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Temperature Dependence and Decay Kinetics of the High-Energy Band in DPH Fluorescence

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The temperature dependence and associated decay kinetics of the high energy band centered at 380 nm in the fluorescence spectrum of 1,6-diphenyl-1,3,5-hexatriene in a nonpolar solvent were investigated. Below 150 K the emission intensity at 380 nm relative to that of a sharp band near 400 nm was very small. Above this temperature the intensity at 380 nm increased with increasing temperature. The fluorescence decay analysis of this band showed two components with decay times of 62 ps and 13.4 ns. The 62 ps component was assigned to the prompt fluorescence of the S2 (1Bu*) state and 13.4 ns component to a delayed type fluorescence from the same state after establishment of a thermal equilibrium with lower S1 (1Ag*) state.

When a molecule is used as a fluorescence probe in biological systems it is essential that its photophysical properties are clearly understood so that the information provided by the probe is correct and most useful. 1,6-Diphenyl-1,3,5-hexatriene (DPH) has been employed as a fluorescence probe for the elucidation of the order and dynamics of membrane systems.[1-3] It is representative of the diphenyl derivatives of linear polyenes and considerable data about their photophysical properties have been reported.[4,5] It has been adequately shown that DPH has anomalous fluorescence properties.[6] The fluorescence properties of DPH has a large Stokes shift and its radiative lifetime when estimated from Strickler–Berg relationships[7] is far smaller than that obtained experimentally.[8] A large number of investigations of DPH photophysics have been directed at understanding origin of these anomalies. It has been shown that the 1Ag* state is the emitting state.[9] Birks and co-workers[10] suggested that the fluorescence occured from the symmetrically forbidden 1Ag*–1Ag transition, which borrowed intensity from the dipole allowed 1Bu*–1Ag transition. They proposed that the energy difference between the 1Bu* and 1Ag* state and conformational changes in the excited state were controlling factors for the photophysical processes of diphenylpolyenes such as diphenylbutadiene (DPB) and diphenyloctatetraene (DPO). While this rationalization appears to be reasonable, Alford, Palmer,[11,12] Itoh and Kohler[13] in their investigations of the fluorescence spectra of DPH and its derivatives assigned a band near 380 nm to the 1Bu*–1Ag transition. Furthermore, recent picosecond transient absorption studies on DPH suggested a thermal equilibrium exists between the S2 and S1 states.[14] In this report we present a detailed study of the temperature dependence and fluorescence decay kinetics of the high enegry band.

Materials and Methods

all-trans-1,6-Diphenyl-1,3,5-hexatriene (Sigma Chemical Co. St. Louis Mo.) was purified by vacuum sublimation. Its purity and photochemical decomposition was checked by measuring its absorption spectrum before and after any fluorescence measurements. Methylcyclohexane (MCH) (Distilled in glass quality, Caledon Lab, Georgetown, Ont) was used without further purification and shown to be free of any fluorescence impurities.

Fluorescence decay measurements were performed with a frequency doubled Spectra-Physics synchronously pumped and cavity dumped laser system (E_x=295 nm or 310 nm) and methods of time correlated single photon counting techniques. The instrumentation and the methods of data analysis used have been described in detail.[15] The emission bandpass was 4 nm. A Perkin-Elmer MPF-44A spectrofluorometer equipped with a DCSU-2 microprocessor correction unit was used for measurement of the fluorescence spectra. The fluorometer was connected through an analog to digital converter to a Commodore PET minicomputer for the storage and manipulation of the fluorescence spectra. The fluorometer was equipped with a thermostable cell holder. Low temperature ($<$0 °C) spectra were recorded using a NRCC built quartz Dewar assembly the temperature of which was controlled by blowing cold nitrogen gas into the Dewar. The samples in this latter case were in a suprasil quartz tube. The excitation and emission bandpasses were 2 nm and 2 nm respectively. All samples were deoxygenated by flushing with oxygen free nitrogen. Absorption spectra were measured on Cary 118 spectrophotometer.

Results

The fluorescence spectrum of DPH in a MCH glass at 77 K has three sharp bands at 397, 425, and 445 nm and a shoulder at 480 nm (Fig. 1). Above 147 K the MCH solution is liquid. The spectrum at 150 K again has three sharp transitions at 401, 426, and 450 nm which are red shifted relative to the bands in the 77 K spectrum. At 150 K and 77 K the emission intensity between 360 nm and 390 nm was negligible when

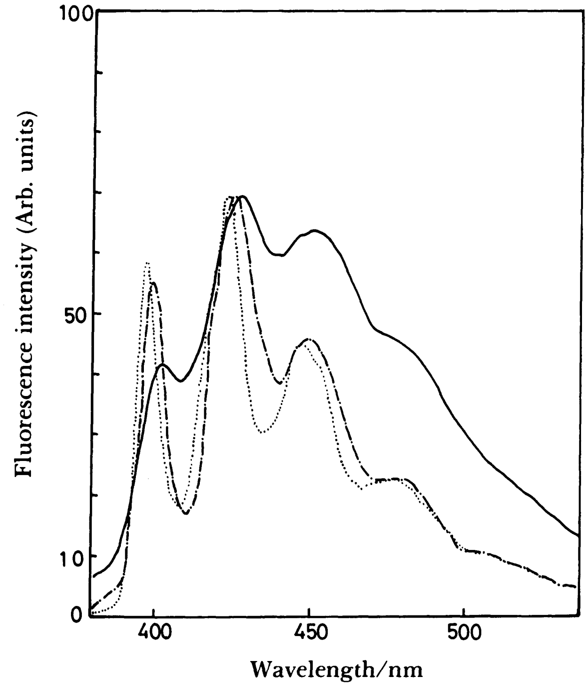

Fig. 1. Fluorescence spectra of DPH in MCH. -----; 77 K, -·-·-; 150 K, —; 293 K.

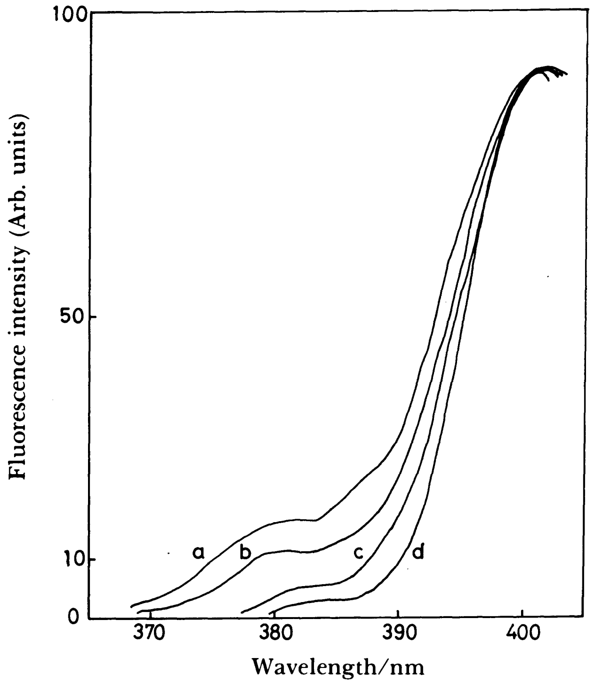

Fig. 2. Temperature dependence of high energy band of DPH fluorescence spectrum in MCH, a; 313 K, b; 279 K, c; 233 K, d; 183 K.

compared to the intensity of the band above 390 nm. As the temperature increased the position of the bands remained constant, however the band widths broadened and the intensities of the long wavelength bands (426, 450, and 482 nm) increased. At the same time, a shoulder became evident at a wavelength below the 400 nm band. Figure 2 shows the high energy edge of the DPH fluorescence spectrum in MCH solution at 183, 233, 279, and 313 K. The emission intensity in this region increased as the temperature increased. The peak position and the band width of the high energy emission band, measured at 279 K was estimated to be 380 nm and 970 cm^{-1} respectively by curve fitting assuming Gaussian band shapes. Because of the broadening of the 400 nm band, it is difficult to determine the precise peak position of the high energy band at high temperature. But this band apparently undergoes a shift to the lower energy side with decreasing temperature. This contrasts with the behavior of the main fluorescence bands above 400 nm.

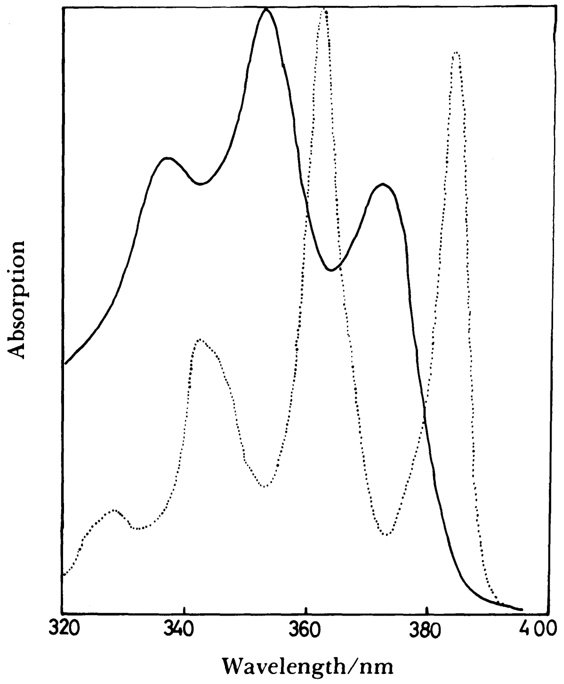

Fig. 3. Absorption spectra of DPH in MCH at 77 K (-----) and 293 K (—). See text.

The absorption spectrum of DPH in MCH at 77 K has sharp bands at 327, 342, 362, and 384 nm and no absorption was detected around 400 nm (Fig. 3). Usually the excitation spectrum is used for the investigation of absorbance at 77 K. But that is correct only when the sample concentration is extremely low. The spectrum shown in Fig. 3 was obtained from the intersection of the straight line $\log(I(\lambda)/I(\lambda_0))$–[DPH] plot of the following equation

$$\log\{I(\lambda)/I(\lambda_0)\} = \log\{\varepsilon(\lambda)/\varepsilon(\lambda_0)\} - \{\varepsilon(\lambda) - \varepsilon(\lambda_0)\}cd$$

where $I(\lambda)$ and $I(\lambda_0)$ are fluorescence intensities excited by λ and λ_0 and ε, c and d are absorption coefficient, DPH concentration and optical path length, respectively.[16] The absorption spectrum of DPH at temperature above 293 K has peaks at 337 nm, at 353 nm and 372 nm which are blue shifted relative to the 77 K

Table 1. Fluorescence Decay Parameters for DPH in MCH at 293 K, A_1 and A_2; Normalized Preexponential Factors, F_1 and F_2; Fractional Contributions

F_m/nm	T_1/ns	T_2/ps	A_1	A_2	F_1	F_2
373	13.38	62	0.167	0.833	0.978	0.022
377	13.38	62	0.268	0.732	0.988	0.012
380	13.38	62	0.357	0.643	0.992	0.008
383	13.38	62	0.375	0.625	0.992	0.008
387	13.38	62	0.449	0.551	0.994	0.006
390	13.38	62	0.557	0.443	0.996	0.004
393	13.38	62	0.817	0.188	0.999	0.001
397	13.40					
400	13.40					
400<	13.40					

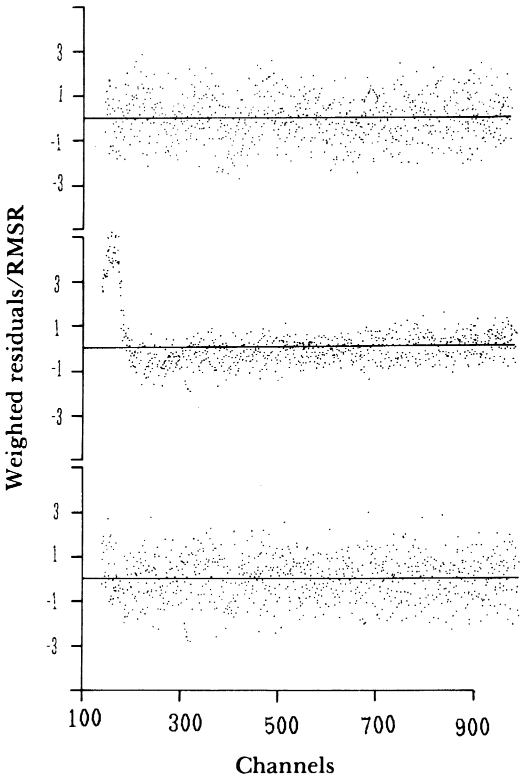

Fig. 4. Plots of weighted residuals/RMSR after iterative convolution of the DPH fluorescence in MCH at 293 K for the best fits (upper) single exponential function at 430 nm, (middle) single exponential function at 380 nm, (lower) double exponential at 380 nm. 10 ps/channel.

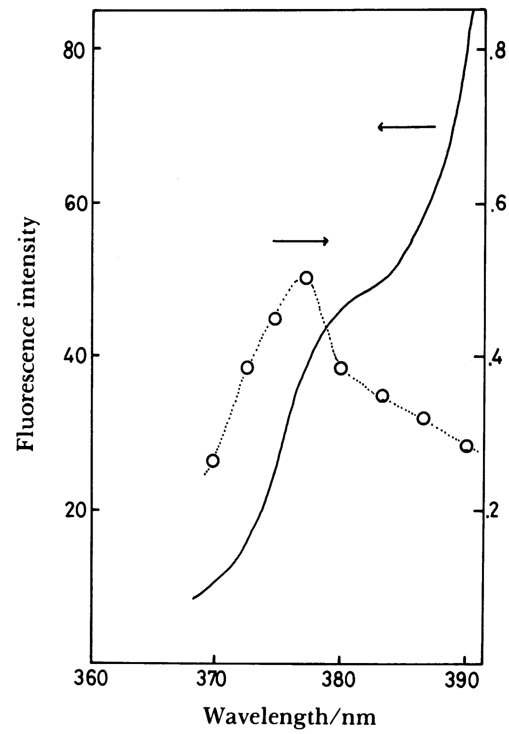

Fig. 5. Fluorescence spectrum of 62 ps component of DPH in MCH at 293 K, solid curve; high energy band of the DPH fluorescence. Broken curve; calculated fluorescence intensities of 62 ps component.

spectrum. As reported earlier[8] these bands undergo small shifts to higher energy with increasing temperature. The emission band at the high energy edge (380 nm) overlaps the lowest energy band of the absorption spectra.

The fluorescence decay of DPH in MCH was measured over the full spectral region. Between 400 nm and 550 nm the fluorescence decayed with single exponential decay kinetics (Fig. 4 upper). Below 400 nm, in the high energy fluorescence spectral region, DPH showed the double exponential decay kinetics (Fig. 4 middle and lower) with lifetimes of 13.38 ns (T_1) and 62 ps (T_2). The fluorescence decay parameters of DPH are summarized in Table 1. Because T_2 is short and its fractional fluorescence is small, close attention was paid to eliminate the possibility that this component was due to light scattering or emission from quartz cuvette or solvent impurities. From the relation between the steady state fluorescence intensity, $F_{ss}(\lambda)$ and the fluorescence decay kinetics, the fast decay component $F_s(\lambda)$ is extracted as follows,

$$F_s(\lambda) = F_{ss}(\lambda)R(\lambda)\ T_2/(R(\lambda)\ T_2 + T_1)$$

where $R(\lambda)$ is the ratio of the normalized preexponential factor. The time resolved spectrum of DPH fluorescence in the high energy spectral region gives a clear peak at 377 nm (Fig. 5). This peak position is coincident with the high energy peak of the steady state DPH fluorescence spectra estimated by a curve fitting procedure. In experiments using the third harmonics (353 nm) from the neodymium laser it was observed that the fluorescence of DPH had a decay component which decayed to 70 percent of the maximum fluorescence within 30 ps after excitation.[17] This component corresponds to the 62 ps component which we observed. The time evolution of the transient absorption spectra of DPH shown by Rullière and Declémy supports the assignment of the fast decay component of DPH fluorescence to the S_2–S_0 transition.[14]

Discussion

Because the 1Ag*–1Ag transition is symmetrically forbidden, it is presumed that the absorption spectrum of DPH is due to the 1Bu*–1Ag transition. Therefore the spectral overlap at temperature 150 K in the 380 nm region would support the assignment of this latter emission band originating from the 0–0 level of the same transition. Further since the 1Ag* state is lower in energy than the 1Bu* state in DPH in nonpolar solvents the 401 nm band in the 150 K fluorescence spectrum can be assigned to the 0–0 band of the 1Ag*–1Ag transition.

We agree that the increase in the 380 nm fluorescence intensity with temperature is indicative, as Alford and Palmer suggested, requires that a thermal equilibrium be established between the S_2 (1Bu*) and S_1 (1Ag*) states. Using the data obtained at several different temperatures it is possible to estimate the energy difference between these states from the relationship proposed by Parker[18]:

$$I(380)/I(401) = A\exp\,(-\,E_0/kT)$$

where $I(380)$ and $I(401)$ represent the fluorescence intensities at 380 nm and 401 nm respectively, A is a temperature independent constant which include a frequency factor and a radiative lifetime, and the other terms, k, and T have their usual definitions. A plot of the natural logarithm of the intensity ratio against $1/T$ is shown in Fig. 6; an excellent straight line was obtained with a slope having a value for E_0=630 cm^{-1} (0.078 eV). This value is coresponds to the energy difference E(1Bu*)−E(1Ag*) of DPH in hexane at zero temperature reported by Itoh and Kohler.[13] The energy difference between the 1Bu* and 1Ag* state can also be estimated from the 0–0 bands of the fluorescence spectra. This gives a value of 840 cm^{-1} which is in close agreement with the value obtained above. It is noteworthy that Cehelnik et al. in a study of the temperature quenching of the fluorescence of DPH in MCH (E_m=400 nm) found a value of 7.1 kJ mol^{-1} (596 cm^{-1}) which is close to the value which we report herein. This is strong evidence that the main nonradiative deactivation pathway of the 1Ag* state is a thermally activated process occuring through the 1Bu* state.

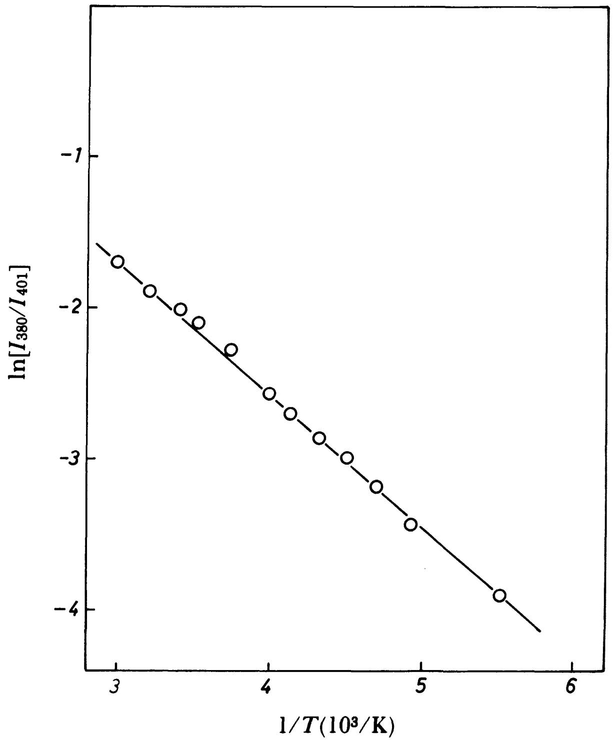

Fig. 6. Plot of ln (I_{380}/I_{401}) against $1/T$, I_{380} and I_{401} are fluorescence intensities at 380 and 401 nm, respectively.

We propose that the fluorescence decay results may be rationalized according to the following scheme. We attribute the 62 ps component, which is only observed at wavelengths below 400 nm, to prompt emission from the initially excited 1Bu* state. We have not been able to detect any evidence of a short component at longer wavelengths. This may be due either to the lack of such a contribution at lower energies or to a very small contribution (<0.2% of a 13.40 ns component) which cannot be detected. The signal 13.4 ns component which is observed above 400 nm is assigned to the emission from the 1Ag* state. The 13.4 ns component, which is observed below 400 nm, is attributed to a delayed type emission from the 1Bu* state after thermal repopulation from the 1Ag* state. At present we cannot rule out the possibility that the fluorescence above 400 nm includes 1Bu* emission. If it did however we might expect to detect a 62 ps component due to the prompt fluorescence from this state. As stated above this might make a negligible contribution to the fluorescence in this region.

The value of the radiative lifetime, τ_r, of DPH from the 1Bu*–1Ag transition, estimated by the Strickler–Berg method was 1.6 ns. The short lifetime, 62 ps, in these experiment is much less than the theoretical value. However, it is reasonable to attribute the shorter value to internal conversion processes between the 1Bu* and 1Ag* states.

In summary we have resolved the temporal behavior of the 380 nm transition and have confirmed the assignment of this band as the 1Bu*–1Ag transition. The model of a thermal equilibrium between the S_2 (1Bu*) and S_1 (1Ag*) excited state is consistent with all the data. We consider that their relationship and the role they play in the photophysical processes in DPH are clarified so that a better understanding of these processes in lipid system where DPH is used as a probe of order and dynamics is possible.

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