ELSEVIER

Biophysical Chemistry 104 (2003) 683–696

Biophysical Chemistry

www.elsevier.com/locate/bpc

Photophysical properties of methyl β-carboline-3-carboxylate mediated by hydrogen-bonded complexes—a comparative study in different solvents

D. Reyman[a,*], M.J. Tapia[b], C. Carcedo[b], M.H. Viñas[c]

[a]*Departamento de Química Física Aplicada, Facultad de Ciencias, C-XIV, Universidad Autónoma de Madrid, Crta Colmenar Viejo Km 15, 28049 Cantoblanco, Madrid, Spain*
[b]*Departamento de Química, Universidad de Burgos, Burgos, Spain*
[c]*Departamento de Sistemas Inteligentes Aplicados, Escuela Universitaria de Informática, Universidad Politécnica de Madrid, Madrid, Spain*

Received 19 February 2003; received in revised form 9 April 2003; accepted 11 April 2003

Abstract

The hydrogen bonding interactions of methyl β-carboline-3-carboxylate (BCCM) in both ground and first singlet excited electronic states have been studied in solvents with different properties in the presence of acetic acid, a hydrogen-bonding donor/acceptor. The methyl ester substituent reduces the pyridinic nitrogen basicity of this β-carboline derivative. This fact has let us study the hydrogen bonding interactions in a higher range of acetic acid concentrations than for other β-carboline derivatives previously studied. Steady and non-steady photophysical studies have been carried out in two non-polar solvents, benzene and *p*-dioxane; and in two polar solvents, acetonitrile and dichloromethane. Six different fluorescence emissions have been isolated corresponding to the uncomplexed BCCM, the protonated species and four different complexes between BCCM and acetic acid whose structures we have tried to elucidate.

Keywords: β-Carbolines; Photophysic; Hydrogen-bonding; Proton transfer; Acetic acid; Organic solvents

1. Introduction

Intermolecular hydrogen bonding is a site-specific interaction between a hydrogen donor and acceptor molecules. It is central to the understanding of the microscopic structure and function of these in many molecular systems since hydrogen bonding is one of the most fundamental processes involved in chemical reactions and living systems (see, e.g. Refs. [1–6] and references therein), i.e. it has been supposed that strong hydrogen bonds play a dominant role in enzyme catalysis [7–9], and especially important are so-called 'charge relay chains' which consist of a sequence of linearly hydrogen bonded molecules with mobile protons.

*Corresponding author. Tel.: +34-91-397-8657; fax: +34-91-397-4512.
E-mail address: dolores.reyman@uam.es (D. Reyman).

0301-4622/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0301-4622(03)00129-7

β-Carboline alkaloids (H-pyrido [3,4-*b*] indol derivatives) (hereafter, BC) have been and still are the aim of many biological, pharmacological and chemical studies due to their presence in many natural compounds [10] and to their pharmacological activity [11–13]. They can interact with a great number of neurotransmitters of the central nervous system [14–16], binding with DNA [17,18] and also possess photocytotoxic and antiviral properties [19–23].

The interactions between BC and these biological receptors are still not well known. Thus, detailed knowledge about physical and chemical properties of BC is necessary in order to understand the biological function of these compounds at the molecular level and the nature of the interactions with DNA under various environmental conditions. These interactions mainly take place through proton transfer or through the formation of proton bridges. So, the study of the ability of these compounds to form proton bridges with other molecules can help us to understand these properties.

BC derivatives present interesting and unusual photophysical properties due to a bifunctional hydrogen bonding character of the BC ring. This is due to the presence in its ring of pyrrolic and pyridinic acid and basic nitrogen atoms, respectively. Furthermore, the acid–base properties of BC are considerably modified in the first singlet excited-state. Upon excitation, the charge density on the nitrogen atoms of the BC ring changes, with the pyridinic nitrogen becoming much more basic and the pyrrolic nitrogen more acidic than in the ground state [24–26].

Recent works suggest the formation of different kinds of complexes between BC and different proton donors/acceptors [27–31]. On the one hand, it has been seen that the formation of these complexes is inhibited by the cationic species formation [32,33], so once the pyridinic nitrogen (N_2) is protonated, the only observed fluorescence corresponds to the protonated species. On the other hand, the interactions between the pyrrolic nitrogen (N_9) and hydrogen acceptors are favored in nonpolar media or with low dielectric constant [34]. However, the formation mechanism of these complexes is still not clear.

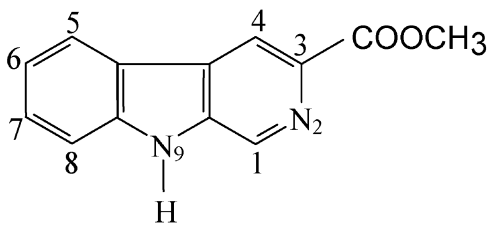


Fig. 1. Methyl β-carboline-3-carboxylate (BCCM) structure.

One of our latest works [35] on a new β-carboline derivative, methyl-β-carboline-3-carboxylate (Fig. 1), shows that the ester subsituent is responsible for the higher acidity of this BC derivative. Thus, we show that the pyridinic nitrogen N_2 of this derivative, hereafter BCCM, is also more acidic (pK_a $[N_2]=4.4$; $pK_a^*[N_2]=6.3$) than the same nitrogen of BC, commonly referred as norharmane, which does not have this ester group (pK_a $[N_2]=7.9$; $pK_a^*[N_2]=14.7$) [36]. The fact that N_2 is less basic in BCCM than in norharmane will allow us to study the hydrogen bonding interactions for a higher range of acetic acid (AcH) concentrations without N_2 protonation.

In this paper, we will report a detailed investigation of the fluorescent properties of BCCM in different solvents with a view to understanding different aspects of excited-state intermolecular proton-transfer. To do so, we have carried out a systematic study of the hydrogen bonding interactions of BCCM with acetic acid, a hydrogen bonding donor/acceptor, in the presence of different polarity solvents: dioxane, benzene, acetonitrile and dichloromethane.

2. Materials and methods

BCCM was purchased from Sigma. The uvasol grade solvents: glacial acetic acid (AcH) from Riedel–deHaën, benzene, dioxane and dichloromethane from Fluka and acetonitrile from Aldrich. All the reagents were used without further purification and the solvents were degassed with bubbling nitrogen. Diluted solutions of BCCM in mixtures of benzene, *p*-dioxane and acetonitrile with a percentage in volume of AcH between 0 and 50% were used. Absorption spectra were

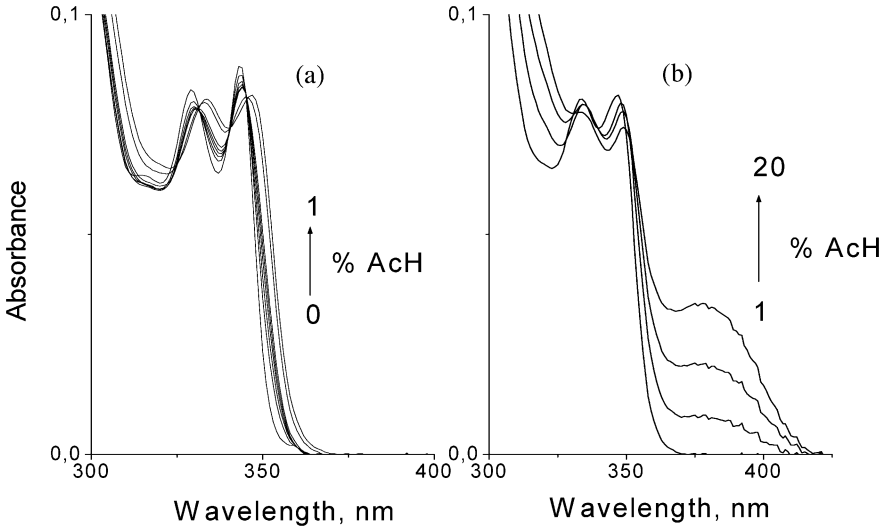


Fig. 2. Absorption spectra of methyl β-carboline-3-carboxylate in benzene with variable concentrations of AcH. (a) 0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.5 and 1% (v/v). (b) 1, 5, 10 and 20% (v/v).

obtained using a Hewlett–Packard spectrophotometer HP 8453. The steady state fluorescence spectra were measured with a Shimazdu spectrofluorimeter RF-5301 PC with a 3 nm bandwidth in excitation and 1.5 nm bandwidth in emission. Fluorescence decays were obtained using the time-correlated single-photon-counting method (Edinburgh Analytical Instruments). The excitation source was a 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 deconvolution and the quality of the fits was 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 °C.

3. Results

3.1. Interactions of BCCM with acetic acid in benzene

3.1.1. Ground state

The results in benzene will be presented in two different AcH concentration interval: 0–1%, where the cationic species (N_2H^+), hereafter C, is still not present in the solution, and the interval 5–50%, where C appears.

In the 0–1% interval, the formation of hydrogen-bonded complexes between BCCM and AcH can be clearly shown by the growth of an absorption shoulder at >350 nm throughout the titration (Fig. 2). The appearance of isosbestic points located at 345 and 331 nm indicates the existence of an equilibrium between two different species.

The cationic species absorption starts at approximately 5% AcH and it is not possible to protonate completely BCCM for an AcH concentration of 50%.

3.1.2. First excited state

Fig. 3 shows the fluorescence spectra as a function of the added AcH concentration in benzene. The uncomplexed BCCM shows a normal Stokes shifted emission maxima at 349 and 365 nm of which the relaxation dynamics were well fitted by single-exponential kinetics with a lifetime of 2.9 ns (Table 1). Increasing the AcH concentration, excitation at 345 nm, and isosbestic point in the 0–1% AcH interval, results in a slight change in the shape and the maxima spectra, together with a progressive decrease of the fluorescence intensity at 365 nm and an increase in the emission at 510

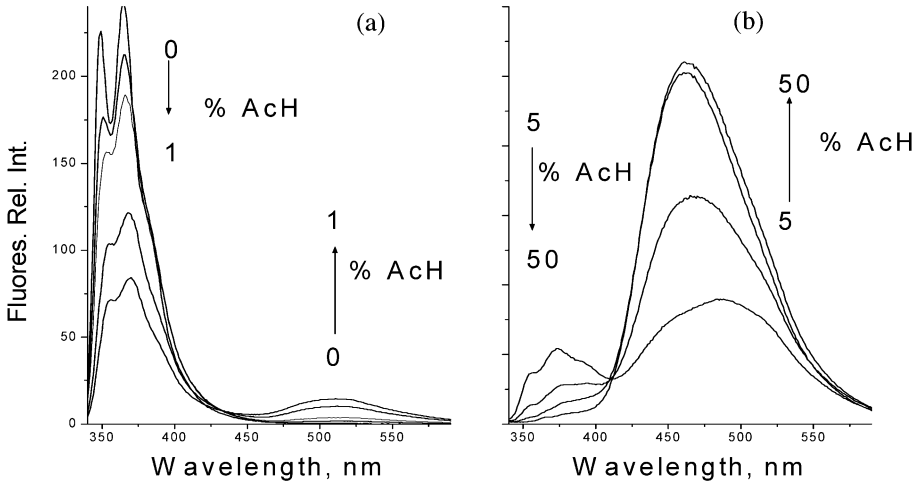


Fig. 3. Emission spectra of BCCM in benzene with variable concentrations of AcH excitation wavelength 329 nm. (a) concentrations of AcH, 0, 0.04, 0.1, 0.5 and 1% (v/v). (b) concentrations of AcH 5, 10, 20 and 50%.

nm. As in other works, the 349–365 nm band is defined as the N component (uncomplexed BCCM) in order to distinguish it from the ≈370 nm component (D1A) associated with the 1:1 complexed BCCM with AcH. These little changes, both in the band shape and the fluorescence maxima, are the consequence of the ground state interactions between BCCM and AcH. It can be considered that for the AcH interval 0–1% both N and D1A are responsible for the emission in the range 340–400 nm. These fluorescences decay mono-exponentially, decreasing the lifetimes with the increase of AcH concentration.

The D2 component (510 nm) fluorescence can be well fitted by a triple-exponential decay rate (Table 1). The shortest decay time is a risetime whose contribution decreases by increasing the AcH concentration. In all cases, there is a decay time of approximately 3.6 ns, and a decay time of approximately 14 ns; the latter can be attributed

Table 1
Decay times (ns) of BCCM in benzene at different AcH concentrations and emission wavelengths; excitation wavelength 345 nm

Wavelengths	τ/ns 0.1% AcH	τ/ns 1% AcH	τ/ns 5% AcH	τ/ns 10% AcH	τ/ns 20% AcH
375	2.4	1.8	0.7	0.4	
χ^2	1.0	1.1	1.0	1.0	
460		2.8 (67)	1.8 (45)	1.2 (17)	1.1 (4)
		3.5 (23)	3.7 (28)	3.5 (27)	3.6 (21)
		14.2 (10)	13.9 (21)	14.6 (56)	14.0 (75)
χ^2	1.00	0.99	1.06	1.10	
520		1.8 (−0.013)	0.6 (−0.012)	0.4 (−0.006)	0.4 (−0.001)
		3.5 (0.050)	3.6 (0.037)	3.7 (0.035)	3.7 (0.030)
		14.0 (0.001)	14.1 (0.001)	13.7 (0.001)	14.0 (0.002)
χ^2		0.99	1.04	1.10	0.98

The fractional intensity can be observed between brackets (pre-exponential factors when there are negative amplitude contributions); χ^2 values are also included for each fit. In the interval between 0 and 0.08% AcH, the τ/ns values recorded at 375 nm were: 0% 2.9 ns, $\chi^2=1.07$; 0.04% 2.7 ns, $\chi^2=0.95$; 0.06% 2.5 ns, $\chi^2=1.08$; and 0.08% 2.4 ns, $\chi^2=1.09$.

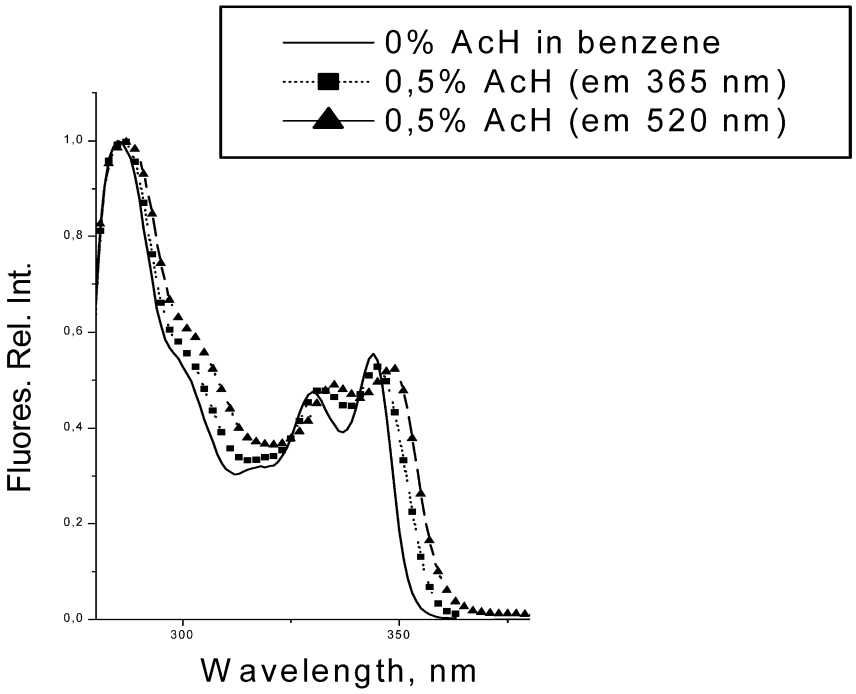


Fig. 4. Excitation spectra of BCCM in benzene with variable concentrations of AcH and recording at two different emission wavelengths.

to the cation emission, whereas the rise time is similar to that found for N and D1A. An attempt to fit the D2 component by a double-exponential decay component rendered a good χ^2 value, but the lifetime at approximately 3 ns increases significantly with AcH concentration.

The *excitation spectra* monitored at 370 nm, are only slightly red shifted (<1 nm) with respect to that obtained from the uncomplexed BCCM (Fig. 4). In contrast, the excitation maximum of the D2 band is shifted by as much as 3 nm, indicating that D1A and D2 bands do not originate from a common ground-state species.

Above 1% AcH, when cationic species emission starts, fluorescence decay was also recorded at 460 nm. For this wavelength, the fluorescence can be well fitted by a triple-exponential decay rate and no risetime was observed (Table 1). The contribution of the shortest decay time decreases by increasing the AcH concentration. In all cases, there is a decay time of approximately 3.6 ns and a decay time of approximately 14 ns. These two can be attributed to the D2 and C emission, respectively, whereas the shortest lifetime has a higher value than that found for N and D1A. An attempt to fit the C band by a double-exponential decay component rendered a good χ^2 value, but the lifetime at approximately 14 ns decreases with the acetic acid concentration.

3.2. Interactions of BCCM with acetic acid in dioxane

3.2.1. Ground state

Comparing the results in dioxane with those in benzene, no isosbestic point is observed in dioxane with increasing AcH concentration. The absorbance increases slightly with acetic acid concentration and when the spectra are normalized, a little shoulder at approximately 350 nm is observed. The cationic species starts to absorb for an acetic acid concentration of approximately 20%.

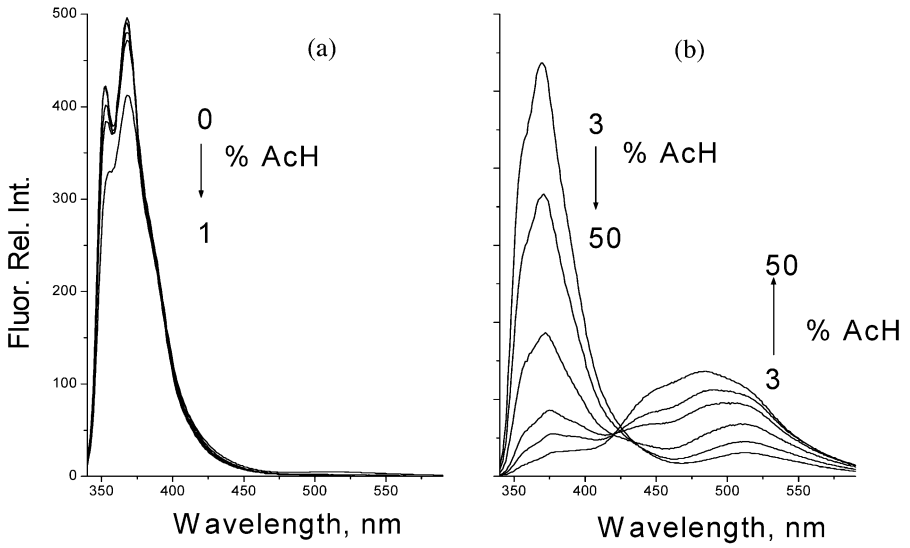


Fig. 5. Emission spectra of BCCM in dioxane with variable concentrations of AcH excitation wavelength 329 nm. (a) concentrations of AcH, 0, 0.04, 0.18, 0.4 and 1% (v/v). (b) concentrations of AcH 3, 5, 10, 20, 30 and 50%.

3.2.2. First excited state

Fig. 5 shows the fluorescence spectra as a function of the added AcH concentration in dioxane. The uncomplexed BCCM shows a normal Stokes shifted emission maxima at 352 and 368 nm (N component) of which the relaxation dynamics were well fitted by single-exponential kinetics with a lifetime of 2.8 ns (Table 2). Upon increasing the AcH concentration, in the 0–1% AcH interval, a very slight change in shape with a progressive decrease of the fluorescence intensity at 368 nm and an increase in the emission at 510 nm are observed. This behavior is similar to that found in benzene although the changes are smaller. As for the relaxation dynamics in dioxane (Table 2), the behavior observed showed some differences with

Table 2
Decay times (ns) of BCCM in dioxane at different AcH concentrations and emission wavelengths; excitation wavelength 345 nm

Wavelengths	τ/ns 3% AcH	τ/ns 5% AcH	τ/ns 10% AcH	τ/ns 20% AcH	τ/ns 40% AcH
380	1.7	1.2	0.7	0.4	
χ^2	0.97	1.01	1.16	1.06	
460			0.8 (43)	0.4 (34)	0.4 (22)
			14.0 (13)	13.4 (23)	14.3 (20)
			3.7 (44)	3.8 (47)	4.0 (57)
χ^2			1.03	1.00	1.12
520	3.7 (0.067)	3.5 (0.055)	3.7 (0.042)	3.8 (0.062)	4.1(0.068)
	1.2 (−0.078)	1.0 (−0.046)	0.7 (−0.026)	0.5 (−0.025)	0.5 (−0.009)
χ^2	0.99	1.15	1.17	1.06	1.12

The fractional intensity can be observed between brackets (pre-exponential factors when there are negative amplitude contributions); χ^2 values are also included for each fit. In the interval between 0 and 1% AcH, the τ/ns values recorded at 380 nm were: 0% 2.8 ns, χ^2= 1.10; 0.18% 2.8 ns, χ^2=1.03; 0.40% 2.7 ns, χ^2=1.08; and 1% 2.5 ns, χ^2=1.16.

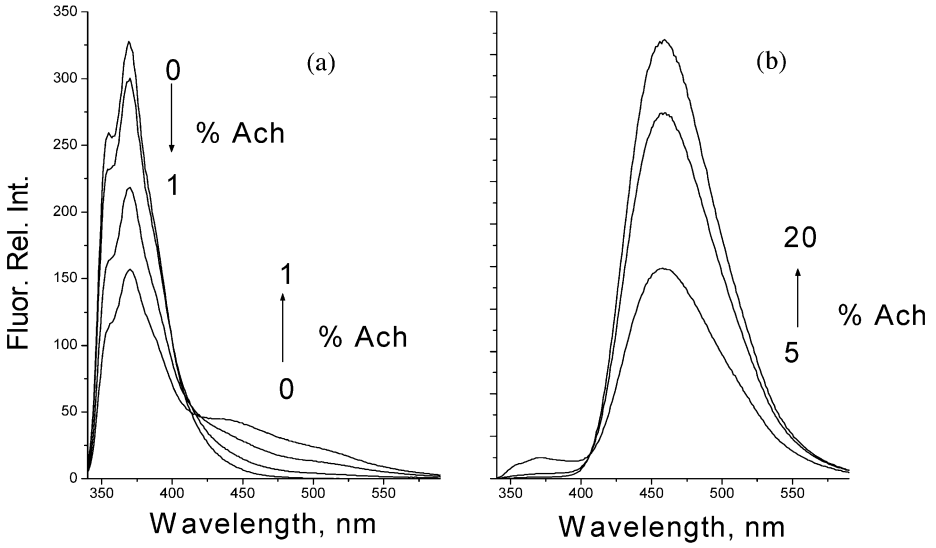


Fig. 6. Emission spectra of BCCM in acetonitrile with variable concentrations of AcH excitation wavelength 329 nm. (a) concentrations of AcH, 0, 0.1, 0.5 and 1% (v/v). (b) Concentrations of AcH 5, 10 and 20%.

that obtained in benzene. Recording at 460 nm, the shortest lifetime has a similar value to that found for N and D1A (in benzene it has a higher value). The D2 component (510 nm) fluorescence can be well fitted by a double-exponential decay rate. The shortest decay time is a risetime similar to that found for N and D1A, whose contribution decreases by increasing the AcH concentration. In all cases, there appears a decay time of approximately 3.6 ns that can be attributed to D2 emission. This good double-exponential fit is due to the low C emission in this solvent.

The *excitation spectra* monitored at 370 nm are only slightly red shifted (<1 nm) with respect to that obtained from the uncomplexed BCCM. In contrast, the excitation maximum of the D2 band is shifted by as much as 2 nm, indicating that D1A and D2 bands do not originate from a common ground-state species.

3.3. Interactions of BCCM with acetic acid in acetonitrile

3.3.1. Ground state

In acetonitrile only an increase of the neutral form absorbance is observed and again, there is no isosbestic point. For 1% AcH the cationic species begins to absorb and for 30%, C is the only species present in solution.

3.3.2. First excited state

As for norharmane, the N component does not shift with AcH concentration (Fig. 6). In this derivative the fluorescence maximum around 405 nm does not appear as in norharmane and although the D2 fluorescence is hidden by cationic emission, the lifetimes confirm its presence (Table 3). The excitation spectra behavior is similar to that found in the other solvents. These spectra are shifted to the red (1 nm) recording emission at 520 nm with respect to those recorded at 365 nm. As the D2 fluorescence intensity is very low for all AcH concentrations, there is no contribution of this component lifetime to the relaxation dynamic recording at 460 nm.

3.4. Interactions of BCCM with acetic acid in dichloromethane

3.4.1. Ground state

The absorbance results in this solvent are similar to those found in acetonitrile and dioxane and

Table 3
Decay times (ns) of BCCM in acetonitrile at different AcH concentrations and emission wavelengths; excitation wavelength 345 nm

Wavelengths	τ/ns 0.5% AcH	τ/ns 1% AcH	τ/ns 5% AcH	τ/ns 10% AcH	τ/ns 20% AcH
380	2.0	1.6	0.7		
χ^2	1.21	1.12	1.18		
460		1.7 (28)	0.8 (6)	0.6 (2)	0.2 (1)
		14.8 (72)	15.0 (94)	14.8 (98)	15.0 (99)
χ^2		0.91	1.09	1.07	1.05
520		4.4 (74)	3.6 (29)	3.6 (15)	4.7 (5)
		15.0 (26)	14.9 (71)	15.1 (85)	15.0 (95)
χ^2		1.19	1.07	1.07	1.08

The fractional intensity can be observed between brackets; χ^2 values are also included for each fit. In the interval between 0 and 0.1% AcH, the τ/ns values recorded at 380 nm were: 0% 2.9 ns, $\chi^2=1.19$; 0.04% 2.8 ns, $\chi^2=1.03$; 0.06% 2.7 ns; $\chi^2=1.00$; and 0.1% 2.6 ns, $\chi^2=1.09$.

again, no isosbestic point is observed. And what is probably more significant is that for 5% AcH, nearly all BCCM is protonated as happened for norharmane in the same solvent mixture.

3.4.2. First excited state

Exciting at 329 nm and depending on AcH concentration, four fluorescences were observed with maxima around 349–365, 382, 462 and 510 nm; however, exciting at 370 nm, only two fluorescences were recorded around 400 and 462 nm (Fig. 7). This maximum at 400 nm was not observed for norharmane in the same solvent. As for the relaxation dynamics in dichloromethane (Table 4), it is similar to that found in acetonitrile. The only difference was observed recording the fluorescence at 460 nm where the shortest lifetime remains constant with the increase of AcH concentration, while in acetonitrile it decreases with AcH.

In dichloromethane, the relaxation dynamics were also recorded exciting at 370 nm. From the results shown in Table 4, we can associate a lifetime of 3.0 ns for the species emitting at 400 nm. As in acetonitrile, no risetime was registered in dichloromethane.

From the *fluorescence excitation spectra*, also in dichloromethane, D1A and D2 components do not originate from a common ground-state species.

4. Discussion

Recent spectroscopic studies [27,28] on the interactions of N_9-methyl harmane (N_9-methyl-1-methyl-9H-pyrido[3,4-*b*] indole, MHN) where the pyrrolic nitrogen is blocked, and therefore the hydrogen interactions only occur through pyridinic nitrogen, have shown three different MHN/hydrogen bonded complexes (1:1, 1:2 and 1:3) in cyclohexane in the presence of a *proton donating compound* as hexafluoropropan-2-ol. These three interactions take place through pyridinic nitrogen. The 1:1 complex lifetime value was very close to that of MHN in cyclohexane (2.1 ns) and the measured lifetimes for 1:2 and 1:3 complexes were 3.7 and 14–15 ns, respectively.

Furthermore, in the presence of *hydrogen bond acceptors* as tetrahydrofuran, *N*,*N*-dimethylformamide, the UV-Vis absorption and fluorescence spectra were shifted to the red by the interaction of harmane (pyrrolic nitrogen deblocked) with these acceptors. In spite of the fact that two different species contributed to fluorescence emission, the decay is monoexponential. The lifetimes of the decays were very close to that of the non-complex harmane, 2.9 ns. Therefore, the 1:1 interactions of both hydrogen donors and acceptors with BC derivatives produce very small shifts in

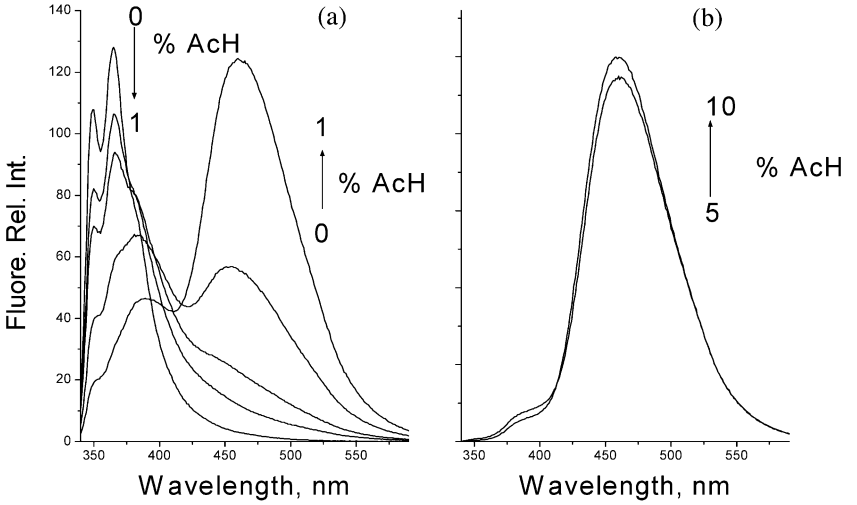


Fig. 7. Emission spectra of BCCM in dichloromethane with variable concentrations of AcH excitation wavelength 329 nm. (a) concentrations of AcH, 0, 0.08, 0.12, 0.5 and 1% (v/v). (b) Concentrations of AcH, 5 and 10%.

the absorption and emission spectra and a small lifetime reduction.

Other recent studies [29] of BC in cyclohexane in the presence of trace amounts of AcH, showed an equilibrium in the ground state between three different compounds, uncomplexed BC and 1:1 BC/AcH, 1:2 BC/AcH complexes. This 1:2 BC/AcH complex consists of a triple-hydrogen bonding formation where a fast excited-state proton-transfer (ESPT) takes place through a conduit of relayed hydrogen bonds. The relaxation dynamics for the uncomplexed and 1:2 BC/AcH complex were well fitted to a monoexponential decay kinetic with lifetime of 2.8 and 5.9 ns in cyclohexane, respectively.

Hydrogen-bonding interactions between the pyrrolic NH group of BC and the π-delocalized electrons of the benzene derivatives have also been proposed [34,37]. At low concentrations of benzene, the NH/π interactions only produce an enhancement in the fluorescence around 360 nm in cyclohexane. Furthermore, at higher concentrations, fluorescence quenching is observed. In addition, harmane and pyridine form a 1:1

Table 4
Decay times (ns) of BCCM in dichloromethane at different AcH concentrations and emission wavelengths; excitation wavelength 345 nm

Wavelengths	τ/ns 0.1% AcH	τ/ns 0.5% AcH	τ/ns 1% AcH	τ/ns 5% AcH	τ/ns 10% AcH
380	1.9	1.6	1.3		
χ^2	1.26	0.96	1.18		
460		1.5 (24)	1.5 (22)	1.4 (17)	1.5 (10)
		14.6 (76)	14.7 (78)	13.7 (83)	13.9 (90)
χ^2		1.05	1.15	0.97	0.98
520			3.7 (87)	3.6 (73)	3.6 (47)
			14.2 (13)	14.2 (27)	14.0 (53)
χ^2			1.00	1.02	1.00

The fractional intensity can be observed between brackets; χ^2 values are also included for each fit. In the interval between 0 and 1% AcH, the τ/ns values recorded at 380 nm were: 0% 2.4 ns, $\chi^2=1.01$; 0.04% 2.1 ns, $\chi^2=1.06$; and 0.06% 2.0 ns, $\chi^2=1.09$.

hydrogen-bonded complex in both the ground and excited states whose stability diminishes as the polarity and hydrogen–bonding ability of the solvent increase [38].

In our case, we have worked with a compound whose pyridinic nitrogen is approximately three orders of magnitude less basic than the pyridinic nitrogen of norharmane. This has allowed us to study the BCCM/AcH interactions for a wide range of AcH concentrations before the formation of cationic species.

Comparing BCCM results with those obtained for norharmane [30] in the same solvents studied in this work, it can be observed that the interactions between the fluorophore and AcH are weaker in the case of BCCM than for norharmane. Some fluorescences, such as that at 425 nm (observed for norharmane in acetonitrile) does not appear and nevertheless, in this solvent D2 emission was registered in both systems, which makes us conclude that there is no connection between these two species (425 nm and D2), and the first one is not responsible for D2 formation.

On the other hand, D2 emission appears at low AcH concentration in all the solvents studied in this work and its formation is only possible in the presence of two AcH molecules. For that, we believe that a double equilibrium should be established between BCCM and two AcH molecules interacting with the two BCCM nitrogen atoms since the first addition of AcH.

On the contrary, Carmona et al. [31] have recently shown, that for harmane (β carboline derivative with a methyl group in the position 1 of the β-carbolinic ring) in mixtures of cyclohexane/toluene and in the presence of a proton donor as hexafluoropropanol, only a 1:3 hydrogen-bonded proton transfer complex is the responsible for the zwiterionic species formation, Z, (D2 for us, 1:2 BCCM/AcH complex). So, it seems that for Z (or D2) formation in the presence of a proton donor, it is necessary the formation of a long enough donor-chain to overcome the steric hindrance of the methyl group in the position 1 in the β-carbolinic ring and, once the chain bends it can interact with the pyrrolic nitrogen forming Z (D2).

In our case, BCCM has not the steric hindrance of the methyl group. This means that D2 can be formed by a 1:2 complex as was proposed by Chou et al. [29]. Moreover, the acetic acid molecule is both a proton donor and acceptor, so two separate acetic acid molecules can interact simultaneously with a BCCM molecule through the two nitrogen atoms, giving rise to D2 formation. So, we believe that D2 can be formed in different ways.

The excitation spectra confirm that D2 is not formed from N but for another species with a spectrum similar to N in which there is a hydrogen-bonding formation between BCCM and AcH (Fig. 4).

A good linear fitting is obtained by plotting the reciprocal of absorbance at 353 nm vs. $1/[\mathrm{AcH}]$, before the cationic species absorption starts (from 0 to 1% AcH), confirming the 1:1 relation between BCCM and AcH in the ground state complex. The values of the complex formation constants, K_{AD}, at 25 °C for the different systems studied are 3.2, 8.3, 24.9 and 57.6 M^{-1} in dioxane, acetonitrile, dichlorometane and benzene, respectively. These values are lower than those obtained for norharmane in the same solvents: 12.6, 14.2, 34.7 and 357.3 M^{-1}, although they maintain the same tendency. Benzene (π base) is the solvent with a more favored complex formation.

As we also observed for norharmane, the reciprocal of the fluorescence decay time of BCCM at 370 nm depends linearly on the acetic acid concentration: $1/\tau = k + k_{\mathrm{a}}\,[\mathrm{AcH}]$ where k includes both the radiative and non-radiative depopulation constants and k_{a} represent the quenching constant by AcH. From the fitting, the k_{a} values are 1.1×10^{8} M^{-1} s^{-1}, 2.1×10^{8} M^{-1} s^{-1}, 2.8×10^{8} M^{-1} s^{-1}, 3.0×10^{8} M^{-1} s^{-1} in dioxane, benzene, acetonitrile and dichlorometane, respectively. In the last three solvents, the values found for these depopulation dynamics are very similar and related to the hydrogen acceptor ability of BCCM pyridinic nitrogen.

Another noticeable fact is the Stern–Volmer plot (F_0/F vs. AcH concentration) upward curvature, concave towards the y-axis (Fig. 8). This feature is characteristic when both static and dynamic quenching occur for the same fluoro-

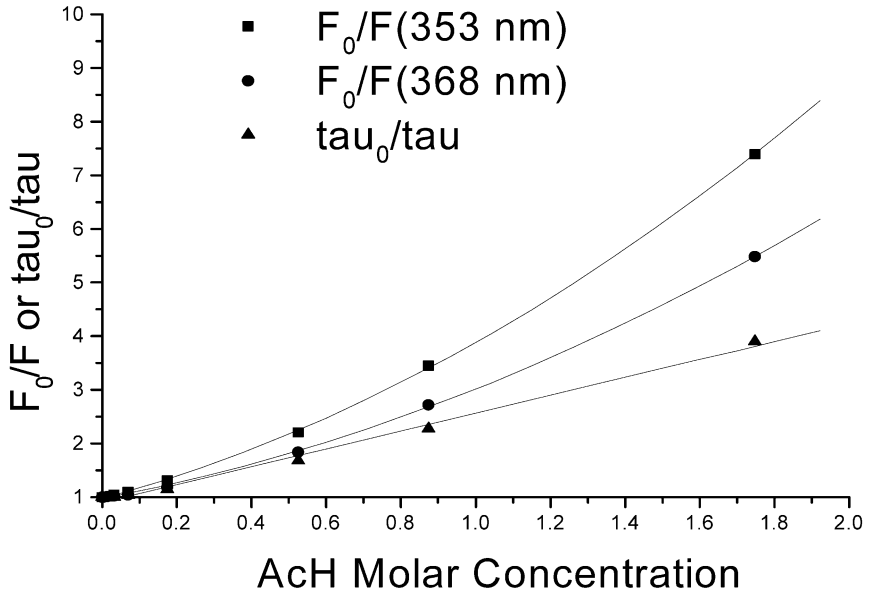
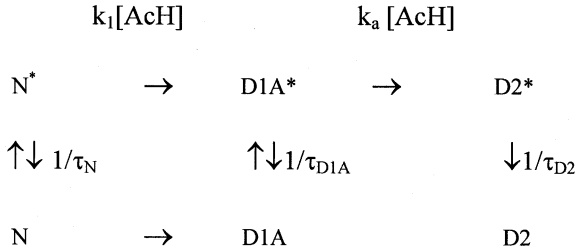


Fig. 8. Quenching of BCCM by AcH in dioxane exciting at 329 nm and recording the fluorescence at 353 and 368 nm (F_0 and F are the fluorescence intensities in the absence and presence of quencher and τ_0 and τ are the lifetimes of BCCM in the absence and presence of the quencher, respectively).

phore. Apart from that, we observed a rise time when the D2 component fluorescence was recorded, indicating that this emission was originated from another species in the first excited state, a fact that is corroborated by the excitation spectra. The problem is to identify this species since all species except for the cationic one, have very similar lifetimes. We propose the following scheme to explain the behavior observed in this system:

Scheme 1 explains the observed behavior in the systems BCCM/AcH in the interval between 0 and 1% AcH. In this interval, a 1:1 complex is formed in the ground state between BCCM pyrrolic nitrogen and AcH, which produces a slight red shift of the absorption spectrum. The possible D1A and D2 structures are presented in Fig. 9. On the one hand, D2* would be formed only in the first excited state and principally from D1A*. This



Scheme 1

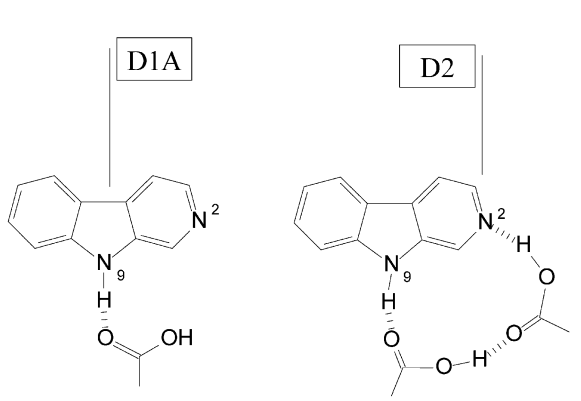


Fig. 9. Structures of D1A and D2.

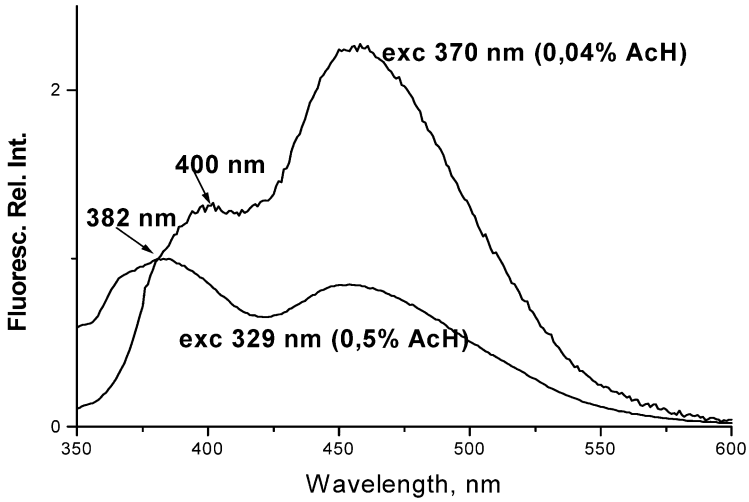


Fig. 10. Emission spectra of BCCM in dichloromethane with different excitations at 329 and 370 nm.

would explain the upward curvature of the Stern–Volmer plot and the observed risetime when the fluorescence decay was recorded at 520 nm. On the other hand, to explain the N lifetime decrease with AcH concentration, N and D1A must also be connected in the first excited state.

For AcH concentrations higher than 1%, other interactions take place in the ground state. However, as they cause little changes in the absorption spectra and are strongly overlapped with the cationic species absorption, it is not possible to reliably clarify the stoichoimetries of these complexes. On the other hand, the D1A stability diminishes as the polarity and hydrogen–bonding ability of the solvent increase, which could explain that by increasing the AcH concentration in solution and therefore by increasing the polarity and hydrogen–bonding ability of the media, D2 fluorescence disappears. In sufficiently polar solvents, the hydrogen-bonding interaction occurs mainly between the pyridinic nitrogen of BCCM and AcH and so, the 1:1, 1:2 and 1:3, MHN/hydrogen-bonded complexes as proposed by Carmona et al. [31] can be formed.

Recently, we have been working also with BCCM in aqueous solution acidifying it both with acetic and sulfuric acid [35]. The absorption maxima are only slightly shifted due to solvent polarity, and the most significant fact is the loss of the vibrational structure and the 20 nm red shift of the emission attributable to the N emission. This means that stronger interactions between BCCM and water are produced in the first excited state than in the ground state. This new species that results from the interaction between BCCM and water, has a fluorescence maximum around 380 nm and a lifetime of approximately 3.2 ns that does not depend on the AcH concentration. Apart from this species, in aqueous solution, only the cationic species emission is observed whose absorption maximum and fluorescence lifetime are similar to those found at 460 nm in dioxane, benzene, dichloromethane and acetonitrile (approx. 14 ns). Apart from that, the emission spectrum shape in aqueous solution at acid pH is constant, no matter if the solution is acidified with acetic or sulfuric acid, which leads us to attribute the fluorescence with a maximum at 460 nm to C emission.

The fluorescence at approximately 380 nm observed in aqueous solution, was also recorded for BCCM in dichloromethane as was previously observed for other BC derivatives in the same solvents [39,40]. In dichloromethane another fluorescence around 400 nm was clearly recorded (Fig. 10). The structures of the species responsible

for these emissions could also be related to hydrogen-bonding interactions (probably established in the ground state) between the pyridinic nitrogen of BCCM and one, two or three AcH molecules. However, it is not possible to reliably clarify the stoichoimetries of these complexes since both their absorption and fluorescence are strongly overlapped with those of the other species. What can be foreseen is that hydrogen donor solvents will favor the formation of these species.

Therefore, four different complexes have been observed in a short range of AcH concentrations before the protonation of BCCM. The structures of D1A and D2 seem to be sufficiently justified (Fig. 9). The structures of the species that emit around 380 and 400 nm, could be attributed to interactions between the pyridinic nitrogen and AcH, but it is still not known how many AcH molecules participate in these complexes. In order to clarify these interactions, NMR studies are currently being carried out and will be presented in future publications.

Acknowledgments

We thank the Junta de Castilla y León and the European Union for financial support (BU09/01 project).

References

[1] V. Guallar, A. Douhal, M. Moreno, J.M. Lluch, DNA mutations by proton and charge transfer in the low-lying excited singlet electronic states of the DNA base pairs: a theoretical insight, J. Phys. Chem. A 103 (1999) 6251–6256.

[2] P.-T. Chou, C.-Y. Wei, G.-R. Wu, W.-S. Chen, Excited-state double proton transfer in 7-azaindole analogues: observation of molecular-based tuning proton-transfer tautomerism, J. Am. Chem. Soc. 121 (1999) 12186–12187.

[3] M. Chachisvilis, T. Fiebig, A. Douhal, A.H. Zewail, Femtosecond dynamics of a hydrogen-bonded model base pair in the condensed phase: double proton transfer in 7-azaindole, J. Phys. Chem. A 103 (4) (1998) 670–673.

[4] A. Douhal, M. Moreno, J.M. Luch, On the theoretical reports on 7-azaindole base-pair phototautomerization, Chem. Phys. Lett. 324 (2000) 75–78.

[5] G.M. Chaban, M.S. Gordon, The ground and excited state hydrogen transfer potential energy surface in 7-azaindole, J. Phys. Chem. A 103 (1998) 185–189.

[6] C.F. Chapman, M. Maroncelli, Excited-state tautomerization of 7-azaindole in water, J. Phys. Chem. US 96 (21) (1992) 8430–8441.

[7] C. Foces-Foces, A. Echevarría, N. Jagerovic, I. Alkorta, J. Elguero, O. Klein, et al., A Solid-state NMR, X-ray diffraction and ab initio computational study of hydrogen-bond structure and dynamics of pyrazole-4-carboxylic acid chains, J. Am. Chem. Soc. 123 (2001) 7898–7906.

[8] N.S. Golubev, G.S. Denisov, V.A. Gindin, S.S. Ligay, H.-H. Limbach, S.N. Smirvov, The role of short hydrogen bonds in mechanism of enzymatic action, J. Mol. Struct. 322 (1994) 83–91.

[9] P.A. Frey, Strong hydrogen bonding in molecules and enzymatic complexes, Magn. Reson. Chem 39 (2001) S190–S198.

[10] J.R.F. Allen, B.R. Holmstedt, The simple β-carboline alkaloids, Phytochemistry 19 (1980) 1573–1582.

[11] B.T. Ho, W.M. McIsaac, K.F. Walker, V. Estevez, Inhibitors of monoamine oxidaxe. Influence of methyl substitution on the inhibitory activity of β-carbolines, J. Pharm. Sci. 57 (1968) 269–274.

[12] D. Fekkes, A. Tuiten, I. Bom, L. Pepplinkhuizen, Pharmacokinetics of the beta-carboline norharman in man, Life Sci 69 (18) (2001) 2113–2121.

[13] C. Szantay, G. Blusko, K. Honty, G. Dörnyei, in: A. Brossi (Ed.), The Alkaloids, Chapter 2, 27, Academic Press, New York, 1986.

[14] W.E. Muller, K.J. Fehske, H.O. Borbe, U. Wollert, C. Nanz, On the neuropharmacology of harmane and other β-carbolines, Pharm. Biochem. Behav. 14 (1981) 693–699.

[15] S.M. Husbands, R.A. Glennon, S. Gorgerat, R. Gough, R. Tyacke, J. Crosby, et al., β-Carboline binding to imidazoline receptors, Drug Alcohol Depend 64 (2001) 203–208.

[16] R.H. Dodd, β-Carbolines and memory: an overview, Eur. Bull. Cognit. Psychol 12 (5–6) (1992) 484–499.

[17] Z. Taira, S. Kanzawa, C.h. Dohara, S. Ishida, M. Matsumoto, Y. Sakiya, Intercalation of six β-carboline derivatives into DNA, Jpn. J. Toxicol. Environ. Health 43 (2) (1997) 83–91.

[18] G. Duportail, Linear and circular dichroism of harmine and harmaline interacting with DNA, Int. J. Macromol. 3 (1981) 188–192.

[19] R.A. Larson, K.A. Marley, R.W. Tuvenson, M.R. Berenbaum, carboline alkaloids mechanisms of phototoxicity to bacteria and insects, Photochem. Photobiol 48 (1988) 665–674.

[20] J.B. Hudson, E. Graham, N. Towers, Antiviral effect of harmine, a photoactive β-carboline alkaloid, Photochem. Photobiol 43 (1986) 21–26.

[21] T. Uezono, W. Maruyama, K. Matsubara, M. Naoi, K. Shimizu, O. Saito, et al., Norharman, an indoleamine-derived beta-carboline, but not Tryp-P-2, a gamma-carboline induces apoptotic cell death in human

neurobastoma SH-SY5Y cells, J. Neural Transm 108 (8–9) (2001) 943–953.

[22] M. Beljanski, M.S. Beljanski, Selective-inhibition of in vitro synthesis of cancer CNA by alkaloids of beta-carboline class, Exp Cell Biol. 50 (2) (1982) 79–87.

[23] M. Beljanski, M.S. Beljanski, 3 alkaloids as selective destroyers of the proliferative capacity of cancer-cells, IRCS Med. Sci. Biochem. 12 (7) (1984) 587–588.

[24] A. Dias, A.P. Varela, M. da, G. Miguel, A.L. Macanita, R.S. Becker, Carboline photosensitizers. 1. Photophysics, kinetics and excited-state equilibria in organic solvents, and theoretical calculations, J. Phys. Chem. 96 (1992) 10290–10296.

[25] J. Seixas de Melo, A.L. Macanita, Three interconverting excited species: experimental study and solution of the general photokinetic triangle by time-resolved fluorescence, Chem. Phys. Lett. 204 (5–6) (1993) 556–562.

[26] A. Dias, A.P. Varela, M. da, G. Miguel, R.S. Becker, H.D. Burrows, et al., β-Carbolines. 2. Rate constants of proton transfer from multiexponential decays in the lowest singlet excited state of harmine in water as a function of pH, J. Phys. Chem. 100 (1996) 17970–17977.

[27] C. Carmona, M. Galán, G. Angulo, M.A. Muñoz, P. Guardado, M. Balon, Ground and singlet excited state hydrogen bonding interactions of betacarbolines, Phys. Chem. Chem. Phys. 2 (2000) 5076–5083.

[28] C. Carmona, M. Balón, M. Galán, G. Angulo, P. Guardado, M.A. Muñoz, Kinetic study of hydrogen bonded exciplex for of n_9-methyl harmane, J. Phys. Chem. A 105 (2001) 10334–10338.

[29] P.-T. Chou, Y.-I. Liu, G.-R. Wu, M.-Y. Shiao, W.-S. Yu, Ch.-Ch. Cheng, et al., Proton-transfer tautomerism of β-carbolines mediated by hydrogen-bonded complexes, J. Phys. Chem. B 105 (2001) 10674–10683.

[30] M.J. Tapia, D. Reyman, M.H. Viñas, C. Carcedo, J.J. Camacho, Hydrogen-bonding interactions of norharme in mixtures of acetic acid with benzene, *p*-dioxane and acetonitrile, Phys. Chem. Chem. Phys. 4 (2002) 3676–3684.

[31] C. Carmona, M. Balon, M. Galán, P. Guardado, M.A. Muñoz, Dynamic study of excited state hydrogen-bonded complexes of harmane in cyclohexane–toluene mixtures, Photochem. Photobiol 76 (3) (2002) 239–246.

[32] D. Reyman, M.H. Viñas, Temperature effect on excited-state proton transfer reactions of β-carboline in different acetic-acid mixtures, Chem. Phys. Lett. 301 (1999) 551–558.

[33] D. Reyman, M.H. Viñas, J.M.L. Poyato, A. Pardo, Proton transfer dynamics of norharman in organic solvents, J. Phys. Chem. A 101 (5) (1997) 768–775.

[34] M.A. Muñoz, O. Sama, M. Galán, P. Guardado, C. Carmona, M. Balón, Interactions between betacarboline and benzenoid π bases: FTIR evidence for the formation of NH-π hydrogen bonds, J. Phys. Chem. B 103 (1999) 8794–8798.

[35] M.J. Tapia, D. Reyman, M.H. Viñas, C. Carcedo, J.M.L. Poyato, Acid–base equilibria of methyl β-carboline-3-carboxylate in aqueous solution, J. Lum. 101 (2003) 227–234.

[36] R. Sakurovs, K.P. Ghiggino, Excited state proton transfer in β-carboline, J. Photochem. 18 (1982) 1–8.

[37] M.A. Muñoz, O. Sama, M. Galán, P. Guardado, C. Carmona, M. Balón, Hydrogen bonding NH/π interactions between betacarboline and methyl benzene derivatives, Spectrochim. Acta Part A 57 (2001) 1049–1056.

[38] M. Balon, M.A. Muñoz, P. Guardado, C. Carmona, Hydrogen-bonding interactions between harmane and pyridine in the ground and lowest excited singlet states, Photochem. Photobiol 64 (3) (1996) 531–537.

[39] D. Reyman, A. Pardo, J.M.L. Poyato, Phototautomerism of beta-carboline, J. Phys. Chem.-US 98 (41) (1994) 10408–10410.

[40] D. Reyman, M.H. Viñas, J.J. Camacho, Steady-state and time-resolved study of the proton-transfer fluorescence of harmine and 2-methyl-harmine in organic solvents, J. Photochem. Photobiol. A Chem 120 (1999) 85–91.