other words, barring unusual steric effects in donor molecules, one can in the present instance measure the effects of alkyl substitution on the tendency of complex formation equally well in terms of variations in ΔG, ΔH or ΔS.

It is particularly intersting that as ΔH values become more negative, corresponding decreases in ΔS are observed. The simultaneous decrease of these terms may serve as an indication of physical restraints imposed upon the complex components as the strength of the bond between them increases. van de Stolpe[6], studying the formation of a number of such iodine complexes, reports values of $\Delta S_{25°C}$ which are all of the order of magnitude of 4 to 5 e.u. It is apparent from Table 7 that the ΔS values for iodine complex formation with monoalkylbenzenes show a definite downward trend as the number of alkyl substituents at the aromatic uncleus increases. In all cases, both ΔH and $T\cdot\Delta S$ terms have an appreciable influence on the magnitude of ΔG for complex formation. The changes in ΔG with increasing alkyl groups at the benzene ring are consistent with the anticipated electronic influences[15,16] of alkyl substituents on the π-electron density of the donor nucleus.

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Fluorescence of Phototautomeric Lumichrome in Pyridine–Dioxane

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Lumichrome (7,8-dimethylalloxazine) exhibits two fluorescence emission maxima at 440 and 540 nm in pyridine-dioxane. These emission band maxima are attributable to radiative decays from the excited states of lumichrome and its flavin tautomer, 7,8-dimethylisoalloxazine, respectively. The growth of the latter can be followed upon excitation of the former with a 2-nanosecond light pulse generated from the nitrogen plasma discharge lamp. The excited state tautomerism results from proton transfer from N-1 to N-10 position during the lifetime of the lumichrome singlet excited state. The rate depends on the concentration of general base, pyridine, and it is an order of magnitude slower than diffusion-controlled processes.

Introduction

In our previous reports[1,2], we described the phototautomerism of lumichrome (7,8-dimethylalloxazine, **1**, Scheme 1) in pyridine–dioxane and acetic acid–ethanol mixtures, as studied by fluorescence intensity and nanosecond time-resolved methods. Lumichrome exhibits two fluorescence emission maxima in these mixtures; for example, 440 and 540 nm, in pyridine–dioxane. The latter is due to the isoalloxazine tautomer (**2**, a flavin) formed in the excited state from the alloxazine **1** singlet during its lifetime as the result of pyridinecatalyzed transfer of proton from N-1 to N-10.[2-4]

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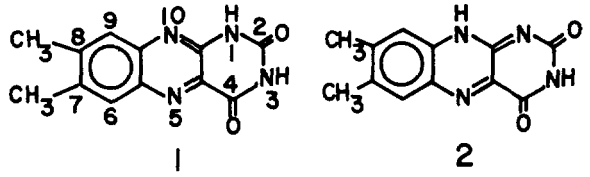
In the present paper, we report nanosecond time-resolved fluorescence measurements of the phototautomerism of **1** in pyridine-dioxane mixture in order to estimate the rate constant for the tautomeric reaction, which will be compared with the data extractable from steady state fluorescence measurements.

Materials and Methods

Lumichrome was obtained and purified as described previously,[2,5] and as a gift from Professor J. Koziol. Pyridine and *p*-dioxane, spectroquality, were obtained from Matheson, Coleman and Bell, and were used without further purification.

Steady state fluorescence measurements were carried out on a Perkin-Elmer MPF 44B spectrofluorometer. Fluorescence decays were measured on an SLM 480 subnanosecond phase-modulation fluorometer[3] and a nanosecond time-resolved spectrofluorometer constructed in this laboratory according to Badea and Georghiou[6], as described elsewhere.[1] Decay curves were deconvoluted by the phase-plane method.[7]

Nanosecond time-resolved spectra of lumichrome were also recorded on the time-resolved spectrofluorometer(boxcar averager) by using a free-running Optitron model NR-11 nitrogen discharge lamp with a repitition rate of 5KHz and FWHM of about 2nsec.[1]



Scheme 1.

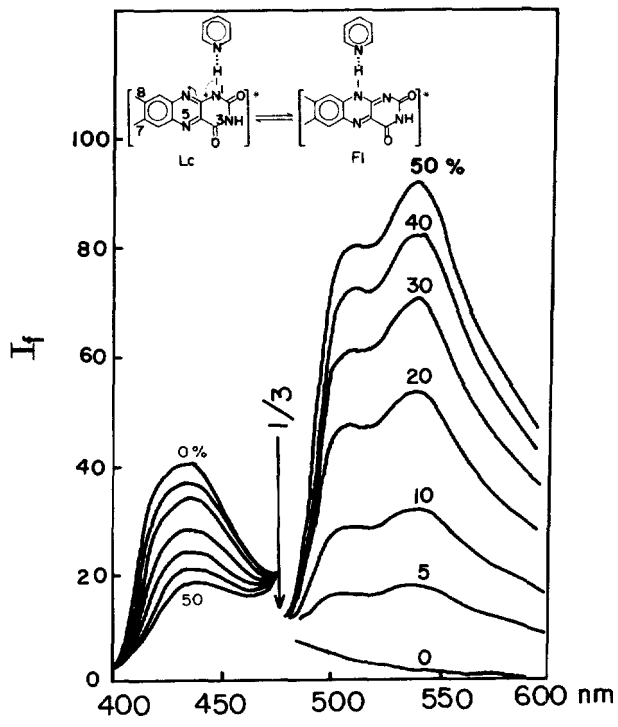


Figure 1. Corrected fluorescence spectra of lumichrome in dioxane as a function of pyridine concentration at 298 °K. Note that the sensitivity scale was reduced to 1/3 for the region of wavelength longer than 475 nm. The emission maxima at 440 and 540 nm are due to the tautomeric equilibrium between two species shown at the top inset: Lc, lumichrome 1; Fl, flavin 2. ordinate I_f, flucrescence intensity in arbitrary unit. λ_{ex} = 385 nm.

Results

Series of fluorescence spectra of the lumichrome (**1**, Lc) solution recorded as a function of pyridine concentration are displayed in Figure 1. At 0% pyridine, **1** shows a typical fluorescence due to the emission from its excited state, Lc*. As pyridine concentration increases, the Lc fluorescence decreases while the emission with maximum at 540 nm grows. The latter is due to the flavin tautomer, **2**(Fl).[2] The rise and decay of the 440 nm fluorescence are indistinguishable for a reliable deconvolution, indicating that the lifetime of lumichrome fluorescence is less than 2 ns(Figure 2A and 2B). However, the 540 nm emission decays much slower than the 440 nm emission (curve a in Figure 2; monitored at 560 nm to minimize contamination of the lumichrome fluorescence), yielding

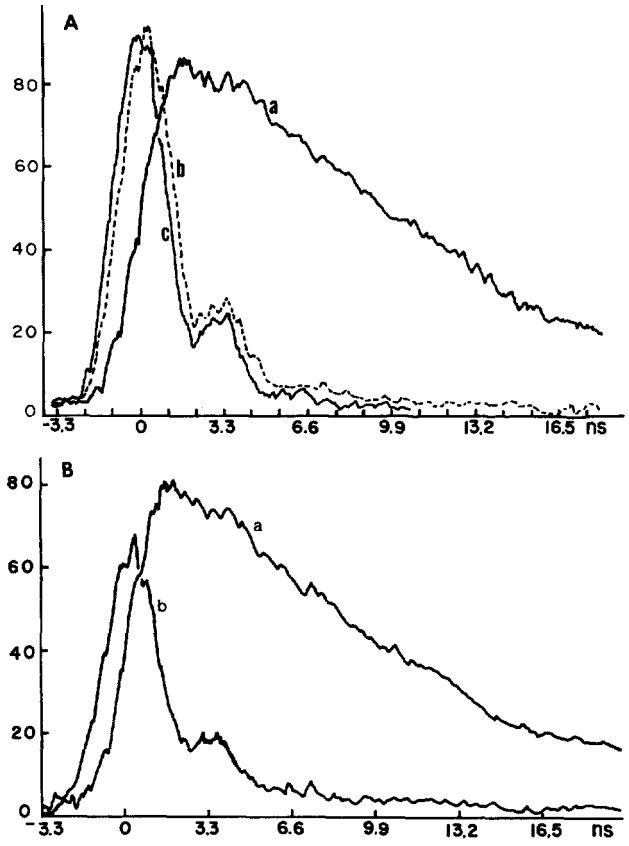


Figure 2. (A) The rise and decay curves for the fluorescence of lumichrome in 5% pyridine-dioxane at 298 °K. λ_{ex} = 349 nm. Cuver **a**, emission at 560 nm; **b**, emission at 440 nm; **c**, pulse excitation profile. (B) The rise and decay curves for the fluorescence of lumichrome in 20% pyridine-dioxane. λ_{ex} = 349 nm. Curve **a**, fluorescence at 560 nm; **b** fluorescence at 440 nm.

fluorescence lifetime of 10 ± 1 ns (phase shift value 8.8 ± 0.7 ns). The long fluorescence lifetime of the 540 nm emission is consistent with the emission being due to the flavin tautomer of lumichrome. As required, the corrected excitation spectrum of **1** with respect to both emissions was identical to the absorption spectrum of **1**, suggesting that the flavin tautomer with emission at 540 nm was formed during the lifetime of the lumichrome singlet excited state.

The growth of the long lifetime component(*i.e.* 540 nm emission) can be directly followed by time-resolved fluorescence measurements of the lumichrome solution in the presence of pyridine. Series of such time-resolved spectra are shown in Figure 3. In these spectra, the spectrum **a** was resolved by using pluse excitation of 1.1 ns prior to the maximum pulse intensity(*i.e.*, -1.1 ns), and the spectrum **b** was the result of scanning the fluorescence spectrum using the pulse max-

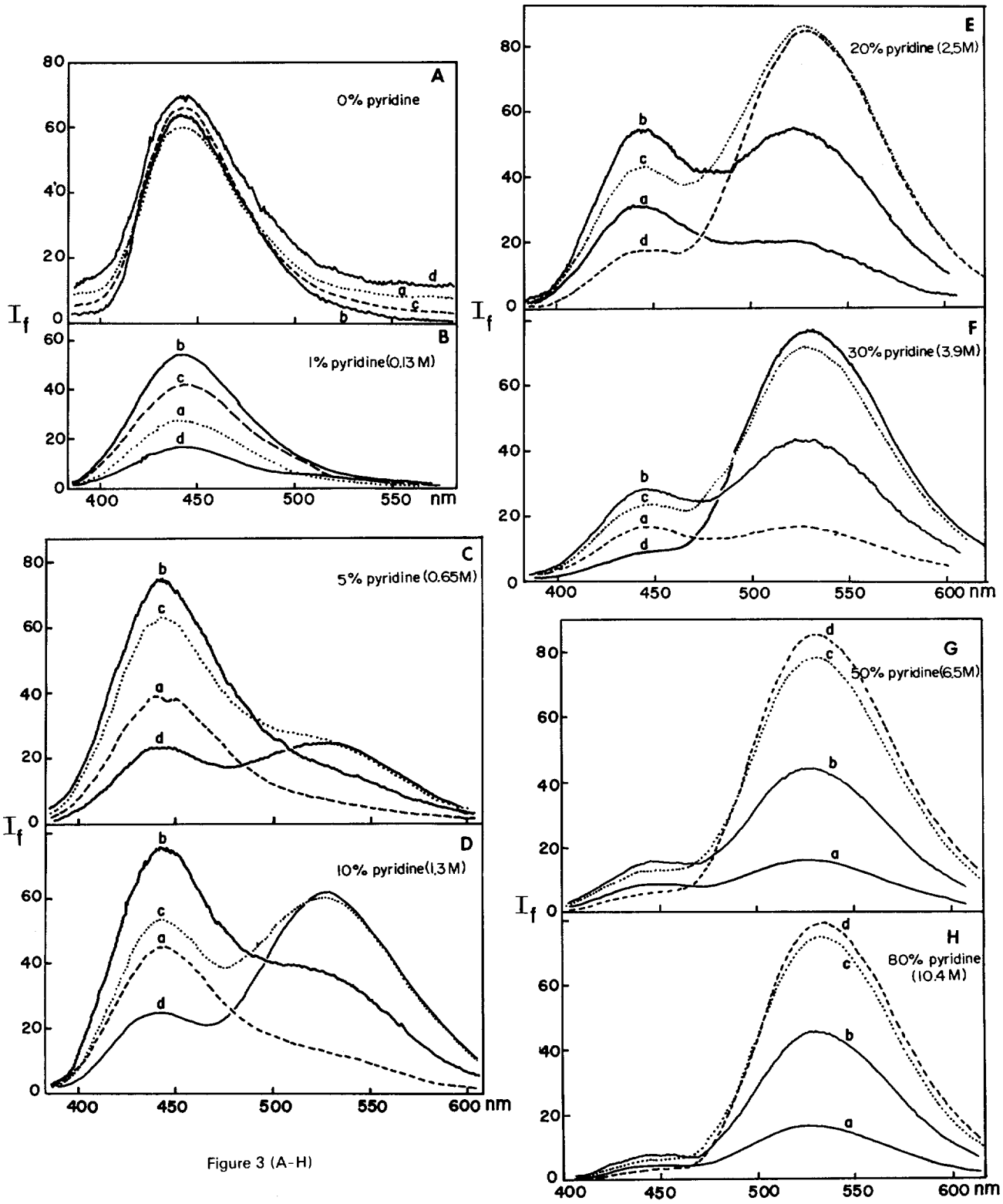


Figure 3 (A-H)

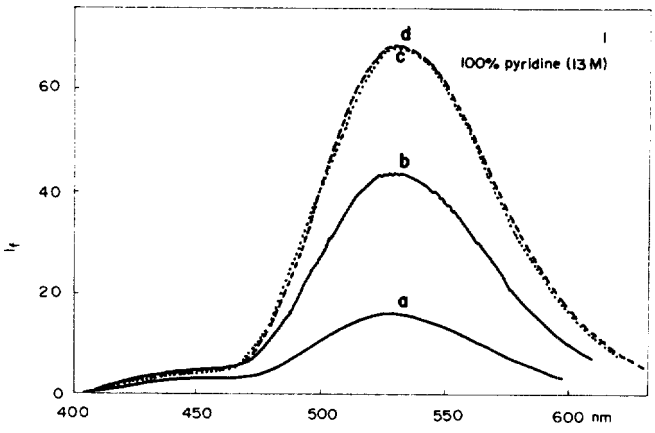


Figure 3. The time-resolved fluorescence spectra of lumichrome (absorbance at 385 nm = 0.8) in dioxane as a function of pyridine concentration at 298 °K. λ_{ex} = 349 nm. Curve **a**, -1.1 ns; **b**, 0 ns; **c**, 1.1 ns; **d**, 2.2 ns. Zero ns disignates pulse excitation maximum in the intensity-time profile of the Optitron NR-11 pulse radiator from nitrogen plasma discharge, and -1.1 and 1.1 ns designate 1.1 ns prior to and after the pulse excitation maximu, respectively. For each time-resolved spectrum shown, the pulse excitation intensity was not normalized. (A) 0% pyridine, (B) 1% pyridine (0.13 *M*), (C) 5% pyridine (0.65 *M*), (D) 10% pyridine (1.3 *M*), (E) 20% pyridine (2.5 *M*), (F) 30% pyridine (3.9 *M*), (G) 50% pyridine (6.5 *M*), (H) 80% pyridine (10.4 *M*), (I) 100% pyridine (13 *M*).

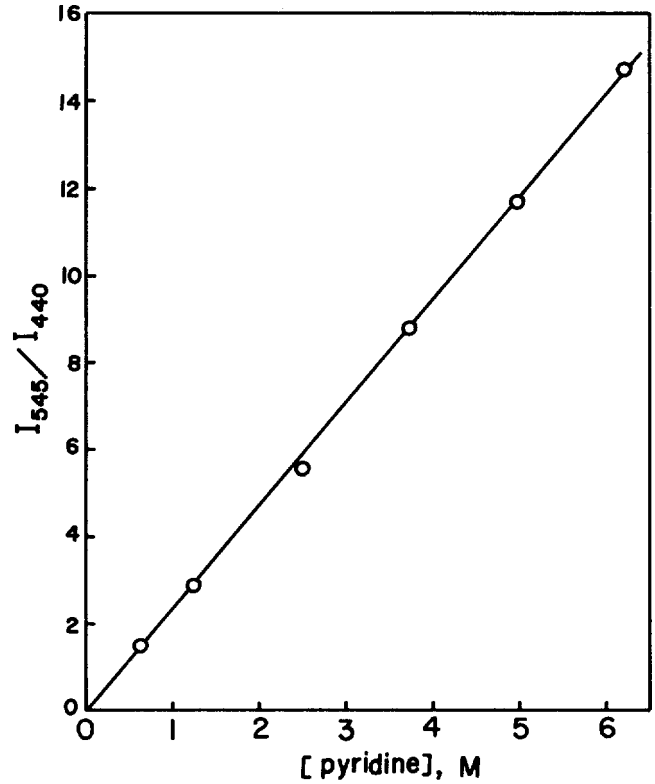


Figure 4. Ratios of the steady state fluorescence intensity (I_f) at 545 nm to I_f at 440 nm for lumichrome in dioxane at 298 °K as a function of pyridine concentration. λ_{ex} = 385 nm. Slope = 2.30 M^{-1}

imum(*i.e.* 0 ns) as the excitation source, and so on. Very little fluorescence at 540 nm evolved during 3 ns after pulse excitation of **1** in 0% and 1% pyridine-dioxane(Figure 3A and B),

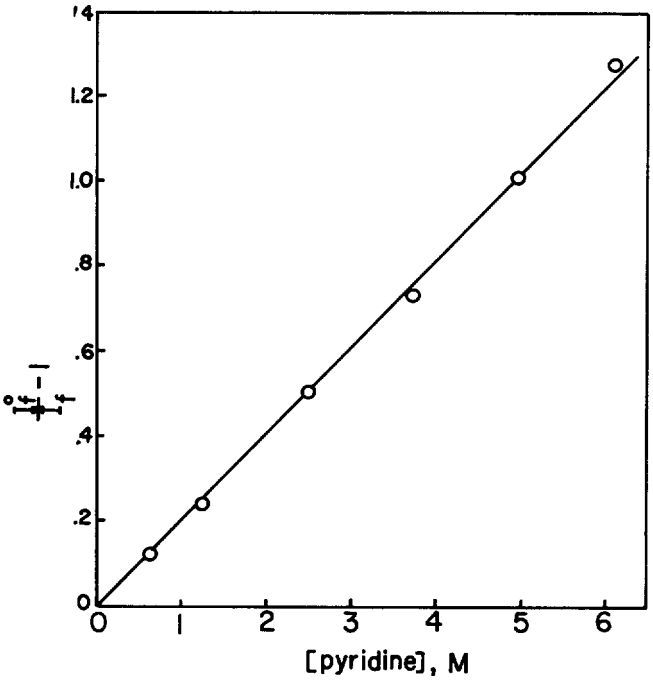


Figure 5. The Stern-Volmer plot for the lumichrome fluorescence at 440 nm in dioxane as a function of pyridine concentration at 298 °K. Slope (K_{SV}, Stern-Volmer quenching constant) = 0.2 M^{-1}.

in agreement with the steady state result shown in Figure 1. However, the 540 nm fluorescence grows faster with increasing pyridine concentrations and time, as shown in Figure 3C-I. In fact, at high concentrations of pyridine, the 540 nm emission becomes predominant(Figure 3G-I) and practically no emission from the excited state of lumichrome occurs at 440 nm in 100% pyridine(Figure 3I).

As shown in Figure 1 and 3, the 540 nm fluorescence emission increases in intensity with pyridine concentrations at the expense of the 440 nm fluorescence. Thus, the fluorescence intensity ratio, I_{545}/I_{440}, is linearly proportional to pyridine concentration(Figure 4), and quenching of the 440 nm emission follows the Stern-Volmer kinetics(Figure 5). From the slope of plot shown in Figure 5, the quenching constant of $K_{SV} = 0.2\ M^{-1}$ can be calculated.

Discussion

Lumichrome exhibits fluorescence emission maximum at 440 nm in dioxane. In the absence of any added pyridine, the lumichrome fluorescence emission spectrum is mirror image to the long wavelength absorption spectrum, suggesting that no emitting species other than the excited state lumichrome itself is formed in pure dioxane. However, in the presence of pyridine, the absorption spectrum of **1** remains unchanged, but a yellow fluorescence evolves with maximum at 540 nm(Figure 1, and 3). The 540 nm emission is due to fluorescence from the excited state of isoalloxazine tautomer **2**, which is formed in equilibrium with the lumichrome excited state during less than 2 nanosecond lifetime of the latter.[1] In addition, the Stern-Volmer plot of the 440 nm fluorescence as

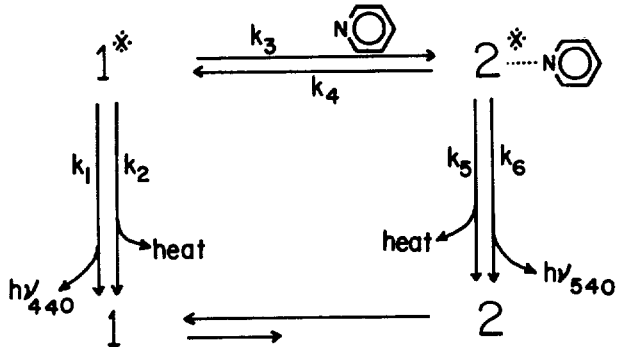
a function of pyridine concentration in dioxane exhibits a straight line(Figure 5), as the lumichrome fluorescence at 440 nm is dynamically quenched producing the flavin tautomer fluorescence at 540 nm(Figure 4) as a result of proton transfer from N-1 to N-10 position catalyzed by the quencher, pyridine. In the range of concentration of pyridine less than 3 M in dioxane, ground state complexes between **1** and pyridine may be disregarded for yielding **2** in the ground state, in contrast to the formation of anionic species of **1** and **2** in strongly alkaline solutions of alloxazines.[4] A log-log plot of fluorescence quantum yield ratio, ϕ_1/ϕ_2, versus pyridine concentration, according to Birks[8], yielded a straight line, with slope equal to 1.03. This result is consistent with a 1:1 stoichiometry for the quenching(and catalysis) reaction of the lumichrome excited state by pyridine.

The above results can be represented in terms of the reactions of lumichrome in its ground and excited states shown in Scheme 2. An approximate value of the rate constant, k_3 can be calculated from the slope of plot shown in Figure 4 and by knowing the fluorescence lifetime(equal to the reciprocal of $k_1+k_2+k_3$) of 1.2 ns in dioxane; $k_3=3.3\times10^8 M^{-1}s^{-1}$. The reverse rate constant, k_4, can also be calculated from the slope of plot shown in Figure 5; $k_4=6.9\times10^7 s^{-1}$.

The forward rate constant, k_3, in the Scheme 2 can also be estimated from the nanosecond time-resolved spectral data on the basis of the following derivative-intensity expression[9]:

$$\frac{\Delta I_{540}/\Delta t}{I_{540}} + \frac{1}{\tau_{540}} \cdot \frac{I_{540}}{I_{440}} = k_3 \text{ (pyridine)}$$

where $\Delta I_{540}=I_{540}(t_2, \text{ns})-I_{540}(t_1, \text{ns})$ represents fluorescence(540 nm) intensity changes in the time-resolved spectra(*e.g.*, Figure 3) between two time periods (t_1, t_2). I_{540} and I_{440} are fluorescence intensities normalized to the constant excitation pulse intensity at 540 and 440 nm, respectively, Δt is time interval(t_2-t_1) in nanosecond, and τ_{540} is the fluorescence lifetime at 540 nm which is due to the decay of **2*** in dioxane. In calculating the rate constant k_3 from the above expression, it is assumed that the proportionality factor[9] for the conversion of I_{540} and I_{440} to the respective concentrations, (**2***) and (**1***), is unity. Plotting the left hand side of the above equation as a function of pyridine concentration yielded slope equal to



Scheme 2.

$k_3=4.5\times10^8 M^{-1}s^{-1}$, which compares closely with the steady state value, $3.3\times10^8 M^{-1}s^{-1}$, *vide supra*. The difference between the two values may well be due to experimental errors including approximation on the intensity-concentration proportionality factor and lifetime of **2*** at 540 nm. The rate constant(k_3) for proton transfer in the tautomeric reaction **1*** → **2*** in pyridine is also close to the time-resolved value obtained in acetic acid-ethanol mixture in which the acid acts as a bifunctional catalyst[1,2], but it is an order of magnitude less than the diffusion-controlled rate constant, $k_{\text{diffusion}}=6\times10^9 M^{-1}s^{-1}$, under our experimental conditons, *i.e.* room temperature and dioxane as solvent.

Although we have not deconvoluted the rise time of **2*** in the excitation profile of lumichrome (**1**) in the presence of pyridine, both steady state and time-resolved values of K_3 are consistent with the fact that the rise time of **2*** is apparently slower than that of the excitation pulse itself, as can be seen from Figuer 2.

In conclusion, we suggest that the excited lumichrome, **1***, undergoes a tautomeric proton shift from N-1 to N-10 position, producing the excited flavin tautomer, **2***, catalyzed by pyridine acting as the general base. Both steady state and nanosecond time-resolved fluorescence studies yielded rate constant of $3\text{-}4\times10^8 M^{-1}s^{-1}$ for the phototautomeric proton shift. This rate constant is an order of magnitude lower than diffusion-controlled processes.

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