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Steady-state and time-resolved study of the proton-transfer fluorescence of harmine and 2-methyl-harmine in organic solvents

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Abstract

Excited-state reactions involved in the fluorescence of 2-methyl-harmine have been investigated in different binary mixtures of acetic acid and dichloromethane. The fluorescence decay of these compounds are performed as a function of acetic acid concentration and emission and excitation wavelengths. A two-stage model of reaction is proposed in order to explain a triple-exponential decay for the cation fluorescence. It is suggested that polar solvents block the π indole ring inhibiting the zwitterion formation.

Keywords: Proton-transfer fluorescence; 2-Methyl-harmine; Organic solvents

1. Introduction

The dynamics of tautomerization process is not well understood up to now. This subject has a considerable interest since Watson and Crick [1] suggested the possible role of the rare tautomeric forms of the nucleotides in causing genetic mutations.

β-Carboline derivatives are widespread in nature [2]. They are present in about 26 plant families, cells, animal tissues, human urine [3] and human lenses [4]. The mechanism by which the β-carbolines are formed constitutes an important area in the study of the chemistry of vision [5]. These compounds, being hallucinogenic [6], present an extensive pharmacological activity, since they inhibit the monoamino oxidasa enzyme [7], they can interact with a great number of neurotransmitters of the central nervous system [8] and they also posses photocytotoxic and antiviral properties [9,10]. It is also known that these compounds bind selectively with DNA [11] and form complexes with flavins [12]. The interactions between β-carbolines and these biological receptors are not well known up to now, so the study of the ability of β-carbolines to form hydrogen bonds can help us to understand these properties. On the other hand, Beljanski [13] has found that some β-carboline derivatives can destroy selectively and completely the proliferative capacity of various types of cancer cells, which is enhanced upon excitation with UV radiation [9]. This behavior makes the study of photophysicochemical properties of these derivatives even more interesting.

Proton-transfer processes of β-carboline derivatives in different organic solvents have been the subject of many of our works [14–21]. In the last studies, we showed that the proton-transfer of a β-carboline derivative as Norharmane (Norh) are strongly influenced by the solvent nature [17,20]. So, when a polar solvent (acetonitrile or ethanol) surrounds to Norh, only the neutral (360 nm) and cation (450 nm) fluorescences were observed in the presence of acetic acid (AcH). Nevertheless, when the solvent is non-polar as *p*-dioxane or benzene, neutral (FN, N_1 N_2H), cation (FC, N_1H^+ N_2H) and zwitterion (500 nm, FZ, N_1H^+ N_2) fluorescences were observed in presence of AcH, where N_1 and N_2 represent the pyridinic and pyrrolic nitrogen, respectively. The zwitterionic and neutral form are tautomeric species. In dichloromethane–AcH mixtures, four fluorescence bands were recorded: FN, FC, FZ and another with maximum around 400 nm (FP) which we have tentatively ascribed with the formation of a hydrogen bond between the C–H group of dichloromethane and the pyridinic nitrogen of Norh. However, it is noteworthy that this band appears in presence of AcH, but in pure dichloromethane it has not

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been observed. By analogy with the behavior of 7-hydroxyflavone [22], this band could be associated with the formation of a complex between β-carboline and dichloromethane.

On the other hand, we also carried out nanosecond fluorescence decay measurements of Norh in dichloromethane–AcH mixtures. Thus, exciting the neutral form, we only recorded two fluorescence lifetimes although the steady-state fluorescence spectra showed three different fluorescence maxima. Exciting the cationic species, we measured three different lifetimes and we only observed two maxima in the steady-state fluorescence spectra.

The physicochemical behavior observed for this compound is so complex that it has not been fully explained yet. To learn more about the kinetic of excited state proton transfer (ESPT) in Norh, we have undertaken a study of steady-state and time-resolved fluorescence of two β-carboline derivatives: harmine and 2-methyl-harmine (2MHarmine). These compounds are 'quasitautomeric' forms (Fig. 1), therefore, the study of photophysicochemical behavior of them can help us to give answers to some questions raised in previous works [17,20]. Is the structure of the species with fluorescence maximum at 400 nm related with the interaction between the pyridinic nitrogen and dichloromethane, or correspond to another structure? Do FN and FP have the same lifetimes? Recording FC we obtained a tri-exponential decay, are these lifetimes observed in other β-carboline derivatives? 2MHarmine has methylated the pyridinic nitrogen and there is no possibility of interaction with the C–H group of dichloromethane. In this way, the comparative study of harmine and 2MHarmine can aid us to clarify the structure of the species with fluorescence maximum at 400 nm.

2. Experimental

Harmine and 2MHarmine were purchased from Sigma-chemie. These alkaloids were of the best available quality and were used as received. Glacial acetic acid and uvasol grade dichloromethane (2CM) were used as solvents without further purification.

Solutions of H and 2MHarmine 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 an hydrogen nanosecond flash lamp: repetition rate 40 kHz and excitation pulse width less than 1 ns. Fluorescence decays were analyzed with the method of non-linear 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 carried out at 25.0°C.

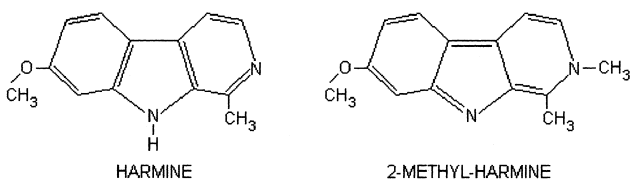

Fig. 1. Chemical structures of harmine, 2-methyl-harmine.

3. Results

3.1. Absorption spectra

In dichloromethane solutions (0% AcH), only the neutral species (N) of harmine is present in the ground state, Fig. 2. The absorption spectrum of this form shows two maxima around 330 and 318 nm for $S_0 \rightarrow S_1$ electronic transition and another around 298 nm for $S_0 \rightarrow S_2$ electronic transition. With increasing AcH concentration in solution, a new band appears corresponding to cationic species (C) of this compound. At a concentration of 5% AcH, the neutral species has disappeared from the solution. An isosbestic point can be observed around 308 nm.

In dichloromethane solutions (0% AcH) of 2MHarmine, only one form absorbs with maxima around 424, 354 and 294 nm corresponding to electronic transitions $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_3$, respectively. This species and the zwitterionic form (Z) of harmine are canonical forms of a resonance hybrid. Thus, we will denominate it as zwitterion. With increasing AcH concentration, a new band appears corresponding to the cationic species of this compound. At a concentration of 0.5% AcH, the zwitterionic species has

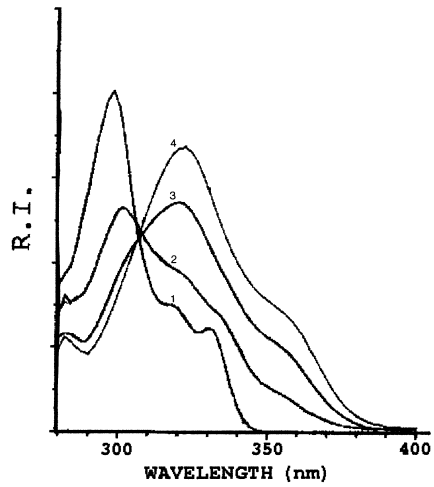

Fig. 2. Absorption spectra of harmine at different % AcH–dichloromethane mixtures. 0%, 0.5%, 2% and 5% of AcH.

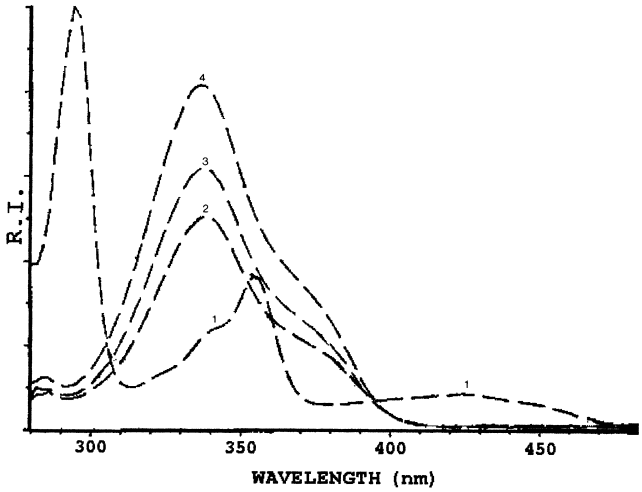

Fig. 3. Absorption spectra of 2MHarmine at different % AcH–dichloromethane mixtures. 0%, 0.5%, 2% and 5% of AcH.

disappeared from the solution, however, the absorbance of this new band increases with the AcH concentration up to 5%, Fig. 3.

3.2. Steady-state fluorescence spectra

The fluorescence spectra of Fig. 4 were obtained for the same harmine concentration in solution, the same exciting wavelength (330 nm) and different AcH concentrations (0%, 0.25%, 0.5%, 2% and 5%) in dichloromethane. For these solutions: (a) only the N fluorescence is observed (343 and 358 nm maxima, FN) in dichloromethane solutions, 0% AcH. (b) Two new species fluoresce with maxima around 386 (FP) and 500 nm (FZ) when small quantities of AcH are added into solution (0.5% AcH). (c) For 2% AcH, the maxima observed are around 405 and 500 nm. (d) For 5% AcH, the maxima are around 425 (FC) and 500 nm. (e) For 20% AcH (it is not included in Fig. 4) only the maximum around 425 nm is observed.

In order to clarify whether the three maxima at 386, 405 and 425 nm correspond to the same or different species (spectral shift by solvent effect), we have excited the 2% AcH dichloromethane/AcH solutions at different wavelengths: 290, 307, 330 and 370 nm, Fig. 5. Exciting with wavelengths at 290 and 307 nm, we are mainly exciting N and two fluorescence maxima are observed at 396 and 500 nm. At 370 nm only C absorbs and two fluorescence bands are recorded at 425 and 500 nm. Finally, at 330 nm, we are exciting N and C and two fluorescence maxima appear at 405 and 500 nm. From these results, we believe that the 405 nm maximum appears by overlapping of the FP and FC, and conclude that there are four fluorescent species. (a) In absence of AcH, exciting N, FN is recorded. (b) In presence of AcH, exciting N, FP and FZ appear. (c) Exciting C, FC and FZ are recorded. Unambiguously, FN corresponds to the fluorescence of N, FC and FZ with the fluorescence of C and Z, respectively, and FP could correspond to species related with the hydrogen bonding C–H–N between the pyridinic nitrogen and C–H group of dichloromethane.

The fluorescence spectra of Fig. 6 were obtained for the same 2MHarmine concentration in solution, the same exciting wavelength (330 nm) and different AcH concentrations (0%, 0.25%, 0.5%, 2% and 5%) in dichloromethane. For all these solutions, the fluorescence spectra show two maxima which correspond to Z fluorescence (around 500 nm) and C fluorescence (425 nm). For this compound, no maximum at 386 or 405 nm was observed. For all solutions studied, exciting with any wavelength between 290 and 370 nm region, only the 425 and 500 nm maxima were recorded.

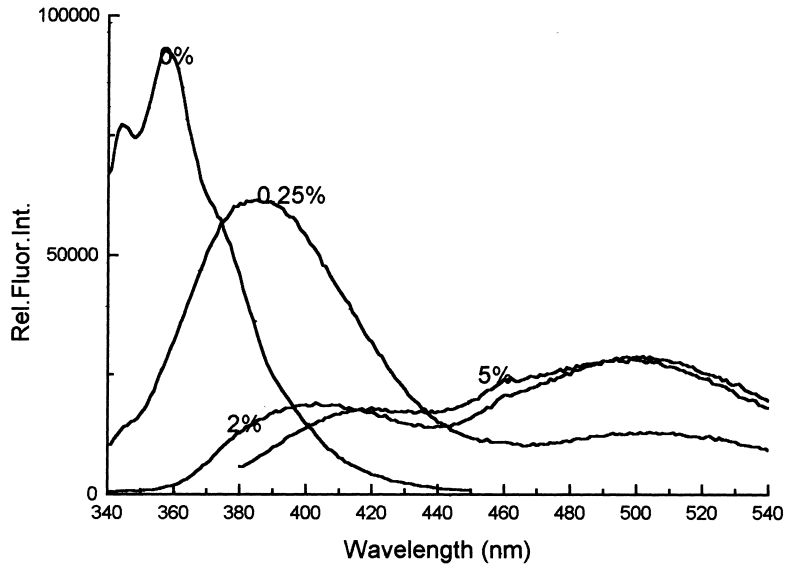

Fig. 4. Fluorescence spectra of harmine at different % AcH–dichloromethane mixtures (0%, 0.25%, 2% and 5% of AcH, $\lambda_{exc.} = 320$ nm).

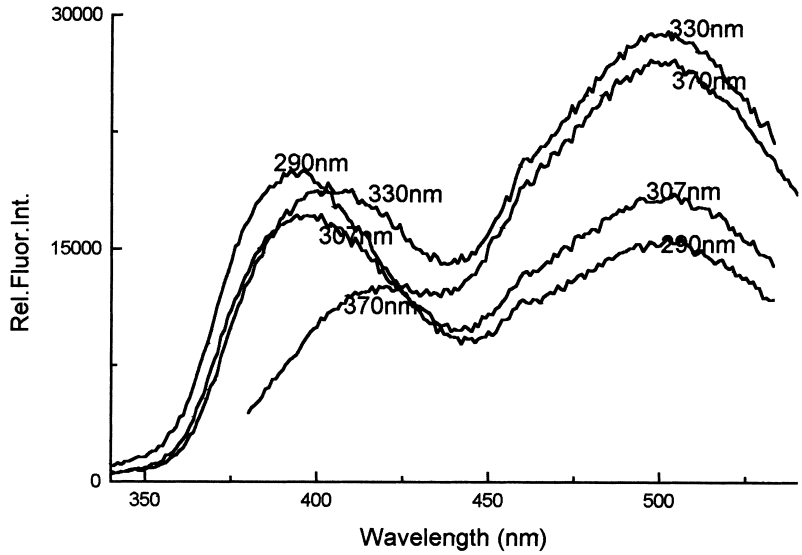

Fig. 5. Fluorescence spectra of harmine at different excitation wavelengths 290, 307, 330 and 370 nm (2% AcH).

3.3. Fluorescence lifetimes

In the analysis of exponential decay curves, we have considered mainly the residuals in order to fit the fluorescence decay at one, two or three exponentials.

From the steady-state fluorescence spectra, we have selected three different AcH–dichloromethane mixtures for the 2MHarmine derivative (0%, 0.5%, and 5%) and four mixtures for the harmine derivative (0%, 0.5%, 2% and 5%).

The results obtained from analysis of decay curves at different AcH concentration are given in Tables 1 and 2 for the harmine and 2MHarmine derivatives, respectively.

Harmine exhibits a single-exponential fluorescence decay of 3.7 ns in dichloromethane solutions, Table 1. We associate this lifetime to emission of N – in these conditions, only this species fluoresces. For 0.5% AcH, recording the fluorescence at 386 or 500 nm, an acceptable fit is obtained using a single-exponential fluorescence decay. From the steady-state fluorescence spectra, we attribute the short-lived component with the FP emission and the long-lived component with FZ.

For 2% and 5% AcH, exciting at 330 nm and recording at 405 or 410 nm, only an acceptable fit was obtained using a triple-exponential. Exciting at 370 nm for 2% AcH or at any wavelength for 5% AcH, we are not exciting N and we have only recorded good residuals fitting the fluorescence decay to a triple-exponential. Hence, we can not attribute the short-lived component to FP since this fluorescence band corresponds to a species which is generated from N in S_1 state. Excepting for 5% AcH solution, recording the fluorescence decay at 500 nm, a single-exponential fluorescence decay of around 14 ns was obtained.

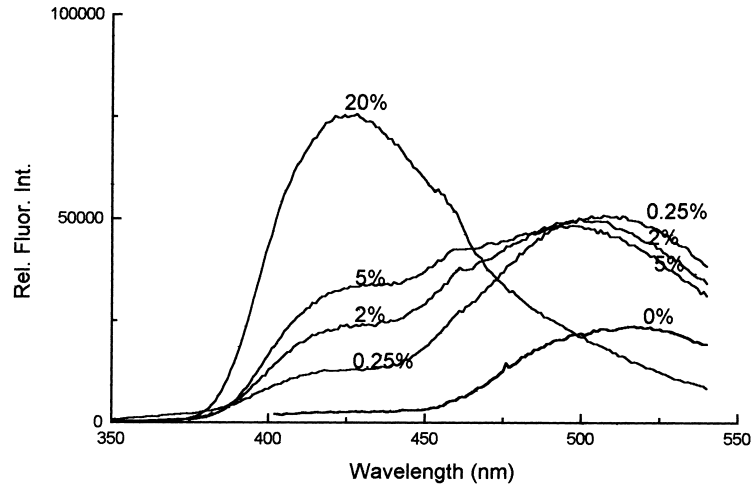

Fig. 6. Fluorescence spectra of 2MHarmine at different % AcH–dichloromethane mixtures: 0%, 0.25%, 2%, 5% and 20% of AcH. $\lambda_{exc.} = 320$ nm.

Table 1
Decay parameters of harmine in dichloromethane solutions at different AcH concentrations

AcH (%)	λ_{ex}	λ_{em}	Amplitude	Time (ns)	% Total emission	χ^2
0	330	350	$A_1 = 0.27$	$\tau_1 = 3.7$		0.98
0.5	330	386	$A_1 = 0.50$	$\tau_1 = 2.0$		1.00
0.5	330	500	$A_1 = 0.08$	$\tau_1 = 12.6$		1.09
2	307	380	$A_1 = 0.91$	$\tau_1 = 1.1$		0.71
2	330	405	$A_1 = 0.88$	$\tau_1 = 0.8$	70	0.83
			$A_2 = 0.15$	$\tau_2 = 1.8$	27	
			$A_3 = 0.002$	$\tau_3 = 12.2$	3	
2	370	425	$A_1 = 0.98$	$\tau_1 = 0.5$	49	0.93
			$A_2 = 0.21$	$\tau_2 = 1.8$	38	
			$A_3 = 0.01$	$\tau_3 = 11.3$	13	
2	307	500	$A_1 = 0.07$	$\tau_1 = 14.3$		1.09
2	330	500	$A_1 = 0.07$	$\tau_1 = 14.0$		1.03
2	370	500	$A_1 = 0.07$	$\tau_1 = 14.9$		1.09
5	330	410	$A_1 = 0.73$	$\tau_1 = 0.6$	44	0.93
			$A_2 = 0.15$	$\tau_2 = 3.1$	45	
			$A_3 = 0.008$	$\tau_3 = 14.4$	11	
5	330	500	$A_1 = 0.03$	$\tau_1 = 3.2$	9	1.01
			$A_2 = 0.06$	$\tau_2 = 14.4$	91	

Table 2
Decay parameters of 2MHarmine in dichloromethane solutions at different AcH concentrations

AcH (%)	λ_{ex}	λ_{em}	Amplitude	Time (ns)	% Total emission	χ^2
0	420	500	$A_1 = 0.06$	$\tau_1 = 17.0$		1.18
0.5	330	410	$A_1 = 0.27$	$\tau_1 = 1.1$	30	1.78
			$A_2 = 0.19$	$\tau_2 = 3.6$	70	
0.5	330	500	$A_1 = 0.03$	$\tau_1 = 3.4$	11	1.02
			$A_2 = 0.05$	$\tau_2 = 17.3$	89	
5	330	410	$A_1 = 0.31$	$\tau_1 = 0.7$	22	0.87
			$A_2 = 0.19$	$\tau_2 = 3.4$	63	
			$A_3 = 0.01$	$\tau_3 = 14.0$	15	
5	330	500	$A_1 = 0.02$	$\tau_1 = 2.2$	4	1.05
			$A_2 = 0.05$	$\tau_2 = 17.7$	96	

For 2Mharmine derivative and 0% AcH in dichloromethane, we have excited and recorded with wavelengths at 420 and 500 nm, respectively. In these conditions, only the zwitterionic form absorbs and emits. We have measured a fluorescence lifetime for this species of around 17.0 ns, Table 2. Between 0.5% and 5% AcH, the steady-state fluorescence spectra show only two maxima independent of excitation wavelength. Therefore, we have only excited with wavelength at 330 nm and recorded at 410 and 500 nm where C and Z emit, respectively. Recording at 410 nm, we have not obtained good residuals when we have fitted to a single-exponential the fluorescence decay. For the solution 0.5% AcH in dichloromethane, the best fit is a bi-exponential with fluorescence lifetime around 1.1 (30%) and 3.6 ns (70%), and for the solution 5% AcH in dichloromethane a triple-exponential decay with lifetimes around 0.7 (22%), 3.4 (63%) and 14 ns (15%) were recorded (Table 2). Recording the emission at 500 nm, we have observed good residuals when we have fitted the fluorescence decay to a bi-exponential. Nevertheless, no negative pre-exponentials were obtained in these fittings.

4. Discussion

If we compare the steady-state fluorescence spectra of harmine and 2MHarmine in 0.5% AcH–dichloromethane mixtures, we can observe a maximum at 386 nm in the harmine spectra, which does not appear in those of 2MHarmine. On the other hand, comparing the fluorescence spectra of harmine and Norh [17,20] in these same conditions, we observed three maxima for Norh (FN, FP and FZ) and two maxima for harmine (FP and FZ) – in this case, the neutral species does not fluoresce. This result agrees with the fact that the pyridinic nitrogen of harmine is more basic than that of Norh [23–25]. Both results confirm our initial hypothesis which correlated FP with the formation of a hydrogen bonding between the C–H group of dichloromethane and the pyridinic nitrogen of Norh [20]. Recently, this same type of interaction has been found between 5-deazalumichrome and 1, 2 dichloroethane in the presence of acetic acid [26].

This last comparison between the fluorescence spectra of harmine and Norh, also corroborates our hypothesis where we assigned the same fluorescence lifetimes for FN and FP.

In the harmine case, contamination of FP by FN does not exist, as the neutral form does not fluoresce. We measured a lifetime around 2 ns, which we can correlate with the 1.4 ns [20] value, recorded in the same conditions, for Norh.

The photophysicochemical behavior found for harmine in AcH–dichloromethane mixtures is similar to that observed for Norh in these same mixtures. Two new fluorescence bands (FP and FZ) appear in presence of small amounts of AcH in solutions, and the fluorescence lifetime of FP decreases with AcH concentration while that of FZ slightly increases from 12.6 to 14 ns. We would like to note that the methylation of 1 position of Norh ring increases the lifetime of FZ. So, the τ_{FZ} measured for Norh was around 3.4 ns and that corresponding to harmine and 2MHarmine were around 14.0 and 17.0 ns, respectively. These results agree with those showed by Dias et al. [27]. They recorded in methanol solutions τ_{FZ} values of 5.4, 23 and 28 ns for Norh, Harmane (Norh which has methylated the 1 position) and harmine, respectively.

Just like Norh, harmine presents a tri-exponential decay exciting and recording where only C absorbs and emits, respectively. In Norh case, we supposed the existence of two cationic species, C1 and C2. C2 is solvated in such a fashion that zwitterion formation is not facile and C1 is solvated suitably in order to yield the zwitterion. The third lifetime can be explained considering an equilibrium C1–Z in S_1 state.

In 2MHarmine case, only C and Z emit between 0.5% and 5% of AcH in dichloromethane. We also found a short component recording the fluorescence where only C emits. C1→Z reaction is not reversible in 0.5% AcH–dichloromethane mixtures, therefore, we observed a bi-exponential decay gathering the fluorescence at 410 nm. At 5% AcH, the process is reversible, so we obtained a triple-exponential decay.

Recording the fluorescence at 500 nm where mainly the zwitterion emits, the fits of fluorescence decay of harmine and 2MHarmine do not show any negative amplitude. In a previous work [20], we considered that C1 is a precursor of Z in S_1 state and this last is not present in the ground-state. Therefore, the fluorescence decay for Z should be bi-exponential with pre-exponentials of different sign and same magnitude [28]. In the Norh case, we obtained negative pre-exponentials in the interval between 0.25% and 2% of AcH in dichloromethane recording the fluorescence at 540 nm. Thus, we explained the behavior observed for this derivative in S_1 state with the following mechanism: P←N→Z in S_1 state, where P represents the species with fluorescence FP. Considering $\tau_P = \tau_N$, this mechanism can explain a single-exponential decay recording FN or FP, and a bi-exponential recording FZ with pre-exponential decay for FZ as is obtained in this work for harmine in 2% AcH solutions. In this point, we wish to leave open these two possibilities and invite to realize picosecond measures of the systems studied in this work.

The pK_a values for Norh, harmine and 2MHarmine in aqueous solution, indicate that the excited state zwitterion should predominate in alkaline solution ($pK_aCZ\,(S_1) = 4.1$). Nevertheless, its fluorescence does not appear up to a pH around 12. Ghiggino and Sakuros [29] considered that only at a pH greater than 10, the diffusion-controlled quenching is significant and zwitterion emission occurs. However, we believe that this behavior could be explained considering that polar solvents block the π indole ring and this situation inhibits the Z formation. So, in water, ethanol, or acetonitrile, the zwitterion fluorescence only is observed in strongly basic media. In non-polar media as benzene, dioxane and carbon tetrachloride or slightly polar as dichloromethane and chloroform, the Z fluorescence is recorded even in acid solutions. For 50% AcH–dichloromethane mixtures, the medium is sufficiently polar, the solvent-cage [30,31] blocks the proton-transfer reaction and consequently only C emission is observed.

We have explained the time-resolved results by assuming a two-stage model of reaction in the excited state. This concept is very similar to the scheme proposed for 7-azaindole [32–35], alloxazines [36] and 1-azacarbazole [37]. In the 7-azaindole case, the steady-state fluorescence spectra for this compound, present two bands corresponding to normal and tautomeric forms. Recording the normal fluorescence, 20% of this emission decays with a time around 70 ps [24]. This short component is attributed to a small population of 7-azaindole that undergoes excited-state tautomerization. The remaining 80% corresponds to fluorescence of 7-azaindole molecules that are blocked by unfavorably solvation for executing excited-state tautomerization (τ around 910 ps). Normal and tautomeric species are formed from distinct ground-state species as different excitation spectra were recorded for normal and tautomeric bands. β-Carboline derivatives show a fluorescence dynamic in dichloromethane–AcH mixtures more complex than 7-azaindole. Together with the phototautomerism, exciting N, a proton-transfer process also occurs exciting C. On the other hand, the excitation spectra of FC and FZ are nearly identical, implying similar absorption of species responsible for these two emissions.

In conclusion, in this work it has been confirmed that the species with fluorescence around 400 nm is related with the interaction between the pyridinic nitrogen and dichloromethane and, on the other hand, the same fluorescence lifetime for FN and FP has been obtained. However, although some questions have been answered, the mechanism of Z formation is still unclear and requires further studies. Subnanosecond time-resolved experiments and effects of temperature and viscosity of the solvent on the excited-state proton transfer could aid us to obtain more information about the photophysicochemical behavior of these derivatives.

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