PHOTOPHYSICAL PROPERTIES OF SOME COUMARIN DERIVATIVES: 5,7-DIMETHOXYCOUMARIN, 4′,5′-DIHYDROPSORALEN, 8-METHOXYPSORALEN (8-MOP) AND 8-MOP($^{4',5}_{5',6}$)THD C_4-CYCLOMONOADDUCT (THD ≡ THYMIDINE)

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Summary

The photophysical properties of a 4′,5′-C_4-cyclomonoadduct (F-2) of 8-methoxypsoralen (8-MOP) with thymidine were compared with those of 5,7-dimethoxycoumarin (DMC), 4′,5′-dihydropsoralen (DHP) and 8-MOP. The magnitudes of the fluorescence quantum yields are in the order DMC > DHP ⩾ F-2 > 8-MOP, which is exactly the opposite of the order for the temperature dependence of the fluorescence in ethanol. For DMC, DHP, 8-MOP and F-2 the ratios of the phosphorescence quantum yields to the fluorescence quantum yields are 0.056, less than 0.01, 0.68 and 5.6 and the phosphorescence lifetimes are 0.88 s, 0.88 s, 0.76 s and 1.2 s respectively, in ethanol at 77 K. The phosphorescence-to-fluorescence ratio increases sharply while the phosphorescence lifetime decreases by the external heavy-atom effect. At 77 K, the fluorescence maximum is very red shifted in isopentane compared with that in ethanol, and phosphorescence is not observed in isopentane. The solvent dependence of the fluorescence of the compounds is probably due to a change in the rate of internal conversion rather than to intersystem crossing.

1. Introduction

Furocoumarins such as psoralens are important drugs used in the photochemotherapy of psoriasis [1] and vitiligo [2], and they have been used more recently as molecular probes for studying nucleic acid structure [3]. However, possible deleterious effects such as carcinoma development in hairless mice as well as possible liver injury from the use of 8-methoxypsoralen (8-MOP) have been reported [4]. Psoralens are also the cause of some forms of dermatitis resulting from skin contact with certain plants or vegetable materials such as fig leaves and celery.

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The photosensitizing ability of psoralens has been generally correlated with their photoreactivity towards pyrimidine bases of DNA [5]. The biological effects of psoralen plus UVA treatment appear to be mediated primarily by the formation of cross-links in DNA. The cross-links are caused by the formation of cyclobutane adducts which arise from [2+2]-photocycloaddition of the psoralen 3,4- and 4′,5′-double bonds with the 5,6-double bond of two pyrimidine bases in opposite strands of the DNA. It is necessary that one psoralen molecule intercalated into duplex DNA successively absorbs two photons to form a cross-link. The 4′,5′-monoadducts absorb light up to 380 nm whereas 3,4-monoadducts do not absorb light above 320 nm. It is thus obvious that after the first quantum of near-UV light has been absorbed by the psoralen itself to form a monoadduct the second quantum must be absorbed by a 4′,5′-monoadduct rather than a 3,4-monoadduct to form a cross-link. It is therefore very important to understand the physical and chemical properties of the 4′,5′-monoadduct for the elucidation of the photobiological activity of furocoumarins.

The photophysical properties of the lowest excited states (singlet and triplet) of furocoumarins and coumarins have been extensively investigated in recent years [6, 7]. These studies indicate that the lowest excited singlet state of 8-MOP is a π,π^* state [7], and the fluorescence from this state is strongly dependent upon the nature of the solvent [8]. In aprotic non-polar solvents, such as 3-methylpentane, the fluorescence is too weak to be detected, while in protic polar solvents fluorescence with moderate intensity is observed. These results were interpreted in terms of the position of the energy level of an n,π^* triplet state relative to that of the lowest energy π,π^* singlet state. These solvent effects were attributed to the efficiency of $^1(\pi,\pi^*)-^3(n,\pi^*)$ intersystem crossing varying with the solvent polarity. However, by comparing the temperature dependence and the solvent dependence of the steady state fluorescence with those of the triplet formation and on considering the time-resolved fluorescence spectra and energy-resolved fluorescence decays in polar solvents, it is found that the solvent

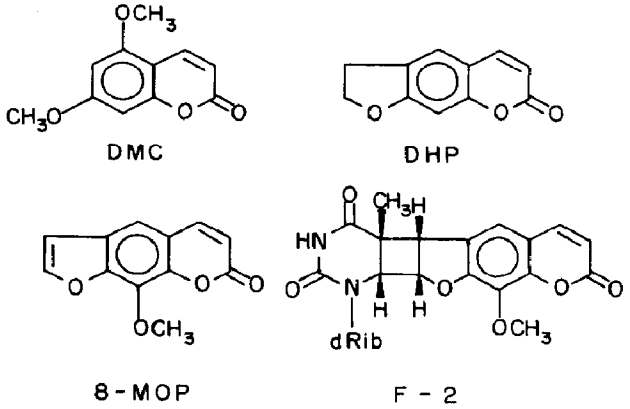


Fig. 1. Structures of 5,7-dimethoxycoumarin (DMC), 4′,5′-dihydropsoralen (DHP), 8-MOP and 8-MOP$\langle^{4'}_{5},^{5}_{6}\rangle$THD C_4-cyclomonoadduct (F-2) (THD ≡ thymidine).

dependence of fluorescence in psoralens has little to do with the change in singlet–triplet intersystem crossing but is a consequence of the changes in $S_1 \rightsquigarrow S_0$ internal conversion [9].

In continuation of our studies on the nature of the ability of furocoumarins as photosensitizers [10 - 16], the solvent and temperature dependence of emission from furocoumarins (Fig. 1) was examined and the photophysical properties of these compounds at 77 K are reported.

2. Experimental details

2.1. Materials

8-MOP and thymidine were purchased from the Sigma Chemical Company and were used without further purification. DMC (Aldrich Chemical Company) was purified by recrystallization from ethanol and DHP was synthesized according to the previously reported method [17] and recrystallized from methanol. The *cis-syn*-8-MOP$\langle^{4',5}_{5',6}\rangle$THD monoadducts were prepared by photolysis of a mixture of 8-MOP and thymidine in a dry film state, as described previously [15] and were isolated according to the method reported in ref. 16. Tetramethylethylene (TME) was obtained from the Aldrich Chemical Company and was used as received. Kiesel Gel_{254} (Merck) and Kiesel Gel G (Merck) were used for silica-gel thin-layer chromatography and column chromatography respectively. Chromatographic and spectroscopic grade solvents were used for high performance liquid chromatography and emission spectroscopy respectively.

2.2. Methods

UV–visible spectra were recorded on a Cary 17 spectrophotometer. Fluorescence and phosphorescence spectra were recorded on an Aminco–Bowman spectrophotofluorometer with an Aminco *XY* recorder at room temperature and at 77 K with modification of the cell compartment. A cylindrical chopper with a maximum rotating frequency of 10 000 rev min^{-1} with two windows opposite to each other was used to isolate phosphorescence from other emissions. The phosphorescence lifetime was measured with this instrument, using a mechanical shutter to cut off the excitation light, in conjunction with a Tektronix 5115 storage oscilloscope. The polarization of the emission was obtained with a Glan–Prism polarizer and was corrected by the Azumi–McGlynn formulation [18]. The concentration employed in the measurement of the luminescence spectra was always less than 10^{-4} M. Recorded emission spectra were corrected for the changes in the response characteristics of the photomultiplier tube (1P21, S-4 spectral response) and the monochromator of the instrument as a function of wavelength. Once the spectra had been corrected it was possible to determine the phosphorescence-to-fluorescence quantum yield ratios and the fluorescence yields. The fluorescence quantum yields of the furocoumarins in ethanol at room temperature were determined relative to DMC [19] (Φ_f =

0.65 in ethanol at room temperature) and the quantum yield of each compound in a given solvent was determined relative to its value in ethanol, by the following relationship:

$$\Phi_f = \Phi_f^r \frac{I_s}{I_r} \frac{A_r}{A_s} \left(\frac{n_s}{n_r}\right)^2$$

where Φ_f^r represents the fluorescence quantum yield of the reference, I_s,I_r and A_s,A_r are the fluorescence intensities and the absorbances of the sample and the reference respectively and n_r,n_s are refractive indices of the solvents for the reference and the sample. The phosphorescence-to-fluorescence quantum yield ratios were estimated by the following correlation:

$$\frac{\Phi_p}{\Phi_f} = \frac{\text{area of phosphorescence}}{\text{area of fluorescence}}$$

During the quantum yield determinations, the absorbance at the excitation wavelength was kept as low as possible, usually below 0.3, in order to minimize errors due to the front surface imprisonment and inner-filter effects. The Aminco–Bowman instrument was employed to estimate the temperature dependence of the fluorescence: the temperature of the sample cell was continuously varied from 25 °C to about −150 °C by flowing cold nitrogen gas through it and maintained to within ±2 °C for recording the spectra. The temperature of the sample solution was measured with a copper–constantan thermocouple. Fluorescence lifetimes were measured by the single-pulse third harmonic (355 nm) from a mode-locked picosecond Nd:YAG laser. The fluorescence was monitored with a photomultiplier (Hamamatsu R1328U) connected to a Tektronix 7104 equipped with a 7A29 plug-in unit. A detailed description of the instrument for the lifetime measurements is given elsewhere [20].

3. Results and discussion

Table 1 shows the absorption and fluorescence maxima and fluorescence quantum yields of the four compounds in various solvents at 25 °C. The absorption and fluorescence maxima are blue shifted as the polarity of the solvent decreases. The fluorescence quantum yields also decrease with decreasing solvent polarity and are very small in the aprotic solvent ethyl ether, indicating that the proton-donating ability of the solvent significantly affects the dynamics of the excited state. The very low fluorescence quantum yield in aprotic non-polar solvents is expected since the internal conversion process is enhanced because of the proximity of the lowest energy n,π^* and π,π^* singlet states. These phenomena are general for nitrogen heteroaromatics and aromatic carbonyl compounds that possess a lowest energy n,π^* singlet state which is close to the lowest energy π,π^* state [21, 22]. As the energy gap between two lowest singlet states becomes smaller, the rate of

TABLE 1

Absorption maxima λ^{A}_{max}, fluorescence maxima λ^{F}_{max} and fluorescence quantum yields Φ_f of DMC, DHP, F-2 and 8-MOP in various solvents at room temperature

Solvent	λ^{A}_{max} (nm)				λ^{F}_{max} (nm)				Φ_f			
	DMC	*DHP*	*F-2*	*8-MOP*	*DMC*	*DHP*	*F-2*	*8-MOP*	*DMC*	*DHP*	*F-2*	*8-MOP*
H_2O	327	336	329	303	450	410	465	505	0.76	0.83	0.044	0.0028
MeOH	324	333	328	300	432	405	446	465	0.71	0.30	0.007	0.0022
EtOH	324	333	327	299	425	402	432	460	0.65[a]	0.23	0.005	0.0020[b]
i-PrOH	324	333	327	298	417	400	428	460	0.56	0.23	0.003	0.0012
Et_2O	318	330	325	294	390	397	410	—[c]	0.07[d]	0.06	0.001	—[c]

[a]This value was used as the reference value for the Φ_f of the other compounds in ethanol, and the value in ethanol for each compound was used as the reference for the measurement of the Φ_f in the other solvents.

[b]Reported as 0.0013 in ref. 9.

[c]At the concentration used for this experiment the fluorescence maximum was not observed.

[d]In 2-methylpentane, the quantum yield of DMC is 0.003.

radiationless decay, especially internal conversion, increases while the fluorescence quantum yield becomes lower, and the temperature dependence of the fluorescence increases. The higher the fluorescence quantum yield, the less sensitive is the fluorescence to a change in temperature. It is suggested that the energy gap between the two lowest π,π^* and n,π^* excited singlet states in ethanol decreases for these compounds in the following order: DMC > DHP ≫ F-2 > 8-MOP. The fluorescence of DMC and DHP is greatly enhanced on decreasing the temperature in 2-methylpentane, in contrast to the behaviour in ethanol (see Figs. 2 and 3); this is expected from the dramatic decrease in the fluorescence quantum yield in 2-methylpentane relative to that in ethanol. The solvent and temperature dependence of the fluorescence of 8-MOP is also consistent with the results reported previously [8, 9]. The magnitude of the fluorescence quantum yields decreases in the following order: DMC > DHP ≫ F-2 > 8-MOP. This order is exactly opposite to that of the temperature dependence of the fluorescence in ethanol. On lowering the temperature, the fluorescence maximum of F-2 in ethanol first red shifts slightly with an enhancement of its intensity and then undergoes a large blue shift with a reduction in its intensity (see Fig. 4). The fluorescence spectra in polar hydrogen-bonding solvents are complicated by the appearance of emission from hydrogen-bonded species, which is quite important at low temperatures. The decrease in fluorescence intensity on decreasing the temperature further below −47 °C suggests that the fluorescence quantum yield of hydrogen-bonded species is less than that of non-

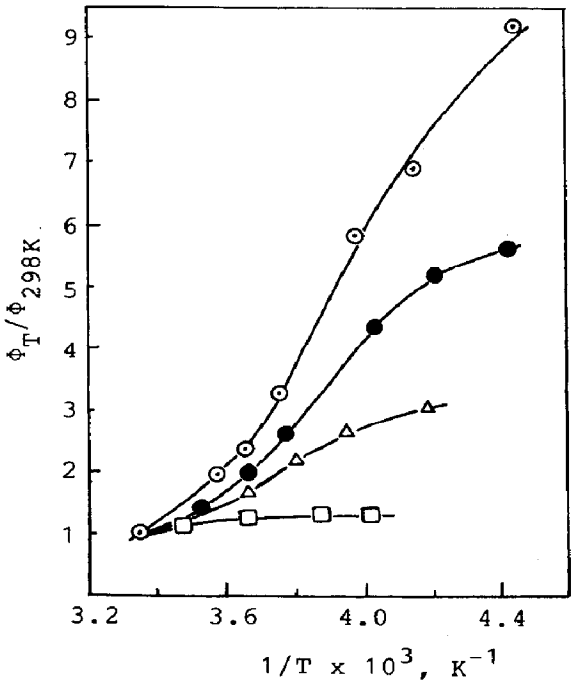
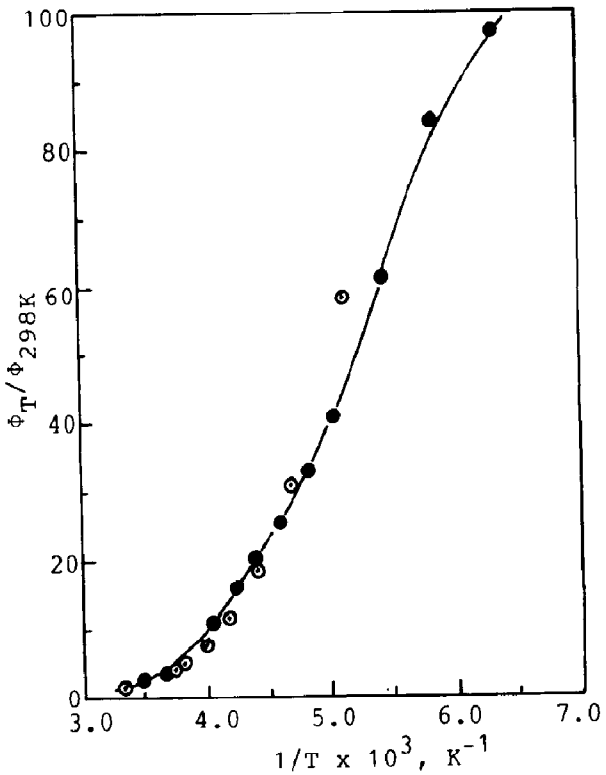


Fig. 2. Temperature dependence of fluorescence in ethanol: 8-MOP (⊙), F-2 (●), DHP (△) and DMC (□).



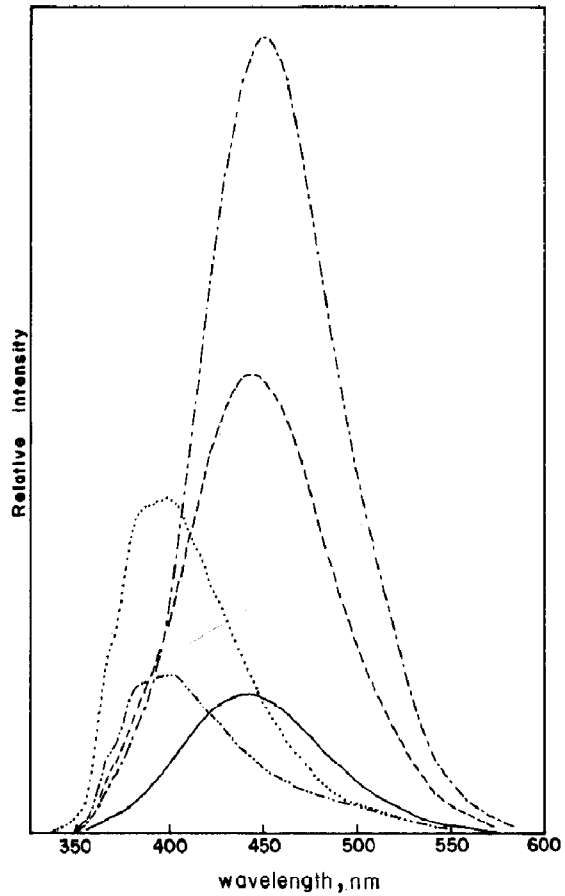


Fig. 3. Temperature dependence of fluorescence in 2-methylpentane: ●, DMC; ⊙, DHP.

Fig. 4. Temperature dependence of fluorescence of F-2 in ethanol: ——, 25 °C; — · —, −47 °C; — — —, −60 °C; ....., −100 °C; — ·· —, −110 °C.

hydrogen-bonded species. In hydrocarbon solvents, the fluorescence maxima of DMC and DHP at low temperatures undergo large red shifts on reducing the temperature further below −90 °C and −120 °C respectively (see Figs. 5 and 6). At these temperatures, aggregation was not observed under the present experimental conditions (the solution was about 4×10^{-6} M). The fluorescence maxima of DMC and DHP at these low temperatures coincide with their fluorescence maxima in isopentane at 77 K.

Table 2 shows some photophysical properties of the four compounds in various solvents at 77 K. The ratios of the phosphorescence quantum yields to the fluorescence quantum yields for DMC and DHP are very small and are not affected significantly by the polarity of the solvent. The ratio for F-2 is much greater than that for DMC and DHP but is much less than that of 8-MOP in both ethanol and ether and significantly decreases as the solvent is changed from ethanol to ethyl ether. If the intersystem crossing quantum yield of F-2 in ether–ethanol solution is similar to that in ethanol, the decrease in the ratio is due to an increase in the rate of $T_1 \rightsquigarrow S_0$ intersystem crossing with decreasing solvent polarity, as in the case of the solvent

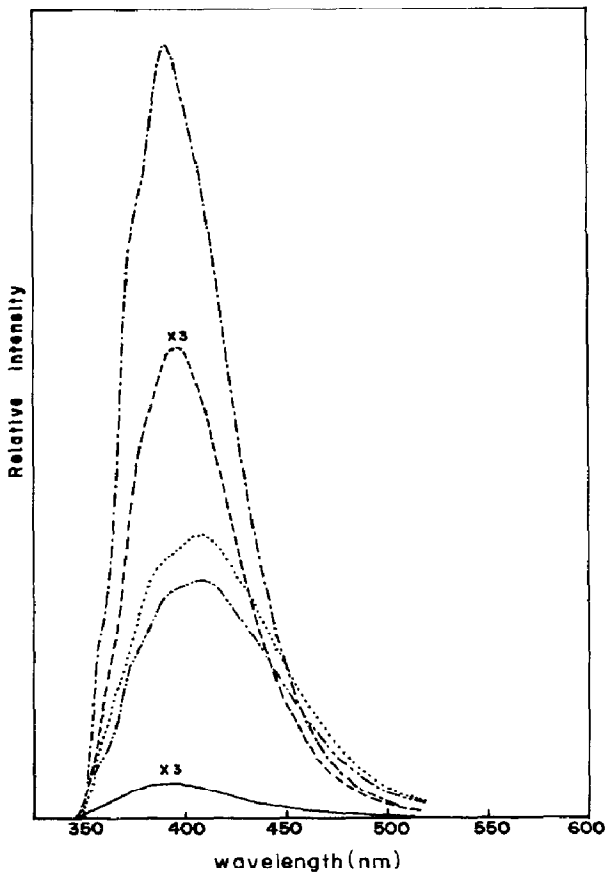


Fig. 5. Temperature dependence of fluorescence of DHP in 2-methylpentane: ——, 25 °C; — — —, −33 °C; — · —, −77 °C; — ·· —, −92 °C; ·····, −120 °C.

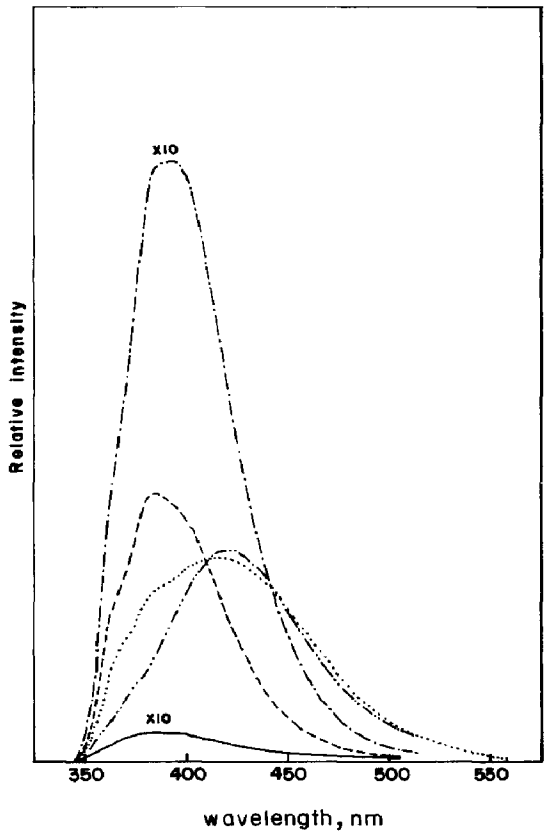


Fig. 6. Temperature dependence of fluorescence of DMC in 2-methylpentane: ——, 25 °C; — · —, −45 °C; — — —, −102 °C; ·····, −127 °C; — ·· —, −132 °C.

dependence of the fluorescence. The ratio of the phosphorescence quantum yields to the fluorescence quantum yields for the four compounds in the external heavy-atom solvent is about four to ten times greater than that in ethanol, indicating that the external heavy atom greatly enhances the rate of intersystem crossing of the compounds. The phosphorescence lifetimes are very long and are similar for DMC, DHP and 8-MOP. The phosphorescence lifetime of F-2 is greater than that of the other compounds, and is quite similar to that of psoralens having $-OCH_3$ or $-OH$ groups at the 5- or 7-positions. The lifetime is also decreased substantially by the external heavy atom. The polarization of the phosphorescence 0–0 band of F-2 (−0.06) is slightly negative polarized, while it is highly negative polarized for DMC (−0.21) and slightly positive polarized for 8-MOP (0.06).

In contrast to the shift of the fluorescence maximum on varying the polarity of the solvent in the solution, the fluorescence of DMC, DHP and 8-MOP is greatly red shifted and becomes structureless as the solvent changes from ethanol to isopentane (see Fig. 7 and Table 2). The phosphorescence of the compounds in the hydrocarbon solvent was not observable within our instrumental sensitivity. A similar abnormal spectral shift of the fluorescence and dramatic decrease in phosphorescence in a hydrocarbon solvent was

TABLE 2

Fluorescence maxima λ^{F}_{max}, phosphorescence 0–0 bands λ^{P}_{0-0}, phosphorescence-to-fluorescence quantum yield ratios Φ_p/Φ_f, polarizations P_{0-0} of the phosphorescence 0–0 bands and phosphorescence lifetimes τ_p in various solvents at 77 K

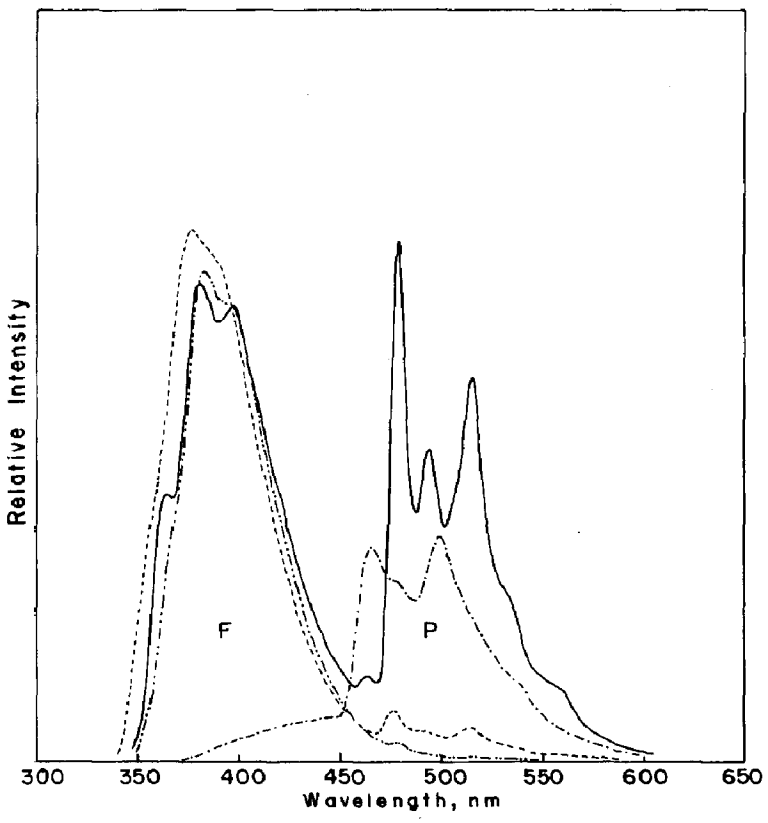
Solvent	λ^{F}_{max} (nm)	λ^{P}_{0-0} (nm)	Φ_p/Φ_f	τ_p (s)	Φ_{isc}[a]	P_{0-0}
DMC						
EtOH	375	476	0.0056	0.88	0.072[b]	−0.21
Et_2O	372	476	0.048	0.87		
Isopentane	428	—	—	—		
EEM[c]	377	476	0.50	0.50		
DHP						
EtOH	382	479	<0.01	0.88	0.068[b]	
Et_2O	376	477	0.014	0.81	0.068[d]	
Isopentane	425	—	—	—		
EEM[c]	382	479	0.17	0.42		
8-MOP						
EtOH	440	466	5.6	0.76	0.04[e]	0.06
Et_2O	440	463	8.5	0.80		
Isopentane	475	—	—	—		
EEM[c]	440	465	23.5	0.31		
F-2						
EtOH	380	478	0.68	1.2	0.076[f]	−0.06
EtOH–Et_2O[g]	378	475	0.26			
EtOH–EtI[h]	380	478	4.08			

[a] At room temperature.
[b] From ref. 23.
[c] Ethanol:ethyl iodide:methanol, 16:5:4 by volume.
[d] From ref. 13.
[e] From ref. 24.
[f] Value for psoralen–thymine 4′,5′-monoadduct in water from ref. 25.
[g] 2:5 by volume.
[h] 2:1 by volume.

observed for coumarin [26]; the observed red shift of the emission bands of coumarin in isopentane was accounted for in terms of the change in the permanent dipole moment of the excited singlet and triplet states relative to its ground state [25]. The same rationale may be applied to the abnormal spectral shift of the three compounds in isopentane.

Based on the spectroscopic data at room temperature and 77 K, the reactivity of the excited singlet state of the four compounds decreases in the following order: DMC > DHP ≫ F-2 > 8-MOP.

The fluorescence quenching of these four compounds by TME was determined to provide a model for the interaction between psoralens in the excited singlet state and DNA bases, especially thymine, which leads to cycloaddition. The results are shown in Table 3. The Stern–Volmer quenching constants $k_q\tau_1$ decrease in the order DMC > DHP > F-2 > 8-MOP, which is



Fluorescence quenching of DMC, DHP, F-2 and 8-MOP by tetramethylethylene in ethanol at room temperature

TABLE 3

Fluorescence quenching of DMC, DHP, F-2 and 8-MOP by tetramethylethylene in ethanol at room temperature

Compound	$k_q\tau$ (M^{-1} s^{-1})	$k_q \times 10^{-8}$ (s^{-1})	τ (ns)	
			EtOH	H_2O
DMC	5.10	7.1	7.2[a]	7.22 0.02
DHP	1.41	4.8[b]	—	2.94 0.01
F-2	0.36	5.8	0.62	1.81 0.01
8-MOP	0.24	6.2	0.39[c]	1.28 0.004[d]

[a] From ref. 10.
[b] Calculated using the lifetime in water.
[c] From ref. 9.
[d] This is considerably smaller than the value reported in ref. 27 (1.9 ns).

exactly the same order observed for the discussed photophysical properties. This result is consistent with the differences between the fluorescence lifetimes. Since the reaction of an excited state is limited by its lifetime, if the excited state has a long lifetime the reaction is more probable. DMC pos-

sesses a considerably longer excited singlet lifetime (7.2 ns) than the other compounds. Psoralens are more likely to undergo reactions with the substrate through the triplet state in solution because the triplet lifetime is generally much longer than the singlet lifetime. When psoralens are intercalated into DNA, however, the restrictions imposed by the short lifetime of the excited state can be overcome because the diffusional prerequisite for the photoreaction of psoralen with DNA bases is relaxed. Thus the singlet reaction must be a predominant path if psoralen is intercalated into DNA. This is strongly supported by the sharp decrease in the fluorescence intensity of psoralens when they are intercalated into DNA. The interior of the DNA helix is less polar than water [28] and the triplet formation quantum yields of psoralens generally decrease on lowering the solvent polarity [9, 29]. These observations suggest that psoralens are unlikely to undergo photoreactions through the triplet state when they are intercalated into DNA.

From these results it is suggested that both the second-stage photocycloaddition of 4′,5′-monoadduct and DNA bases yielding interstrand cross-links and the photocycloaddition at the 4′,5′-double bond forming a 4′,5′-monoadduct proceed via a singlet excited state.

Acknowledgments

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