Photophysical Properties of Khellin-Dimethylfumarate C_4-Cyclomonoadduct

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The fluorescence intensity of khellin-dimethylfumarate C_4-cycloadduct (KDF) is very sensitive to temperature and to the nature of solvents, especially hydrogen-bonding ability. The fluorescence quantum yields of KDF in ethanol and isopentane at 77K are 0.73 and 0.54, respectively, both of which are much larger than the room temperature values. The phosphorescence lifetime is very long and decreases with decreasing the solvent polarity. The phosphorescence and fluorescence quantum yield ratio is very small and decreases with decreasing solvent polarity. The solvent relaxation plays an important role in the excited states of KDF. The internal conversion is a major decay process of the excited singlet state of KDF in all the solvents used at room temperature.

Introduction

The furochromones khellin(I) and visnagin(II), two photobiologically active compounds isolated from *Ammi visnaga*,[1] closely resemble psoralen in structure and khellin has been used to sensitize λ-phages by 360nm light.[2] Recent observations[3,4] indicate that oral administration of khellin and subsequent exposure to sunlight or long wavelength UV(UVA) induces repigmentation in vitiligo. Compared with the usual psoralen photochemotherapy recommended for vitiligo, kehllin 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 is not clear yet, a [2+2]-photocycloadduct between khellin and thymine was isolated[5] and *cis-syn* stereochemistry similar to 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,[6,7] photophysical properties of the furochromone have not been reported yet. If two successive photoreactions, as bifunctional psoralens,[8,9] are to occur in the biological condition, photophysical properties of the monoadduct of khellin and thymine are very important in understanding the second-step photoreaction of khellin and DNA.

In this study, we report the photophysical properties of khellin-dimethylfumarate monoadduct (KDF, III) as a model for the monoadduct of khellin and thymine.

Materials and Methods

Materials. Khellin was purchased from Sigma Chemical Company and recrystallized from methanol. The khellin-dimethylfumarate C_4-monoadduct was prepared according to the following procedure; one gram of khellin and 2.7g of dimethylfumarate (molar ratio = 1:10) were dissolved in 20m*l* of dichloromethane and the solution was deoxygenated by bubbling with nitrogen gas for about 30 min. The deoxygenated solution was irradiated for about 24h in a Rayonet Photochemical Reactor (The Southern New England Ultraviolet Co.) Model RPR-100 equipped with four RUL-350nm fluorescent lamps. Almost all of khellin disappeared. The photocycloadduct formed quantitatively was isolated by flash silica gel column chromatography (Kiesel Gel G,230-240 mesh, Merck) and purified by recrystallization in methanol. Quinine bisulfate (Aldrich) 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 the other solvents were purified according to literature procedures. Doubly distilled and deionized water was used for spectroscopic study.

Methods. Ultraviolet-visible spectra were recorded on a Cary 17 spectrophotometer and emission spectra were measured on an Aminco-Bowman spectrophotofluorometer with an Aminco-XY recorder at room temperature and at 77K with modification of 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 to cut off the excitation light, in conjunction with a Tektronix 5115 storage oscilloscope. The polarized excitation and emission spectra were obtained with a Glan-Prism polarizer and the polarized spectra were corrected by the Azumi-McGlynn formulation.[10] In the measurements of polarized spectra the short-wavelength cut-off filters[11] were employed on the exciting and emitting paths to avoid the interference of scattered light. The temperature of sample cell was continuously varied from 25°C to about −150°C by flowing cold nitrogen gas and maintained to within ±2°C for recording the temperature dependent fluorescence spectra. The temperature of sample solutions was measured with a copper-constant an 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. The corrected spectra 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 following relationship;

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

where Φ_f^r represents fluorescence quantum yield of reference and I_s,I_r and A_s,A_r are areas integrated under fluorescence

Table 1. Absorption (λ^{A}_{max}) and Fluorescence (λ^{f}_{max}) Maxima, and Fluorescence Quantum Yield of KDF in Various Solvents at Room Temperature

Solvent	λ^{A}_{max}, nm	λ^{f}_{max}, nm	ϕ_f[a]
H_2O	295	490	0.0016
H_2O–MeOH[b] 9:1			0.0017
4:1			0.0020
1:1			0.0031
1:4			0.0038
MeOH	291	485	0.0038
EtOH	291	478	0.0069
CH_3CN	284	432	0.0010
CH_2Cl_2	285	430	0.0016
Et_2O	278,302 (sh)	—[c]	—
n-Hexane	274,301(sh)	—[c]	—
Isopentane	275	442[d]	5.5×10^{-4}[d]

[a] With λ_{ex}=320 nm, relative to quinine bisulfate (ϕ_f=0.55 at room temperature) in 1N H_2SO_4. [b] Ratios by volume. [c] Not observed at 25°C. [d] At 0°C.

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), Fluorescence Quantum Yield (ϕ_f), and Phosphorescence Lifetime (τ_p) of KDF at 77K

Solvent	λ^{f}_{max}, nm	λ^{p}_{max}, nm	ϕ_p/ϕ_f	ϕ_f[a]	τ_p, sec
EtOH	447	462	0.070	0.73	1.9
Et_2O	442	—	—	0.60	—
2-MeP[b]	435	477	0.029	—	1.3
Isopentane	435	475	0.038	0.54	—
EEM[c]	455	468	0.104	—	—

[a] Relative to 9,10-diphenylanthracene (ϕ_f=1.0 in ethanol at 77K). [b] 2-Methylpentane. [c] Ethyl iodide: ethanol: methanol = 5:16:4 by volume.

spectra and absorbances at exciting wavelength of sample and reference, respectively, and n_r, n_s are referactive indice of solvents of reference and sample. In quantum yield determination, absorbance at 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 also employed for measurement of low temperature (77K) fluorescence quantum yields relative to 9,10-diphenylanthracene (ϕ_f=1.0 in ethanol at 77K). Fluorescence quantum yields at 77K were also calculated by the same mehod used for room temperature, assuming that the relative values of the optical densities and refractive indice of all the solutions at 77K were the same as at room temperature.[12] The ratios of the phosphorescence to fluorescence quantum yields were estimated by the following correlation; ϕ_p/ϕ_f = (area of phosphorescence)/(area of fluorescence).

Results and Disscussion

The solvent dependence of the absorption and fluorescence of KDF at room temperature is shown in Table 1. The absorption maximum of KDF is gradually blue-shifted with decreasing the polarity of solvents and a big blue-shift of fluorescence maximum is observed when solvent is changed from ethanol to acetonitrile. The fluorescence quantum yield of KDF in protic solvents is decreased as the hydrogen-bonding ability of solvent increases, for example, from ethanol to water and it is increased in aprotic solvents as the dipole moment of solvent increases. The fluorescence maximum of KDF was significantly red-shifted as the polarity of solvent decreases and the quantum yield of its fluorescence is very small in isopentane ($\phi_f \lesssim 10^{-4}$) at room temperature. The observation suggests that the (n, π^*) and (π, π^*) singlet states are reversed in energy in both dichloromethane and isopentane. The possible presence of the lowest lying (n, π^*) singlet state in aprotic-nonpolar solvent is also suggested by the appearance of a shoulder at about 305nm absent in protic solvents and a long-wavelength side low-intensity tail in the room temperature absorption spectrum. Fluorescence quantum yields are greater in protic than in aprotic solvents. In aprotic nonpolar solvents, fluorescence quantum yield is too weak to determine the emission maxima at room temperature. This may be due to the closeness of the lowest excited singlet (π, π^*) and (n, π^*) states in aprotic nonpolar solvents. It is possible that the closeness of these states in KDF, which is model for the monoadduct of khellin and thymine, also plays an important role in the second-step photoreaction of khellin and DNA.

The solvent and temperature dependence of fluorescence in several nitrogen heterocyclic and aromatic carbonyl compounds which possess a lowest energy (n, π^*) state have been extensively investigated by Lim *et al.* in recent years, both theoretically[13,14] and experimentally.[7,15,16] Since the quantum yields of fluorescence and triplet formation for these compounds decrease sharply with increasing temperature, and the temperature dependence of both processes is quantitatively very similar, the observed temperature effects were therefore attributed to a process other than fluorescence or intersystem crossing, that is, to the thermal enhancement of $S_1 \rightsquigarrow S_0$ internal conversion.

Figure 2 shows the solvent and temperature dependence of fluorescence of KDF. The results are consistent with the dependence of energy gap between the lowest (n, π^*) and (π, π^*) singlet states upon the nature of solvents. The fluorescence of KDF in a hydrocarbon is very sensitive to temperature change and increases sharply with lowering temperature.

The emission spectrum shifts continuously to the blue in 2-methylpentane, while in ethanol the emission maximum shifts first to the blue and then to the red, with decreasing temperature. The fluorescence maximum in ethanol at 131K is consistent with that at 77K, and in 2-methylpentane, the emission maximum at 140K also coincides with that at 77K.

To interpret the observed temprature dependence of fluorescence intensity, it is assumed that the rate constant of the radiative process, k_f, is independent of temperature and the temperature dependent processes are the $S_1 \rightsquigarrow S_0$ 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 change under experimental conditions, the quantum yield of fluorescence can be written as

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

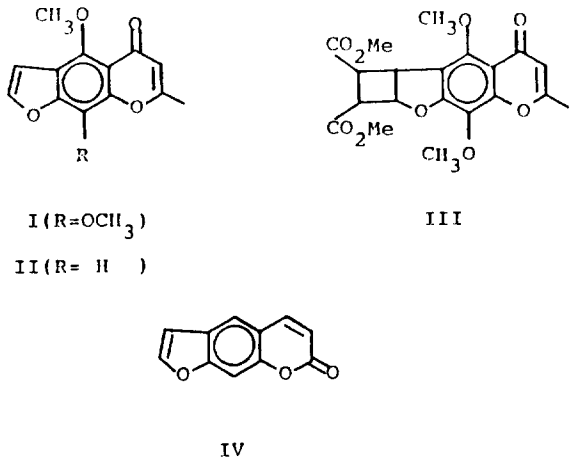

Figure 1. Structure of Khellin(I), Visnagin(II), Khellin-Dimethylfumarate monoadduct(III) and Psoralen(IV).

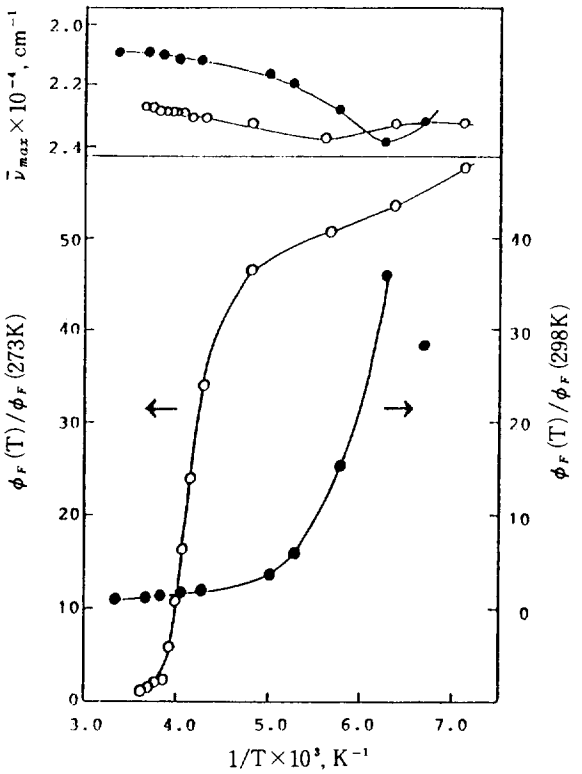

Figure 2. Temperature dependence of fluorescence quantum yield and maximum of KDF in 2-methylpentane(-O-) and ethanol (-●-).

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,[13,14] the process mainly affecting the temperature dependence of fluorescence is internal conversion at relatively high temperature, while 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

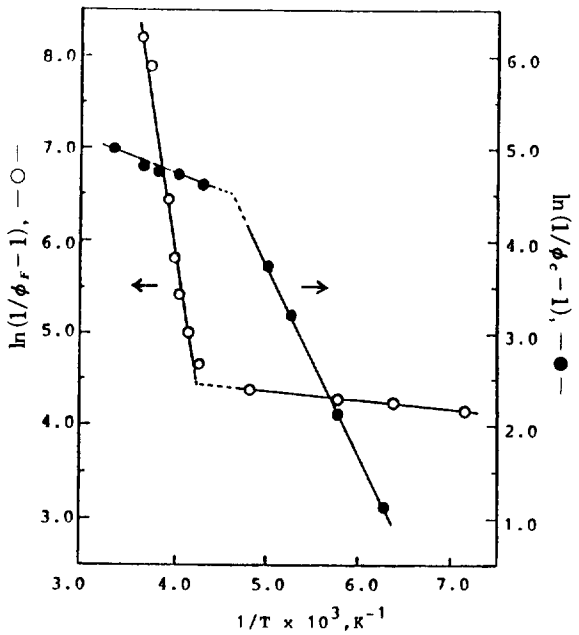

Figure 3. Temperature dependence of fluorescence of KDF in 2-methylpentane (-O-) and ethanol (-●-).

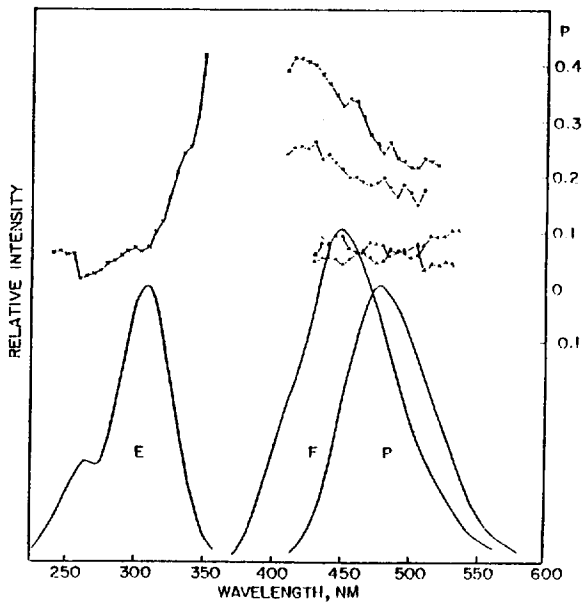

Figure 4. Fluorescence excitation (-×-, λ_{em} =450nm), emission (-×-, λ_{ex}=350nm; -●-, λ_{ex}=320nm) polarization and phosphorescence (-△-, λ_{ex}=350nm; -▲-, λ_{ex}=320nm) polarization of KDF in ethanol at 77K.

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

Figure 3 shows plots of ln [1/Φ_f)-1] versus 1/T for KDF. The E_1 and E_2 values obtained for KDF are 3.3 kJ/mol and 16.7 kJ/mol in ethanol and 55.2 kJ/mol and 836 J/mol in 2-methylpentane. The relative magnitude of the appearent activation energies appears to reflect their relative importance

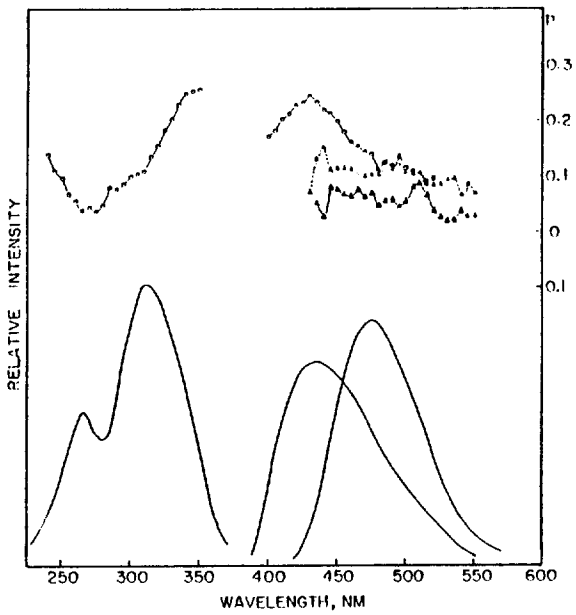

Figure 5. Fluorescence excitation (-O-, λ_{em}=460nm), emission (-O-, λ_{ex}=320nm) and phosphorescence (-Δ-, λ_{ex}=320nm; ··▲··, λ_{ex}=270nm) polarization of KDF in isopentane at 77K.

in the excited state dynamics with changing tempeature.

The ratio of the phosphorescence to fluorescence quantum yield increases from 0.07 in ethanol to 0.1 in EEM (ethyl iodide–ethanol–methanol, 5/16/4 by volume) solution containing a heavy atom suggesting the enhancement of intersystem crossing by an external heavy atom. The ratio of the phosphorescence to fluorescence quantum yield decreases from 0.07 in ethanol to 0.03 in 2-methylpentane. The fluorescence maximum shifts to the blue as solvent changes from ethanol to 2-methylpentane, while the phosphorescence maximum shifts to the red. However, in the triplet state manifold the lowest triplet state in hydrocarbon solovents is not perceived to be (n, π^*) state, since the energy difference between the fluorescence and phosphorescence maxima increases from 726 cm^{-1} in ethanol to 2024 cm^{-1} in 2-methylpentane and phosphorescence lifetime in 2-methylpentane is still great (1.3 s). The high fluorescence quantum yield in 2-methylpentane is also expected from the upward curvature in its temperature dependence, even at low temperature.

Figures 4 and 5 show the emission and excitation spectrum in ethanol and isopentane, respectively, at 77K. In contrast to emission properties of thioxanthone[18] and chromone,[19] KDF shows very weak unstructured phosphorescence and it is highly overlapped with fluorescence. These different spectral features imply that the behavior of excited state of KDF is very different from that of thioxanthone and chromone. As in the case of chromone, no evidence was found that the lowest excited singlet and triplet states in ethanol and isopentane at 77K have the (n, π^*) configuration. However, the room temperature absorption an fluorescence spectra imply that the lowest excited singlet state of KDF in hydrocarbon solvents is (n, π^*) state as was previously pointed out from the spectral data at room temperature in contrast to high fluorescence quantum yield in isopentane at 77K. From the different spectral data at room temperature in contrast to high fluorescence lifetime of KDF, we now suggest the (π, π^*) state as the lowest energy triplet state of KDF, despite of the slightly positive polarized phosphorescence.

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