43.9	0.82	56.1	–	0
AMT	4.1	4.5	1.73	95.5	–	0

a) Concentration $\approx 10^{-4}$ dm^3 mol^{-1} except AMT (2.8×10^{-5} dm^3 mol^{-1}).

Table 2
Fluorescence decay time constants (τ_1, τ_2, τ_3) and relative initial amplitudes (P_1, P_2, P_3) of linear furocoumarins and models in ethanol [a]

Molecule	τ_1 (ns)	P_1(%)	τ_2 (ns)	P_2(%)	τ_3 (ns)	P_3(%)
7-HC	4.31	100		0		0
DMDHA	4.28	5.5	1.70	94.5	-	0
Ps	4.01	8.4	1.52	5.2	0.20	86.4
8-MOP	6.95	5.9	1.52	23.1	0.41	71
5-MOP	3.48	100		0		0
5/8-DiMOP	5.28	3.1	1.18	11.6	0.43	85.3
AMT	4.05	2.7	1.08	97.3		0

[a] Concentrations $(6 \pm 3) \times 10^{-5}$ dm^3 mol^{-1}.

Table 3
Fluorescence decay time constants (τ_1, τ_2, τ_3) and relative initial amplitudes (P_1, P_2, P_3) for the molecule psoralen in ethanol/water mixtures

Ethanol(%)	τ_1(ns)	P_1(%)	τ_2(ns)	P_2(%)	τ_3(ns)	P_3(%)
100	4.0	8.4	1.58	5.2	0.20	86.4
80	4.54	5.7	1.31	9	0.27	85.3
50	4.9	1.9	1.18	8.5	0.42	89.6
40	5.18	10.6	1.17	24.2	0.44	65.2
35	5.0	2.3	0.86	31.3	0.42	66.4
30	4.74	4.6	0.82	48.7	0.45	46.7
20	4.33	7.3	0.84	92.7		0
15	4.33	4.7	0.85	95.3	-	0
10	4.15	17.1	0.88	82.9	-	0
5	4.43	5.8	0.9	94.2	-	0
1	4.11	5.3	0.91	94.7	-	0
0	4.08	4.6	0.89	95.4		0

cules show only two components in this solvent.

The interaction of psoralen triplet states with DNA bases such as thymine and also with amino acids such as tryptophan have previously been reported and the rather fast ($\approx 10^8$–10^9 dm^3 mol^{-1} s^{-1}) quenching rate constant interpreted in terms of electron transfer processes [11]. To investigate the possibility of such interactions with S_1 state of FC molecules, we have studied the effect of tryptophan and thymine on the fluorescence lifetimes of 8-MOP, Ps and AMT in water and the results show that for tryptophan and thymine concentrations in the region of 1.9×10^{-2} mol dm^{-3} there was no appreciable increase in the fluorescence decay rates of the three FC molecules studied.

4. Discussion

The observation of 3 and 2 fluorescence lifetimes of the several FC studied in both ethanol and water respectively (except for 5-MOP) is somewhat surprising since emission solely from the lowest excited singlet state is normally expected. A reasonable explanation is that the coumarin emits and that the benzofuran (or furan) forms a solvent exciplex which also emits. In addition in ethanol as solvent, some type of ground-state complex is formed which gives rise to the third component in this solvent. In an attempt to confirm such speculations we report the fluorescence emission of two coumarins, 7-HC and DMDHA, in both EtOH and H_2O and, as can be seen, a single emission is observed for both coumarins in water and for 7-HC in

ethanol. We therefore suggest that this emission is specifically associated with the coumarin part of the molecule and that the other short lifetime(s) in water and ethanol for the FC is associated with the furan ring presumably via solvent complex formation. In a further attempt to assign the two remaining fluorescence lifetimes in EtOH and one in H_2O, we report in table 3 the fluorescence lifetimes of the parent FC, psoralen, in EtOH/water mixtures. As can be seen, the amount of the very short component (τ_3) decreases with decreasing EtOH concentration and falls to zero at about 20% or below. Thus we suggest that the τ_3 component is associated with some benzofuran–ethanol complex, such a suggestion being consistent with our preliminary observation of only two fluorescence decays of FC in benzene solution.

In a further attempt to interpret the observation of more than one fluorescence lifetime we have measured the fluorescence emission spectra of psoralen in ethanol alone, as a function of exciting wavelength at liquid nitrogen temperature (77 K). In general exciting at less than 300 nm gave rise to an emission peak at ≈395 nm, while excitation wavelengths greater than 310 nm gave rise to additional peaks at 456 and 490 nm. We believe the latter observations imply we are selectively exciting the lowest excited singlet with the longer wavelength excitation which is at least consistent with the above suggestions. This effect can also be observed by monitoring the fluorescence excitation spectra of psoralen in ethanol at liquid nitrogen temperature. Thus with the emission wavelength at 395 nm the excitation spectra show a main band at 290 nm while with the emission wavelength set at 456 or 490 nm the excitation spectra show a main band at ≈335 nm.

It is of interest to speculate on the possible photobiological consequences of our observation of more than one fluorescence-emitting species. The question immediately arises as to whether photobinding to DNA involves one or both of such species. Thus, for example, if photobinding involves short-wavelength excitation and if triplet formation involves longer-wavelength excitation ($S_0 \rightarrow S_1$), then there would be advantages in avoiding the excitation $S_0 \rightarrow S_1$ because no therapeutic value arises and possible damage via singlet oxygen could occur from the triplet state generated via S_1. That is, this assumption would suggest that it would be worth investigating using the shorter region of wavelengths of UVA for PUVA, say <350 nm, so as to substantially avoid S_1 (and hence T_1) formation.

In future work we intend to obtain fluorescence lifetimes for angular furocoumarins and investigate the effect of DNA and HSA on such lifetimes.

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