ACIDITY CONSTANTS OF β-CARBOLINES IN THE GROUND AND EXCITED SINGLET STATES

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Summary

The ground state ionization constants $pK_a(S_0)$ of β-carboline and three of its derivatives (harmane, harmine and 2-methylharmine) were measured spectrophotometrically in aqueous solutions. The corresponding $pK_a(S_1)$ of the first excited singlet state was calculated by means of the Förster cycle from the absorption and emission spectra. It was also established that protonated β-carboline cations are responsible for photoabsorption and fluorescence in acidic solutions. However, in alkaline solutions excitation of the neutral species leads to formation of the protonated β-carboline cations by rapid proton transfer from the solvent. A zwitterion species is produced by diffusion-controlled quenching of the excited state cation by OH^- ions.

1. Introduction

The fluorescence of derivatives of β-carboline (9H-pyrido[3,4-b]indole) has been widely reported. Some of these derivatives have been reported to be hallucinogens [1], and their behaviour as inhibitors of monoamine oxidase has been carefully studied [2]. β-carboline (Fig. 1(a)) is also the fluorescent product in the Denckla–Dewey determination of tryptophan [3].

The acid–base equilibria of β-carboline (norharmane) and its derivatives harmane (1-methyl-β-carboline), harmine (1-methyl-7-methoxy-β-carboline) and 2-methylharmine (Figs. 1(b), 1(c) and 1(d)) were investigated in this work.

The ground state ionization constants $pK_a(S_0)$ were determined by standard UV spectrophotometric methods [4]. Photoabsorption curves for buffered solutions of the various species were recorded for pH values at least 2 units distant from the estimated $pK_a(S_0)$ of the substance being examined.

Reviews of the chemical properties and reactivities of β-carboline indicate that the ground state $pK_a(S_0)$ of the nitrogen in the 2 position of the
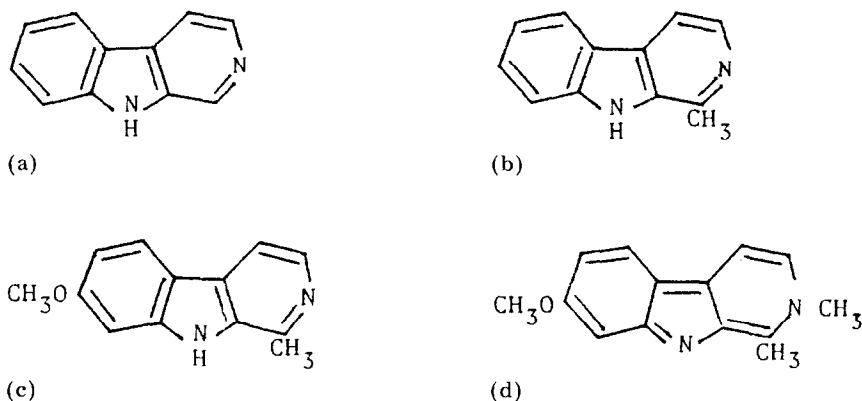
Fig. 1. The structures of (a) norharmane, (b) harmane, (c) harmine and (d) 2-methylharmine.

pyridine ring is 7.9 [5], whereas that of the nitrogen in the 9 position of the pyrrole ring of 2-methyl-β-carboline is 10.9 [6]. Förster and Weller first established that the acid–base behaviour of most organic molecules is strongly affected by electronic excitation [7]. It has been reported that the pK_a values of related pyridine derivatives increase by 5 - 7 units on electronic excitation [8], and it is known that the acidity of pyrrole derivatives increases in the excited state [9].

To obtain further information concerning excited state acid–base equilibria for aqueous solutions of these molecules, we have carried out fluorescence studies of β-carbolines under various pH conditions.

2. Experimental details

2.1. Materials

Norharmane, harmine hydrochlorides and 2-methylharmine were obtained from the Sigma Chemical Co. and harmane hydrochloride was obtained from Fluka AG. Buchs S.S. The buffer solutions were as follows: 0.1 M mixtures of acetic acid and sodium acetate for pH 3 - 4; 1/15 M mixtures of Na_2HPO_4 and KH_2PO_4 for pH 5 - 8; 0.1 M mixtures of glycine and NaOH for pH 9 - 13.

2.2. Apparatus

The UV absorption spectra were recorded using a Cary 219 spectrophotometer. Uncorrected fluorescence emission and excitation spectra were recorded using a Perkin–Elmer MPF-44A spectrofluorometer. The pH values were measured directly with an accuracy to 0.01 pH units using a Radiometer 26 pH meter.

All the experiments were carried out using thermostatted solutions at a temperature of 25 ± 0.1 °C.

2.3. Determination of pK_a values

The ground state ionization constants $pK_a(S_0)$ were calculated using the standard formula [4]

$$pK_a(S_0) = pH - \log\left(\frac{A_{BH^+} - A}{A - A_B}\right) \qquad (1)$$

where A, A_{BH^+} and A_B are the absorbances of the unknown solution, a solution of pure BH^+ and a solution of pure B respectively. All these solutions have the same overall concentration.

The absorbance values used to calculate the $pK_a(S_0)$ corresponded to wavelengths exhibiting significant variations in absorbance when the pH was modified. We have also chosen pH values that imply a concentration of protonated species in the range 15% - 85% of the overall concentration to guarantee the existence of both B and BH^+ in the solution.

The $pK_a(S_1)$ values for the first singlet excited state were estimated using the following relation derived from the Förster cycle [10]:

$$pK_a(S_1) - pK_a(S_0) = \frac{0.625}{T}\ \overline{\Delta\nu} \qquad (2)$$

where $\overline{\Delta\nu}$, which is expressed in reciprocal centimetres, is the difference between the energy of the 0-0 electronic transition in the free base B and the corresponding energy in the protonated molecule BH^+ and T is the absolute temperature.

The energy of the 0-0 transition cannot be obtained directly from the recorded absorption or emission spectra. To overcome this difficulty it was estimated by taking the average of the energy corresponding to the least energetic maximum of the absorption spectrum and the energy corresponding to the most energetic maximum of the fluorescence spectrum. This procedure was used in cases where both spectra were recorded. In cases where one of these spectra was not recorded, the 0-0 transition energy was approximated by the least energetic maximum of the absorption spectrum or the most energetic maximum of the fluorescence spectrum. However, the results are poorer under these conditions.

3. Results and discussion

3.1. Electronic absorption spectra

The absorption spectra of the β-carbolines for a wide pH range (1 - 13) are shown in Figs. 2 - 5. The invariance of the spectra obtained at low pH values suggests that at pH 1 they correspond to protonated β-carboline cations. Hypsochromic shifts corresponding to the transformation from the cation to the neutral species occur in slightly alkaline solutions. However, this shift is bathochromic in 2-methylharmine because the protonation site is at the nitrogen in the pyrrole ring. A new red-shifted absorption band due to β-carboline anions produced by the release of the pyrrolic proton
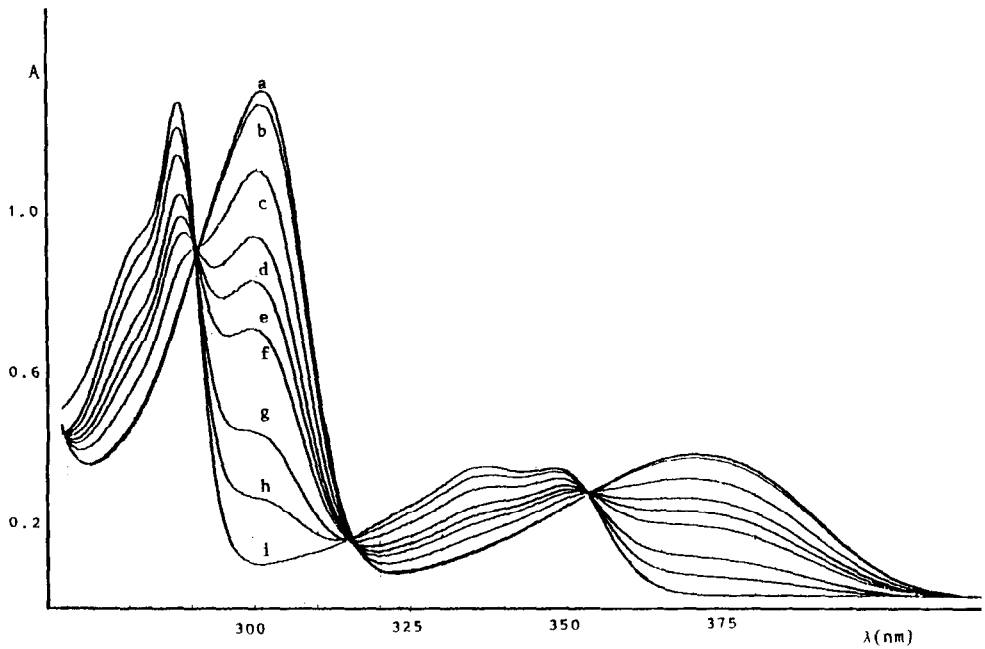
Fig. 2. Absorption spectrum of norharmane (concentration, 5×10^{-5} M; ionic strength, 0.1): curve a, pH 1.35; curve b, pH 5.04; curve c, pH 6.40; curve d, pH 6.81; curve e, pH 7.01; curve f, pH 7.23; curve g, pH 7.64; curve h, pH 8.03; curve i, pH 13.00.
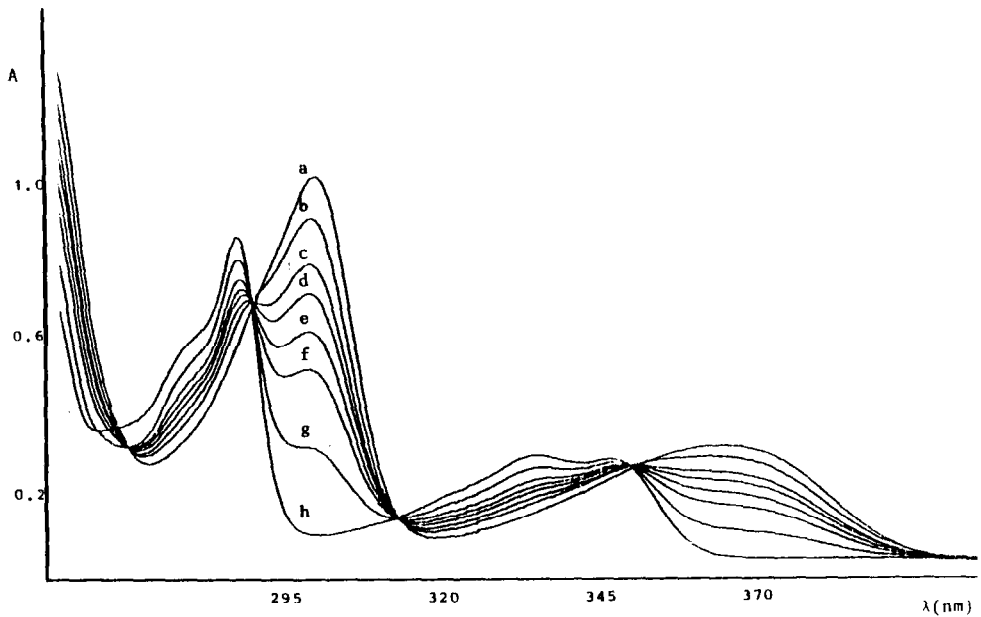

Fig. 3. Absorption spectrum of harmane (concentration, 5×10^{-5} M; ionic strength, 0.1): curve a, pH 1.50; curve b, pH 6.82; curve c, pH 7.24; curve d, pH 7.42; curve e, pH 7.64; curve f, pH 7.82; curve g, pH 8.29; curve h, pH 13.00.
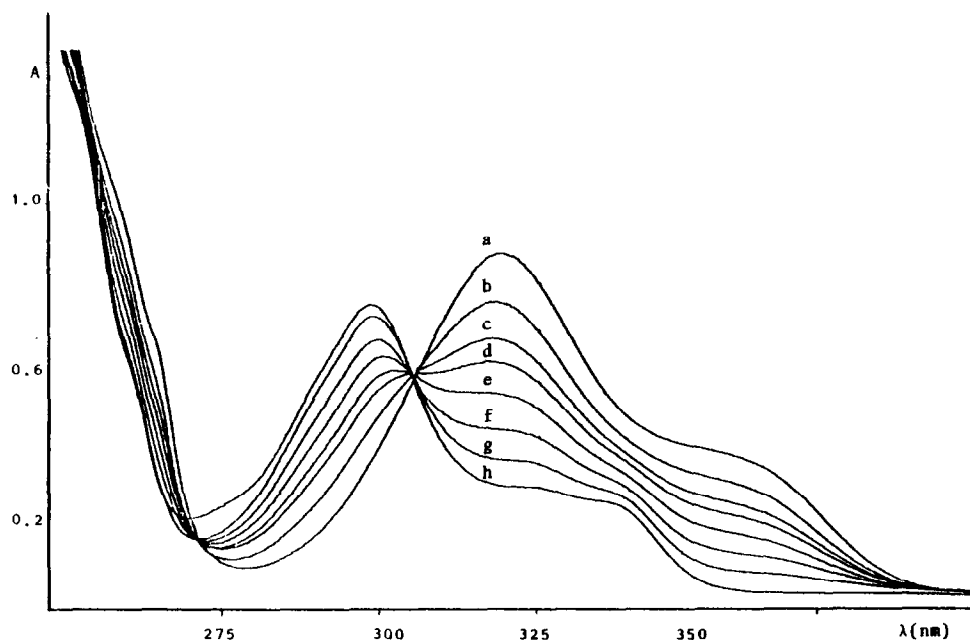
Fig. 4. Absorption spectrum of harmine (concentration, 5×10^{-5} M; ionic strength, 0.1): curve a, pH 1.04; curve b, pH 7.44; curve c, pH 8.04; curve d, pH 8.24; curve e, pH 8.41; curve f, pH 8.81; curve g, pH 9.03; curve h, pH 12.50.

TABLE 1

Ground and excited state ionization constants for β-carboline acid–base equilibria (see Fig. 12)

Compound		$pK(S_0)$	$pK(S_1)$
Norharmane	pK_{CN}	7.2	13.0
	pK_{CZ}	10.9 [5]	4.1 [5]
	pK_{NA}	14.5 [10]	8.0
	pK_{ZA}	10.8[a]	16.9
Harmine	pK_{CN}	8.0	12.9
	pK_{CZ}	11.5	6.0
	pK_{NA}	14.5 [10]	8.8
	pK_{ZA}	11.0	15.7
2-methylharmine	pK_{CZ}	11.5	6.0
Harmane	pK_{CN}	7.7	12.8

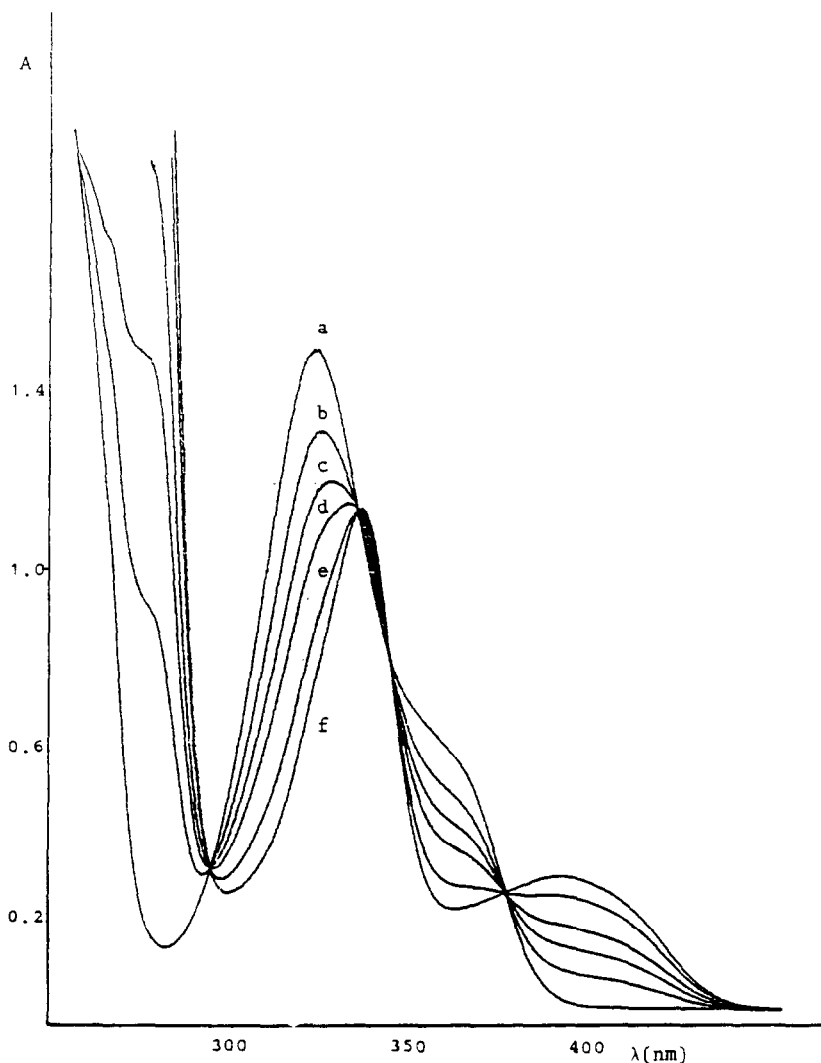[a]Obtained by difference: $pK_{ZA} = pK_{CN} + pK_{NA} - pK_{CZ}$.

Fig. 5. Absorption spectrum of 2-methylharmine (concentration, 5×10^{-5} M; ionic strength, 0.1); curve a, pH 12.85; curve b, pH 12.25; curve c, pH 11.73; curve d, pH 11.47; curve e, pH 11.08; curve f, pH 9.07.

appears in strongly alkaline solutions (pH 14) (Fig. 6). The $pK_a(S_0)$ values determined from eqn. (1) are summarized in Table 1.

3.2. Fluorescence spectra

The fluorescence spectra of the β-carbolines for a wide range of pII (1 - 13) are shown in Figs. 7 - 10. λ_{ex} was chosen at the second isosbestic point to guarantee absorption by BH^+ and/or B. At pH 1 the fluorescence spectra are due to the β-carboline cations ($\lambda_{max}(1)$) (Table 2). The characteristic
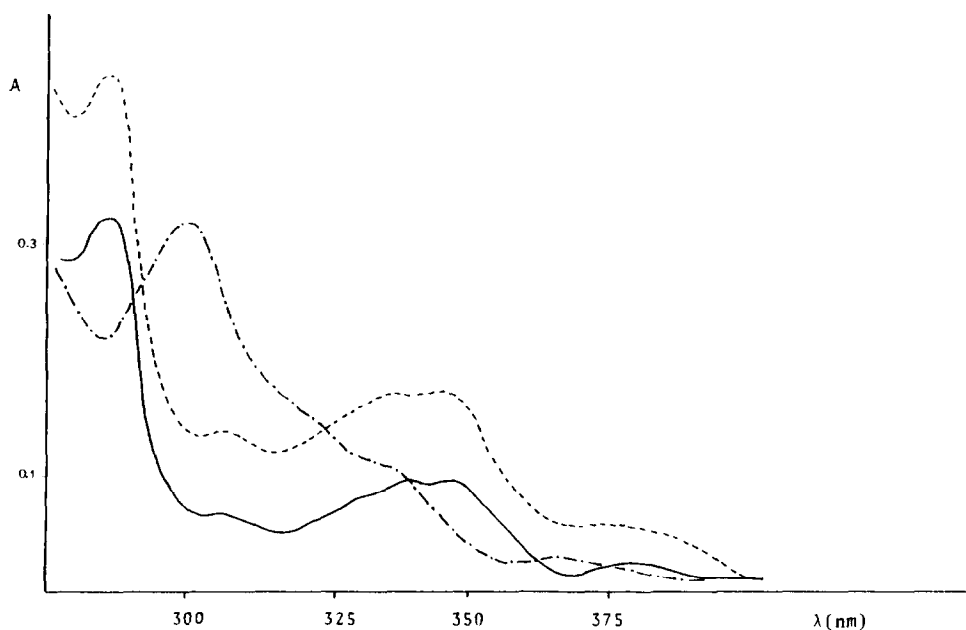
Fig. 6. Absorption spectra of β-carbolines at pH 14: ———, norharmane; — — —, harmane; — · —, harmine.

TABLE 2

Wavelengths of the absorption and fluorescence band maxima

Compound	Spectrum	$\lambda_{max}(1)$ (HBH⁺) (nm)	$\lambda_{max}(2)$ (HB) (nm)	$\lambda_{max}(3)$ (B⁻H⁺) (nm)	$\lambda_{max}(4)$ (B⁻) (nm)
Norharmane	Absorption	370	348		390
	Fluorescence	445	385	510	
Harmine	Absorption	355	336		370
	Fluorescence	418	373	476	
Harmane	Absorption	364	346		375
	Fluorescence	430	378	483	
2-methylharmine	Absorption	360		392	
	Fluorescence	422		482	
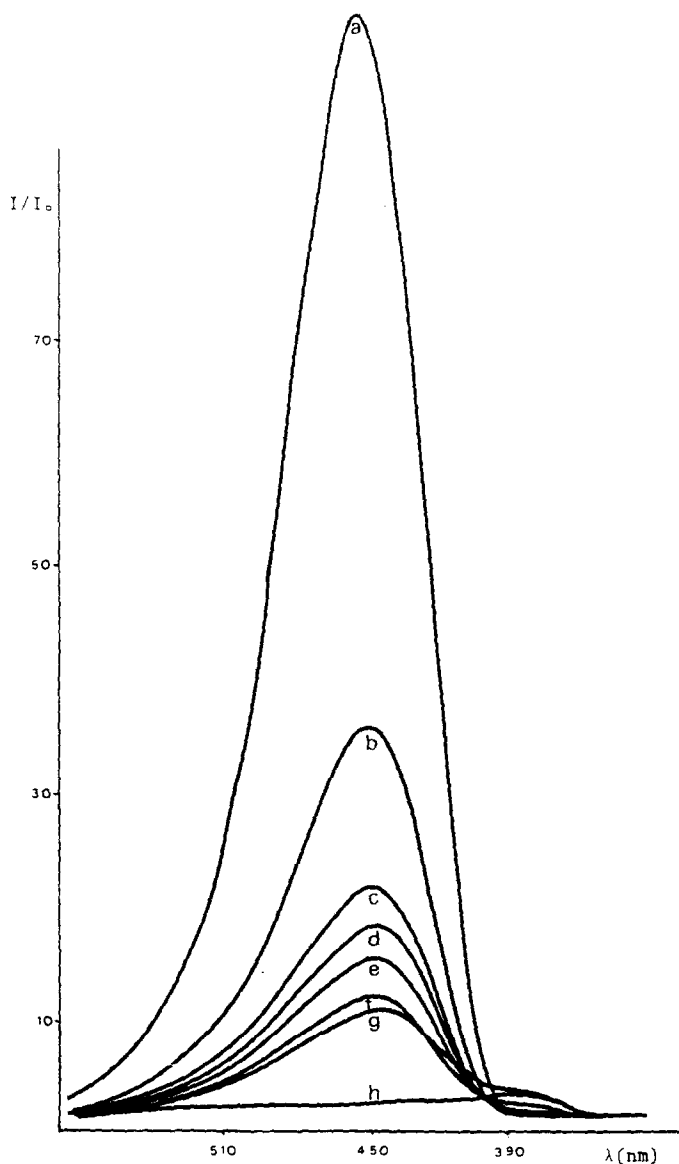
Fig. 7. Fluorescence spectrum of norharmane (λ_{ex} = 315 nm; concentration, 5 × 10⁻⁵ M; ionic strength, 0.1): curve a, pH 1.50; curve b, pH 6.40; curve c, pH 6.80; curve d, pH 7.01; curve e, pH 7.23; curve f, pH 7.64; curve g, pH 8.03; curve h, pH 13.00.

emission of slightly alkaline solutions of norharmane, harmane and harmine is that of the protonated species, suggesting that proton transfer to the initially excited neutral species occurs rapidly. With a further increase in pH the intensity of the fluorescence is markedly reduced and two new emission

Fig. 8. Fluorescence spectrum of harmane (λ_{ex} = 312.5 nm; concentration, 5×10^{-5} M; ionic strength, 0.1): curve a, pH 1.00; curve b, pH 6.82; curve c, pH 7.24; curve d, pH 7.64; curve e, pH 7.82; curve f, pH 8.29; curve g, pH 13.00.

maxima, $\lambda_{max}(2)$ and $\lambda_{max}(3)$, appear (Fig. 11). Identical excitation spectra were recorded at emission wavelengths throughout the three bands, indicating that all the emitting species have a common ground state precursor. The fluorescence maxima for solutions of each species are summarized in Table 2. We believe that these results can be explained using the scheme for excited state acid–base equilibria illustrated in Fig. 12 [6].

The emission band $\lambda_{max}(2)$ observed in alkaline solutions of norharmane, harmane and harmine can be assigned to fluorescence from the neutral species because its spectral characteristics are the "mirror image" of the excitation spectra and are similar to the fluorescence observed from β-carbolines in cyclohexane (Fig. 13). The low intensity of these emissions in aqueous solution and the strong fluorescence from the cations suggest that protonation of the neutral species in the excited state is very rapid. $\lambda_{max}(3)$ must therefore be due to the formation of the zwitterion as proposed in Fig. 12.

The fluorescence properties of the 2-methyl-β-carbolines were studied to provide confirmation of this description. These compounds may only
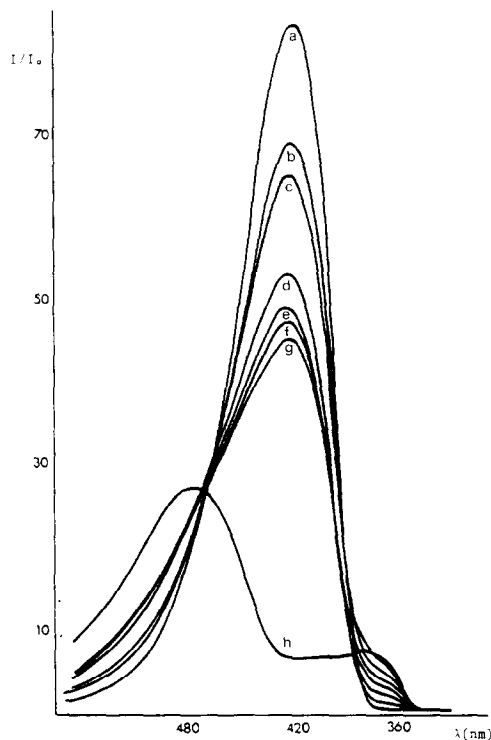
Fig. 9. Fluorescence spectrum of harmine (λ_{ex} = 305 nm; concentration, 5×10^{-5} M; ionic strength, 0.1): curve a, pH 1.00; curve b, pH 8.40; curve c, pH 8.80; curve d, pH 7.44; curve e, pH 7.84; curve f, pH 8.04; curve g, pH 8.21; curve h, pH 12.50.

exist as cations except in alkaline solutions where the proton from the nitrogen in the pyrrole ring is released, resulting in the formation of a zwitterion species. The fluorescence observed in acid solutions is similar to that of the β-carboline cations. However, only one new emission band, with a maximum close to $\lambda_{max}(3)$, appears as the pH is increased. The fluorescence spectra of these zwitterion species closely resemble the emission bands of β-carbolines at $\lambda_{max}(3)$.

The well-known Förster relation (eqn. (2)) was used to obtain information concerning the magnitude $pK_{CN}(S_1)$ (Fig. 12) of the excited state ionization constant of the cation–neutral species equilibrium. Equation (2) was also used to obtain the $pK_{CZ}(S_1)$ values from the spectra of the 2-methyl derivatives. These values of the excited state pK_a for the cation–zwitterion equilibrium are lower than the calculated values of $pK_{CN}(S_1)$, indicating that the excited state zwitterion species should predominate in alkaline solutions.

In strongly alkaline solutions (pH > 14) the β-carbolines show a new band in the absorption spectra. This suggests the existence of a new kind
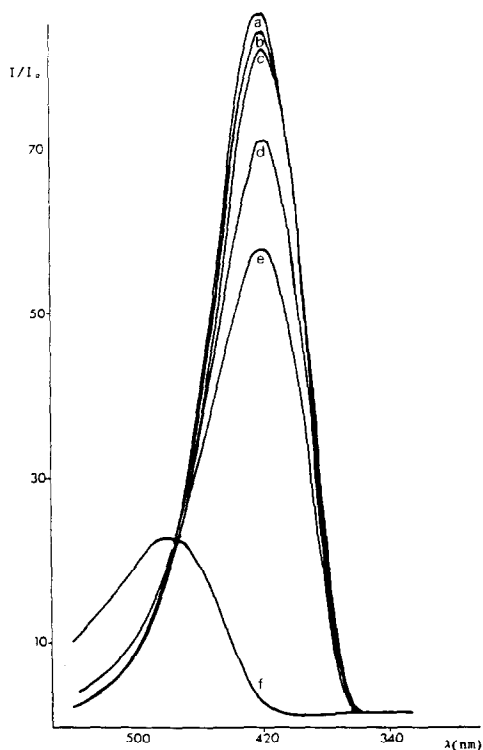
Fig. 10. Fluorescence spectrum of 2-methylharmine (λ_{ex} = 345 nm; concentration, 5 × 10^{-5} M; ionic strength, 0.1): curve a, pH 1.00; curve b, pH 8.40; curve c, pH 9.00; curve d, pH 10.00; curve e, pH 11.02; curve f, pH 12.40.

of molecular species corresponding to the anionic molecule produced by releasing the proton from the pyrrolic nitrogen.

Excitation of the anion produces emission at $\lambda_{max}(3)$ characteristic of the zwitterion species, suggesting that rapid protonation of the excited state anion occurs [5]. Since no anion emission can be detected, $pK_{ZA}(S_1)$ and $pK_{NA}(S_1)$ can only be estimated from the Förster equation (eqn. (2)) by using the energy differences in the absorption spectra (Fig. 6) for the respective species. It has been claimed that consideration of absorption energies only in the Förster equation may give $pK(S_1)$ values that err significantly towards the ground state $pK_a(S_0)$ values [11]. The $pK_{ZA}(S_1)$ values found in this work (Table 1) indicate that the excited state anion probably abstracts a proton from a water molecule to form the zwitterion.

The results for $pK_a(S_1)$ are summarized in Table 1. The values of $pK_{CN}(S_0)$ and $pK_{CN}(S_1)$ for harmane are only estimates because of the unavailability of data for 2-methylharmane.
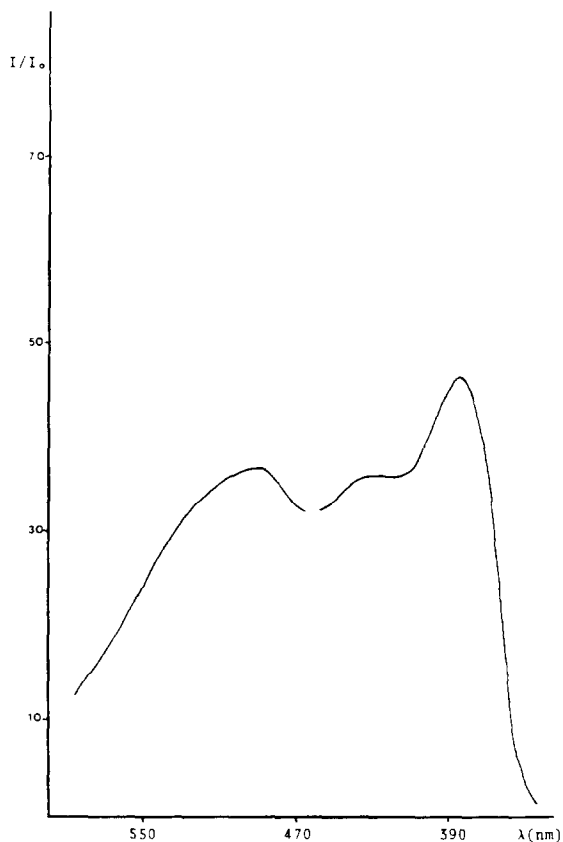
Fig. 11. Fluorescence spectrum of β-carboline solution at pH 13 (the sensitivity is approx·imately 50 times greater than that in Fig. 7, curve h).
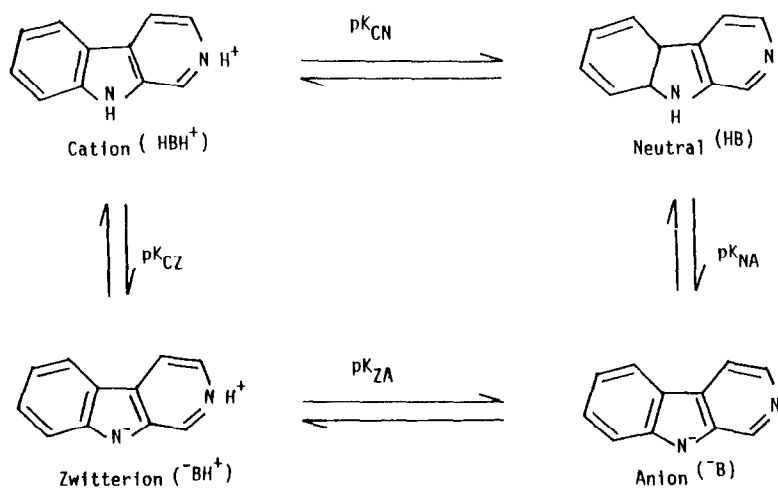

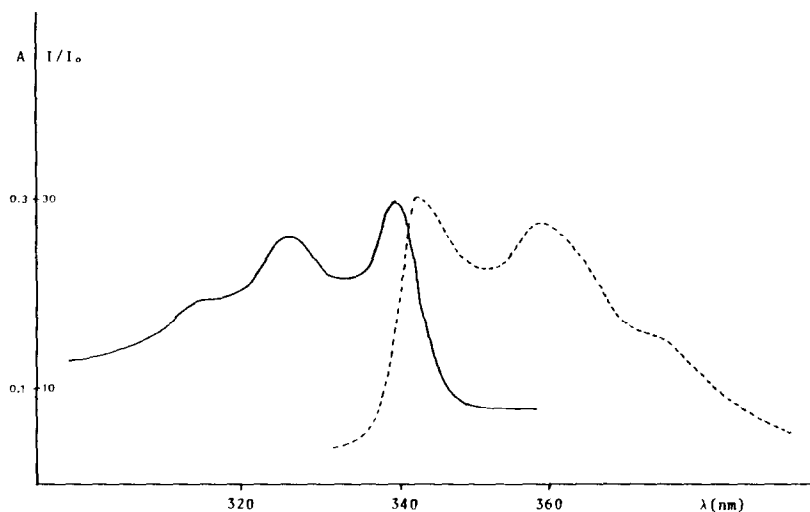Fig. 12. An acid–base equilibrium scheme for β-carbolines.

Fig. 13. "Mirror image" of the emission (— — —) and absorption (———) spectra of norharmane in cyclohexane.

4. Conclusion

The ground and excited state pK_a values for the acid–base equilibria present in aqueous solutions of β-carbolines are summarized in Table 1. It is apparent that these molecules exhibit quite unusual excited state acid–base behaviour. In particular, the formation of a cation and a previously unreported zwitterion species via proton transfer from the solvent is a major process following photoabsorption in alkaline solutions.

The results reported in this paper together with those published previously [5] indicate that β-carbolines exhibit an acid–base behaviour similar to that observed for other nitrogen heterocycles [12, 13].

The basicity of neutral β-carbolines is much larger in the first excited singlet S_1 than in the ground state in acid–base equilibria involving the nitrogen in the pyridine ring, whereas the $pK_a(S_1)$ values for equilibria involving the nitrogen in the pyrrole ring are much lower than the ground state values [9, 14].

The substituent effect on the acid–base properties of the β-carbolines in the ground state is rather small, being in order of basicity CH_3O- > CH_3- > $H-$. This behaviour can be explained by the stabilization of the protonated form in the presence of electron-donating substituents. The influence of the substituents on the pK_a values of the excited states does not appear to take place in the same direction as in the ground state.

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