Electronic spectroscopy of the β-carboline derivatives nitronorharmanes, nitroharmanes, nitroharmines and chloroharmines in homogeneous media and in solid matrix

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Dedicated to Prof. Dr. Rosa M. de Lederkremer on occasion of her 70th anniversary

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Abstract
Electronic spectra (absorption, fluorescence and phosphorescence emission spectra) of nitro-β-carboline derivatives (nitronorharmanes, nitroharmanes and nitroharmines) in acetonitrile and in methanol solution at 298 K and in solid matrix at 77 K have been recorded and compared with those of the corresponding β-carbolines and chloroharmine derivatives. The electronic absorption spectra of triplet transients of these β-carbolines, the triplet lifetime, τ_T, and the intersystem crossing quantum yield, φ_{isc}, were measured by Laser Flash Photolysis at 298 K. The dynamic properties of the lowest excited singlet and triplet states (τ_f, τ_p, φ_f and φ_p) have been measured in the same experimental conditions. From these data, the radiative and radiationless rate constants (k^o_f , k_{isc} and k_{ic}) were derived. The intramolecular spin – orbital coupling effect on the spectroscopic data and photophysical rate constant were also analyzed.

Keywords: β-Carbolines, Nitro-β-carbolines, Spectroscopic and photophysics

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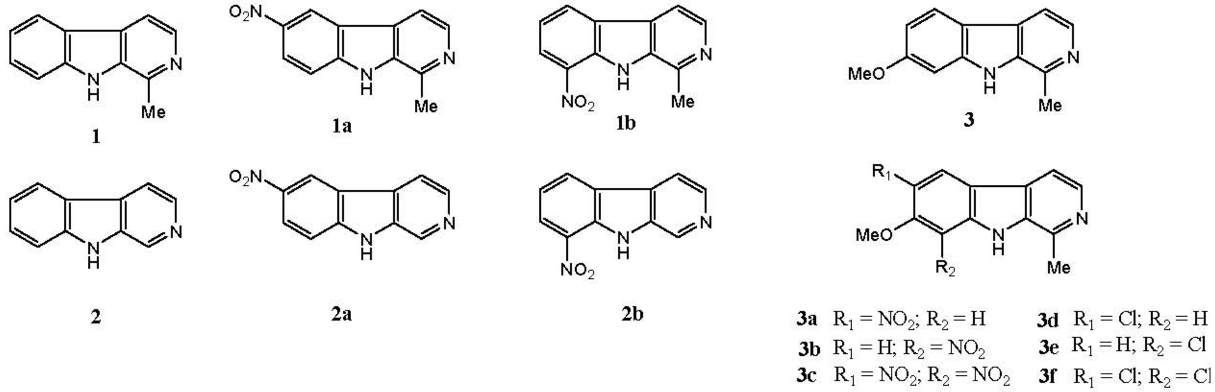
Introduction

As part of our study of the photochemistry of β-carbolines, 9H-pyrido[3,4-*b*]indoles,[1] and their potential use as matrices (photosensitizers) in matrix assisted ultraviolet laser desorption / ionization mass spectrometry (UV-MALDI MS),[2] we decided to examine the behavior of substituted β-carbolines. We have recently described commercial β-carbolines (norharmane, harmane and harmine) as quite efficient UV-MALDI matrices.[2] As substituted β-carbolines, nitro-β-carbolines were selected because, as it is known, for aromatic molecules, nitro group as substituent might induce modifications of the acid-base properties in the ground and electronic excited state, on the nature (π,π*; n,π*), multiplicity (singlet, S_1; triplet, T_1), lifetime (τ_{S1}; τ_{T1}) and efficiency of population (φ) of the electronic excited states without affecting much their photostability.[3,4]

As it is well known, UV-MALDI MS[5,6] has become a versatile and important tool for the determination of molecular masses and structure of macromolecules such as synthetic polymers[7] and biopolymers.[8,9] The principle of UV-MALDI involves ionizing macromolecules for MS analysis by diluting them in a suitable matrix of small organic molecules which absorb the UV-laser photons. Thus, the knowledge of the photochemistry and photophysics of the potential UV-MALDI matrices is essential for the comprehension of the UV-MALDI processes which occur after the electronic excitation of the matrix.

The effect of heavy atoms on the photophysical properties of aromatic compounds has been of great interest.[3,4,10] An halogen with high atomic number (heavy atom) attached directly to an aromatic compound can reduce significantly the fluorescence quantum yield and this effect is called the intramolecular heavy atom effect (HAE).[10-15] The spin-orbital coupling mechanism of the heavy atom enhances the rate of intersystem crossing to the triplet state.[12,15,16] Bromine and iodine atoms, as heavy atoms, have been preferentially used in order to study both intramolecular and intermolecular HAE.[3,4,10-16] Furthermore, carbonyl and nitro groups attached to aromatic compounds also show a spin – orbital coupling mechanism that reduces significantly the fluorescence quantum yield of the aromatic compound and enhances the intersystem crossing process.[3,4]

In the present work the effect of the nitro group as substituent on the fluorescence (φ_f, τ_f, τ^0_f, k^0_f) and phosphorescence (φ_p, τ_p, k^0_p) parameters of several β-carboline derivatives is investigated: i) in acetonitrile and in methanol at 298 K; ii) in the same solvents in the presence of added mineral acids at 298 K; iii) in a mixture of isopropanol - ethyl ether (1:1; v:v) at 77 K and iv) in a mixture of isopropanol - ethyl ether (1:1) with added mineral acids at 77 K by using time correlated single photon counting technique and phosphorescence lifetime spectroscopy. Also, the electronic absorption spectra of the triplet transient, the triplet lifetime (τ_T) and the intersystem crossing quantum yield (φ_{isc}) of the β-carboline derivatives were investigated by Laser flash photolysis technique at 298 K. Scheme 1 shows the β-carboline derivatives studied and in order to compare the effect of a light atom as substituent (light substituent = H) and a spin - orbital coupling group as substituent (heavy substituent = NO_2) in the same β-carboline moiety. Thus, the spectroscopic data for harmane, norharmane and harmine were also studied and described briefly in the present paper. In the case of harmine, the properties of nitroharmine derivatives **3a** – **c** are also compared with that of the chloroharmine derivatives **3d** – **f**.



Scheme 1. β-Carbolines studied.

Results and Discussion

It is known,[3,4] in the absence of photochemical reactions, the fluorescence quantum yield φ_f of an emitting substance is given by the ratio of rate constants shown in eq. 1:

$$\varphi_{f} = k^0_f/[k^0_f + k_{isc} + k_{ic}] = k^0_f \tau_f = \tau_f / \tau^0_f \qquad \text{eq. 1}$$

where the subscripts denote respectively the processes of fluorescence (f), intersystem crossing (isc) and internal conversion (ic). The parameters τ_f and τ^0_f represent respectively the fluorescence and radiative lifetime. When a spin – orbital mechanism operates due to the presence of chlorine atom or a nitro group attached to the aromatic moiety, the rate constant k_{isc} is increased, and hence both τ_f and φ_f are reduced significantly as k_{isc} grows larger. Also, the spin – orbital mechanism affects the absorption spectra of nitro aromatic compounds where a strong enhancement of ε ($S_0 \rightarrow T_1$) is observed but not on ε ($S_0 \rightarrow S_1$).[3,4] Because of the relationship between ε ($S_0 \rightarrow T_1$) and k^0_p and between ε ($S_0 \rightarrow S_1$) and k^0_f ($k^0_f = 1/\tau^0$) it is expected that k^0_p but not k^0_f will be influenced by spin – orbital perturbation. Therefore, a study of the fluorescence and phosphorescence spectroscopy, the corresponding φ_f and φ_p and the comparison of the photophysical rate constants, k^0_f and k^0_p, is essential in order to understand the spin – orbital mechanism. The much higher values of φ_p reflect both a greater efficiency of population of T_1 (k_{isc} is enhanced) and a greater efficiency of emission from T_1 (k^0_p is enhanced more than k_{st}).

Absorption Spectroscopy

The absorption spectra of β-carbolines were recorded in acetonitrile and in methanol at 298 K and generally they showed two bands in the 290-330 and 320 -360 nm region (Figure 1a). Previous spectroscopic studies and theoretical calculations have been carried out[1c-1e,17] allowing for unambiguous assignment of the lowest-lying excited electronic states of β-carbolines as ^1L_a

and ^1L_b in the C_{2v} symmetry point group with the short (z) axis and long (y) axis respectively in the plane of the molecule. Comparing the position and oscillator strength of the bands located in the 300 and 330 nm region for chloroharmines with that of harmine, it is possible to assign them as ^1L_a (S_2-S_0) and ^1L_b (S_1-S_0) electronic transitions too (Figure 1a).

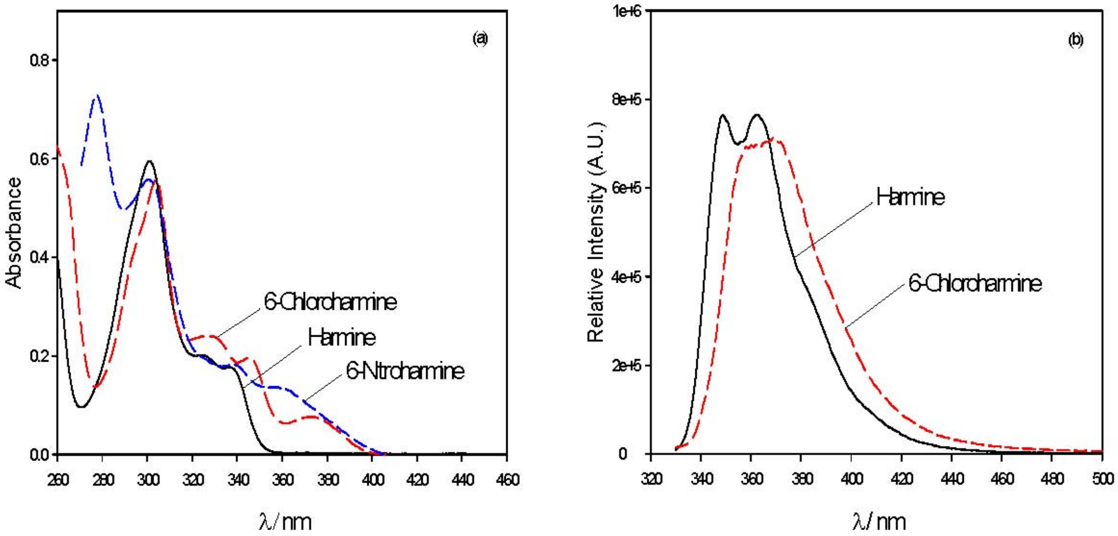


Figure 1. (a) Absorption spectra of harmine, 6-chloroharmine and 6-nitroharmine in acetonitrile (Concentration: $1x10^{-5}$ $mol.dm^{-3}$) at room temperature. (b) Fluorescence emission spectra of harmine and 6-chloroharmine in acetonitrile (Concentration: $1x10^{-5}$ $mol.dm^{-3}$; λ_{exc}: 310 nm) at 298 K.

The absorption spectra of the nitro β-carboline derivatives recorded in acetonitrile and in methanol at 298 K exhibit a noticeable bathochromic shift of the ^1L_b band ranging between 20 and 40 nm compared to that of the β-carbolines **1-3** (Tables 1 and 2; Fig. 1a). This large red shift accounts for the in-plane conjugation of the nitro substituents and it can be interpreted by the mesomeric effect induced by the nitro group bonded to the β-carboline moiety (Tables 1 and 2). The absorption spectra of chloroharmines (**3d-3f**) show a minor bathochromic shift of the ^1L_b band ranging between 4 and 16 nm. This moderated red shift is due to a minor mesomeric effect of the chlorine atom attached to the harmine moiety (Tables 1 and 2). In general, the absorption spectra of chloroharmine and nitro-β-carbolines show a small bathochromic shift of the ^1L_a electronic transition bands with respect to non-substituted β-carbolines **1**, **2** and **3** and this moderate red shift ranges from 2 to 6 nm.

Table 1. Spectroscopic data (λ_{max}(abs) and λ_{max}(em) [nm]), fluorescence quantum yield (φ_f) and fluorescence lifetime (τ_f [ns]) of β-carbolines and substituted β-carbolines in MeCN and in MeCN + $HClO_4$ at 298 K

Compd.	MeCN				MeCN + H^+			
	λ_{max}(abs)	λ_{max}(em)	φ_f	τ_f	λ_{max}(abs)	λ_{max}(em)	φ_f	τ_f
1	344	369	0.23	4.3	366	433	0.75	22.4
1a	364	*NF*[a]	-	-	362	446	0.005	-
1b	394	*NF*	-	-	384	440	0.006	-
2	346	369	0.25	3.9	368	434	0.72	22.0
2a	366	*NF*	-	-	366	446	0.004	-
2b	396	*NF*	-	-	392	442	0.006	-
3	332	362	0.39	5.1	360	416	0.64	7.2
3a	360	*NF*	-	-	356	417	0.003	-
3b	372	*NF*	-	-	363	425	0.003	-
3c	432	*NF*	-	-	376	422	0.005	-
3d	336	364	0.06	2.0	360	426	0.10	2.1
3e	342	366	0.04	1.3	364	429	0.19	2.0
3f	348	360	0.04	1.1	358	435	0.07	-

[a] *NF*: non fluorescent.

Table 2. Spectroscopic data (λ_{max}(abs) and λ_{max}(em) [nm]), fluorescence quantum yield (φ_f) and fluorescence lifetime (τ_f [ns]) of β-carbolines and substituted β-carbolines in MeOH and in MeOH + $HClO_4$ at 298 K

Compd.	MeOH				MeOH + H+			
	λ_{max}(abs)	λ_{max}(em)	φ_f	τ_f	λ_{max}(abs)	λ_{max}(em)	φ_f	τ_f
1	350	375	0.30	5.3	370	430	0.70	21.2
1a	352	*NF*[a]	-	-	375	432	0.09	-
1b	390	*NF*[a]	-	-	384	436	0.11	-
2	352	379	0.31	6.0	376	445	0.60	24.2
2a	342	*NF*[a]	-	-	364	*NF*	-	-
2b	392	*NF*[a]	-	-	392	*NF*[a]	-	-
3	338	367	0.40	5.4	364	415	0.45	6.8
3a	342	*NF*[a]	-	-	360	*NF*	-	-
3b	398	*NF*[a]	-	-	360	*NF*[a]	-	-
3d	342	369	0.10	2.6	368	420	0.10	2.0
3e	348	372	0.07	1.1	373	427	0.05	2.0
3f	352	360	0.05	0.9	363	430	0.04	2.5

[a] *NF*: non fluorescent.

The absorption spectra of β-carbolines (**1** – **3**) and chloroharmines (**3e** – **3f**) were recorded in MeOH and in MeCN doped with $HClO_4$. When the absorption spectra of β-carbolines and chloroharmines in neutral conditions are compared with those spectra recorded in acidic media a bathochromic shift (around 20 nm) is observed for the lowest-lying energy bands (see Tables 1 and 2). The spectral change is attributed to the formation of a cationic species due to an acid-base equilibrium process in the electronic ground state, as shown in Figure 2 for norharmane.

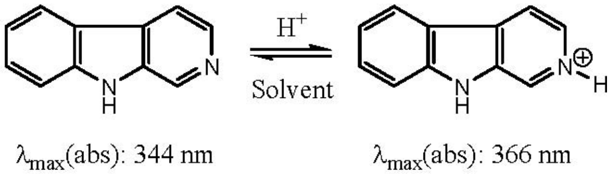


Figure 2. Acid-base equilibrium of norharmane in the electronic ground state.

This behavior could be attributed to the fact that a change of the lowest-lying electronic transition takes place upon protonation of the pyridine nitrogen atom. Therefore, the lowest energy band of the neutral β-carbolines, which is a π,π* electronic transition, is red shifted because the HOMO increases its electron density. When protonation takes place the *n* electron pair at pyridine N increases its σ character and the probability of its interaction (overlapping) with the π HOMO is higher. Then, a red shift is expected for the lowest energy band in the absorption spectra. This kind of behavior upon protonation of the β-carboline moiety was first observed and rationalized by Aullo and Tomas Vert for norharmane, harmane and harmine.[17]

Instead, the comparison of the λ_{max}(abs) data collected in Tables 1 and 2 for the nitro-β-carbolines and its cationic species shows a minor blue shift of 2 to 10 nm. This behavior is attributed to the fact that an increase of ΔE for the lowest-lying electronic transition takes place after protonation of the pyridine nitrogen atom. Therefore, the lowest energy band of the neutral nitro-β-carbolines has, in some extent, an n,π* character. Thus, when protonation occurs, a blue shift is expected for the lowest energy band in the absorption spectra.[3,4]

Emission spectroscopy

Emission at 298 K. The β-carbolines **1**, **2** and **3** are good fluorescent chromophores with φ_f values ranging from 0.20 to 0.40 and τ_f values of the order of 4 - 6 ns (see Tables 1 and 2).[1e,18] These compounds display mirror symmetry when the fluorescent emission and absorption spectra are compared (spectra of harmine (**3**) are shown in Figures 1a and 1b).[1e] The fluorescence lifetimes (τ_f) of the corresponding cationic species were measured in MeCN and in MeOH doped with $HClO_4$ by the TC-SPC technique; τ_f values of 22 ns were obtained for compounds **1** and **2** while for compound **3** the τ_f value was 7.2 ns (Tables 1 and 2). Pardo *et al.*[18] have measured the φ_f and τ_f values of compounds **1**, **2** and **3** in ethanol and in acidic media. Comparison of our results measured in methanol with those of Pardo, we can conclude that the φ_f values of compounds **1**, **2** and **3** in methanol are similar and show the same trend while the τ_f

values of these compounds are higher than those reported by Pardo.

The absorption and fluorescence spectra of chloroharmines (**3d – 3f**) recorded in acetonitrile and in methanol at 298 K display excellent symmetry as is shown in Figures 1a and 1b. This indicates that the geometries of the molecules are quite similar in their ground and excited states. Tables 1 and 2 show that the λ_{max}(fluo) of mono-chloroharmine derivatives **3d-3e** display a minor bathochromic shift ranging from 2 to 6 nm in comparison to harmine (**3**). This effect depends on the position and number of the chlorine atoms attached to the harmine moiety. This effect is due to a mild in-plane conjugation of the chlorine atom with the harmine moiety. For the dichloroderivative **3f** a minor hypsochromic shift was observed (Tables 1 and 2). We have also measured the fluorescence quantum yield (φ_f) and the fluorescence lifetime (τ_f) for compounds **3**, **3d** – **3f** in MeCN and in MeOH (see Tables 1 and 2). As it was expected the intramolecular quenching of the fluorescence emission (intramolecular heavy atom effect, HAE) of chloro-β-carbolines **3d** – **3f** increases as the number of chlorine atoms attached to the β-carboline moiety is increased. This trend is evidenced through the φ_f values of compounds **3d** – **3f** that decrease an 80 – 90 % relative to the φ_f value of harmine (**3**). A similar trend is observed when the τ_f values are analyzed (Tables 1 and 2).

As it is expected, the nitro-β-carbolines are not fluorescent chromophores in MeCN and in MeOH due to the nitro group attached to the β-carboline moiety where a high spin – orbital coupling mechanism operates. This behavior means that these molecules dissipate efficiently the energy from S_1 through radiationless processes (internal conversion and/or intersystem crossing processes).

The emission fluorescence spectra of β-carbolines **1-3**, chloro- and nitro-β-carbolines were recorded in MeCN doped with $HClO_4$, showing a broad band centered between 416 and 446 nm depending on the substituent attached to the β-carboline moiety (Table 1). When the solvent used was MeOH doped with acid, a similar behavior was observed for the β-carbolines except for compounds **2a**, **2b**, **3a** and **3b**, where no fluorescence emission was detected (Table 2). The comparative analysis of the φ_f values in acetonitrile acidic conditions show that the cationic species of harmane, norharmane and harmine are excellent fluorophores (φ_f 0.75, 0.72 and 0.64). It is noteworthy to point out that the cationic species of chloro- and nitro-β-carboline derivatives show a fluorescence emission quantum yield that diminishes in a significant extent due to a high spin-orbital coupling mechanism ((intramolecular fluorescence quenching; see Table 1). This trend is clearly depicted if the φ_f values are compared: **3**, $\varphi_f = 0.64$; **3d**, $\varphi_f = 0.10$; **3b**, $\varphi_f = 0.003$. Also, the τ_f values for compounds **3**, **3d** and **3e** show a decreasing trend due to the HAE of chlorine atoms attached to the harmine moiety. The φ_f and τ_f values in methanolic acidic conditions show a similar trend for the β-carboline derivatives analyzed except for compounds **2a**, **2b**, **3a** and **3b**, where no fluorescence emission was detected.

Emission at 77 K

We have measured the fluorescence and phosphorescence emission spectra and the spectroscopic parameters (φ_f, φ_p and τ_p) of compounds **1**, **2**, **3**, **1a**, **1b**, **2a**, **2b**, and **3a-3f** in solid matrix at 77 K and the data are collected in Table 3 ((a) isopropanol-ethyl ether (1:1)). Compounds **1**, **2** and **3** are good fluorescent and phosphorescent chromophores at 77 K showing the λ_{max}(fluo) at 360 nm and the λ_{max}(phosph) around 410 nm. The φ_f values of these compounds are high, ranging between 0.67 and 0.91. These compounds deactivate their excited triplet state through a moderate phosphorescence emission (φ_p: 0.36) and a radiationless pathway competes in the deactivation of the excited triplet state in a great extent. Taking into account that the shape of the phosphorescence emission spectra is "naphthalene-like" and the phosphorescence lifetimes (τ_p) are on the time scale of seconds we concluded that the electronic excited triplet state is most likely to be a π,π^* electronic transition.[3]

The nitroderivatives **1a**, **1b** and **3a-c** do not show emission in solid matrix at 77 K. Thus, the triplet excited states deactivate efficiently through a radiationless pathway. Instead, nitronorharmane derivatives (**2a** and **2b**) show φ_p values lesser than that of harmane (**2**) indicating that these compounds deactivate efficiently the excited triplet state through a radiationless process while the radiative deactivation takes place to a lesser extent. In the case of nitronorharmane derivatives (**2a** and **2b**) the shape of the phosphorescence emission spectra are "benzophenone-like"; due to the presence of the nitro group attached to the β-carboline moiety it resembles the phosphorescence emission spectra of benzophenone.

Comparison of the λ_{max}(phosph) values of the nitro-harmane derivatives with that of harmane a bathochromic shift is observed due to the in-plane conjugation of the nitro group with the β-carboline moiety (Table 3 (a)).

It is interesting to note that compounds **3d** and **3e** show fluorescence and phosphorescence emissions in solid matrix at 77K and again, the spin – orbital coupling mechanism operates because the φ_f values diminish significantly in comparison with φ_f value of harmine (see Table 3 (a)). In this connection, it is expected that an enhancement of the intersystem crossing process from S_1 to T_1 for compounds **3d** and **3e** takes place; this process is thermodynamically favored as can be seen from the values of $\Delta E(T_1\text{-}S_1)$ shown in Table 3. A bathochromic shift (25 nm) of the λ_{max}(phosph) is observed for 6-chloro- and 8-chloroharmine which is due to the in-plane conjugation of chlorine atom with the harmine moiety. The chloro-β-carboline derivatives show φ_p values higher than that of harmine indicating that these compounds deactivate efficiently the excited triplet state through phosphorescence radiation. Taking into account the τ_p values and the shape of the electronic phosphorescence emission spectra we concluded that the electronic excited triplet state is mostly π,π^* for compounds **3d** and **3e.** The dichloroharmine derivative **3f** does not show emission in solid matrix at 77 K.

We have also studied the fluorescence and phosphorescence emission spectra of the cationic species of **1**, **2**, **3**, **1a**, **1b**, **2a**, **2b**, and **3a-3f** in solid matrix at 77 K (Table 3; (b) isopropanol-ethyl ether (1:1) doped with $HClO_4$). In general, the cationic species show a significant phosphorescence emission as a broad band centered between 445 to 483 nm depending on the compound studied.

Table 3. Spectroscopic data (λ_{max}(fluo) and λ_{max}(phosph) [nm]), fluorescence quantum yield (φ_f), phosphorescence quantum yield (φ_p) and phosphorescence lifetime (τ_p [s]) of β-carbolines and β-carboline derivatives in solid matrix at 77 K

(a) Isopropanol-ethyl ether (1:1)

Compound	λ_{max}(fluo)	λ_{max}(phosph)	$\Delta E(T_1\text{-}S_1)^a$	φ_f	$\varphi_p{}^b$
1	357	419	- 0.57	0.84	0.36
2	360	419	- 0.56	0.67	0.36
2a	-	435	-	-	0.10
2b	-	436	-	-	0.18
3	361	405	- 0.61	0.91	0.37
3d	363	430	- 0.75	0.60	0.53
3e	369	425	- 0.64	0.28	0.73

(b) Isopropanol-ethyl ether (1:1) doped with $HClO_4$ before freezing

Compound	λ_{max}(fluo)	λ_{max}(phosph)	φ_f	$\varphi_p{}^c$
1	-	477	-	0.63
1a	-	475	-	0.24
1b	-	475	-	0.31
2	-	477	-	0.62
2a	-	477	-	0.20
2b	-	477	-	0.50
3	-	460	-	0.56
3a	-	455	-	0.72
3b	-	445	-	0.58
3c	-	430	-	0.16
3d	-	483	-	0.33
3e	-	472	-	0.17

[a] Values calculated in eV. [b] τ_p: **1**, 4.8; **2**, 4.6; **3**, 2.5; **3d**, 0.38; **3e**, 0.55. [c] τ_p: **1+H$^+$**, 5.7; **2+H$^+$**, 4.4; **3+H$^+$**, 3.1; **3d+H$^+$** 0.84; **3e+H$^+$**, 0.61.

The cationic species of compounds **1**, **2** and **3** have high φ_p values and this indicates that the phosphorescence emission is the efficient deactivation pathway of the excited triplet state. Instead, for the cationic species of the chloro-derivatives **3d** and **3e** a radiationless pathway deactivates the excited triplet states and competes efficiently with the phosphorescence emission. From the τ_p values for cationic species of **1**, **2**, **3**, **3d** and **3e** we concluded again that the excited triplet states are most likely to be π,π*. The dichloroharmine derivative **3f** does not show emission in solid acidic matrix at 77 K (Table 3 (b)).

In acidic conditions, the φ_p values of the cationic species of the nitro-β-carbolines **1a**, **1b**, **2a** and **2b** are smaller than that of cationic species of compounds **1** and **2**. Instead, for the cationic species of compounds **3a** and **3b** the φ_p values are quite similar to that of the cationic harmine. We concluded that these compounds deactivate the excited triplet state through a radiative (phosphorescence emission) process as efficiently as cationic harmine (**3**) do, in competition with the radiationless process.

Recently, Varela *et al.*[19] described the phosphorescence spectra, φ_p and τ_p at 77 K for the neutral and cationic forms of compounds **1**, **2** and **3**. The values obtained and the conclusions reached are similar to those discussed above.

Photophysical parameters and triplet transient absorption spectroscopy

In order to calculate the different rate constants of the deactivation of the lowest singlet excited state (k^0_f, k_{ic} and k_{isc}) of β-carbolines **1**, **2** and **3** and chloroharmines **3d**, **3e** and **3f** we measured the φ_{isc} of these compounds in acetonitrile and methanol by Laser Flash Photolysis at room temperature and the φ_{isc} values are collected in Table 4. These values were measured following the absorbance of the triplet transient of 1-cyanonaphthalene (triplet energy acceptor) formed at 500 nm after excitation with a laser pulse at $\lambda = 355$ nm in the presence of benzophenone, which was chosen as a reference ($\varphi_{isc} = 1$), and in the presence of the β-carboline derivatives. 1-Cyanonaphthalene was chosen as a triplet energy acceptor because the triplet energy is located at 57 Kcal / mol[20] and the energy of the triplet excited states of benzophenone and β-carboline derivatives are higher than 65 Kcal / mol. Therefore, the triplet energy transfer process from the triplet excited state of the substrates (and reference) to the triplet excited state of the energy acceptor (1-cyanonaphthalene) is thermodynamically feasible.

In the absence of photochemical reaction, it is known that equation 2 is valid for any organic chromophore, in particular, β-carboline derivatives:

$$1 = \varphi_f + \varphi_{isc} + \varphi_{ic} \qquad \text{eq. 2}$$

Taking into account that the quantum yield of fluorescence (φ_f) and the quantum yield of intersystem crossing (φ_{isc}) were measured independently by different spectroscopic methods in methanol and in acetonitrile at 298 K, the φ_{ic} values are easily derived from equation 2 and these data are shown in Table 4. In this connection, we are now able to calculate the radiative and radiationless deactivation rate constants of the lowest singlet excited states (k^o_f, k_{isc} and k_{ic}) of β-carbolines **1**, **2** and **3** and chloroharmines **3d**, **3e** and **3f** using eq. 1 and the relationships: $\varphi_{isc} = k_{isc}.\tau_f$ and $\varphi_{ic} = k_{ic}.\tau_f$. These data are collected in Table 4.

From the analysis of the k^o_f, k_{isc} and k_{ic} values, we can conclude that the lowest singlet excited state of β-carbolines **1**, **2** and **3** is efficiently deactivated through the fast inherent fluorescence emission process (k^o_f) in competition with the intersystem crossing channel (k_{isc})

which is highly favored, owing to the high rigidity and planarity of the β-carboline structures.[21] Also, the internal conversion process (k_{ic}) competes in the deactivation of the lowest singlet excited state of compounds **1**, **2** and **3** in a lesser extent.

The k_{isc} values of compounds **3**, **3d**, **3e** and **3f** show that the intersystem crossing process is an efficient deactivation pathway of the lowest singlet excited state of these compounds and is enhanced as the number of chlorine atoms attached to the β-carboline moiety is increased. This behavior can be rationalized due to a large HAE effect of the chlorine atoms. Also, the k_{ic} value of compounds **3**, **3d**, **3e** and **3f** increase slightly from harmine to chloroharmine derivatives and this is another competitive radiationless process that deactivates the lowest singlet excited state of these compounds in a lesser extent.

Table 4. Photophysical parameters (φ_{isc}, φ_{ic}, τ_T [ns], $k^o{}_f$ [s^{-1}], k_{isc} [s^{-1}] and k_{ic} [s^{-1}]) of β-carbolines **1**-**3** and chloroharmines **3d**-**3f** at 298 K

(i) MeCN

Compound	φ_{isc}[a]	φ_{ic}[b]	τ_T	$k^o{}_f$	k_{isc}	k_{ic}
1	0.47	0.30	471	$5.3x10^7$	$9.1x10^7$	$4.2x10^7$
2	0.54	0.21	877	$6.4x10^7$	$1.3x10^7$	$1.5x10^7$
3	0.50	0.11	173	$7.6x10^7$	$9.8x10^7$	$2.2x10^7$
3d	0.53	0.41	-	$3.0x10^7$	$2.7x10^8$	$2.1x10^8$
3e	0.55	0.41	-	$3.3x10^7$	$4.2x10^8$	$3.2x10^8$
3f	0.50	0.43	-	$6.0x10^7$	$4.5x10^8$	$3.9x10^8$

[a] Measured by Laser Flash Photolysis at room temperature. [b] $\varphi_{ic} = 1-\varphi_f - \varphi_{isc}$

(ii) MeOH

Compound	φ_{isc}[a]	φ_{ic}[b]	τ_T	$k^o{}_f$	k_{isc}	k_{ic}
1	0.48	0.22	556	$5.7x10^7$	$1.1x10^8$	$5.1x10^7$
2	0.57	0.21	250	$4.2x10^7$	$1.5x10^8$	$5.4x10^7$
3	0.49	0.04	113	$8.9x10^7$	$9.6x10^7$	$7.8x10^6$
3d	0.54	0.36	-	$3.0x10^7$	$2.1x10^8$	$1.4x10^8$
3e	0.56	0.37	-	$3.3x10^7$	$5.1x10^8$	$3.4x10^8$
3f	0.52	0.34	-	$6.0x10^7$	$5.8x10^8$	$3.8x10^8$

[a] Measured by Laser Flash Photolysis at room temperature. [b] $\varphi_{ic} = 1-\varphi_f - \varphi_{isc}$

We have also measured the photophysical parameters (φ_{isc} and τ_T) and the electronic absorption spectra of the triplet transients of the nitro-β-carbolines in acetonitrile and in methanol at room temperature by Laser Flash Photolysis. Taking into account that the nitro-β-carbolines do not show any fluorescence emission at room temperature and are photostables upon irradiation, the φ_{ic} value can be easily derived according to the following relationship: $\varphi_{ic} = 1 - \varphi_{isc}$. Therefore, the measured spectroscopic and photophysical data and the derived internal conversion quantum yield values (φ_{ic}) are collected in Table 5.

Table 5. Spectroscopic data (λ_{max}(abs) [nm]), intersystem crossing quantum yield (φ_{isc}), internal conversion quantum yield (φ_{ic}) and triplet lifetime (τ_T [ns]) of nitro-β-carbolines in acetonitrile and in methanol by Laser Flash Photolysis at 298 K

Compd.	MeCN				MeOH			
	φ_{isc}	φ_{ic}[a]	τ_T	λ_{max}(abs)	φ_{isc}	φ_{ic}	τ_T	λ_{max}(abs)
1a	0.66	0.34	365	525	0.53	0.47	370	530
1b	0.27	0.73	137	500	0.43	0.57	135	505
2a	0.55	0.45	520	515	0.58	0.22	520	515
2b	0.37	0.63	150	520	0.45	0.55	145	520
3a	0.41	0.59	-	-	0.27	0.73	-	-
3b	0.39	0.61	-	-	0.39	0.61	-	-
3c	0.10	0.90	-	-	0.11	0.89	-	-

[a] $\varphi_{ic} = 1 - \varphi_{isc}$

The analyses of the φ_{isc} and φ_{ic} values of nitro-β-carboline derivatives allow us to conclude that the lowest singlet excited state of these compounds (not experimentally detected), which have a fleeting lifetime (τ_f lesser than 1 ns), is efficiently deactivated through both radiationless processes: the intersystem crossing (isc) and internal conversion (ic) channels. It is noteworthy to point out that the φ_{isc} values of 6-nitro-β-carbolines are higher than that of the 8-nitro-β-carboline regioisomers. This fact could be rationalized taking into account that 8-nitro-β-carboline forms an intramolecular hydrogen bond whereas the 6-nitro regioisomer does not, as is depicted in figure 3.

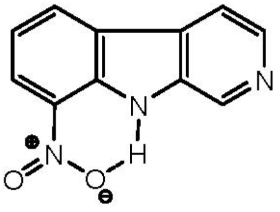


Figure 3. Intramolecular hydrogen bond in 8-nitro-norharmane (**2b**).

Therefore, the nitro group is forced to be in-plane with the β-carboline moiety and the enhancement of the rigidity of the molecule favored the radiationless deactivation of the lowest singlet excited state through the internal conversion channel in competition with the intersystem crossing channel. Of course, this behavior is not possible for 6-nitro-β-carbolines and hence, the trend observed is opposite to that of the 8-nitro regioisomers. The analysis of the triplet transient lifetimes of the nitro-β-carbolines shows a similar trend when the 6-nitro- and 8-nitro-β-carboline derivatives are compared. Finally, the electronic absorption triplet transient spectra of the nitro-β-carbolines were recorded by Laser Flash Photolysis at room temperature and showed a broad band from 420 to 600 nm with a λ_{max}(abs) centered around 500 nm. Similar results were obtained in methanol solution (Table 5).

Conclusions

In the present study, the radiative and radiationless rate constants (k^{o}_{f}, k_{isc} and k_{ic}) of harmane (**1**), norharmane (**2**), harmine (**3**) and chloroharmines (**3d** – **3f**) in acetonitrile and in methanol at room temperature were derived from the photophysical parameters: τ_f, φ_f, φ_{isc} and φ_{ic}. Also, the $\Delta E(T_1 - S_1)$ values and the φ_f and φ_p values were measured in solid matrix at 77 K. An important heavy atom effect for compounds **3d** – **3f** on the photophysical and spectroscopic data was observed in comparison to harmine. Therefore, a noticeable intramolecular quenching of the fluorescence emission spectra at room temperature was observed due to the chlorine atoms attached to the harmine moiety and the τ_f and φ_f parameters decrease while the k_{isc} parameter increases proportionately. Measurements at 77 K showed that these compounds are still fluorescent chromophores while an important phosphorescence emission was observed too. A comparative analysis of the photophysical rate constants and the shape of the fluorescence and phosphorescence emission spectra of β-carbolines and chloroharmines allow us to conclude that the lowest singlet and triplet excited states of compounds **1**, **2**, **3** and **3d** – **3f** are to be π,π* electronic states.

Nitro-β-carbolines are not fluorescent chromophores and the fleeting lowest singlet excited state (n,π*) deactivates efficiently through the intersystem crossing and internal conversion pathways. The former pathway operates preferentially for 6-nitro-β-carbolines while the latter is observed when 8-nitro-β-carbolines are studied. In general, the nitro-β-carboline derivatives show triplet transient absorption spectra between 420 and 600 nm with a λ_{max}(abs) centered around 500 nm and the τ_T values ranged between 135 and 520 ns depending on the position of the nitro group attached to the β-carboline moiety. Taking into account that the nitro-β-carbolines show φ_{ic} values higher than the corresponding β-carbolines, laser induced optoacoustic spectroscopy (LIOAS) of nitro-β-carbolines in homogeneous media at 298 K are in progress in our laboratory in order to analyze the thermal deactivation of the electronic excited states.

β-Carbolines and nitro-β-carbolines constitute two families of 9H-pyrido[3,4-*b*]indole compounds, both photostable showing a quite different deactivation process from the electronic excited state.

As the competition between radiative and radiationless deactivation processes of the electronic excited state of the UV-MALDI matrix may strongly affect the efficiency of the desorption/ionization of the analyte in UV-MALDI-MS experiments,[2] comparative studies using β-carbolines and nitro-β-carbolines as matrices are in progress in our laboratory.

Experimental Section

General Procedures

Materials. Harmane (**1**), norharmane (**2**) and harmine (**3**) were purchased from Aldrich Chemical Co. Benzophenone and 9-cyanonaphthalene of high quality were purchased from Aldrich Chemical Co. and were used without further purification. Acetonitrile, methanol, ethyl ether and iso-propanol HPLC grade (Merck) were used as purchased without any further purification. Water of MilliQ grade, perchloric acid and sulfuric acid of analytical grade were used. Quinine sulfate and *p*-terphenyl were used as purchased from Aldrich Chemical Co. and Sigma. The chloro-β-carbolines and the nitro-β-carbolines were synthesized according to the procedures reported elsewhere.[22]

Equipment. The absorption measurements were performed with a spectrophotometer Hewlett Packard HP5. The spectrofluorometer employed in this study was a Hitachi F-500. The quantum yields at room temperature were determined relatively to the quantum yield of Quinine sulfate in HClO 0.1 N (QS). The general method followed for the measurements has been previously described.$_4$[23]

The fluorescence lifetimes were measured by the time correlated single photon counting technique with an Edinburgh 0B 900 nanosecond fluorescence spectrometer. The resulting decay curves were analyzed by deconvoluting a single exponential with the Lamp function as it was described elsewhere. Total luminescence emission spectra (fluorescence and/or phosphorescence) were measured in the same spectrophotometer at 77 K. The conventional flash apparatus was used in order to measure the phosphorescence lifetimes. The fluorescence quantum yields at 298 K and at 77K and the phosphorescence quantum yields at 77 K were determined according to the procedure previously described in detail.[2323]

Laser flash photolysis experiments were performed by using a pulsed frequency quadrupled Nd:YAG laser (J. K. Lasers HY750, 15 ns FWHM, maximum energy 75 mJ at 1.06 µm). The energy of a single 355 nm pulse was monitored with a beam splitter and a pyroelectric energy meter (Laser Precision Corp.). Laser induced transient transmission changes were monitored, perpendicular to the laser beam, by using a Xe arc lamp (ILC UV 33P). The analysing light passed through a 2 mm wide section of the excited sample and was then focused at the entrance slit of a 0.25 m, double grating monochromator (PTI f/4, spectral resolution 3 nm). A photomultiplier (Hamamatsu R936) and a Tektronix R-7912 transient digitizer interfaced to a PC

IBM Asyst. Programs were used to acquire and process the signals. The intersystem crossing quantum yields at room temperature were determined relatively to the quantum yield of benzophenone using 1-cyanonaphthalene as a triplet energy acceptor. The triplet lifetimes (τ_T) were measured at the maximum wavelength of the electronic absorption spectra of the triplet transients.

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