Proton Transfer Dynamics of Norharman in Organic Solvents

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Excited-state reactions involved in the fluorescence of norharman have been investigated in different binary mixtures of acetic acid with dichloromethane, dioxane, and benzene. Time-resolved fluorescence measurements have revealed the existence of a new species which has not been observed from the steady-state spectra. The complex decay kinetics of norharman and the interactions with the surrounding environment are discussed in detail.

1. Introduction

Owing to its great importance in chemistry and biology, excited-state proton transfer is a subject of intense research.[1−6] It is well-established that the ionization constants of organic bases and acids in the electronically excited state differ by several orders of magnitude from those observed in the ground state.[7−9] On the other hand, it is also well-known that these acid−basic properties vary as a function of solvent characteristics.

Norharman (Figure 1) presents interesting photophysical properties. It has been proposed as a fluorescence standard[10,11] and as a photosensitizer of biological interest.[12] In the ground state the electronic charge density is highest in the midplane of the molecule—the pyrrole ring. Upon excitation to S_1, the electronic charge density migrates to both ends of the molecule, especially to the pyridinic nitrogen.[13]

In a previous study,[14] we reported that the photophysical photochemical properties of norharman are strongly influenced by the solvent. In this paper, we studied proton transfer spectroscopy of this compound in dichloromethane (2CM), chloroform (3CM), carbon tetrachloride (4CM), ethanol, and acetonitrile in the presence of different amounts of acetic acid (AcH). For 2CM or 3CM and in the interval of AcH concentrations between 0.25 and 5%, neutral and cationic species absorb and four different species fluoresce. Thus, when the neutral form is excited, three species emit: neutral (λ_{em} = 360 nm), zwitterion (λ_{em} = 500 nm), and a novel species with fluorescence maximum around 400 nm. This last fluorescence had not been observed up to now. When the cationic form is excited, two species emit: cation (λ_{em} = 450 nm) and zwitterion. Similar behavior was observed in 2CM−formic acid mixtures. However, only the neutral and cationic species fluoresce in 2CM−H_2SO_4 mixtures.

On the contrary, in ethanol or acetonitrile solutions[14] for different AcH concentrations, only the fluorescence of neutral and cationic forms were recorded—no excited state proton transfer reactions were observed in these media.

In aqueous solution, norharman shows an interesting proton transfer spectroscopy.[15] At the interval between pH = 1 and 10, only the cationic species emits. At pH = 12.3, the neutral species only absorbs and three species emits: neutral, cationic, and zwitterionic. The fluorescence lifetimes (τ) of these species are τ_N < 300 ps, τ_C = 22 ns, and τ_Z = 1.6 ns, respectively. However, no proton transfer in the S_1 state was observed in pure ethanolic solutions of norharman.[16,17] In pure methanol solutions, an equilibrium between neutral−cation and neutral−zwitterion was observed in the S_1 state.[12]

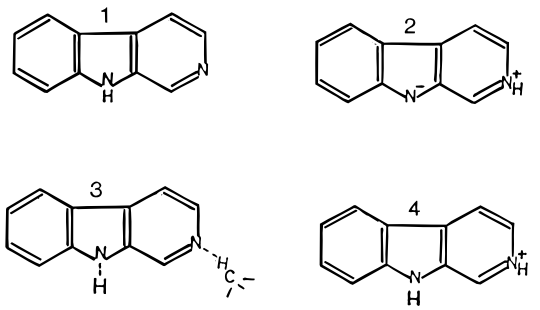

Figure 1. Chemical structures of norharman detected in S_1 state in AcH−dichloromethane mixtures: 1, neutral form; 2, zwitterionic form; 3, form with fluorescence maximum around 400 nm; 4, cationic form.

In this paper, we present a detailed study of the dynamics of excited state proton transfer reactions of norharman in 2CM−AcH mixtures. In order to explain the complex behavior for this system, we have applied nanosecond fluorescence decay measurements. These have revealed the existence of a new species which had not been observed from the steady state spectra. In order to know the role of the solvent in this system, we have obtained the steady state fluorescence of norharman in dioxane−AcH and benzene−AcH mixtures.

The photophysical properties of norharman that we have presented in a previous work,[14] together with the present results, suggest that this compound could be used as a fluorescence probe in biological systems and for the study of solvation dynamics.

2. Experimental Section

Norharman was purchased from SIGMA-Chemie. This alkaloid, of the best available quality, was used as received. Glacial acetic acid, uvasol grade 2CM, and benzene were used as received. Dioxane (uvasol grade) was dried using 4 Å molecular sieves and distillation.

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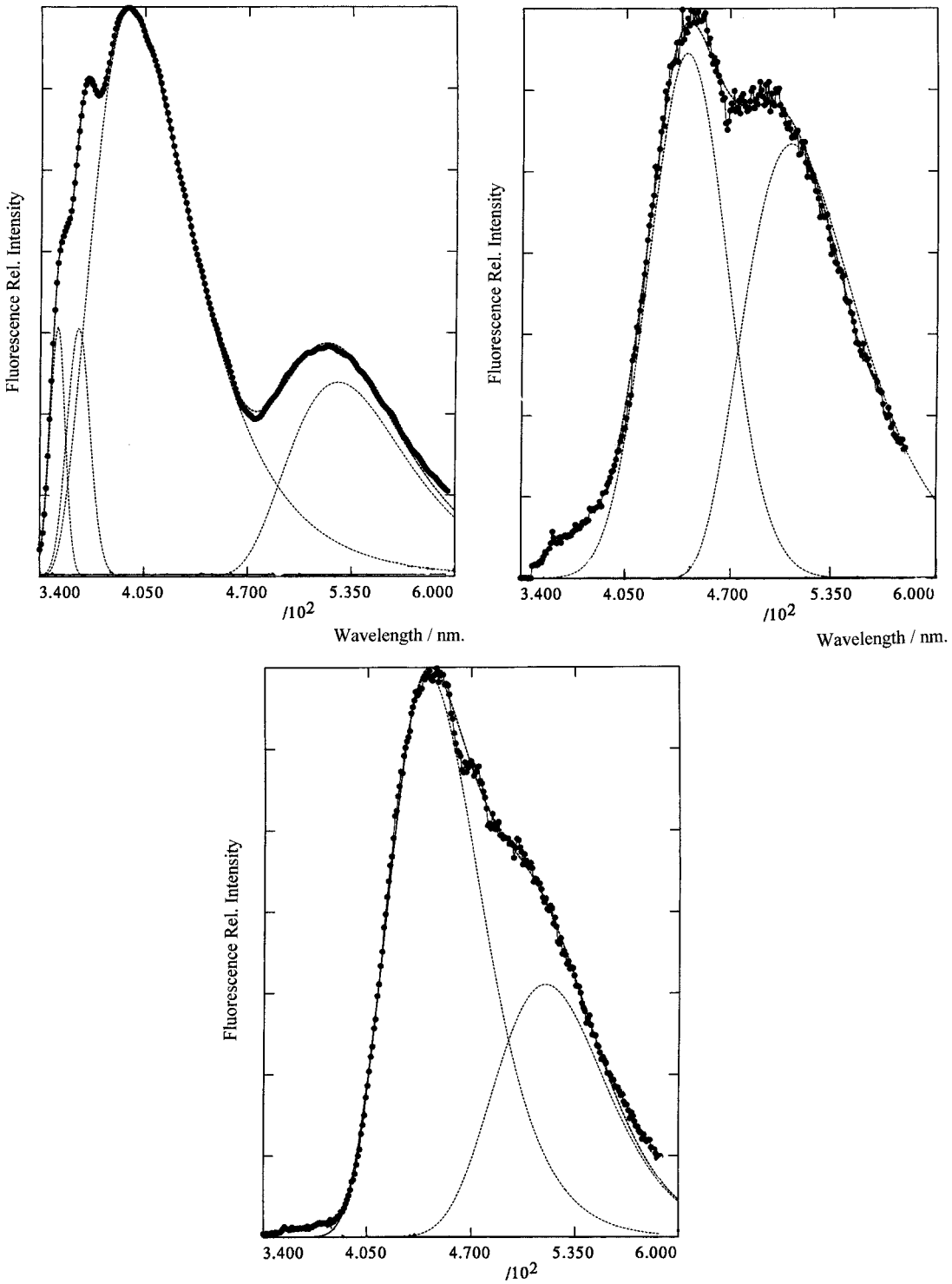


Figure 2. Fluorescence emission spectra of norharman for different %AcH−dichloromethane mixtures: A, top left, 0.5% AcH; B, top right, 5% AcH; C, bottom, 20% AcH. $\lambda_{exc} = 320$ nm. These two last solutions showed similar profiles with $\lambda_{exc} = 360$ nm. ●, Fluorescence spectra; - -, individual components (bands) of the fluorescence profile.

The solutions of norharman were prepared by setting the concentration around 10^{-4} M. Absorption spectra were obtained using a Cary-17 spectrophotometer.

The steady state and time-resolved emission were recorded with the same electronic equipment. Fluorescence spectra were not corrected.

Fluorescence decays were obtained using the time-correlated single-photon-counting method (Edinburg Analytical Instruments). The excitation source was a hydrogen nanosecond flash lamp: repetition rate of 40 kHz and excitation pulse width of less than 1 ns. The excitation wavelengths were chosen in order to excite neutral or cation species of norharman.

Fluorescence decays were analyzed with the method of nonlinear least-squares iterative reconvolution, and the quality of the fits were judged by the value of the reduced chi-square (χ^2) and the autocorrelation function of the residuals.

All experiments were obtained at 25.0 °C.

Resolution of complex fluorescence profiles of norharman into their individual components has been performed by using

TABLE 1: Decay Parameters, at Different Emission Wavelengths and AcH Percent, of Norharman in Dichloromethane Solutions (λ_{exc} = 320 nm)[a]

	λ_{em} = 355 nm		λ_{em} = 400 nm		λ_{em} = 540 nm	
AcH, %	*a*	τ, ns	*a*	τ, ns	*a*	τ, ns
0	$a_1 = 0.42$	$\tau_1 = 2.4$	$a_1 = 0.40$	$\tau_1 = 2.5$		
	$\chi^2 = 0.75$		$\chi^2 = 0.90$			
0.25	$a_1 = 0.53$	$\tau_1 = 1.9$	$a_1 = 0.56$	$\tau_1 = 1.8$	$a_1 = -0.10$	$\tau_1 = 1.2$
	$\chi^2 = 0.75$		$\chi^2 = 0.65$		$a_2 = 0.11$	$\tau_2 = 3.7$
					$\chi^2 = 1.05$	
0.5	$a_1 = 0.63$	$\tau_1 = 1.6$	$a_1 = 0.71$	$\tau_1 = 1.4$	$a_1 = -0.08$	$\tau_1 = 1.0$
	$\chi^2 = 0.93$		$\chi^2 = 1.18$		$a_2 = 0.10$	$\tau_2 = 3.5$
					$\chi^2 = 1.04$	
2	$a_1 = 1.00$	$\tau_1 = 1.0$	$a_1 = 1.15$	$\tau_1 = 0.8$	$a_1 = -0.50$	$\tau_1 = 0.4$
	$\chi^2 = 1.15$		$a_2 = 0.01$	$\tau_2 = 14.8$	$a_2 = 0.90$	$\tau_2 = 3.5$
			$\chi^2 = 1.30$		$a_3 = 0.005$	$\tau_3 = 21.0$
					$\chi^2 = 1.06$	

[a] Parameters: a = amplitude; τ = decay time.

TABLE 2: Decay Parameters, at Two Emission Wavelengths and Different AcH Percent, of Norharman in Dichloromethane Solutions (λ_{exc} = 360 nm)[a]

	λ_{em} = 450 nm			λ_{em} = 540 nm		
AcH, %	a	τ, ns	% tot. emissn	a	τ, ns	% tot. emissn
0.25%	$a_1 = 0.10$	$\tau_1 = 1.5$	47	$a_1 = -0.30$	$\tau_1 = 1.4$	17
	$a_2 = 0.01$	$\tau_2 = 17.1$	53	$a_2 = 0.60$	$\tau_2 = 3.3$	80
		$\chi^2 = 1.10$		$a_3 = 0.005$	$\tau_3 = 17.8$	3
					$\chi^2 = 1.06$	
0.5%	$a_1 = 0.12$	$\tau_1 = 1.2$	45	$a_1 = -0.30$	$\tau_1 = 0.8$	8
	$a_2 = 0.01$	$\tau_2 = 17.6$	55	$a_2 = 0.80$	$\tau_2 = 3.2$	88
		$\chi^2 = 1.08$		$a_3 = 0.005$	$\tau_3 = 19.8$	4
					$\chi^2 = 1.03$	
2%	$a_1 = 0.33$	$\tau_1 = 0.4$	22	$a_1 = 0.13$	$\tau_1 = 3.4$	88
	$a_2 = 0.04$	$\tau_2 = 2.1$	14	$a_2 = 0.003$	$\tau_2 = 19.1$	12
	$a_3 = 0.02$	$\tau_3 = 19.0$	64		$\chi^2 = 1.21$	
		$\chi^2 = 1.17$				
5%	$a_1 = 0.45$	$\tau_1 = 0.3$	21	$a_1 = 0.23$	$\tau_1 = 3.8$	89
	$a_2 = 0.04$	$\tau_2 = 3.3$	20	$a_2 = 0.007$	$\tau_2 = 16.6$	11
	$a_3 = 0.02$	$\tau_3 = 19.1$	59		$\chi^2 = 1.10$	
		$\chi^2 = 1.05$				
10%	$a_1 = 0.18$	$\tau_1 = 0.7$	23	$a_1 = 0.19$	$\tau_1 = 4.7$	90
	$a_2 = 0.05$	$\tau_2 = 4.7$	43	$a_2 = 0.005$	$\tau_2 = 21.6$	10
	$a_3 = 0.01$	$\tau_3 = 18.8$	34		$\chi^2 = 1.19$	
		$\chi^2 = 1.12$				
20%	$a_1 = 0.96$	$\tau_1 = 0.1$	13	$a_1 = 0.15$	$\tau_1 = 6.0$	92
	$a_2 = 0.06$	$\tau_2 = 5.2$	42	$a_2 = 0.004$	$\tau_2 = 22.0$	8
	$a_3 = 0.02$	$\tau_3 = 17.1$	45		$\chi^2 = 1.21$	
		$\chi^2 = 1.10$				

[a] Parameters: a = amplitude; τ = decay time.

the fitting program described in refs 18 and 19. A log-normal fitting function was used to represent the fluorescence bands.

3. Results and Discussion

We have selected seven different 2CM−AcH mixtures in order to obtain their fluorescence decay at different wavelengths of emission. These mixtures are 0, 0.25, 0.5, 2, 5, 10, and 20% of AcH in 2CM. Figure 2 shows some steady state fluorescence spectra of norharman in 2CM−AcH mixtures. Each spectrum has been decomposed into different fluorescence bands in order to get the emission wavelength where only one species fluoresces.

Exciting at 320 nm the mixtures of 0, 0.25, and 0.5% AcH concentrations in 2CM, we can consider that we are mainly pumping the neutral species. Nevertheless, for 2% AcH in 2CM and this wavelength, we cannot exclude the possibility of cation absorbance. In these conditions, three different species emit (Figure 2A) and we have collected the nanosecond fluorescence decays at 355, 400, and 540 nm.

We have also excited at 360 nm all solutions (where only the cationic form absorbs), and we have recorded at 450 and 540 nm, where cation and zwitterion emit, respectively (Figure 2B,C).

The analysis of the decay curves appears to be extremely sensitive to nonrandom noise. We have considered the autocorrelation function of the residuals in order to fit the fluorescence decay with one, two, or three exponential functions (Tables 1 and 2).

Exciting at 320 nm and recording at 355 nm, only the neutral form emits (Figure 2A). The fluorescence decay fits to a monoexponential function in all solutions, and the fluorescence lifetime of the neutral species (τ_N) decreases with AcH concentration (Table 1).

Exciting at 320 nm and recording at 400 nm, two species emit: neutral and another species (Figure 2A) assigned to a phototautomer in a previous work.[14] With AcH concentrations of 0, 0.25, and 0.5%, the fluorescence decay is also a monoexponential with approximately the same lifetime as that at 355 nm (Table 1). For 2% AcH concentration, the fluorescence decay is a biexponential with lifetimes 0.8 and 14.8 ns.

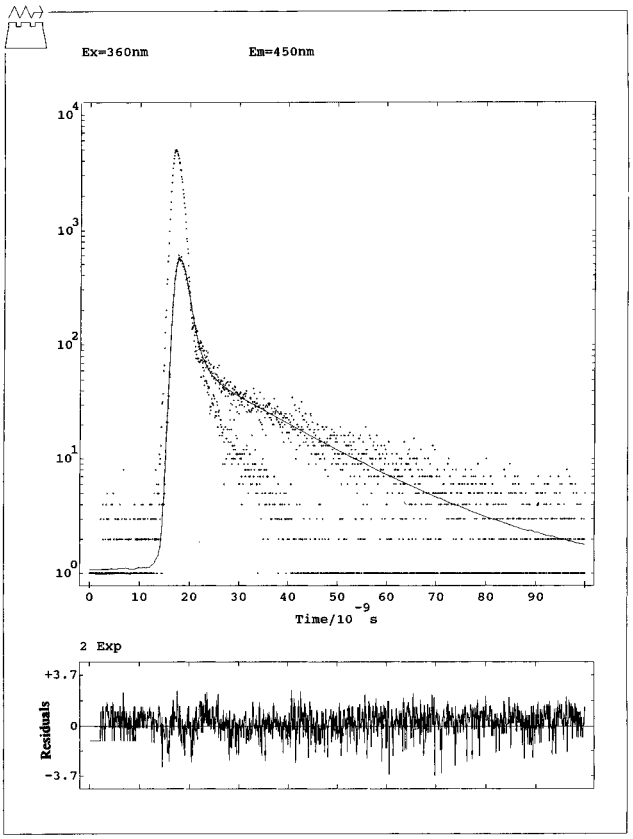


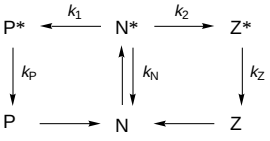
Figure 3. Excitation pulse profile and fluorescence decay and residuals plots of norharman in dichloromethene containing 0.5% acetic acid. The excitation and emission wavelengths are 360 and 450 nm, respectively.

The long component corresponds to the cation (τ_C) since we are also exciting this species.

Exciting at 320 nm and recording at 540 nm, only the zwitterion emits (Figure 2A). Up to 0.5% AcH concentration, the fluorescence decay is a biexponential. For the solution at 2% AcH, the fluorescence decay is a triple-exponential with lifetime values of 0.4, 3.6, and 25.0 ns (Table 1). The longest lifetime corresponds to τ_C.

From the steady state fluorescence spectra, we can consider two posibilities when exciting the neutral form: N* → P* and N* → Z* or N* → P* → Z*, where N*, P*, and Z* represent the neutral form, the species with fluorescence around 400 nm, and the zwitterion in S_1 state, respectively. Nevertheless, from the results of Table 1, P* is not an intermediate to yield Z* from N*, if this occurs, the fluorescence decay of Z* should be a triple-exponential and only a biexponential decay was obtained. Thus, we have studied the following mechanism of reaction:

SCHEME 1



where an asterisk indicates the lowest excited singlet state of $\pi\pi^*$. k_P, k_N, and k_Z are rate constants which includes both radiative and nonradiative depopulation mechanisms for P*, N*, and Z*, respectively.

The following kinetic equations describe the system represented in Scheme 1.

$$-\mathrm{d[N^*]}/\mathrm{d}t = (k_N + k_1 + k_2)[\mathrm{N^*}] \quad (1)$$

$$\mathrm{d[P^*]}/\mathrm{d}t = k_1[\mathrm{N^*}] - k_P[\mathrm{P^*}] \quad (2)$$

$$\mathrm{d[Z^*]}/\mathrm{d}t = k_2[\mathrm{N^*}] - k_Z[\mathrm{Z^*}] \quad (3)$$

Integration of eqs 1−3, subject to the initial boundary conditions $[\mathrm{N^*}] = [\mathrm{N^*}]_0$, $[\mathrm{P^*}] = 0$, and $[\mathrm{Z^*}] = 0$ at $t = 0$, yields

$$[\mathrm{N^*}] = [\mathrm{N^*}]_0 \mathrm{e}^{-kt} \quad (4)$$

$$[\mathrm{P^*}] = k_1[\mathrm{N^*}]_0/(k_P - k)[\mathrm{e}^{-kt} - \mathrm{e}^{-k_P t}] \quad (5)$$

$$[\mathrm{Z^*}] = k_2[\mathrm{N^*}]_0/(k_Z - k)[\mathrm{e}^{-kt} - \mathrm{e}^{-k_Z t}] \quad (6)$$

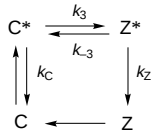
where $k = k_N + k_1 + k_2$, $\tau_N = 1/k$, $\tau_P = 1/k_P$, and $\tau_Z = 1/k_Z$.

Thus, we excluded the equilibria P* ⇄ N* and N* ⇄ Z* because, for all AcH concentrations, we have obtained a monoexponential decay for the fluorescence of neutral species.

Recording at 400 nm, we have observed a single decay with the same lifetime as the neutral species, when a biexponential decay was expected from eq 5. We can explain this behavior by considering $k = k_P$. Nevertheless, the monoexponential decay for P* can also be explained whether we consider a rapid equilibrium N* ⇄ P*, where k_1 and k_{-1} are the rate constants of the direct and inverse steps, respectively. In this case, (k_1 and k_{-1}) ≫ (k_N, k_P, and k_2) leading to $\tau_N = 1/(k_1 + k_{-1})$. Therefore τ_N should have a value below the resolution time of the used instrumental device. The measured value for τ_N is around 1 ns, suggesting $\tau_N = \tau_P$.

When we excite at 360 nm, the steady state fluorescence spectra show only two maxima around 450 and 510 nm for all solutions studied (Figure 2B). From these spectra we can consider the following kinetic scheme for two species which are coupled in the excited state:

SCHEME 2



where C represents the cationic form and Z the zwitterionic one. An asterisk indicates the lowest excited singlet state of $\pi\pi^*$. k_C and k_Z are rate constants which include both radiative and nonradiative depopulation mechanisms for C* and Z*, respectively.

The following kinetic equations describe the system represented in Scheme 2:

$$-\mathrm{d[C^*]}/\mathrm{d}t = (k_C + k_3)[\mathrm{C^*}] - k_{-3}[\mathrm{Z^*}] \quad (7)$$

$$\mathrm{d[Z^*]}/\mathrm{d}t = -(k_Z + k_{-3})[\mathrm{Z^*}] + k_3[\mathrm{C^*}] \quad (8)$$

The solution to these kinetic equations has been described previously.[1,7] Integration of eqs 7 and 8, subject to the initial boundary conditions $[\mathrm{C^*}] = [\mathrm{C^*}]_0$, $[\mathrm{Z^*}] = 0$ at $t = 0$, yields

$$[\mathrm{C^*}] = [\mathrm{C^*}]_0/(\gamma_2 - \gamma_1)[(\gamma_2 - X)\mathrm{e}^{-\gamma_1 t} + (X - \gamma_1)\mathrm{e}^{-\gamma_2 t}] \quad (9)$$

$$[\mathrm{Z^*}] = k_3[\mathrm{C^*}]_0/(\gamma_2 - \gamma_1)[\mathrm{e}^{-\gamma_1 t} - \mathrm{e}^{-\gamma_2 t}] \quad (10)$$

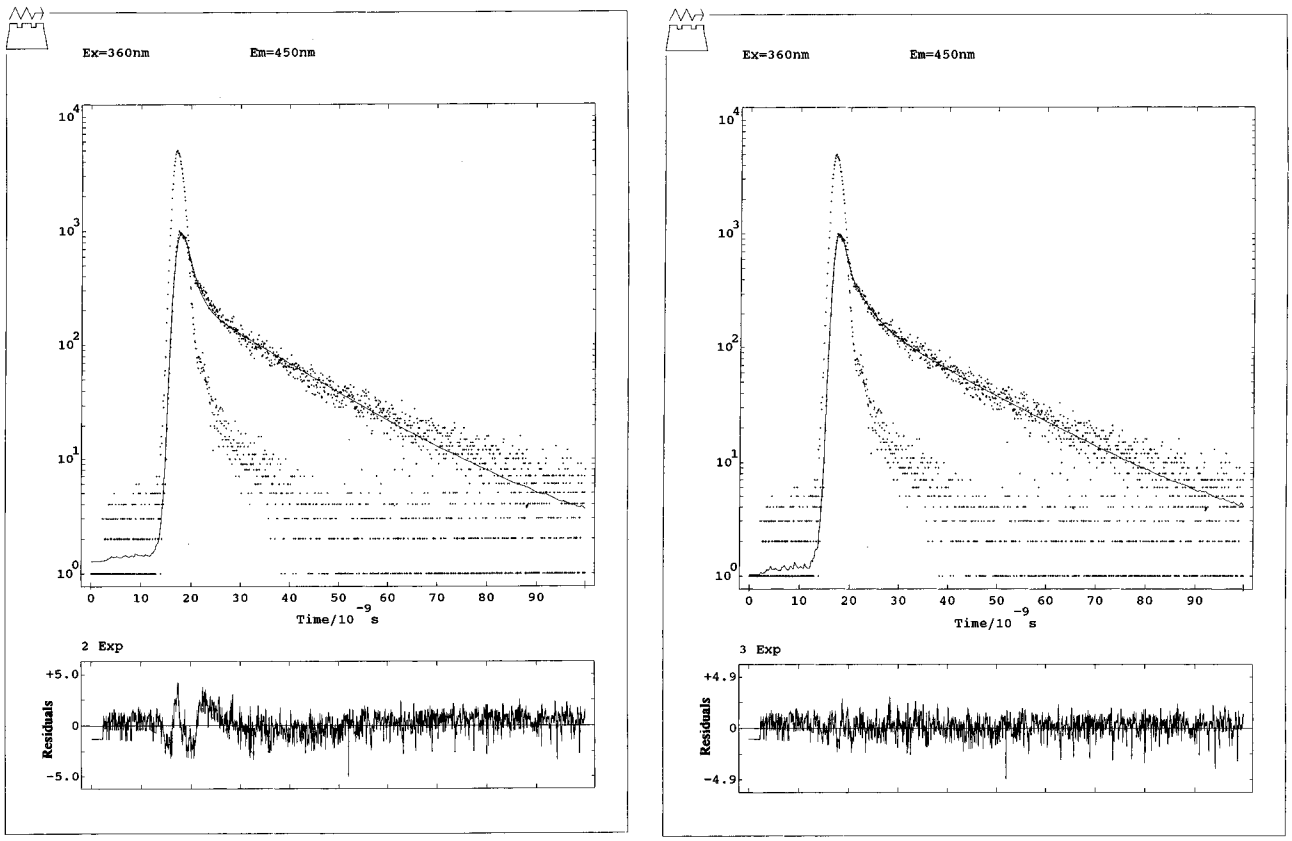


Figure 4. Excitation pulse profile, fluorescence decay, and and residuals plots of norharman in dichloromethene containing 5% acetic acid. The excitation and emission wavelengths are 360 and 450 nm, respectively. A, left, Decay curve analyzed for two components. Parameters obtained: $a_1 = 0.15$, $\tau_1 = 1.8$ ns (44% on total emission) and $a_2 = 0.02$, $\tau_2 = 16.5$ ns (56%), $\chi^2 = 1.19$ and bad residuals. B, right, Decay curve analyzed for three components. Parameters obtained: $a_1 = 0.45$, $\tau_1 = 0.3$ ns (21% on total emission), $a_2 = 0.04$, $\tau_2 = 3.3$ ns (20%), and $a_3 = 0.02$, $\tau_3 = 19.1$ ns (59%), $\chi^2 = 1.05$ and good residuals.

where $\gamma_{1,2} = {}^1/_2[(X + Y) \pm \{(Y - X)^2 + 4k_3k_{-3}\}^{1/2}]$, $X = k_C + k_3$, $Y = k_Z + k_{-3}$, $\gamma_1 = 1/\tau_1$, and $\gamma_2 = 1/\tau_2$.

From steady state fluorescence spectra, at 450 nm only the cation emits, and recording at 540 nm, only the zwitterion emits. According to eqs 9 and 10, we can predict a biexponential decay for C* when the process is reversible in the S_1 and a single exponential for C* when no back-reaction occurs. The decay from Z* shall be always biexponential and with preexponentials of different sign and same magnitude. Nevertheless, there are three different fluorescence lifetimes in Table 2. We can associate the fluorescence lifetime around 3.5 ns with zwitterion emission. This last fluorescence lifetime increases with the polarity of the solution (3.3, 4.7, and 6.0 ns for 0.25, 10, and 20% of AcH in 2CM, respectively). This result agrees with the 5.4 ns value measured in methanol by Dias et al.[12]

For the 0.25 and 0.5% AcH solutions, it is clear that the fluorescence decay is not a monoexponential (Figure 3). It is possible to obtain a good autocorrelation function of the residuals with a biexponential decay, and we got a short lifetime around 1.5 ns which decreases with AcH concentration and a long lifetime around 17 ns. Recording the zwitterion fluorescence ($\lambda_{em} = 540$ nm), the fluorescence decay fits to a triple-exponential with lifetimes around 1, 3, and 18 ns (Table 2).

From 2 to 20% of AcH in 2CM, and recording the cation emission, the fluorescence decay fits to a triexponential (Figure 4). The shortest lifetime (less than 1 ns) decreases with AcH concentration. On the contrary, the 3 ns component increases, while the longest lifetime value remains constant with AcH concentration (Table 2).

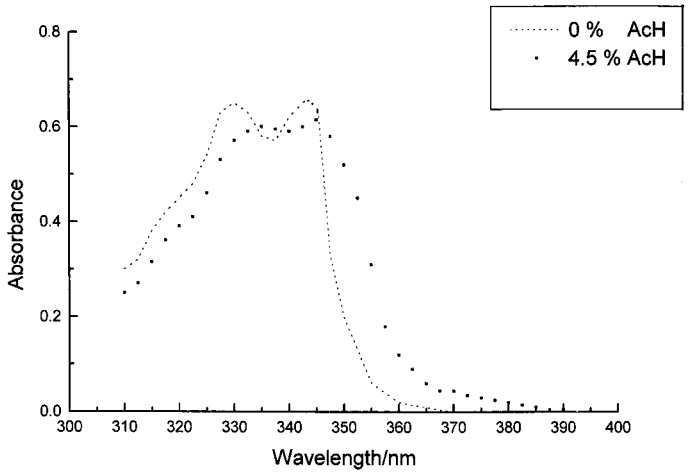


Figure 5. Absorption spectra of norharman in different AcH−dioxane mixtures.

From these results, it is clear that the system studied is more complex than the system represented in Scheme 2. We consider that the cationic species C* ($\tau_C \approx 17$ ns) does not yield Z* ($\tau_Z \approx 3.5$ ns) but that there is another form (with τ around 1 ns) that produces the zwitterion. We suggest that this one could be the cation with a different solvation shell. Hence, for 0.25 and 0.5% AcH concentrations in 2CM, where there is no back-reactions in the S_1 state, the decay at 450 nm is a biexponential due to the fluorescence of both cations. For 2−20% of AcH, the process is reversible and we observed a triple fluorecence decay.

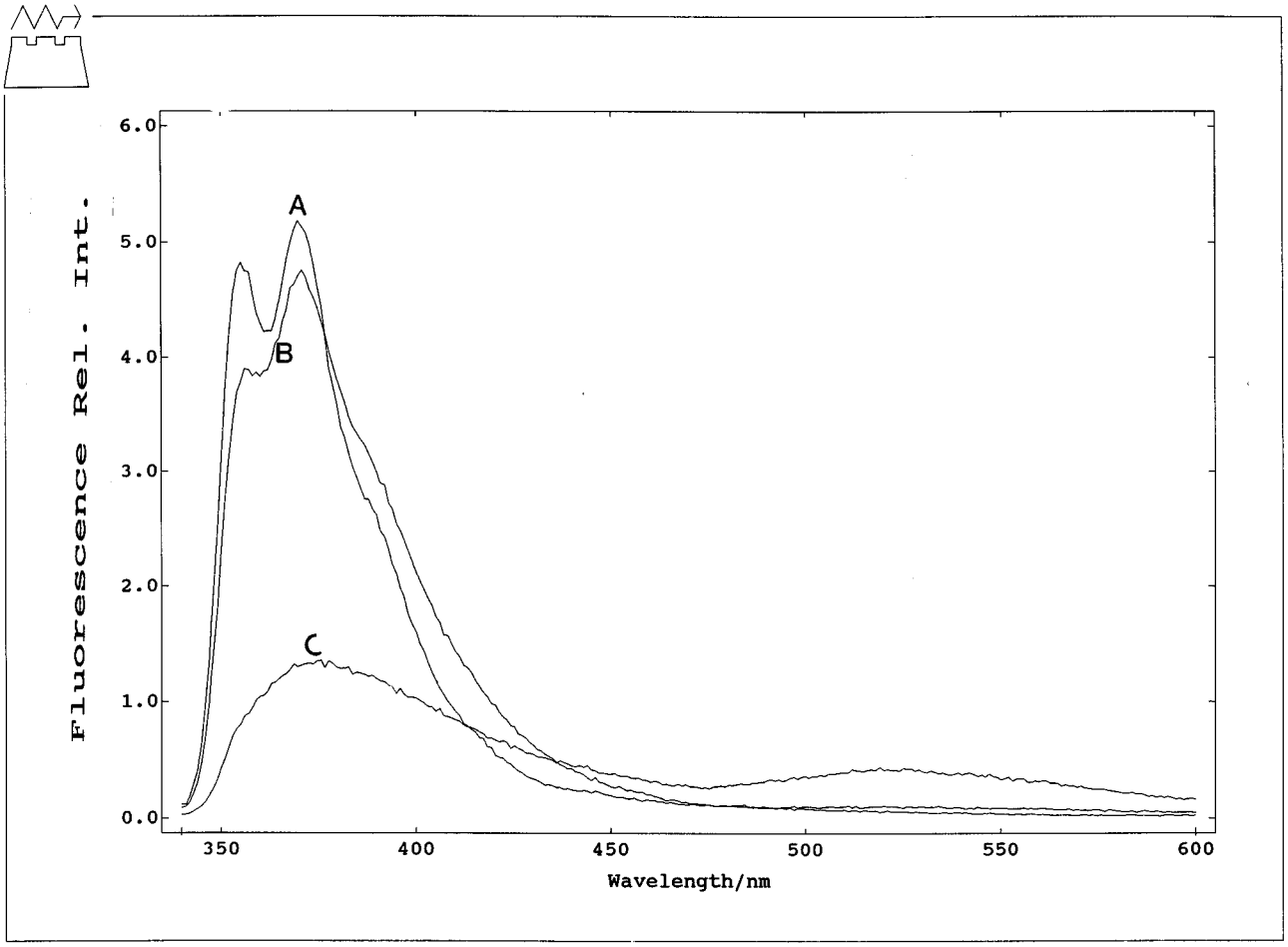


Figure 6. Fluorescence spectra of norharman in different AcH−dioxane mixtures: A, 0% AcH; B, 0.5% AcH; C, 4.5% AcH. Excitation wavelength 320 nm.

Recently,[14] we have associated the fluorescence maximum at 400 nm with the hydrogen bond formation between the C−H group of halomethane and the pyridinic nitrogen of norharman. On the other hand, this maximum and that at 510 nm appear with the presence of AcH in 2CM and disappear when AcH concentration is larger than 50%. Normally, we should observe only the maximum around 510 nm in pure AcH solutions. On the contrary, we have only observed the maximum at 450 nm (cation fluorescence). In order to explain this special behavior of norharman in 2CM−AcH mixtures and the interaction between 2CM and norharman, we have obtained the fluorescence spectra of this compound in binary mixtures of dioxane and benzene with AcH.

For 0, 0.5, and 4.5% AcH concentration in dioxane, the absorption spectra of norharman show a shoulder near 360 nm which corresponds to the cationic form absorbance (Figure 5). Thus, norharman is mainly in the neutral form in the ground state. Exciting these solutions at 320 nm, the fluorescence intensity of the neutral form decreases with the AcH concentration (Figure 6). The zwitterion fluorescence also appears in these mixtures, and nervertheless, these spectra do not present maxima at 400 nm.

For 0, 0.5, and 4.5% AcH concentration in benzene, the absorption spectra present an equilibrium between the neutral and the cation similar to those observed in 2CM−AcH mixtures (Figure 7). No 400 nm maximum was observed in the fluorescence spectra, and the zwitterion emission was predominant in contrast with the cation emission (Figure 8).

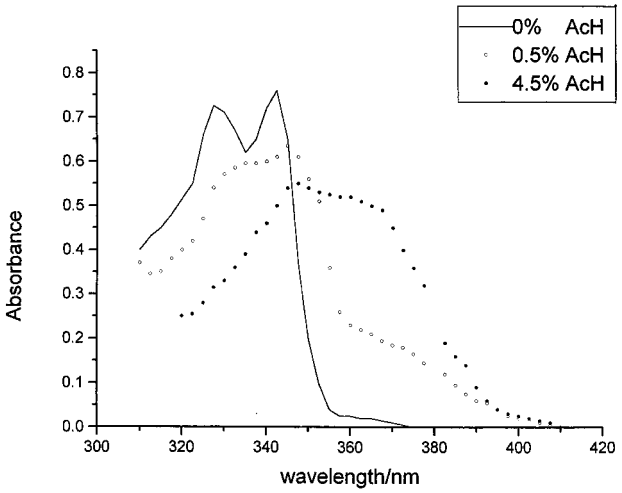


Figure 7. Absorption spectra of norharman in different AcH−benzene mixtures.

These results confirm Scheme 1, where P* is not an intermediate to yield Z* from N*, since the fluorescence of Z* is observed in binary mixtures of dioxane or benzene in AcH, and, nevertheless, the emission of P* does not appear in the fluorescence spectra.

In other mixtures as ethanol−AcH and acetonitrile−AcH, the fluorescence maxima at 400 and 520 nm do not appear and only an equilibrium neutral−cation was observed.

There are different types of excited state proton transfer reactions, some of which would be expected to be relatively

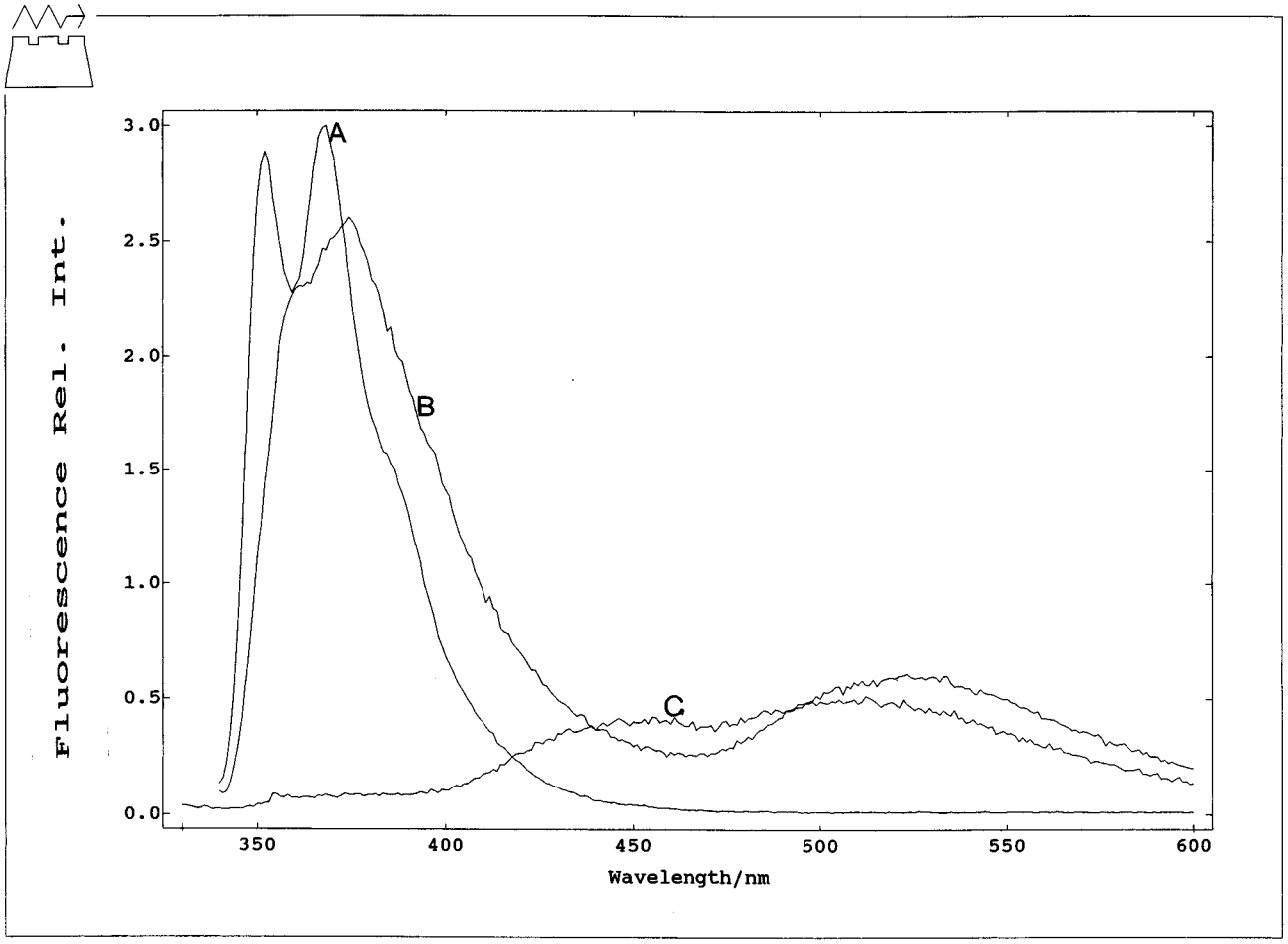


Figure 8. Fluorescence spectra of norharman in different AcH−benzene mixtures: A, 0% AcH; B, 0.5% AcH; C, 4.5% AcH. Excitation wavelength 320 nm.

inert to solvent perturbations and some totally dependent on the nature of the solvent.[20−22] According to the results obtained, solvation dominates the dynamic of transfer proton reactions of norharman. Kasha et al.[20−22] denominate this fact as solvent−cage perturbations of proton transfer spectroscopy.

The proton transfer process of norharman is strongly modified by the nature of the solvent. When the solvent−cage is apolar as dioxane or benzene: neutral, cation, and zwitterion fluorescences are observed in presence of AcH. In 2CM, a hydrogen bond between the C−H group of 2CM and the pyridinic nitrogen of norharman is formed (H-bonded associations of 2CM with pyridine are well-known[23,24]). Thus, in 2CM−AcH mixtures, four fluorescence maxima are observed: neutral, cation, zwitterion, and the species related with this hydrogen bond.

When a polar solvent surrounds the norharman, this solvent−cage blocks the proton transfer reaction and only neutral and cation fluorescences are observed. 2CM and AcH are completely miscible, so the mixture can be varied from slightly polar (pure 2CM) to highly polar (pure AcH). Thus, from AcH concentrations larger than 50% only the emission of cation is observed.

Norharman absorbs at longer wavelength (330 nm) than most protein residues (300 nm), and its fluorescence shows great sensitivity to H-bonding interactions. Thus, the fluorescence behavior of norharman displayed in nonpolar and polar media with carboxylic acid, together with the spectral difference between the fluorescence bands of neutral, phototautomer, cation, and zwitterion species, suggests the possibility of using this compound as a molecular probe to recognize carboxylic acid funtions in weakly polar solvents. These properties might be used in probing biological systems, and the related solvation dynamics constitute an interesting topic in physical chemistry.

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