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Solvent Effect on the Emission Spectra and the Fluorescence Quantum Yields of Psoralens

Hitoshi Matsumoto*,[a] and Akihiko Isobe[b]

School of Pharmacy, Tokushima University of Arts and Science,[a] Yamashiro-cho,
Tokushima, 770, Japan and Laboratory of Biology, Gumma Prefectural
Women's College,[b] 2-19-16, Bunkyo-cho, Maebashi,
Gumma, 371, Japan

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The emission spectra and the fluorescence quantum yields of psoralens were measured in various solvents. We found that these compounds show a large Stokes shift, which can be explained in terms of intermolecular charge transfer interaction between a solute molecule and a solvent molecule in an excited singlet state. Psoralens have a larger fluorescence quantum yield in hydroxylic solvents than in non-hydroxylic solvents. These were in the following order: TMP$\approx$5-MOP$>$PS$\approx$8-MOP$\approx$8-iso-AOP. We postulate that the fluorescence quantum yield is increased by the hydrogen bonding between a solute molecule and a solvent molecule in an excited singlet state. In addition, the fluorescence efficiency was enhanced by the presence of an electron-donating substituent group. The peak wavelengths of the phosphorescence spectra were little affected by the different solvents.

Keywords——psoralens; photosensitizing agent; fluorescence spectra; phosphorescence spectra; solvent effect; Stokes shift; fluorescence quantum yield

8-Methoxypsoralen (8-MOP) and 4,5′8-trimethylpsoralen (TMP) have been used for a long time to treat vitiligo,[1] and more recently for the photochemotherapy of psoriasis.[2] However, it has been reported that psoralens induce skin erythema and skin cancer in mice and guinea pigs,[3] and have other adverse biological effects.[4] It is believed that the skin-sensitizing potency is correlated with their photoreactivity to pyrimidine bases of DNA *via* cycloaddition,[1,5] and their electronic spectra have been investigated in order to elucidate the mechanism of the photocycloaddition.

The absorption spectra of psoralens were studied in detail by several investigators.[6] On the other hand, Song *et al.*[7] reported on the fluorescence and phosphorescence spectra of these compounds in EtOH solution at 77 K, and Sasaki *et al.*[8] on the fluorescence spectra of 8-MOP in various solvents at room temperature. However, no detailed work on solvent effects on the emission spectra of psoralens has been reported, except for 8-MOP. In this paper, the fluorescence and phosphorescence spectra of psoralen (PS), TMP, 5-methoxypsoralen (5-MOP), 8-MOP, and 8-iso-amylenoxypsoralen (8-iso-AOP) in various solvents are reported.

Experimental

Materials——PS, 5-MOP, and 8-MOP were the same as those used in a previous paper.[6b] TMP and 8-iso-AOP were generously supplied by Santen Seiyaku and Nippon Shyoji Co., Ltd., respectively. The former was purified by recrystallization from EtOH, mp 233—234° (lit. 234.5—235°),[9] and the latter from ether, mp 102° (lit. 102°).[10] Commercial quinine sulfate was recrystallized from distilled and deionized water before use, mp 205°.

Solvents——MeOH was purified according to the method given in the literature.[11] Other solvents used (methylcyclohexane, cyclohexane, dioxane, benzene, chloroform, *n*-BuOH, acetone, and acetonitrile) were commercial products of spectrograde.

Measurements——The absorption spectra were measured with a Hitachi 200-20 spectrophotometer. The fluorescence spectra were measured with a Shimadzu RF-502 recording spectrofluorophotometer which was automatically corrected with rhodamine B. The fluorescence quantum yields (ϕ_F) were determined according to the method of Parker and Rees,[12] and quinine sulfate in 1 N H_2SO_4 was used as the standard reference.[13] The phosphorescence spectra were measured with a Hitachi MPF-2A fluorescence spectrophotometer with a phosphorescence attachment. The recorded phosphorescence spectra were not corrected for the response characteristic of the instrument. The concentration of psoralens ranged from 10^{-4}—10^{-5} M for the fluorescence spectra and 10^{-3}—10^{-4} M for the phosphorescence spectra. The solutions were excited at 340 nm. All solvents were checked for emission deriving from impurities.

Results and Discussion

Fluorescence Spectra

Fig. 1 shows the fluorescence spectra of PS, TMP, 5-MOP, 8-MOP, and 8-iso-AOP in cyclohexane, MeOH, and acetonitrile solutions at room temperature (besides the ones shown in the figure, the fluorescence spectra in dioxane, benzene, chloroform, *n*-BuOH, and acetone were also measured). The fluorescence maxima (λ^f_{max}) in various solvents are summarized in Table I along with the absorption maxima (λ_{max}) of the longest wavelength bands. It can be seen that the fluorescence spectra were very broad in any solvent and the λ^f_{max} were located at around the 410—510 nm region. The positions of λ^f_{max} were considerable shifted towards the longer wavelength region when the solvent was changed from non-polar to polar and when an alkoxy group was introduced into PS. Song *et al.*[7] reported that the λ^f_{max} values of PS, TMP, 5-MOP, and 8-MOP in EtOH at 77 K were 409, 416, 427, and 440 nm, respectively. These values are located at the shorter wavelength region by 36—73 nm as compared with those in MeOH or *n*-BuOH at room temperature. Thus, the positions of λ^f_{max} were very sensitive to the nature of the solvent, the substituent group, and the temperature. On the other hand, positions of λ_{max} showed little change with different solvents and substituent groups.

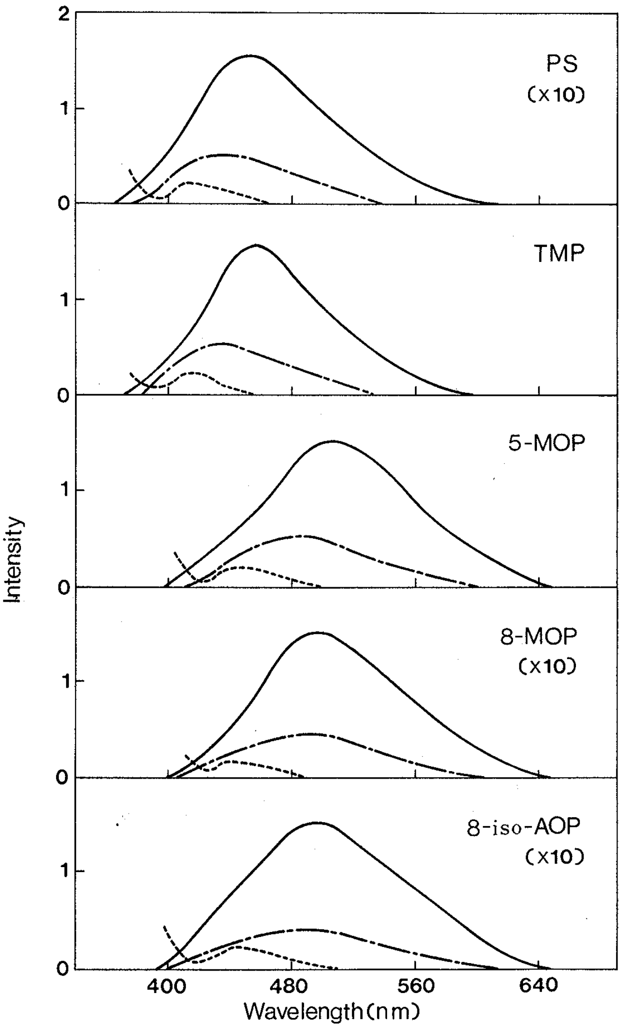

Fig. 1. Fluorescence Spectra of Psoralens in Various Solvents at Room Temperature

——: MeOH, —·—: acetonitrile, ------: cyclohexane.

Stokes Shift

Table II shows the Stokes shifts

TABLE I. $\lambda_{\max}$ of Longest Absorption Bands and $\lambda^{f}_{\max}$ for Psoralens in Various Solvents at Room Temperature

Solvent	PS		TMP		5-MOP		8-MOP		8-iso-AOP	
	$\lambda_{\max}$ (nm)	$\lambda^{f}_{\max}$ (nm)	$\lambda_{\max}$ (nm)	$\lambda^{f}_{\max}$ (nm)	$\lambda_{\max}$ (nm)	$\lambda^{f}_{\max}$ (nm)	$\lambda_{\max}$ (nm)	$\lambda^{f}_{\max}$ (nm)	$\lambda_{\max}$ (nm)	$\lambda^{f}_{\max}$ (nm)
Cyclohexane	332[a]	410	338	410	335[b]	430	340[a]	440	340	440
Dioxane	331[a]	420	336	420		460	340[a]	470	340	470
Benzene	331[a]	418	336	416		450	340[a]	460	340	460
Chloroform	332	420	338	420		442	340	442	340	442
n-Butanol	329	440	335	442		485		488		488
Acetone		426	335	426		452		452		452
Methanol	326[a]	445	334	452		500		498		498
Acetonitrile	327[a]	436	335	436		480		482		482

a) Reference 6 *b*.
b) Value obtained by MO calculation (reference 6 *b*).

($\Delta\lambda=\lambda^{f}_{\max}-\lambda_{\max}$ or $\Delta\nu=\nu_{\max}$ (the wave numbers of the absorption maxima) $-\nu^{f}_{\max}$ (the wave numbers of the fluorescence maxima)) for psoralens in various solvents, along with the dielectric constants and Z–values[14] of the solvents. In the calculations for 8-MOP and 8-iso-AOP, the values (340 nm and 24912 cm^{-1}) in non-polar solvents were used as $\lambda_{\max}$ and $\nu_{\max}$ because they were not clear in polar solvents, and for 5-MOP the values (335 nm and 29851 cm^{-1}) estimated from MO calculation were used because they were not clear in any solvent. As may be seen in Table II, the Stokes shifts were much larger than those generally observed. They were remarkable for alkoxy substituted psoralens in MeOH solution.

TABLE II. Stokes Shifts for Psoralens in Various Solvents

Solvent	DC[a]	Z-Value[b]	$\Delta\lambda$, nm ($\Delta\nu$, cm^{-1})				
			PS	TMP	5-MOP	8-MOP	8-iso-AOP
Cyclohexane	2.023		78(5730)	72(5196)	95(6595)	100(6685)	100(6685)
Dioxane	2.209		89(6401)	84(5952)	125(8112)	130(8135)	130(8135)
Benzene	2.284		87(6288)	80(5724)	115(7629)	120(7673)	120(7673)
Chloroform	4.806	63.2	88(6310)	82(5776)	107(7227)	102(6788)	102(6788)
n-Butanol	15.8	77.7	111(7668)	107(7227)	150(9232)	148(8920)	148(8920)
Acetone	20.70	65.7		91(6377)	117(7727)	112(7288)	112(7288)
Methanol	32.63	83.6	119(8203)	118(7816)	165(9851)	158(9332)	158(9332)
Acetonitrile	37.5	71.3	109(7645)	101(6915)	145(9018)	142(8665)	142(8665)

a) Dielectric constants of the solvents at 20 or 25°.
b) Reference 14.

Such large Stokes shifts have also been found for indoles and proteins,[15] and various mechanisms have been proposed: dipole-dipole interaction,[15c] formation of an exciplex of charge transfer type between a solute molecule and a solvent molecule,[15d,j] hydrogen bonding,[15g] and solvent-lattice relaxation.[15k] In addition, it was reported by Nagakura *et al.*[16] that the intermolecular charge transfer complexes between tetracyanobenzene and benzenes show large Stokes shifts.

In the case of psoralens, the Stokes shifts were large in cyclohexane solution, which has a very small dielectric constant, and there was no correlation between the shifts and the dielectric constants of the solvents. Although psoralens can form hydrogen bonds to hydroxylic solvents at the carbonyl (2-position) and ethereal (1- and 1′-positions) oxygens, for 8-MOP and 8-iso-AOP the hydrogen bonding at the ethereal oxygens is sterically hindered. However, the

Stokes shifts were similar in magnitude to those of 5-MOP, in which the hydrogen bonding is not hindered. On the other hand, there was a parallel relationship between the Stokes shifts and the Z-values (Table II). Song *et al.*17) reported that PS has charge transfer character in an excited singlet state (but not in an excited triplet state). Judging from the above experimental results and discussion, we consider that intermolecular charge transfer in an excited singlet state may be a major cause of the large Stokes shifts for these compounds.

Fluorescence Quantum Yield

The values of ϕ_F of psoralens were very small in any solvent at room temperature (Table III). However, these values were larger in the hydroxylic solvents than in the non-hydroxylic solvents. Thus, the fluorescence was more efficient when hydrogen bonding occurred between the solute and the solvent.

In EtOH at 77 K, the lowest excited singlet and triplet states of psoralens which do not have strong charge transfer substituents were assigned as both (π, π^*) states and it was suggested that an $^3(n, \pi^*)$ state is located slightly below a $^1(\pi, \pi^*)$ state.7) Sasaki *et al.*8) reported that, at room temperature, the λ^f_{max} of 8-MOP markedly shifted to the longer wavelength region with increasing polarity of the solvent, and its relative fluorescence quantum yield was increased. In general, a (π, π^*) level becomes lower and an (n, π^*) level higher with increasing polarity of the solvent, and they explained the enhancement of fluorescence efficiency in terms of the inversion of the relative positions of the $^1(\pi, \pi^*)$ and $^3(n, \pi^*)$ states: the $^3(n, \pi^*)$ state becomes higher than the $^1(\pi, \pi^*)$ state in polar solvents at room temperature.

TABLE III. Fluorescence Quantum Yields (ϕ_F) for Psoralens in Various Solvents at Room Temperature

Solvent	$\phi_F(\times 10^{-2})$				
	PS	TMP	5-MOP	8-MOP	8-iso-AOP
Cyclohexane	0	0	0.02	0	0
Dioxane	0	0.02	0.03	0	0
Benzene	0	0.02	0.04	0	0
Chloroform	0.01	0.27	0.15	0.01	0.01
n-Butanol	0.17	1.30	1.51	0.13	0.09
Acetone	0.02	0.15	0.20	0.02	0.03
Methanol	0.16	1.37	1.60	0.12	0.12
Acetonitrile	0.05	0.51	0.58	0.02	0.02

As may be seen in Fig. 1 and Table I, our results on the λ^f_{max} and ϕ_F of 8-MOP and other psoralens in hydroxylic solvents are very similar to the observations of Sasaki *et al.* This suggests that the relative positions of the $^1(\pi, \pi^*)$ and $^3(n, \pi^*)$ states of psoralens, except 8-MOP, are also reversed by hydrogen bonding between the solute and the solvent. If such an inversion occurs in hydroxylic solvents, the process of intersystem crossing changes from $^1(\pi, \pi^*) \rightarrow {}^3(n, \pi^*)$ (in the non-hydroxylic solvents) to $^1(\pi, \pi^*) \rightarrow {}^3(\pi, \pi^*)$ (in the hydroxylic solvents), with an increase in fluorescence.

Menger *et al.*18) suggested that the basicity of 8-MOP in the excited singlet state is stronger than that in the ground state. Other psoralens used will also have a similar character because their chemical structures are analogous to that of 8-MOP. If this postulate is accepted, the hydrogen bonding ability of these psoralens is expected to be larger in the excited singlet state than in the ground state. On the other hand, as already stated, charge transfer was also involved in the interaction between psoralens and the solvent. However, it is known that fluorescence is quenched by a charge transfer interaction.19) Therefore, the hydrogen bonding in the excited singlet state may play the main role in the enhancement of ϕ_F.

Clearly the ϕ_F values of TMP and 5-MOP in the hydroxylic solvents were about 10 times that of PS (Table III). This may be explained as being due to the increase of the hydrogen bonding ability because of the presence of electron donating groups such as a methyl or methoxy group. However, the ϕ_F values of 8-MOP and 8-iso-AOP, each substituted with an alkoxy group, were similar to that of PS. This is thought to be due to the steric hindrance to hydrogen bonding between the ethereal oxygen and the solvent.

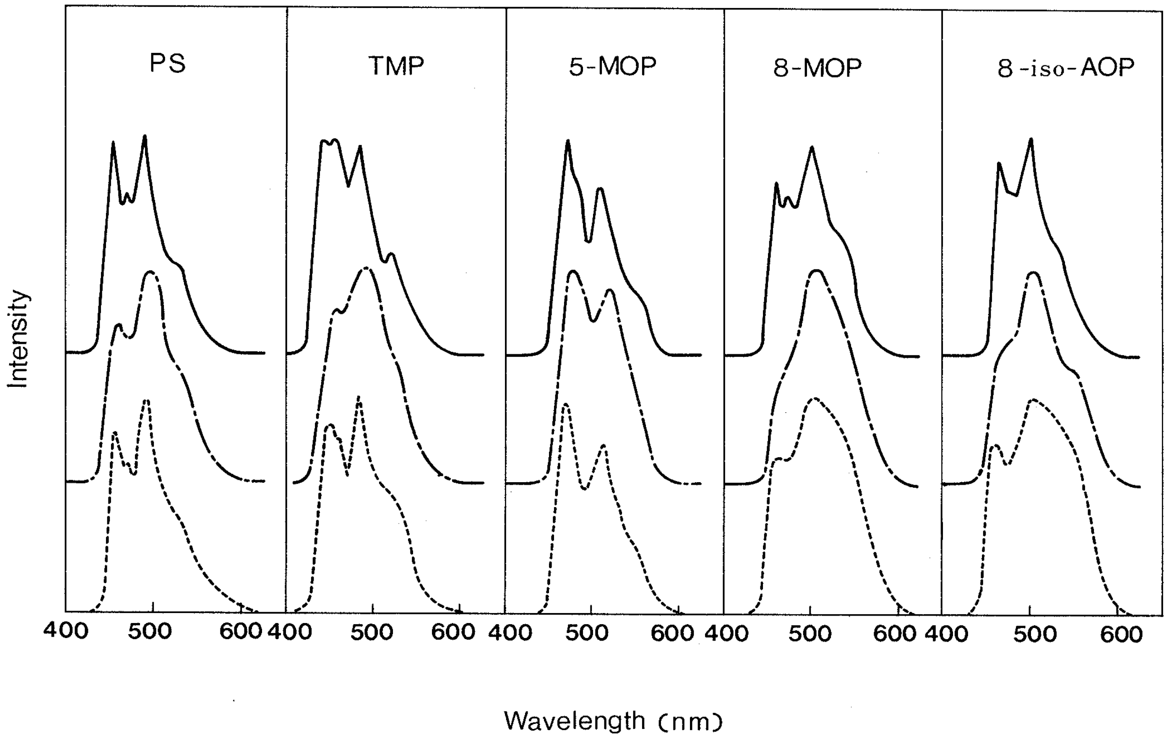

Fig. 2. Phosphorescence Spectra of Psoralens in Various Solvents at 77 K

——: MeOH, —·—: dioxane, ------: methylcyclohexane.

Table IV. Peak Wavelengths (nm) for the Phosphorescence Spectra of Psoralens in Various Solvents at 77 K

Compound	Methylcyclohexane	Dioxane	Benzene	Methanol
PS	460	462	460	458
	470			470
	494	494	492	490
	530$^{s)}$	530$^{s)}$	528$^{s)}$	526
TMP	452			448
	462	460	460	456
	488	488	490	478
	524	526$^{s)}$	520$^{s)}$	518
5-MOP	475	480	480	480
	513	512	512	512
	550$^{s)}$	550$^{s)}$		550$^{s)}$
8-MOP	465	470$^{s)}$	465$^{s)}$	460
				472
	500	500	512	500
				532$^{s)}$
8-iso-AOP	460	465$^{s)}$	460	460
	496	500	500	494
				530$^{s)}$

s: shoulder.

Phosphorescence Spectra

Fig. 2 shows the phosphorescence spectra of psoralens in methylcyclohexane, dioxane, and MeOH solutions at 77 K (the spectra in benzene solution are not shown). Their peak wavelengths are summarized in Table IV. In MeOH solution, the phosphorecence spectra of these compounds exhibit relatively sharp vibrational structures. However, very broad phosphorescence spectra appeared for dioxane and benzene solutions. This may be due to the charge transfer interaction between psoralens and the solvents.[20] In methylcyclohexane solution, the phosphorescence spectra of 8-MOP and 8-iso-AOP were very broad. As may be seen in Table IV, the positions of their peak wavelengths were little affeced by the different solvents. This result may be explained in terms of localization of the excitation energy at the 3,4-double bond of the pyrone moiety in the excited triplet state.[7] On the other hand, the positions of the peak wavelengths of 5-MOP were situated at a somewhat longer wavelength region than those of the other compounds.

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