Direct Observation of Excited State Intramolecular Proton Transfer Kinetics in 3-Hydroxyflavone

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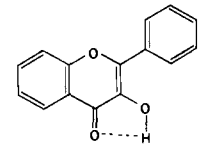
Abstract: The use of both steady state and ultrafast time-resolved fluorescence spectroscopy has enabled a detailed analysis of the kinetics of excited state intramolecular proton transfer in 3-hydroxyflavone. These investigations have revealed an interesting solvent dependence of the mechanism for proton transfer. In hydrocarbon solvents it is suggested that the proton transfer occurs across a pre-existing intramolecular hydrogen bond. In methanol, however, an encounter complex mechanism is proposed to account for the observed results. The activation energy for the excited state proton transfer, in both situations, has been measured. The characteristics of excited state prototropism in 3-hydroxyflavone in these solvents have been found to differ greatly from those found in earlier studies of excited state intramolecular proton transfer in other systems, including methyl salicylate and salicylamide. In methanol and cyclohexane only one ground state species exists. However, in a rigid PMMA matrix there is evidence for the existence of more than one ground state conformer. In this case the interpretation of the results is complex, and a quantitative analysis, in terms of activation energies for proton transfer, becomes impossible. It is believed that this work represents the first direct observation of a "rise time" for the fluorescence of a tautomer resulting from excited state intramolecular proton transfer. The radiationless processes which may contribute to the deactivation of the excited tautomeric state are discussed and measurements of the activation energies for some of these processes are reported. The interesting viscosity dependence of the excited state tautomer fluorescence decay time has been interpreted in terms of a radiationless process associated with torsional motion of the two-ring system.

Developments in the field of ultrafast time-resolved fluorescence spectroscopy have recently led to considerable progress in the study of excited state proton transfer processes.[1-13] Systems in which these processes occur commonly exhibit dual emissions—attributable to the normal excited state and the tautomer resulting from the proton transfer process.[1-3,14-21] Studies of *inter*molecular proton transfer have routinely revealed rise times for the tautomer emission,[4-13] however, these have not been observed where the transfer occurs *intra*molecularly.[1-3,14,19] This difference may result from the need, in the intermolecular case, for the donor and acceptor to diffuse (rotationally and/or translationally) into a suitable conformation. By contrast, intramolecular transfer usually occurs across a pre-existing hydrogen bond.[1-3,15-17] The rate of proton transfer is also strongly dependent on the transfer distance[22] and the greater distance expected in the intermolecular case may contribute to a slower transfer rate.

An interesting example of a molecule capable of intramolecular proton transfer is 3-hydroxyflavone (I). Frolov et al. have observed two intense fluorescence bands for 3-hydroxyflavone in ethanol at 77 K, which they attributed to inter- and intramolecularly hydrogen-bonded species.[23] Sengupta and Kasha have also observed a dual emission in alcoholic solvents, but their interpretation was in terms of an intramolecular excited state proton transfer.[24] The long wavelength emission (ca. 530 nm) was attributed to the resonance stabilized tautomer, II, and the normally Stokes shifted band to the uncharged 3-hydroxyflavone molecule. This interpretation is consistent with the expected changes in the acid-base properties of the carbonyl and hydroxy groups upon excitation.[25-27] A Forster cycle calculation,[28] using



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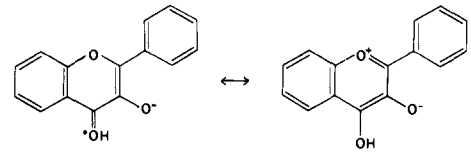

II

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the present results, predicts that the pK_a of the protonated carbonyl group increases by ca. 4.8 upon excitation. The interpretation of Sengupta and Kasha is further supported by the observation of an appreciable deuterium isotope effect on the relative intensities of the two emission bands.[24]

Spectroscopic and chromatographic evidence points to the existence of a relatively weak, intramolecular hydrogen bond between the hydroxy and carbonyl groups of 3-hydroxyflavone in non-hydroxylic solvents, resulting in a five-membered chelate ring.[25,26,29-40] The fluorescence spectrum of 3-hydroxyflavone in 2-methylbutane at room temperature consists of a single, intense band at 520 nm, attributable to the tautomer.[24] When the solution is cooled to 77 K, forming a rigid glass matrix, the emission consists mainly of normal (400 nm) emission with only a very weak tautomer emission. In contrast to the results of other studies of excited state intramolecular proton transfer,[1,3,15-17] the two emission bands in hydrocarbon glass at 77 K exhibit identical excitation spectra, pointing to a common ground state precursor.

Sengupta and Kasha proposed that the full excited state basicity of the carbonyl oxygen was not attained until the phenyl ring became coplanar with the γ-pyrone ring of the 3-hydroxyflavone molecule. Such an interpretation attributes a central role to the viscosity of the medium. According to this model the dramatic change in the fluorescence spectrum in 2-methylbutane, upon going from liquid solution to the rigid glass matrix, arises as a consequence of an out-of-plane conformation being locked in prior to excitation, and the torsional motion of the phenyl ring being restricted or prevented in the rigid matrix.

A major doubt with the interpretation of Sengupta and Kasha is that they attribute the change in the emission spectrum entirely to the change in the solvent viscosity, notwithstanding this being achieved by changing the temperature. In the investigation reported here the effects of solvent, viscosity, and temperature have been distinguished.

Experimental Section

The sample of 3-hydroxyflavone was supplied by Tokyo Kasei as a "Guaranteed Reagent" grade chemical, and its purity was confirmed both spectroscopically and by melting point determination. It was used as supplied. Methanol-*d* (CH_3OD) was purchased from Merck, Sharpe and Dohme and was found to be free from fluorescent impurities. Methanol was redistilled and all hydrocarbon solvents were chromatographically purified by passing them through a column of 60–120 mesh silica gel (B.D.H.). These procedures rendered all solvents free of fluorescent impurities. All measurements were made on solutions ranging in concentration from 5×10^{-5} to 5×10^{-4} M. Solvent viscosities were determined with use of standard viscometric methods.[41]

A solution of 3-hydroxyflavone in a solid poly(methyl methacrylate) matrix (PMMA) was prepared by dissolving 25 mg of α,α′-azobis(isobutyronitrile) (recrystallized from acetonitrile) and 5 mg of 3-hydroxyflavone in 50 mL of freshly distilled monomeric methyl methacrylate (B.D.H.). The mixture was degassed using five freeze–pump–thaw cycles and then left at 30 °C for ca. 100 h. The solidified sample was then baked at 80 °C for ca. 8 h. This procedure has been found to remove any regions of partial polymerization,[42] producing a sample of uniform viscosity throughout.

Fluorescence emission and excitation spectra were recorded on a Perkin–Elmer MPF-44A spectrofluorimeter. Excitation spectra were run on optically dilute solutions (maximum absorbance <0.1) to avoid distortions of the spectrum arising from saturation in the sample and from the optical geometry of the instrument. All excitation and emission spectra reported here are uncorrected.

Time-resolved fluorescence measurements in alcoholic solvents were performed with an ultrafast streak camera/O.M.A. detection system which has been described earlier.[43] Excitation was with a single pulse of the third harmonic of a Nd^{3+}:phosphate glass laser (λ = 351.4 nm, t_p = 6 ps, E = 0.1 mJ). In order to avoid distortions in the fluorescence decay profile by the effects of rotational diffusion, the fluorescence is collected through an analyzer set at an angle of 54.7° from the direction of polarization of the excitation pulse.[44] Right angle detection was used in all time-resolved fluorescence measurements. The longer lived fluorescence decays of 3-hydroxyflavone in hydrocarbon solvents were measured with use of conventional single photon counting with an ORTEC/Applied Photophysics Ltd. nanosecond spectrometer. Excitation was at 360 nm, using an N_2 filled spark discharge lamp. All hydrocarbon solutions were thoroughly degassed with use of five freeze–pump–thaw cycles prior to their fluorescence decay profiles being measured.

Fluorescence decay curves were analyzed, using non-linear least-squares curve fitting, on a NOVA 2-10 computer.[45] Response function deconvolution, for both single photon counting[46] and the streak camera data,[47] was accomplished with the least-squares iterative convolution method.

Accurate control of sample temperatures was achieved with use of an Oxford Instruments DN 704 Variable Temperature Liquid Nitrogen Cryostat with an Oxford Instruments Digital Temperature Controller-DTC2.

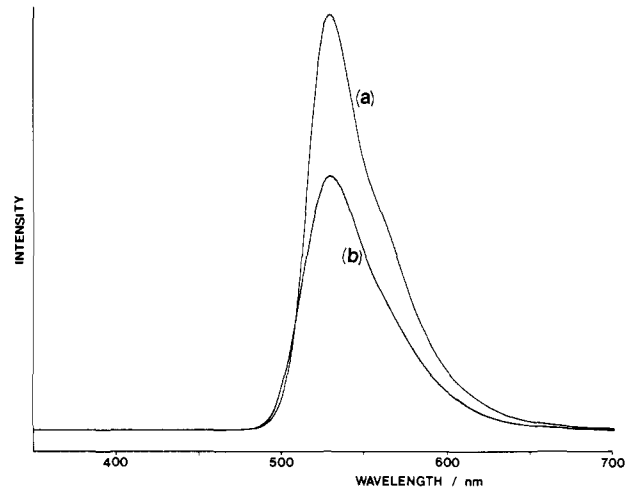

Figure 1. Room temperature fluorescence spectra of 3-hydroxyflavone in (a) Shell Ondina oil 68 (η = 204 cP) and (b) rigid PMMA matrix (η = 10^{13} cP).

Table I. Solvent Viscosities and Tautomer Fluorescence Lifetimes

solvent	viscosity (20 °C), cP	τ_{fl}, ns
75% hexane + 25% Shell Ondina Oil 15 (v/v)	0.713 ± 0.001	4.52 ± 0.03
methylcyclohexane	0.723 ± 0.001	4.58 ± 0.02
Shell Ondina Oil 68	204.0 ± 1	5.05 ± 0.01
PMMA	10^{13} (est)	6.09 ± 0.06

Results and Discussion

a. Viscosity Dependence. The room-temperature fluorescence spectrum of 3-hydroxyflavone in a range of hydrocarbon solvents of varying viscosities consists of a single intense band, peaking at 527 nm and almost identical with that reported in 2-methylbutane (see Figure 1).[24] The solvents used are listed, together

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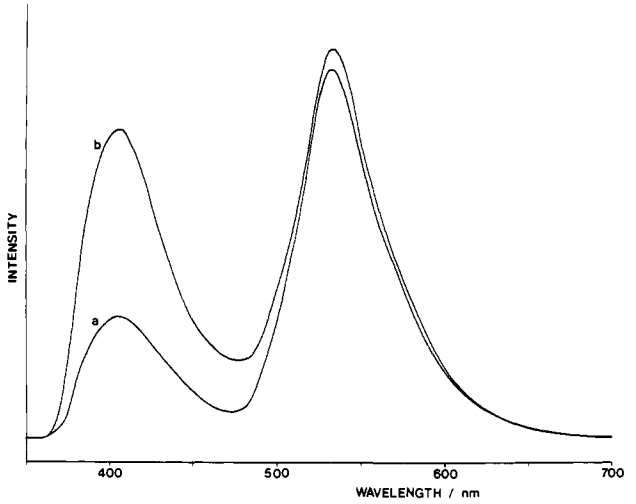

Figure 2. Fluorescence spectrum of 3-hydroxyflavone at room temperature (295 K) in (a) methanol and (b) methanol-*d*.

with their viscosities, in Table I. The emission is clearly attributable to the tautomeric species. No trace of a normally Stokes shifted emission is observed in any of the solvents.

Clearly, the model of Sengupta and Kasha, regarding the viscosity dependence of the rate of proton transfer, is inconsistent with these results. However, as a check, the fluorescence of 3-hydroxyflavone in a solid PMMA matrix has been studied. Although the solvent properties of PMMA are not exactly equivalent to those of the above mentioned hydrocarbons, its dielectric constant of ca. 3.25[48] suggests that it resembles the hydrocarbons more closely than it does methanol. It is useful therefore as a solvent of extremely high viscosity for the present investigations. The subtle solvent effects of PMMA are discussed later. The room temperature fluorescence spectrum in PMMA consists of a single, intense emission at 528 nm (Figure 1). The complete absence of a normally Stokes shifted emission band at this very high viscosity casts serious doubts onto the interpretations of Sengupta and Kasha. They obtained their viscosity change by cooling the solvent to 77 K, forming a rigid glass matrix. The present results suggest that the dramatic change which they observed in the fluorescence spectrum arose solely from the effects of temperature upon the excited state processes in 3-hydroxyflavone.

The tautomer lifetimes for the various solvents listed in Table I suggest a correlation between lifetime and solvent viscosity. This correlation may indicate a radiationless process involving an internal molecular motion, which is inhibited in highly viscous solvents.

b. Solvent Dependence. The fluorescence spectra of 3-hydroxyflavone in methanol and methanol-*d* at room temperature are shown in Figure 2. The long wavelength fluorescence band, attributable to the tautomer, is considerably less intense than in hydrocarbon solvents, and has its maximum at 531 nm in both methanol and methanol-*d*. The short wavelength emission maximum is at 405 nm in both solvents, this band being identified with the non-proton-transferred excited state. Kinetic isotope effects on the rates of proton transfer reactions have been commonly noted,[49,50] and the effect of deuteration on the relative intensities of the two emission bands can, in this case, be interpreted in terms of a decrease in the tautomerization rate constant. The excitation spectra of both emission bands are identical with each other and very similar to the absorption spectrum (Figure 3). No change in these spectra is observed upon deuteration.

These results suggest that both emission bands arise by excitation of a common ground state precursor. Intramolecular proton

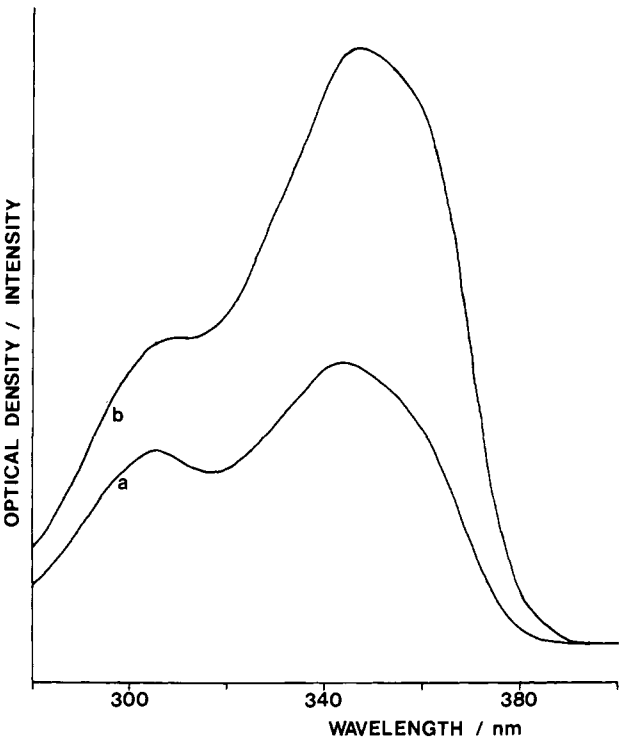

Figure 3. (a) Absorption spectrum of 3-hydroxyflavone in methanol. (b) Excitation spectrum of 3-hydroxyflavone in methanol.

transfer can occur after excitation, but the rate for this process must be substantially slower than in other systems[3] including salicylamide[1,2] and methyl salicylate[14,19] in order to explain the observation of emission from the non-proton-transferred species. An alternative explanation of the present results in terms of the two emission bands arising from excitation of distinct ground state conformers, along similar lines to that proposed for methyl salicylate and other related systems,[1,3,15,16] would require the ground state conformers to have identical absorption spectra. This possibility is considered to be extremely remote. The validity of the former interpretation can be confirmed by time-resolved fluorescence measurements. This interpretation leads to the following differential equations describing the time dependence of the concentrations of the excited state non-proton-transferred species (A) and tautomer (B). In these equations the k_r's, k_{nr}'s,

$$d[A]/dt = (k_{r_A} + k_{nr_A} + k_t)[A] \quad (1)$$

$$d[B]/dt = k_t[A] - (k_{r_B} + k_{nr_B})[B] \quad (2)$$

and k_t are the radiative, total nonradiative, and tautomerization rate constants, respectively. It is assumed that the initial creation of the non-proton-transferred excited state is instantaneous. The boundary conditions, for $t = 0$, are

$$[A] = A_0 \text{ and } [B] = 0 \quad (3)$$

The differential equations are easily solved,[51] yielding the following equations.

$$[A] = A_0 e^{-t/\tau_A} \quad (4)$$

$$[B] = \frac{\tau_A \tau_B k_t A_0}{(\tau_B - \tau_A)}(e^{-t/\tau_B} - e^{-t/\tau_A}) \quad (5)$$

In these equations

$$\tau_A = k_{r_A} + k_{nr_A} + k_t \quad (6)$$

and

$$\tau_B = k_{r_B} + k_{nr_B} \quad (7)$$

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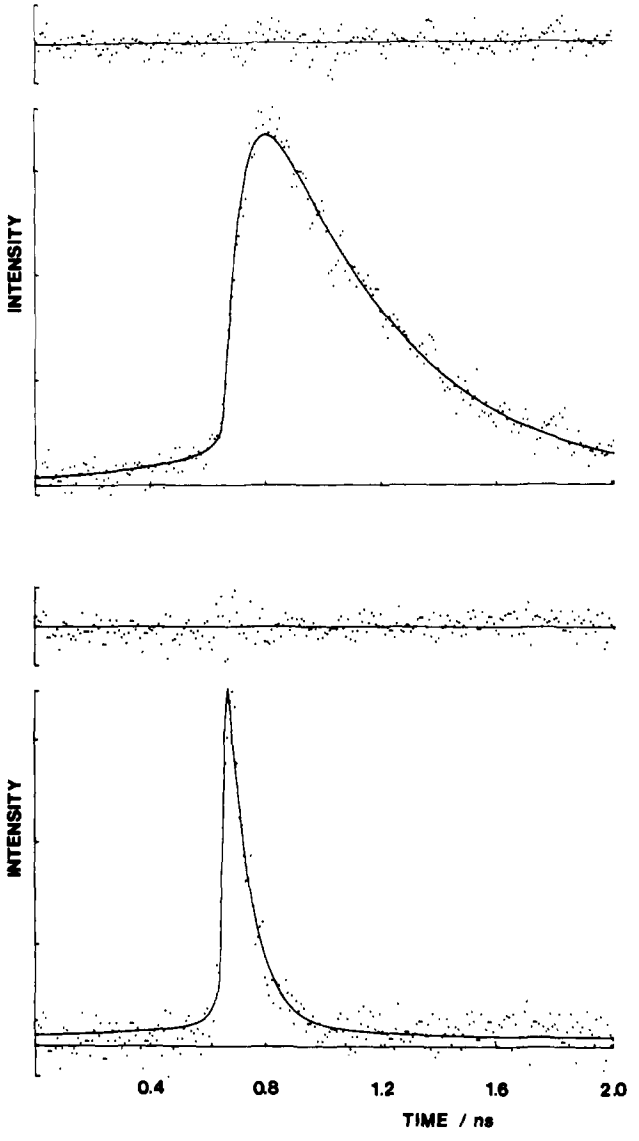


Figure 4. Fluorescence time dependence profiles in methanol-*d* for (a, top) the long wavelength (tautomer) band and (b, bottom) the short wavelength band.

The predicted fluorescence decay of A is simply a single exponential, while the tautomer fluorescence should show an exponential growth and decay. Furthermore, depending upon the relative magnitudes of τ_A and τ_B, the fluorescence decay lifetime of A should equal either the growth or the decay lifetime of the tautomer fluorescence.

The fluorescence time dependences for both emission bands of 3-hydroxyflavone in methanol-*d* are shown in Figure 4. The measurements are easier in the deuterated solvent because of the lower tautomerization rate and the more intense short wavelength emission. The short wavelength emission is well fitted by a single exponential decay, with a lifetime of 69 ± 8 ps. The tautomer fluorescence, however, can only be fitted satisfactorily by a difference of two exponentials (eq 8). The growth lifetime (τ_2) of 65 ± 10 ps is equal, within experimental error, to the decay lifetime of the short wavelength emission. The tautomer emission decay time is 370 ± 25 ps.

The boundary condition, that no excited state tautomer arises

$$I(t) = A[e^{-t/\tau_1} - e^{-t/\tau_2}] + B \quad (8)$$

by direct excitation, was confirmed when a fit of the data in Figure 4b to eq 9 yielded, within experimental error, identical values for the pre-exponentials, A_1 and A_2.

$$I(t) = A_1e^{-t/\tau_1} - A_2e^{-t/\tau_2} + B \quad (9)$$

These kinetic results are in complete accord with the earlier interpretation of the photophysics of 3-hydroxyflavone in methanol and methanol-*d* and provide an upper limit for the tautomerization rate constant of 1.5×10^{10} s^{-1} in the latter. These results contrast with the conclusions of Sengupta and Kasha, who suggested that the short wavelength emission arose from the excitation of 3-hydroxyflavone molecules in which tautomerization was prevented by external hydrogen bonding with the solvent.

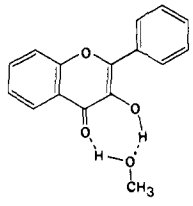
The observation of identical excitation spectra for the two fluorescence bands in hydrocarbon glass at 77 K suggests that the same model applies as was proposed for the alcoholic solvents. This model predicts the following expressions for the fluorescence quantum yields of the non-proton-transferred (A) and tautomer (B) excited states.

$$\phi_A = \frac{k_{r_A}}{k_{r_A} + k_{nr_A} + k_t} = k_{r_A}\tau_A \quad (10)$$

$$\phi_B = \frac{k_t k_{r_B}}{(k_{r_A} + k_{nr_A} + k_t)(k_{r_B} + k_{nr_B})} = k_t k_{r_B} \tau_A \tau_B \quad (11)$$

The total absence of a short wavelength emission in hydrocarbon solvents at room temperature can be explained by a larger value of the tautomerization rate constant, k_t, compared to that in alcohols.

It is highly likely that the weak,[30-37] and hence long, intramolecular hydrogen bond in 3-hydroxyflavone is disrupted by intermolecular hydrogen bonding in solvents such as alcohols. Since tautomerization is not totally prevented in alcohols, the external hydrogen bonding must provide for an alternative mechanism. A study of accurate, space-filling molecular models suggests that an apparently stable encounter complex can be formed, involving a seven-membered chelate ring, between 3-hydroxyflavone and a single methanol molecule (III). The intermolecular hydrogen bonds in such a complex should be considerably stronger, since they are both shorter and more linear, than in the five-membered intramolecularly hydrogen bonded chelate ring. It is envisaged that the proton transfer could occur within the seven-membered chelate ring as either two successive proton transfers or a concerted double proton transfer (Figure 5a). In hydrocarbon solvents the proton transfer is thought to occur directly across the intramolecular hydrogen bond in the five-membered chelate ring (Figure 5b). It is expected that the two proton transfer mechanisms will display different activation energies.



III

c. Temperature Dependence. In many previous studies the distinct ground state precursors of the emission bands have prevented the determination of the activation energy for the excited state proton transfer. In these cases the relative intensities of the normal and tautomeric emissions are affected by changes in the ground state equilibria between the various precursors in addition to changes in the rate constants for the various photophysical processes with temperature. For the present situation, with only a single ground state species, the ratio of fluorescence quantum yields for the long and short wavelength emission bands is given by eq 12. This ratio is proportional to the ratio of the areas, α_A

$$\frac{\phi_B}{\phi_A} = \frac{k_t k_{r_B} \tau_B}{k_{r_A}} = p\frac{\alpha_B}{\alpha_A} \quad (12)$$

and α_B, under the two bands in the emission spectrum; the proportionality constant being p. An Arrhenius type temperature dependence can be assigned to k_t, and assuming that the ratio of

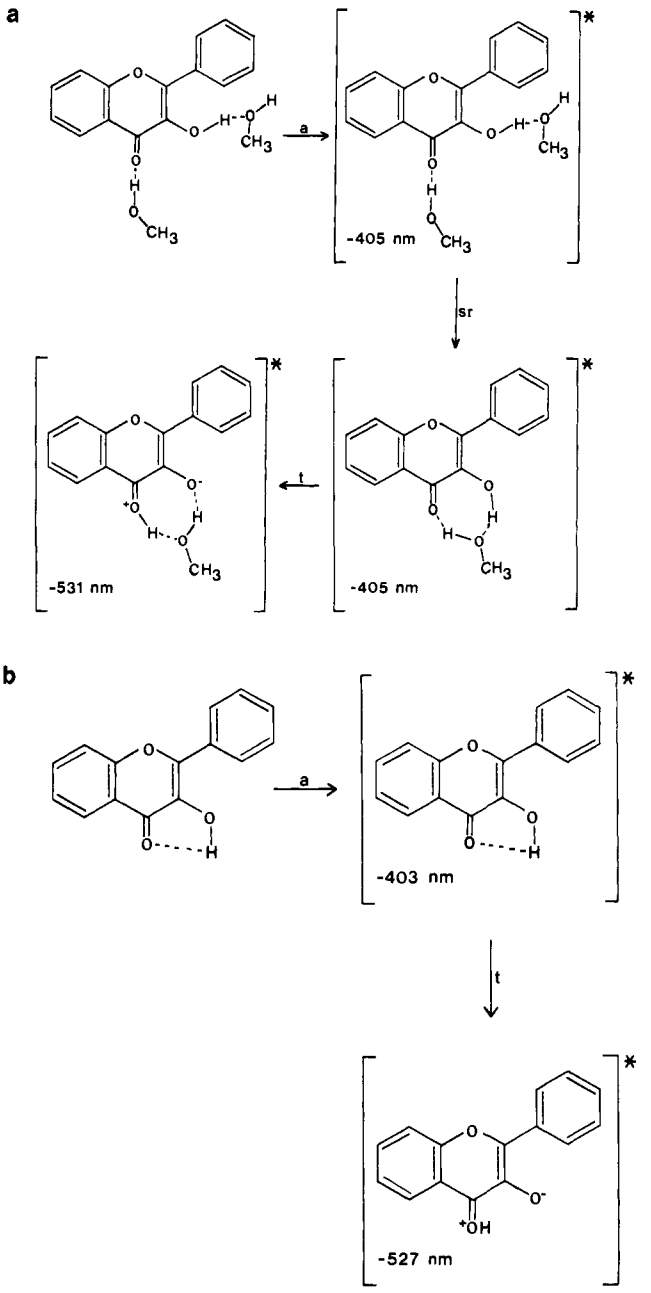


Figure 5. Proposed mechanism for excited state proton transfer in 3-hydroxyflavone in (a) methanol and (b) hydrocarbon solvents. The approximate emission maxima of the various excited states are indicated. The following abbreviations have been used to indicate the various processes involved: a = absorption, sr = solvent reorientation, t = tautomerization.

the radiative rates for the tautomer and the non-proton-transferred species is temperature independent eq 13 is obtained. In this

$$\frac{\alpha_B}{\alpha_A} = C\tau_B e^{-E_t/RT} \tag{13}$$

equation C is a constant containing contributions from the Arrhenius frequency factor, the ratio of the radiative rates, and the proportionality constant, p, as well as a factor resulting from the use of uncorrected spectra. The activation energy for tautomerization is represented by E_t. Equation 14 results from rearrangements and linearization of eq 13.

$$\ln \frac{\alpha_B}{\alpha_A \tau_B} = \ln C - \frac{E_t}{RT} \tag{14}$$

All quantities on the left-hand side of eq 14 are measureable and hence E_t can be determined. The temperature dependences

Table II. Temperature Dependence of 3-Hydroxyflavone in Methanol-*d*

temp, K	α_B/α_A	τ_B, ns	$\tau_{MD'}$, ns
295	1.37 ± 0.04	0.36 ± 0.03	0.37 ± 0.04
265	1.82 ± 0.04	0.68 ± 0.04	0.69 ± 0.05
235	2.56 ± 0.07	1.52 ± 0.04	1.53 ± 0.05
210	3.42 ± 0.11	3.11 ± 0.06	3.11 ± 0.06
180	3.41 ± 0.08	5.20 ± 0.18	5.20 ± 0.18

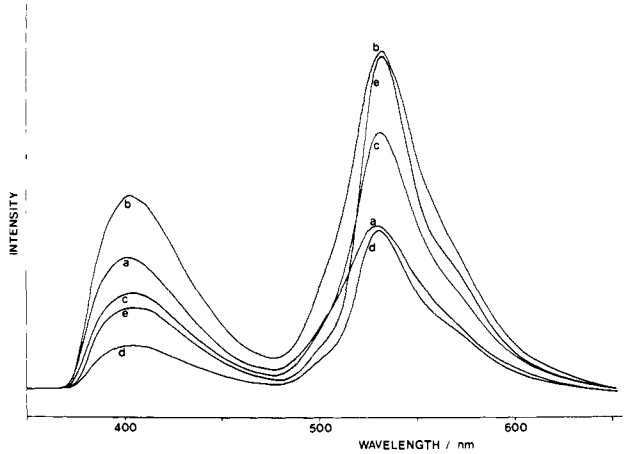


Figure 6. Fluorescence spectrum of 3-hydroxyflavone in methanol-*d* as a function of temperature. Temperatures are (a) 295 K, (b) 265 K, (c) 235 K, (d) 210 K, and (e) 180 K.

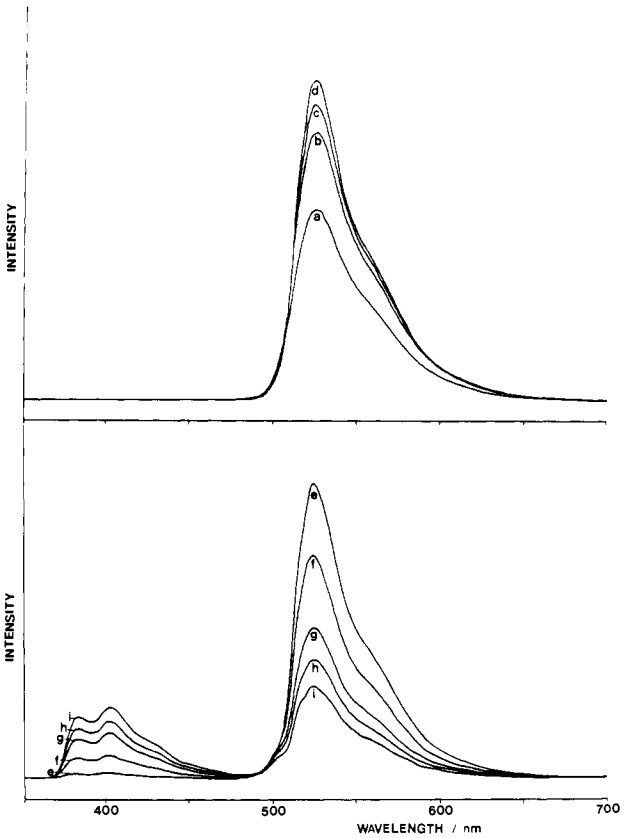


Figure 7. Fluorescence spectrum of 3-hydroxyflavone in methyl cyclohexane as a function of temperature. Temperatures are (a) 295 K, (b) 265 K, (c) 235 K, (d) 210 K, (e) 190 K, (f) 175 K, (g) 165 K, (h) 160 K, and (i) 155 K.

of the emission spectra in methanol-*d* and methylcyclohexane are shown in Figures 6 and 7, respectively. Tables II and III list the values of the ratio α_A/α_B and the fluorescence decay time, τ_B, of the tautomer in the two solvents. In virtually all cases the tautomer fluorescence time profiles are well fitted by eq 8; however, at room

Table III. Temperature Dependence of 3-Hydroxyflavone in Methylcyclohexane

temp, K	α_B/α_A	τ_B, ns
295	*a*	4.58 ± 0.03
265	*a*	6.30 ± 0.06
235	*a*	7.35 ± 0.06
190	25.0 ± 2.1	7.35 ± 0.06
175	7.76 ± 0.37	7.36 ± 0.06
165	2.90 ± 0.13	7.29 ± 0.06
160	1.82 ± 0.07	7.32 ± 0.06
155	1.10 ± 0.04	7.36 ± 0.06

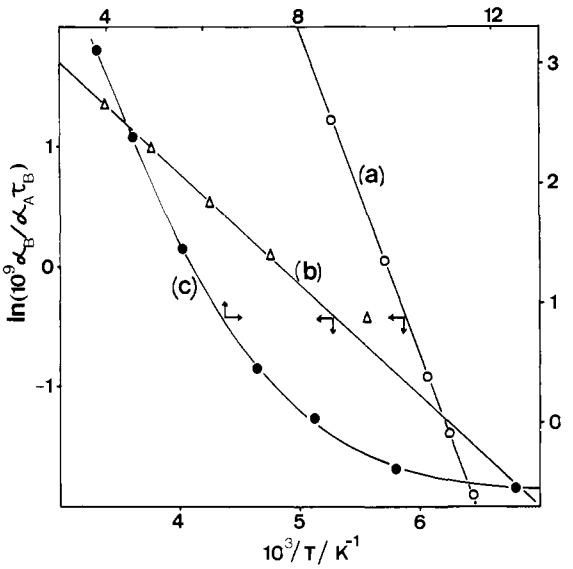


Figure 8. Plot of ln $(10^9\alpha_B/\alpha_A\tau_B)$ vs. $10^3/T$ for 3-hydroxyflavone in (a) methylcyclohexane, (b) methanol-*d*, and (c) PMMA matrix.

temperature in methylcyclohexane the rise time is so short that a good fit to a single exponential is obtained.

Plots of ln $(\alpha_B/(\alpha_A\tau_B))$ vs. T^{-1} for both solvents are shown in Figure 8. The excellent straight line fit of the methylcyclohexane results corresponds to an activation energy of 22.0 kJ mol^{-1}. The straight line fit to the four higher temperature methanol-*d* results corresponds to an activation energy of 7.6 ± 1.0 kJ mol^{-1}. The slight deviation from linearity in this case may be due to quantum mechanical tunneling.[52-54] The large difference in the activation energies strongly supports the earlier proposals regarding the proton transfer mechanisms in the two solvents.

The rates and activation energies for the two mechanisms can be rationalized on the basis of the distance between proton donor and acceptor groups in the two cases. Proton transfer across the long, weak hydrogen bond of the five-membered chelate ring in methylcyclohexane corresponds to the case where the potential minima are widely separated. Accordingly the activation energy is high, and the probability of tunneling is low, consistent with the observed linear Arrhenius plot. The shorter, stronger hydrogen bonds of the seven-membered chelate ring encounter complex in methanol-*d* result in a smaller separation between the potential minima, consistent with a smaller activation energy and the possibility of quantum mechanical tunneling.[22,52-54]

In the classical Arrhenius description the rate constant is determined by an exponential term involving the activation energy and a frequency factor, or "probability" term, reflecting the configurational requirements for the reaction. In methylcyclohexane, where there are no prior configurational requirements to be satisfied, the proton already being held in the intramolecular hydrogen bond, this probability factor should be large. Thus a

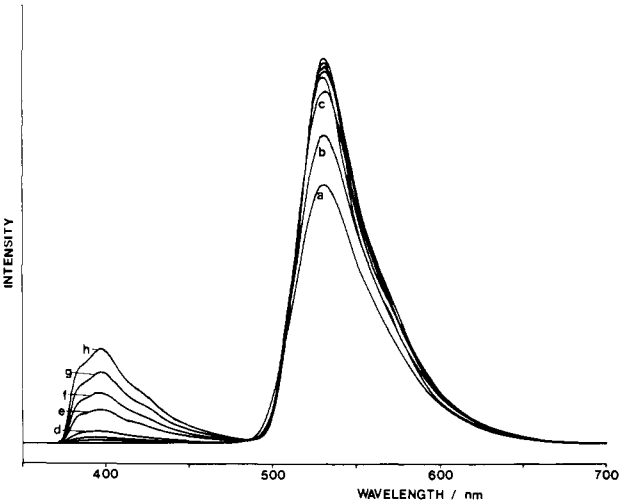
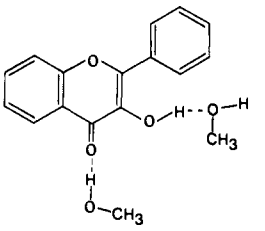


Figure 9. Fluorescence spectra of 3-hydroxyflavone in a rigid PMMA matrix, excited at 340 nm, as a function of temperature. Temperatures are (a) 290 K, (b) 260 K, (c) 220 K, (d) 180 K, (e) 140 K, (f) 120 K, (g) 100 K, and (h) 80 K.

high rate of proton transfer is anticipated, despite the high activation energy. In a solvent such as methanol-*d* there are expected to be many externally hydrogen-bonded structures which do not involve a chelate ring, but nonetheless result in the disruption of the intramolecular hydrogen bond (IV). Proton transfer is not



IV

expected in such structures due to the lack of a reasonably direct pathway for such a process. The rather exacting configurational requirements for the formation of the encounter complex will be manifest in a low probability factor, and hence a lower rate constant than in methylcyclohexane. In the absence of a significant number of pre-existing seven-membered chelate ring complexes, the time for proton transfer could not be less than the solvent rearrangement time.

d. Solid PMMA Matrix. The fluorescence spectra, at various temperatures, of 3-hydroxyflavone in a solid PMMA matrix, excited at 340 nm, are shown in Figure 9. As the temperature is lowered the appearance of a short wavelength emission is noted. The positions of the two emission bands are close to those in methanol and hydrocarbon solvents, arguing against the possibility of the 3-hydroxyflavone being covalently bound to the polymer. Furthermore, if binding did exist it would most likely occur through the hydroxy group and the flavone would be bound to the polymer via an ether linkage. Flavones lacking the 3-hydroxy group do not fluoresce.[29,55] However, many have been found to phosphoresce strongly near 470 nm.[23,56] No phosphorescence has been observed in any of the experiments reported here. These observations indicate that the polymer is acting purely as a solvent and is not covalently bound to the 3-hydroxyflavone. Because

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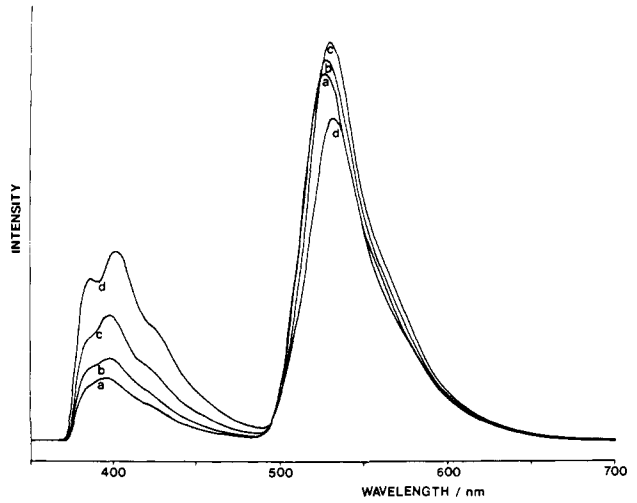
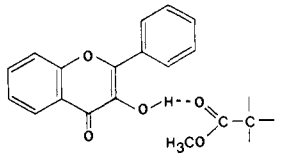


Figure 10. Fluorescence spectra of 3-hydroxyflavone in a rigid PMMA matrix at 120 K as a function of excitation wavelength. Excitation wavelengths are (a) 340 nm, (b) 350 nm, (c) 365 nm, and (d) 370 nm.

of the strong absorption of PMMA at 300 nm and beyond, it is impossible to measure the electronic absorption and excitation spectra of this sample.

The results in Figure 10 illustrate that the emission spectrum depends on the excitation wavelength, suggesting that the two emissions arise, at least in part, by excitation of distinct ground state conformers. As the excitation wavelength is increased the intensity of the short wavelength emission increases relative to that of the long wavelength band, and there is a slight red shift of both emission bands.

Excitation at the longest wavelength (375 nm) results in emission maxima very close to those observed in methanol while shorter wavelength excitation gives rise to emission maxima which correspond closely with those observed in hydrocarbon solvents. These results can be interpreted in terms of a range of solute environments within the PMMA matrix. The environments are envisaged to range from "hydrocarbon-like", in which the solute is predominantly surrounded by alkyl groups, to those in which the solute is surrounded mainly by ester groups. In the more polar environments, in addition to intramolecularly hydrogen-bonded 3-hydroxyflavone, the possibility exists of intermolecular hydrogen bonding between the hydroxy group of 3-hydroxyflavone and the ester groups of the polymer. The ester groups act only as hydrogen bond acceptors and therefore cannot participate in proton transfer, as occurs in the alcohols. Hydrogen bonding to the ester group can, however, stabilize an "open-ring" type of structure (V).



V

Proton transfer is unlikely to occur upon excitation of such a structure due to the lack of a hydrogen-bonded pathway between the hydroxy and carbonyl groups and it should therefore be a precursor of short wavelength emission. In the "hydrocarbon-like" environments all 3-hydroxyflavone molecules should be intramolecularly hydrogen-bonded "closed-ring" structures, and hence capable of proton transfer upon excitation. Earlier results have shown that both emission and absorption spectra of 3-hydroxyflavone are red shifted in going from nonpolar solvents to polar (alcohol) solvents. These observations are consistent with the results in Figure 10, with longer wavelength excitation favoring the 3-hydroxyflavone molecules in polar environments. This causes the observed red shift of both emission bands, and the increase in the relative intensity of the short wavelength band due to the excitation of "open-ring" solute molecules in these environments.

Table IV. Temperature Dependence of 3-Hydroxyflavone in PPMA

temp, K	α_B/α_A	τ_B, ns
295	*a*	6.09 ± 0.06
265	*a*	6.46 ± 0.06
260	145.1 ± 17.8	6.53 ± 0.06[b]
235	*a*	6.78 ± 0.06
220	73.7 ± 9.1	6.88 ± 0.06[b]
210	*a*	6.97 ± 0.06
190	*a*	6.99 ± 0.06
180	30.0 ± 2.6	7.05 ± 0.06[b]
140	11.09 ± 0.59	7.10 ± 0.06[b]
120	7.30 ± 0.34	7.11 ± 0.06
100	4.83 ± 0.21	7.11 ± 0.06[b]
80	4.08 ± 0.18	7.11 ± 0.06[b]

[a] A fluorescence spectrum was not recorded at this temperature. [b] Lifetime has been estimated with use of Figure 11.

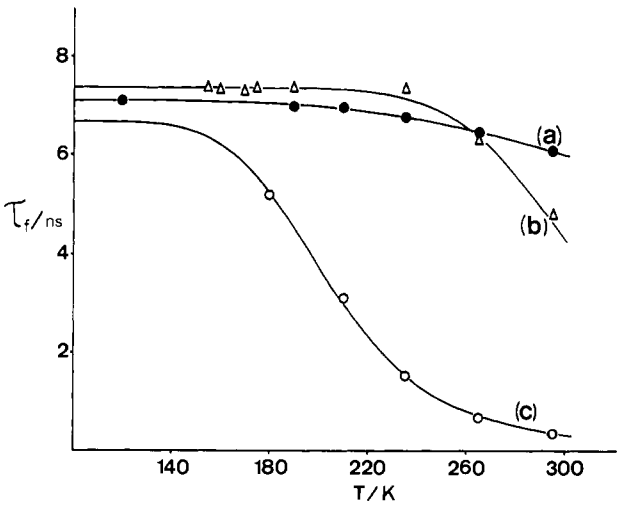


Figure 11. Plot of fluorescence decay time vs. temperature for 3-hydroxyflavone in (a) PMMA matrix, (b) methylcyclohexane, and (c) methanol-*d*. The solid lines are the fits of eq 15 to the data.

The effect of temperature upon the fluorescence spectrum of 3-hydroxyflavone in PMMA is now obviously complex. In addition to affecting the rate of tautomerization and the various nonradiative rates, it can also affect the ground state equilibrium between "open-ring" and "closed-ring" structures in the polar environments of the polymer matrix. Because of the distinct ground state precursors of the two emission bands the previously used temperature dependence analysis is inapplicable in PMMA. This is illustrated by the marked nonlinearity in the plot of ln $(\alpha_B/(\alpha_A\tau_B))$ vs. T^{-1} in Figure 8. The data used for this plot are summarized in Table IV.

e. The Deactivation of the Tautomeric Excited State. The tautomer fluorescence lifetime is temperature dependent in all the abovementioned solvents. As mentioned previously (section a) there is apparently a radiationless process involving an internal molecular motion of the 3-hydroxyflavone molecule, most probably torsional motion of the phenyl and γ-pyrone rings. Such a radiationless process is expected to be important in low viscosity solvents like methanol-*d* and methylcyclohexane. In a rigid PMMA matrix, however, torsional motion of the phenyl ring is prevented, and the radiationless process does not occur.

The fluorescence decay time for the excited tautomer in PMMA is rather insensitive to temperature. The temperature dependence may be analyzed with the simplifying assumptions of a single-temperature-dependent nonradiative process and a temperature-independent radiative rate. Assuming Arrhenius behavior we obtain

$$\tau_B^{-1} = k_0 + Ae^{-E_a/RT} \qquad (15)$$

where k_0 is the sum of all first-order temperature-independent rate constants, A is the Arrhenius frequency factor, E_a is the

Table V. Temperature Dependence of the Tautomer Fluorescence Lifetime

solvent	k_0, s^{-1}	A, s^{-1}	E_a, kJ mol^{-1}
PMMA	$(1.41 \pm 0.03) \times 10^8$	$(2.9 \pm 1.3) \times 10^9$	11.8 ± 1.1
methylcyclohexane	$(1.36 \pm 0.03) \times 10^8$	$(1.6 \pm 1.3) \times 10^{13}$	29.8 ± 2.2
methanol-*d*	$(1.50 \pm 0.12) \times 10^8$	$(1.9 \pm 0.6) \times 10^{12}$	16.2 ± 0.7

activation energy for the temperature-dependent radiationless process, and τ_B is the tautomer fluorescence decay time. The fluorescence decay times in PMMA have been fitted to eq 15 with use of the method of non-linear least squares.[45] The fit is shown in Figure 11. The "best fit" parameter values are shown in Table V. Both the activation energy and frequency factor are small, indicating that the process responsible is a minor one.

In methylcyclohexane the torsional relaxation radiationless process is expected to be quite important while the temperature-dependent process found in PMMA is expected to play only a very minor role. An attempt was made to allow for this latter process in methylcyclohexane by assuming the same parameters as found for PMMA. The lifetimes in methylcyclohexane were adjusted to remove the effect of this process but the resulting data could not be fitted by eq 15. The observation that the low-temperature limiting lifetime is reached at a much higher temperature in methylcyclohexane reinforces our belief that the temperature-dependent process found in PMMA is inoperative in methylcyclohexane.

The torsional relaxation radiationless process is expected to be temperature sensitive, with contributions to its apparent activation energy from the internal energy barrier to torsional motion, and from the energy required for rotational diffusion in the solvent. The fitting of an Arrhenius temperature dependence is clearly only a first approximation. However, if the internal barrier to rotation is considerably larger than the energy required for diffusion, as we believe is the case here,[61] a reasonable estimate will be obtained. Using an Arrhenius model, Karstens and Kobs[60] have measured the apparent activation energy for the radiationless process involving torsional motion of the diethylamine groups in Rhodamine B,[57-60] finding a value of 27.6 kJ mol^{-1}. The best fit of eq 15 to the methylcyclohexane data gives the parameter values listed in Table V. The fit is shown in Figure 11. Hofmann and Thieroff have calculated that 3-hydroxyflavone exists in a twisted conformation with an angle of about 40° between the planes of the phenyl and γ-pyrone rings.[61] Molecular models support this conclusion, indicating that steric hindrance due to substitution at the 3 position would interfere with the attainment of a coplanar configuration. Hofmann and Thieroff further predicted an energy barrier to rotation of 22 kJ mol^{-1} in the ground state. Calculations by Song et al. for the structurally similar pelargonidin point to an increase in bond order in the ring linkage on excitation which would mean a somewhat larger barrier to rotation.[62] In the light of these considerations the theoretical value of 22 kJ mol^{-1} and our measurement of 29.8 kJ mol^{-1} are quite consistent. The calculations of Song et al. further suggest a contraction of the S_1–S_0 energy gap on movement into the coplanar configuration.[62] An enhanced rate of internal conversion might then be anticipated.

The very short tautomer lifetimes observed at higher temperatures in methanol-*d* suggest that a different, very efficient, temperature-dependent, radiationless process is operative. There is no reason to expect that the torsional radiationless process is any less effective in methanol-*d* than in methylcyclohexane. If we now assume that the activation energy and frequency factor for the torsional relaxation radiationless process are the same in methanol-*d* then the effect of this process can be allowed for. In

$$(\tau_{MD}')^{-1} = \tau_{MD}^{-1} - A_{tor}e^{-E_{tor}/RT} \quad (16)$$

this equation τ_{MD}' is the adjusted fluorescence decay time in methanol-*d*, at temperature T, τ_{MD} is the measured decay time, A_{tor} is the frequency factor, and E_{tor} is the activation energy for the torsional relaxation process in methylcyclohexane. The adjusted lifetimes are listed in Table II. Because of the much lower temperature sensitivity in methylcyclohexane this adjustment is a small one. The adjusted lifetimes can now be fitted by eq 15. The fit is shown in Figure 11. The measured activation energy is 16.2 kJ mol^{-1}, significantly different from the 29.8 kJ mol^{-1} found in methylcyclohexane. A reassuring feature of our analysis is that the total temperature-independent rate constant, k_0, is identical, within experimental error, for all three solvents.

Summary and Conclusion

Evidence has been presented which shows the mechanism of excited state intramolecular proton transfer in 3-hydroxyflavone to be dependent upon the solvent. It is suggested that in hydrocarbon solvents the proton transfer occurs across a pre-existing intramolecular hydrogen bond. This mechanism is characterized by a high activation energy, and a high value of the rate constant for proton transfer. In alcoholic solvents, the weak intramolecular hydrogen bond is disrupted, and excited state proton transfer occurs via a seven-membered chelate-ring encounter complex. This mechanism shows a considerably lower activation energy than that proposed for hydrocarbon solvents. The strict configurational requirements for the formation of the encounter complex are reflected in a low-frequency factor, and hence a lower value of the rate constant for proton transfer in this case. In a solid PMMA matrix, excitation of distinct ground state conformers is partly responsible for the observed fluorescence behavior. The interpretation of the results in PMMA is complex, but there appears to be evidence for the existence of "open-ring" and "closed-ring" ground state conformers analogous to those proposed for methyl salicylate and related systems.

It is stressed the for 3-hydroxyflavone in methanol, methanol-*d*, and hydrocarbon solvents all available evidence points to there being only a single gound state conformer. In these solvents both emission bands arise subsequent to excitation of this conformer and their relative intensities are determined purely on kinetic grounds. This is in direct contrast to the situation in many earlier studies of molecules known to undergo excited state intramolecular proton transfer, and to the case of 3-hydroxyflavone in PMMA where the relative intensities of the emission bands are determined by the interplay of kinetic factors and the relative populations of ground state precursors excited.

Attempts have been made to examine the radiationless processes which contribute to the deactivation of the excited state tautomer. In methylcyclohexane the major radiationless transition appears to be associated with an internal molecular motion, probably torsional motion of the phenyl ring, in the excited tautomer. A different radiationless process appears to be primarily responsible for the shorter lifetimes observed in methanol-*d*. The exact nature of this process is yet to be determined.

Acknowledgment. Our thanks are extended to Mr. G. S. Bartlett for machining and polishing the rigid PMMA matrix. The financial assistance of the Australian Research Grants Committee is gratefully acknowledged.

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