Photochemical behaviour of β-carbolines. Part 4.[1] Acid–base equilibria in the ground and excited states in organic media

Miriana C. Biondic and Rosa Erra-Balsells*

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Pabellón 2, 3° p. Ciudad Universitaria, 1428 Buenos Aires, Argentina

The protonation equilibrium of six β-carbolines has been studied in organic media. The pK_a values in acetonitrile were determined by spectrophotometric methods. The pK_a values in the first excited singlet state (pK_a*) were calculated from the Förster cycle and the values compared with those obtained by fluorimetric titration. It is concluded that the acid–base equilibrium is not established during the lifetime of the excited state. The differences between the pK_a of the ground and the electronic excited singlet states in different organic media (MeOH, EtOH, Pr^{i}OH, Bu^{t}OH, MeCN and CH_2Cl_2) were also calculated.

Introduction

The β-carboline alkaloids have been studied mainly because of their biological and pharmacological properties and also because of their photochemical behaviour. They are known to be hallucinogens,[2] to inhibit the monoamine oxidase,[3] to have photosensitizing activity towards a variety of systems, including bacteria, fungi, viruses and insects,[4,5] and to be present in human lenses[6,7] and human urine.[8] Because of their emission properties they have been proposed as fluorescence standards.[9,10] Particular interest has been shown in the study of the acid–base properties of the β-carbolines. Electronic excitation results in significant charge-density changes due to the presence of two different types of nitrogen atoms in their structure (pyridinic and pyrrolic). These acid–base equilibria have been extensively studied in aqueous solution as a function of pH;[11–15] the pK and the first excited state pK* values have been determined,[10–16] together with some kinetic parameters.[17,18] In addition, fluorescence quantum yields and/or lifetimes have been determined in water at different pH values.[16,19] Also the partition coefficient between octan-1-ol and water has been measured,[20] as well as the electronic spectra in microemulsions formed by Aerosol-OT [sodium bis(2-ethylhexyl)sulfosuccinate]–water–cyclohexane.[21]

These alkaloids are extremely sensitive to the addition of acid and bases in organic solvents[22,23] but little work has been carried out in these media. Although the foundations for this type of study were laid down in the 1960s by Kolthoff *et al.*[24] and Coetzee[25] and extended by Bordwell and co-workers[26,27] in the 1980s, as far as we know, there has been no quantitative study of the acidity constant of these alkaloids in organic solvents. We present here the first quantitative study of the deprotonation equilibrium of β-carbolines in a polar aprotic solvent, such as acetonitrile. Furthermore, also for the first time, the pK_a in the S_1 state (pK_a*) in acetonitrile and ΔpK_a between S_0 and S_1 states in different organic solvents were calculated using the Förster cycle.[28] Additionally, the pK_a* values obtained with this method are compared to those obtained by fluorimetric titration.

Results and discussion

pK_a Determination in acetonitrile

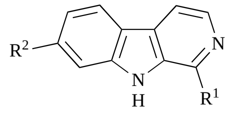
The equilibrium of deprotonation of β-carbolines was studied [eqn. (1)]. In diluted solutions the acidity constant K_a can be expressed as $K_a = [B][H^+]/[BH^+]$. The method used involves relating the acidity of the β-carboline with the acidity of a pH indicator (In), of known pK, by titrating a mixture of both. In this case, the equilibrium established is as shown in eqns. (2) and (3), where B and BH^+ are the β-carboline in the neutral and protonated form, respectively, and In and InH^+ are the indicator as a neutral and protonated form (the + sign in the latter does not mean net charge).

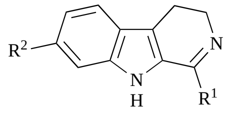
$$BH^+ \rightleftharpoons B + H^+ \qquad (1)$$

$$B + InH^+ \rightleftharpoons BH^+ + In \qquad (2)$$

$$K = [BH^+][In]/[B][InH^+] = K_{aInH}/K_{aBH^+} \qquad (3)$$

As the initial concentrations of the β-carboline ($[B]_0$) and indicator ($[I]_0$) are known, the concentration of the protonated β-carboline ($[BH^+]$) and either of the indicator species can be determined spectrophotometrically and as the K_a of the indicator is also known, the K_a of the β-carboline can be calculated. This method requires that the difference between the pK of the analyte (B) and the indicator (In) must not be higher than 2 pK units. Additionally, as the concentrations are determined spectrophotometrically, the four species involved should show absorption at different wavelengths. These conditions are fulfilled by the system of aromatic β-carbolines (**1**, **2**, **3** and **4**) and neutral red. This indicator exhibits two colour change intervals, one from red (InH^+) to yellow (InH) (pH 14.5 to 16.5) and the other from blue (InH_2^{+2}) to red (pH 5 to 7), with the absorption maxima located at 532, 441 and 608 nm, respectively, in acetonitrile solution. Beer's law was found to hold for these various forms.[29] When a solution of β-carboline in acetonitrile was added to a solution of neutral red in acetonitrile, the equilibrium was displaced from the red form to the yellow one, as can be seen in Fig. 1.





1 Norharmane $R^1 = H, R^2 = H$
2 Harmane $R^1 = CH_3, R^2 = H$
3 Harmine $R^1 = CH_3, R^2 = OCH_3$
4 Harmol $R^1 = CH_3, R^2 = OH$
5 Harmaline $R^1 = CH_3, R^2 = OCH_3$
6 Harmalol $R^1 = CH_3, R^2 = OH$

In the case of the partially hydrogenated β-carbolines (**5** and **6**) the indicator used was bromphenol blue, which also exhibits two colour change intervals, one from yellow (In^-) to blue

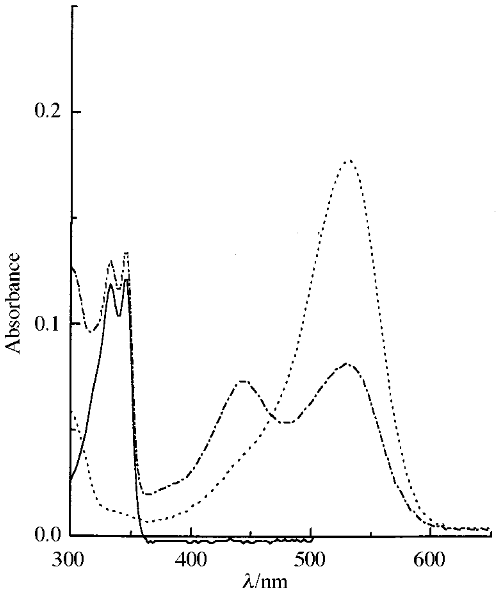


Fig. 1 Absorption spectra in acetonitrile of norharmane **1**, 2.91 × 10^{-5} mol dm^{-3} (——); of neutral red, 6.8 × 10^{-6} mol dm^{-3} (••••••); and of a mixture of both (-•-•-).

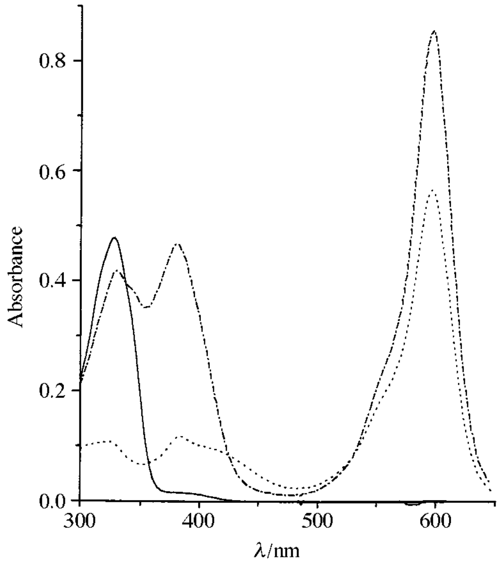


Fig. 2 Absorption spectra in acetonitrile of harmaline **5**, 1.1 × 10^{-5} mol dm^{-3} (——); of bromphenol blue, 1.1 × 10^{-5} mol dm^{-3} (••••••); and of a mixture of both (-•-•-).

(In^{2-}) (pH 16.5 to 18.5) and the other from colourless (In) to yellow (pH 11 to 13), with the absorption maxima located at 408 and 596 nm, respectively, in acetonitrile solution. Beer's law was found to hold for these various forms.[30] When a solution of the alkaloid in acetonitrile was added to a solution of bromphenol blue in acetonitrile, the equilibrium was displaced from the yellow form to the blue one, as can be seen in Fig. 2.

The values obtained are presented in Table 1. It can be seen the pK_a values in acetonitrile follow the trend previously observed in water.[13–15] The difference between the pK_a values measured in water and acetonitrile is similar to that previously observed by Coetzee[25] for aliphatic and aromatic amines and pyridine.

The partially hydrogenated β-carbolines are more basic than the fully aromatic ones, in agreement with previous observations in aqueous medium.[13–15] This can be explained by taking into account the fact that in the latter case the protonation causes localization of the n electrons, producing a loss of delocalization energy in the aromatic system, making the protonation process less favourable.

Table 1 pK_a Values of β-carbolines in acetonitrile

β-Carboline	$pK_{a\,MeCN}$[a]	Indicator	$pK_{a\,H_2O}$[b]	$pK_{a\,MeCN} - pK_{a\,H_2O}$
Norharmane **1**	14.9	NR[c]	7.2	7.7
Harmane **2**	15.4	NR	7.7	7.7
Harmine **3**	15.8	NR	8.0	7.8
Harmol **4**	15.7	NR	8.0	7.1
Harmaline **5**	17.4	BB[d]	10.0	7.3
Harmalol **6**	17.6	BB	10.0	7.6

[a] Error: ±0.2 pH units. [b] pK_a from refs. 13, 14 and 15. [c] NR: neutral red. [d] BB: bromphenol blue.

Table 2 pK_a* Values of β-carbolines in acetonitrile

β-Carboline	$pK_a^*{}_{MeCN}$[a]	$pK_a^*{}_{H_2O}$[b]
Norharmane **1**	23.4	13.0
Harmane **2**	22.7	12.8
Harmine **3**	23.3	12.9
Harmol **4**	22.7	13.6
Harmaline **5**	31.4[c]	19.5
Harmalol **6**	31.3[c]	16.2

[a] Obtained from $pK^* = pK + 0.0021\,\Delta\tilde{\nu}$, where $\Delta\tilde{\nu} = \tilde{\nu}_B - \tilde{\nu}_{BH^+}$, $\tilde{\nu}$ is the average from the frequency maxima of absorption and emission. [b] From refs. 13, 14 and 15. [c] ν_B is the absorption maximum because this species shows no emission.

In the case of the fully aromatic alkaloids, the presence of an electron donor group in the aromatic ring seems to affect slightly the pK_a value, and there is a trend which corresponds to the mesomeric effect of these substituents (methyl, methoxy and hydroxy groups).

It is interesting to point out the fact that some pK_a values obtained in acetonitrile are higher than 14 because acetonitrile is a weaker base, as well as a weaker acid, than water. These facts coupled with the influence of its relatively low relative permittivity, gives acetonitrile a very low autoprotolysis constant[31] (3 × 10^{-29} or less[32]) that makes it a strongly differentiating solvent. It must be kept in mind that although the solvating capability of acetonitrile is lower than that of water it still solvates the ammonium ions significantly, making the homoconjugation equilibrium ($BH^+ + B \rightleftharpoons BH^+B$) between BH^+ and B unimportant.[25]

As a result, the protonated amines are weaker acids in acetonitrile than in water. Although the solvent influence is complex, the dominant factor may be the fact that the proton acceptor ability of acetonitrile is lower than that of water.

pK_a* Determination in acetonitrile

Taking into account that BH^+ absorbs at lower energy values than B, it is expected that B will behave as a stronger base in the electronic excited state. The Förster cycle[28] predicts quantitatively the difference between the pK_a and pK_a* as shown in eqn. (4), where $\Delta\tilde{\nu} = \tilde{\nu}_B - \tilde{\nu}_{BH^+}$, and $\tilde{\nu}$ is the average value obtained

$$\Delta pK_a = pK_a^* - pK_a = 0.021\,\Delta\tilde{\nu} \quad \text{at 298 K} \qquad (4)$$

from the frequency maxima of absorption and emission, except in the case of the partially hydrogenated β-carbolines **5** and **6** where $\tilde{\nu}_B$ is just the frequency of the absorption maximum, as the neutral species does not emit.

The values obtained are presented in Table 2. When the pK_a* values measured in water and acetonitrile are compared, it is observed that the values in acetonitrile are greater than those in water, as was observed for the ground state measurements.

Fluorimetric titration

The results obtained from the Förster cycle indicate that if the neutral form is irradiated at a certain pH, the fluorescence emission of the cation should be observed. This is true if the equilibrium is established during the lifetime of the excited species.

This kind of equilibrium was described in aqueous solutions of norharmane **1**, harmane **2** and harmine **3**.[11,12] This is possible because these alkaloids in the excited state are stronger bases than HO^- and thus they are able to abstract a proton from water. In those cases where the proton must approach the base, though the rate constant is diffusional, the protonation process is slower than the deactivation of the excited state involved. This is the case of the equilibrium between the cationic and zwitterionic forms of harmol **4**[12] in aqueous medium; although the presence of the cation is predicted by the Förster equation at certain values of pH, it is not observed.

In the present study where the solvent is acetonitrile, taking into account the ΔpK_a values, it would be expected that when a mixture of the cationic and neutral species is observed in the ground state, only emission from the cationic species should be observed. In order to check this fact the emission spectra in acetonitrile at different proton concentrations were obtained.

The pK_a^* value was calculated in a graphical way, as can be seen in Fig. 3. If F_0/F (F is the emission intensity at a wavelength where only the cationic species emits and F_0 is F when only the cationic species is present) or F'_0/F' (F' is the emission intensity at a wavelength where only the neutral species emits and F'_0 is F' when only the neutral species is present) are plotted *vs.* pH, the inflection point corresponds to the pK_a^* (considering that the emission intensity is proportional to the fluorophore concentration at $A < 0.1$). In the harmaline **5** case only the cation emission could be followed because, as we stated before,[22] the neutral species does not fluoresce. The proton concentration was calculated from $[H^+] = [BH^+]\ K_a/[B]$, where $[BH^+]$ and $[B]$ were determined by absorption spectroscopy and K_a was determined as before (Table 1).

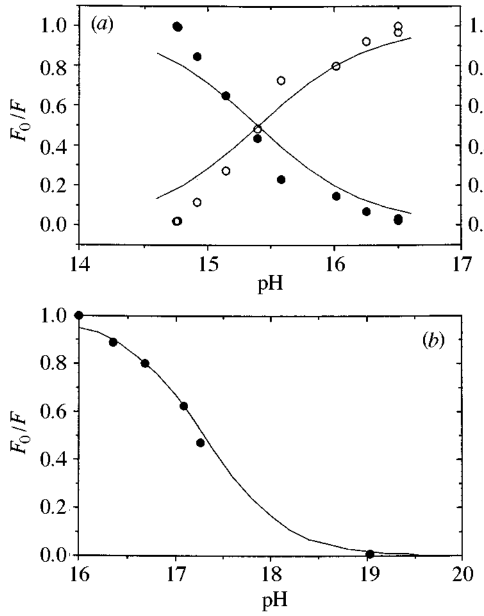


Fig. 3 Fluorimetric titration of β-carbolines in acetonitrile with 0.1 mol dm^{-3} $HClO_4$ in glacial acetic acid; (*a*) norharmane **1** λ_{exc} 316 nm, λ_F (●) 480 nm, $\lambda_{F'}$ (○) 356 nm; (*b*) harmaline **5** λ_{exc} 400 nm, λ_F (●) 560 nm

Table 3 pK_a^* values in acetonitrile obtained by different methods

β-Carboline	pK_a^{*a}	pK_a^{*b}	pK_a^c
Norharmane **2**	23.4	15.4	14.9
Harmaline **5**	31.4	17.3	17.3

[a] From Förster's cycle. [b] Graphical method from fluorimetric titration. [c] Determined in this paper.

The results are presented in Table 3. The values of pK_a^* obtained by fluorimetric titration are similar to those of pK_a rather than the ones predicted by the Förster cycle.

These results could be explained by taking into account that the autodissociation constant of acetonitrile[31] is 26.5 (or higher[32]) and the pK_a^* values of the aromatic β-carbolines are around 24, implying the fact that the latter species cannot abstract a proton from the former. The rate constant of protonation will then be $k_p = k_r\ [H^+]$ and in the best situation, k_r is diffusional. This value in acetonitrile[33] is 2.9×10^{10} dm^3 mol^{-1} s^{-1} and when both species of β-carbolines are detected in the ground state the proton concentration is *ca.* 10^{-15} mol dm^{-3}, thus k_p is *ca.* 3×10^{-5} s^{-1}. If we compare this value with the fluorescence rate constant k_f, which is *ca.* 10^8 s^{-1}, it is clearly seen that in this medium the acid–base equilibrium does not have time to be established during the lifetime of the excited states.

In the case of the partially hydrogenated β-carbolines **5** and **6**, as they are even stronger bases, they may abstract a proton from the acetonitrile. There are no data referring to the rate constant of abstraction of a proton in acetonitrile but this value in water[17] is between 10^7 and 5×10^8 s^{-1}. In this case the non-radiative pathways can compete in the deactivation of the first excited state, as no fluorescence could be detected. As the k_{IC} could be[34] in the order of 10^{11} to 10^{13} s^{-1} again the prototropic equilibrium does not have enough time to be established.

ΔpK_a Calculation

The difference between the pK_a in the ground and excited states was also calculated in different organic solvents as can be seen in Table 4. The values of the frequency maxima are described in Table 5 and elsewhere.[22,23] In the case of the partially hydrogenated β-carbolines, a slight modification needs to be carried out. As the neutral species does not emit, $\Delta pK_a'$ was defined as the difference between the frequency of the neutral and protonated species, where the frequency of the neutral form is obtained from the absorption maxima and the one of the protonated species is obtained from the average of the emission and absorption maxima.[22,23]

When it was possible ΔpK_a and $\Delta pK_a'$ were both calculated for comparison. The values of $\Delta pK_a'$ were always 1 to 1.5 pH units higher but the trend observed was the same as in ΔpK_a. The conclusions of the comparative acid–base study are therefore not affected by using $\Delta pK_a'$ values.

Table 4 ΔpK_a[a] and $\Delta pK_a'$[b] values

β-Carboline		MeOH	EtOH	Pr^iOH	Bu^tOH	MeCN	CH_2Cl_2	H_2O^c
Norharmane **1**	ΔpK_a	7.1	6.9	7.2	7.0	8.5	8.9	5.5
	$\Delta pK_a'$	8.6	8.2	8.0	7.7	9.2	9.8	8.4
Harmane **2**	ΔpK_a	6.2	6.2	6.4	6.2	7.3	7.7	4.9
	$\Delta pK_a'$	7.4	7.2	7.2	7.1	8.1	8.7	7.4
Harmine[d] **3**	ΔpK_a	6.7	6.4	6.3	6.3	7.5	8.6	4.6
	$\Delta pK_a'$	8.3	7.8	7.7	7.6	9.1	9.9	7.8
Harmol[d] **4**	ΔpK_a	6.6	6.6	6.6	6.5	7.6		6.6
	$\Delta pK_a'$	7.8	7.9	7.8	7.7	8.8		8.0
Harmaline[d] **5**	$\Delta pK_a'$	12.6	13.0	12.8	13.0	14.1	13.9	13.6
Harmalol[d] **6**	$\Delta pK_a'$	11.8	12.0	12.4	12.4	13.7	12.9	11.8

[a] Calculated from Förster's cycle. [b] See text for definition. [c] Data from refs. 13, 14 and 15. [d] From refs. 24 and 25.

Table 5 Frequency of absorption (abs) and fluorescence emission (em) maxima of norharmane **1** and harmane **2** in organic solvents

		Neutral species		Cationic species	
β-Carboline	Solvent	$\bar{\nu}_{\mathbf{abs}}$/cm^{-1}	$\bar{\nu}_{\mathbf{em}}$/cm^{-1}	$\bar{\nu}_{\mathbf{abs}}$/cm^{-1}	$\bar{\nu}_{\mathbf{em}}$/cm^{-1}
Norharmane **1**	MeOH	28 571	27 174	26 738	22 222
	EtOH	28 409	27 174	26 596	22 422
	Pr^{i}OH	28 409	27 624	26 596	22 624
	Bu^{t}OH	28 409	27 701	26 738	22 727
	MeCN	28 902	28 249	26 738	22 321
	CH_2Cl_2	29 070	28 249	26 596	22 222
Harmane **2**	MeOH	28 736	27 624	27 174	23 256
	EtOH	28 571	27 624	27 027	23 256
	Pr^{i}OH	28 571	27 778	27 027	23 256
	Bu^{t}OH	28 736	27 855	27 174	23 529
	MeCN	29 070	28 249	27 322	23 095
	CH_2Cl_2	29 240	28 249	27 174	22 989

The ΔpK_a values obtained are all positive. Thus, in the excited state these alkaloids are more basic than in the ground state. Comparing these values with those described by Tomas Vert and co-workers[13–15] in aqueous solution it is concluded that in hydroxylic polar solvents with Taft $\alpha > 0.40$,[35] the ΔpK_a values are 1.5 pH units lower than in non-hydroxylic organic solvents (acetonitrile $\alpha = 0.19$, dichloromethane $\alpha = 0.13$).[35] The basic character is more enhanced in solvents with low hydrogen bonding ability because the electrons of the fluorophore have less interaction with the solvent and consequently they are more readily available. Interactions have previously been found between the alkaloids and the hydroxylic solvents (hydrogen bonding),[36] that compete with the protonation process.

It is noteworthy that the increment in the basicity is more pronounced in the case of the partially hydrogenated β-carbolines. This fact could be explained by taking into account the more flexible structure of the partially hydrogenated pyridinic ring in this kind of compound, together with its iminic character compared with the rigid structure of the full aromatic pyridinic ring.

Experimental

The absorption spectra were recorded with stoppered quartz cells (quartz Suprasil Hellma) of 1 cm length in a Hewlett Packard 8452A diode array spectrophotometer, with a cut-off filter at 265 nm. The temperature was regulated using a thermostatically controlled cell holder and a Lauda RC20 cryostat.

The excitation and emission spectra were performed on a Perkin-Elmer LS-5 spectrofluorometer, whose output is automatically corrected for instrumental response by means of a Rhodamine B quantum counter and equipped with a Hamamatsu R928 photomultiplier tube. The spectra are not corrected.

MS data were obtained with a GC-Varian Aerograph 1400/MS-Varian MatCH-7A/Data System 166.

The β-carbolines **1**, **2**, **3** and **5** were purchased from Aldrich as free bases. Harmol **4** and harmalol **6** were purchased from Fluka. The latter was bought as the hydrochloride salt. The free base was precipitated from an aqueous solution of the hydrochloride salt made alkaline with sodium carbonate. The purity of the alkaloids was checked by TLC, mp and MS.

The indicators neutral red and bromphenol blue were purchased from Mallinckrodt.

The acetonitrile was J. T. Baker HPLC grade and used without further purification. The glacial acetic acid was J. T. Baker HPLC grade and in order to eliminate traces of water, acetic anhydride was added. The other solvents were purified as described elsewhere.[22,37]

Preparation of 0.1 mol dm^{-3} perchloric acid in acetic acid

A mixture of 4.2 ml of 70% analytical grade perchloric acid (E. Merck) with 5 ml of acetic anhydride was diluted up to 500 ml with glacial acetic acid. The titre of the solution was determined titrating with potassium biphthalate, using as indicator few drops of 1% crystal violet in glacial acetic acid.[38]

pK_a Measurements

The method used in acetonitrile is a modification of the method proposed by Bordwell and co-workers[26] in dimethyl sulfoxide. The extinction coefficient of the indicator species of interest was determined by weighing appropriate amounts of the indicator, dissolving it in acetonitrile and recording the absorption spectra at different pH until the maximum concentration of the species was reached. The acid used was 0.1 mol dm^{-3} perchloric acid in glacial acetic acid[38] and the base was 0.1 mol dm^{-3} lithium methoxide in methanol–toluene.[39]

The concentration of β-carboline was determined spectrophotometrically. The extinction coefficients were described elsewhere.[22,23,37]

pK_a* Measurements

The emission spectra were recorded at different proton concentrations.

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