Photophysical Properties of Khellin

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The fluorescence quantum yield of khellin is sensitive to temperature and to the nature of solvents, especially the proton-donating ability in solute-to-solvent hydrogen bonding. The intersystem crossing quantum yields are 0.4 and 0.15 in acetonitrile and ethanol, respectively. The fluorescence quantum yields in ethanol and isopentane at 77 K are 0.61 and 0.07, respectively, both of which are much larger than the values at room temperature. The phosphorescence lifetime is relatively long and decreases with decreasing solvent polarity. The phosphorescence to fluorescence quantum yield ratio is very small and remains unchanged in various solvents. The results suggest that internal conversion is an important decay channel of the excited singlet state of khellin, especially in the hydrogen-bonding hydroxyl solvents.

Introduction

The photosensitizing ability of furocoumarins has been generally correlated with their photoreactivity towards pyrimidine bases of DNA.[1,2] The biological effects of psoralen plus light treatment appear to be mediated primarily by the formation of cyclobutane adducts that arise from the [2+2] cycloaddition of the psoralen 3,4-and 4′,5′-double bonds with 5,6-double bond of pyrimidine bases of DNA.[1-4] The furochromones khellin (I) and visnagin (II), two photobiologically active compounds isolated from *Ammi visnaga*,[5] closely resemble psoralen in structure and khellin has been used to sensitize λ-phages by light of 360 nm.[6] Recent observation[7,8] indicate that oral administration of khellin and subsequent exposure to sunlight or long wave-length UV(UVA) light induces repigmentation in vitiligo. Compared with the usual psoralen photochemotherapy recommended for vitiligo, khellin and UVA treatment appears to be equally effective and has the major advantage that khellin neither produces substantial side effects nor phototoxic erythema reactions. Although the mechanism of action of khellin in vitiligo treatment has not been clearly established, a [2+2]-photocycloadduct between khellin and thymine was isolated[9] and the *cis-syn* stereochemistry similar to the photoadducts between psoralen and pyrimidine bases has been determined. Although the photophysical properties of the lowest excited states (singlet and triplet) of furocoumarins and coumarins have been extensively investigated,[10,11] photophysical properties of the furochromones have not been reported. In this study, we report the photophysical properties of khellin, a furochromone.

Materials and Methods

Meterials. Khellin was purchased from Sigma Chemical Company and recrysrallized from methanol. Quinine sulfate (Aldrich Chemical Co.) was purified by recrystallization from water and 9, 10-diphenylanthracene (Aldrich) was recrystallized from ethanol. The solvents (Merck) methanol, ethanol, acetonitrile, diethyl ether, and isopentane were of spectroquality. 2-Methylpentane (Aldrich) and other solvents of extra pure grade were purified according to the literature procedures. Doubly distilled and deionized water was also used for spectroscopic studies.

Methods. Ultraviolet-visible spectra were recorded on a Cary 17 spectrophotometer. Fluorescence and phosphorescence spectra were recorded on an Aminco-Bowman spectrophotometer with an Aminco-XY recorder at room temperature and at 77 K with modification of the cell compartment. A cylindrical chopper having the maximum rotating frequency of 10,000 rpm 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 (lifetime of shutter, ~40ms) to cut off the excitation light, in conjunction with a Tektronix 5115 storage oscilloscope. The polarized excitation and emission spectra were obtained with Glan-Thompson prism polarizers and the polarization degrees were corrected by the Azumi-McGlynn formula.[12] In the measurements of polarized spectra the short-wavelength cut-off filters[13] were employed on the exciting and emitting paths to avoid the inteference of scattered light. The temperature of sample cells was continously varied from 25°C to about -150C by flowing cold nitrogen gas and maintained to within ±2°C for recording the temperature-dependent fluorescence spectra. The temperature of sample solution was measured with a copper-constantan termocouple. Recorded emission spectra were corrected for the response characteristics of the photomultiplier tube (1P21, S-4 spectral response) and monochromator of this instrument as a function of wavelength. Corrected spectra on wavenumber scale subsequently permitted the determination of fluorescence quantum yields and ratios of the phosphorescence to fluorescence quantum yields. The fluorescence quantum yields at room temperature were determined relative to quinine bisulfate (Φ_f=0.55 in 1.0N H_2SO_4 at room temperature) by the following relationship;

$$\Phi_f = \Phi_f^r \times (I_s/I_r) \times (A_r/A_s) \times (n_s/n_r)^2$$

where Φ_f represents fluorescence quantum yields of reference, and I_s and I_r are areas integrated under fluorescence spectra, A_s, A_r absorbances at exciting wavelength, and n_s n_r refractive indice of solvents of sample and reference, respectively. In quantum yield determinations, concentrations of

Table 1. Absorption (λ^A_{max}) and Fluorescence (λ^F_{max}) Maxima, and Fluorescence Quantum Yield of Khellin in Various Solvents at Room Temperature

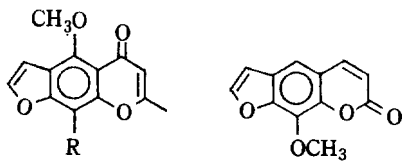
Solvent	λ^A_{max}, nm	λ^F_{max}, nm	Φ_f[a]
H_2O	334	505,470 (sh)	0.0006
H_2O-MeOH[b] 9:1			0.0005
4:1			0.0007
1:1			0.0013
1:4			0.0016
MeOH	331	505	0.0018
EtOH	330	490	0.0036
1-BuOH	328	485	0.0059
CH_3CN	325	457	0.013
CH_2Cl_2	325	450	0.021
Et_2O	320	445	0.0029
n-Hexane	320	440	0.0016

[a] Relative to quining bisulfate (Φ_f=0.55 at room temperature) in 1N H_2SO_4. [b] Ratios by volume.

both sample and reference were adjusted to have similar absorbances of 0.25 at exciting wavelength. Both sample and reference solutions were excited with equal excitation bandwidth (2.5nm) at the same wavelength to eliminate the correction factor for excitation conditions and emission spectra of both solutions were also recorded with equal emission bandwidth (2.5nm). The Aminco-Bowman instrument was also employed for the measurements of low temperature (77 K) fluorescence quantum yields relative to 9, 10-diphenylanthracene (Φ_f=1.0 in ethanol at 77 K). Fluorescence quantum yields at 77 K were also calculated by the same method used at room temperature, assuming that the relative values of the optical densities and refractive indices of solutions at 77 K were the same as at room temperature.[13] The ratios of phosphorescence to fluorescence quantum yields were estimated by the following correlation; Φ_p/Φ_f=(area of phosphorescence)/(area of fluorescence). Since lifetimes of phosphorescence were much longer relative to rotating time of chopper (about 3ms for 180° rotation), decrease of phosphorescence intensity by employment of the chopper could be negligible in this work. Both fluorescence and phosphorescence at 77 K were observed with equal instrumental condition except for employing the chopper for phosphorescence measurements.

Results and Discussion

The absorption and fluorescence spectra of khellin are solvent dependent at room temperature as shown in Table 1. The first absorption and fluorescence mixima of khellin are blue-shifted as the polarity of solvent is decreased from water to n-hexane. While the absorption maximum is gradually blue-shifted as solvent changes from protic to aprotic, the fluorescence maximum is significantly blue-shifted as solvent changes from 1-butanol to acetonitrile. The fluorescence quantum yields of khellin are relatively small in all solvents used and decrease as the ability of solvent to donate a proton in solute-to solvent hydrogen bonding increases in protic solvents, for example, from 1-butanol to water. However, in aprotic solvents the fluorescence yield increases as the dipole moment of solvent increases. The intersystem



I; R=OCH_3
II; R=H
III

Figure 1. Structures of khellin (I), visnagin (II), and 8-methoxypsoralen (III).

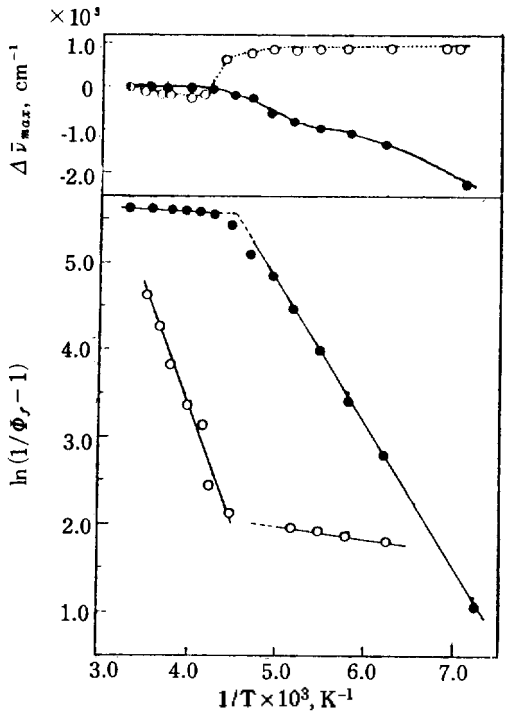


Figure 2. Temperature dependence of fluorescence maximum (upper) and fluorescence quantum yield (lower) of khellin in ethanol (-●-) and 2-methylpentane (-○-). The $\Delta\bar{\nu}_{max}$ is wavenumber of fluorescence maximum at 298 K minus wavenumber of fluorescence maximum at any temperature.

crossing quantum yield (Φ_T=0.4) in aprotic polar solvent, acetonitrile, is much greater than that (Φ_T=0.15) in protic solvent, ethanol and the triplet formation yield in acetonitrile is decreased to about one-fifth by addition of small amount (9%) of water.

This indicates that hydrogen-bonding interaction between solvent and khellin in the excited singlet state enhances the decay rate of radiationless process, $S_1 \rightsquigarrow S_0$ internal conversion. Figure 2 shows the temperature dependence of khellin in ethanol and 2-methylpentane. Since khellin possesses the lowest energy (n, π^*) singlet state which is close in energy to the lowest energy (π,π^*) singlet state, the solvent and temperature dependences of fluorescence have been examined. The closeness of the two states may play an important role in electronic relaxation, and hence in the photochemistry and photobiology of khellin. The fluorescence of khellin is strongly dependent upon temperature. In ethanol, the fluorescence quantum yield of khellin increases slowly first and then rapidly with lowering temperature. If the slow increase of fluorescence at relatively high temperature is attributed to the decrease of S_1-S_0 internal conversion, as expected from

Table 2. Solvent Effect on Fluorescence (λ^{f}_{max}) and Phosphorescence (λ^{p}_{max}) Maxima, Phosphorescence to Fluorescence Quantum Yield Ratio (Φ_p/Φ_f) and Phosphorescence Lifetime (τ_p) of Khellin at ‡‡ K

Solvent	λ^{f}_{max}, nm	λ^{P}_{max}, nm	Φ_p/Φ_f	Φ_f^a	τ_p,s
EtOH	430	485	0.015	0.61	1.4
EPA	430	485	0.017	-	1.3
Et_2	-	-	-	0.19	-
2-Methylpentane	455	513	0.014	-	1.3
Isopentane	467	535	-	0.07	-
EEM[b]	433,475	487,505 (sh)	0.025	-	-

[a] Relative to 9, 10-diphenylanthracene (Φ_f= 1.0 in ethanol at 77 K). [b] Ethyl iodide: ethanol: methanol = 5:16:4 by volume.

large n, π^*-π, π^* energy gap in ethanol, its rapid increase at low temperature may be due to a decrease in intersystem crossing from the lowest excited (π,π^*) singlet state to an (n, π^*) triplet state as in the case of 9-benzoyl-10-cyanoanthracene[14]. As shown in Figure 2, an interesting feature of the steady-state fluorescence of of khellin was observed. The emission maximum blueshifts in ethanol and red-shifts in 2-methylpentane with decreasing temperatue. These shifts of emission spectra coincide with the observation that the emission maxima appear at the much shorter wavelength in ethanol than that in 2-methylpentane at 77 K (see Table 2).

It is assumed that the rate constant of the radiative process, k_f, is independent of temperature and temperature dependent processes are $S_1 \rightsquigarrow S_0$ internal conversion and $S_1 \rightsquigarrow T_n$ intersystem crossing specified by $k_{ic}\exp(-E_1/RT)$ and $k_{isc}\exp(-E_2/RT)$, respectively. Then, in the absence of photochemical changes under the experimental conditions, the quantum yield of fluorescence can be written as

$$\Phi_f = \frac{k_f}{k_f + k_{ic}\exp(-E_1/RT) + k_{isc}\exp(-E_2/RT)}$$

where E_1 and E_2 are the thermal activation energies of internal conversion and intersystem crossing processes, respectively. As expected from the proximity effect[15,16], the process mainly affecting the temperature dependence of fluorescence is internal conversion at relatively high temperature, while it is intersystem crossing at low temperature. Thus, the above equation can be rewritten separately as follows;

$$\Phi_f = \frac{k_f}{k_f + k_{isc} + k_{ic}\exp(-E_1/RT)} \quad \text{(at high temperature)}$$

and

$$\Phi_f = \frac{k_f}{k_f + k_{ic} + k_{isc}\exp(-E_2/RT)} \quad \text{(at low temperature)}$$

Figure 2 shows plots on $\ln[(1/\Phi_f)-1]$ versus $1/T$ for khellin. The calculated values of E_1 and E_2 are 90cal/mol and 3.1 Kcal/mol in ethanol, respectively. In 2-methylpentane E_1 and E_2 are 5.4Kcal/mol and 300cal/mol, respectively. The relative magnitude of the apparent activation energies appears to reflect the relative importance of proximity effect in the excited state dynamics with varying temperature. The error in Φ_f at a smaller value of Φ_f will cause a greater error in E as is obvious from the equation;

$$\frac{d}{d\Phi_f}\ln[(1/\Phi_f)-1] = -\frac{1}{\Phi_f(1-\Phi_f)}$$

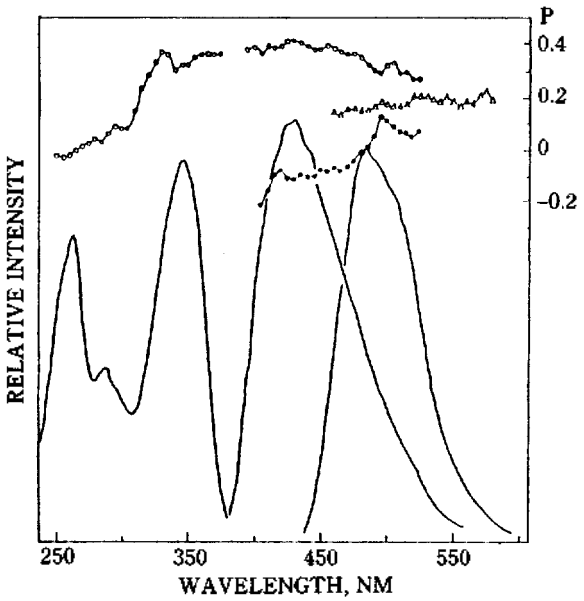


Figure 3. Fluorescence (middle, λ_{ex} = 335 nm), phosphorescence (right, λ_{ex} = 350 nm)emission and fluorescence excitation (left, λ_{em} = 425 nm) spectra of khellin; fluorescence emission (-●-, λ_{ex} = 265 nm; -○-, λ_{ex} = 335 nm) and excitation(-○-, λ_{em} = 425 nm) and phosphorescence (-△-, λ_{ex} = 350 nm) polarization spectra in ethanol at 77 K. P is degree of polarization.

Table 2 shows some photophysical properties of khellin in various solvents at 77 K. The fluorescence maximum of khellin shifts to the red from 430 nm in ethanol to 455 nm in 2-methylpentane, and shifts of the phosphorescence are parallel to those of fluorescence. This spectral shift is in contrast to that in fluid media. The ratio of the phosphorescence to fluorescence quantum yield remains essentially unchanged, when the polarity of solvent decreases from ethanol to 2-methylpentane, while the fluorescence quantum yield decreases dramatically from 0.61 in ethanol to 0.07 in isopentane. The high quantum yield of fluorescence in ethanol can be suspected from the temperature dependence as shown in Figure 2. The ratio of the phosphorescence to fluorescence quantum yield increases from 0.015 in ethanol to 0.025 in EEM (ethyl iodide-ethanol-methanol, 5/16/4 by volume) solution containing a heavy atom. The phosphorescence lifetime is relatively long and slightly decreases with decreasing solvent polarity, suggesting that the lowest triplet state of khellin has the (π,π^*) configuration. Figure 3 represents the emission spectra of khellin with their polarization in ethanol at 77 K. In the fluorescence excitation spectra, the polarization value on the short wavelength side is much lower than that on the longer wavelength side. The fluorescence in ethanol is positively polaized with respect to 335 nm excitation, while negatively polarized with respect to 265 nm excitation. The decrease in the degree of fluorescence polarization with respect to λ_{ex} = 335 nm and the increase with respect to λ_{ex} = 265 nm, respectively, at long wavelength side in ethanol may be ascribed to positively polarized phosphorescence which begins to contribute to some extent in the long wavelength region. The phosphorescence of khellin is highly polarized in ethanol. The positively polarized phosphorescence are often noted for psoralen and its derivatives such as 8-methoxypsoralen, 8-methylpsoralen, and 4,5′,8-trimethylpsoralen,[10] which have the lowest energy (π,

π^*) triplet state.

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References

1. L. Musajo, F. Bordin, G. Caporale, S. Marciani, and G. Rigatti, *Photochem. Photobiol.*, **6**, 711 (1967).
2. L. Musajo, F. Bordin, and R. Bevilacqua, *Photochem. Photobiol.*, **6**, 927 (1967).
3. D. Kanne, K. Straub, H. Rapoport, and J. E. Hearst, *Biochemistry*, **21**, 861 (1982).
4. D. Kanne, K. Straub, J. E. Hearst, and H. Rapoport, *J. Am. Chem. Soc.*, **104**, 6754 (1982).
5. A. Schönberg and A. Sina, *J. Am. Chem. Soc.*, **72**, 1611 (1950).
6. E. Cassuto, N. Gross, L. Bardwell, and P. Howard-Flanders, *Biochem. Biophys. Acta*, **475**, 589 (1977).
7. A. Abdel-Fattah, M. N. Aboul-Enein, G. M. Wassel, and B. S. El-Menshowi, *Dermatologica*, **165**, 136 (1982).
8. B. Ortel, A. Tanew, K. Wolff, and H. Honigsmann, *Photochem. Photobiol.*, **39**, 52s (1984).
9. B. F. Abeysekera, Z. Abramowski, and G. H. N. Towers, *Photochem. Photobiol.*, **38**, 311 (1983).
10. W. W. Mantulin and P. -S. Song, *J. Am. Chem. Soc.*, **95**, 5122 (1973).
11. B. J. Parsons, *Photochem. Photobiol.*, **32**, 813 (1980).
12. T. Azumi and S. P. McGlynn, *J. Chem. Phys.*, **37**, 2413 (1962).
13. C. A. Parker, "Photoluminescence of Solutions," Edited by C. A. Parker, Elsevier, Amsterdam, 1968, pp 128-302.
14. T. -I. Lai and E. C. Lim, *J. Am. Chem. Soc.*, **107**, 1134 (1985).
15. W. A. Wassam, Jr. and E. C. Lim, *J. Chem. Phys.*, **68**, 433 (1978).
16. W. A. Wassam, Jr. and E. C. Lim, *J. Chem. Phys.*, **69**, 2175 (1978).