EXCITED STATE PROTON TRANSFER IN β-CARBOLINE

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Summary

Steady state and time-resolved fluorescence techniques were used to investigate the excited state acid–base equilibria behaviour of β-carboline and 2-methyl-β-carboline in aqueous solutions. In acid conditions the β-carboline cation is responsible for light absorption and fluorescence (λ_{max} = 450 nm; τ_F = 22.0 ns). However, in alkaline solutions excitation of the neutral species leads to the formation of the β-carboline cation by a rapid proton transfer with the solvent. A diffusion-controlled quenching of the excited state cation by OH^- ions produces a zwitterion species which fluoresces with λ_{max} at 510 nm (τ_F = 1.6 ns). The zwitterion is also formed after excitation of the ground state anion at pH 14. pK values for the ground state and excited state acid–base equilibria are reported.

1. Introduction

β-Carbolines (9H-pyrido[3,4-b] indoles) are markedly fluorescent alkaloids which are of considerable pharmacological interest at present, many being hallucinogens [1]. β-Carboline (Fig. 1, I) itself (also called norharman) is the fluorescent product obtained in the widely used Denckla and Dewey determination of the aromatic amino acid tryptophan [2]. Although the intense fluorescence of β-carboline in acid solutions is commonly reported [1, 3, 4], there appear to have been no detailed studies of the fluorescence behaviour or excited state reactivity of this important class of molecules over a wide range of pH.
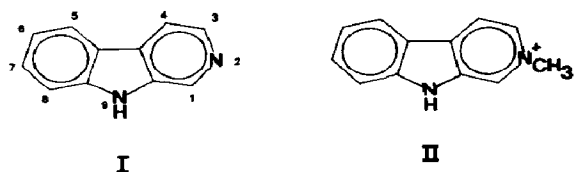
Fig. 1. The structures of β-carboline (I) and 2-methyl-β-carboline (II).

Reviews [3, 5] on the chemical properties and reactivities of these compounds indicate that the ground state pK of the 2-position pyridine ring nitrogen is 7.9 whereas that of the 9-position pyrrole ring nitrogen of 2-substituted β-carbolines (Fig. 1, II) is typically 10.9 [3]. It has been reported that related pyridine derivatives increase their pK by 5 - 7 units on electronic excitation [6] and it is known that pyrrole derivatives become stronger acids in the excited state [7]. Recent molecular orbital calculations suggest that this behaviour may also occur in β-carboline [8] and thus the excited state of this molecule may possibly exist as a zwitterion in slightly alkaline solutions.

In order to obtain further information concerning excited state acid-base equilibria for these molecules in aqueous solution, we have carried out steady state and time-resolved fluorescence studies on β-carboline and its 2-methyl derivative under various pH conditions.

2. Experimental procedure

2-Methyl-β-carboline (II) was prepared from β-carboline (Aldrich) by the method of Spenser [9] and purified by recrystallization from water. The compounds were dissolved in 0.01 M HCl to have an absorbance of approximately 0.1 at 373 nm (25×10^{-6} M). Sodium hydroxide was added to bring the solutions to the required pH, measured on an Athom pHM25 pH meter. Measurements were carried out using air-equilibrated solutions at 25 °C. Degassing of solutions did not significantly affect the fluorescence properties of these molecules.

Uncorrected fluorescence emission and excitation spectra were recorded on a Perkin–Elmer MPF-44A spectrofluorimeter. Fluorescence decay curves were measured on an Ortec–Applied Photophysics Ltd. nanosecond spectrometer using the time-correlated single-photon counting technique. A monochromator was used to select the excitation wavelength and fluorescence emission was isolated with a series of Jena GG optical filters. The fluorescence decay data were transferred to an on-line Nova 2-10 computer and analysed with single- and double-exponential iterative deconvolution programs. The goodness-of-fit was established from the magnitude of the reduced chi-squared value and inspection of weighted residuals [10]. Subnanosecond decay time measurements for emission at wavelengths greater than 400 nm were confirmed using a picosecond laser–streak camera system described previously [11].

3. Results and discussion

The absorption and emission spectra of the β-carboline cation at pH 2 are shown in Fig. 2. Under these conditions the fluorescence decay is single exponential with a lifetime of 22.0 ± 0.1 ns.
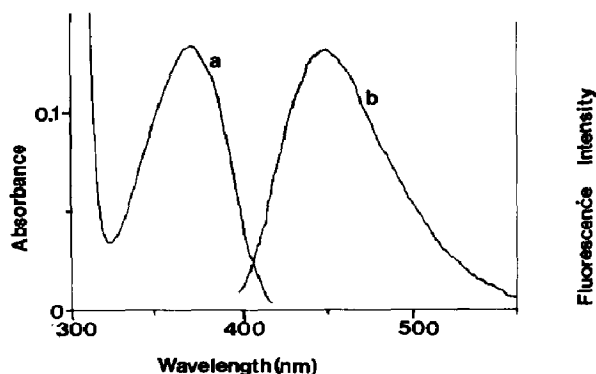
Fig. 2. The absorption spectrum (curve a) and the fluorescence spectrum (curve b) of a β-carboline solution at pH 2.

In slightly alkaline solutions there is a hypsochromic shift of the absorption spectrum, corresponding to the cation–neutral species transformation with a pK of 7.9. However, the emission characteristics remain those of the cation (λ_{max} = 450 nm), suggesting that proton transfer to the initially excited neutral species occurs rapidly. With a further increase in pH the intensity and lifetime of the fluorescence are markedly reduced and two new emission maxima appear at 380 and 510 nm (Fig. 3). Identical excitation spectra were recorded at emission wavelengths throughout the three bands, indicating that the emitting species all had a common ground state precursor. It was observed that the intensity of the 380 nm emission band remained constant between pH 10 and pH 13.

In strongly alkaline solutions (pH 14) a new red-shifted absorption band appears arising from the formation of some β-carboline anion. Emis-
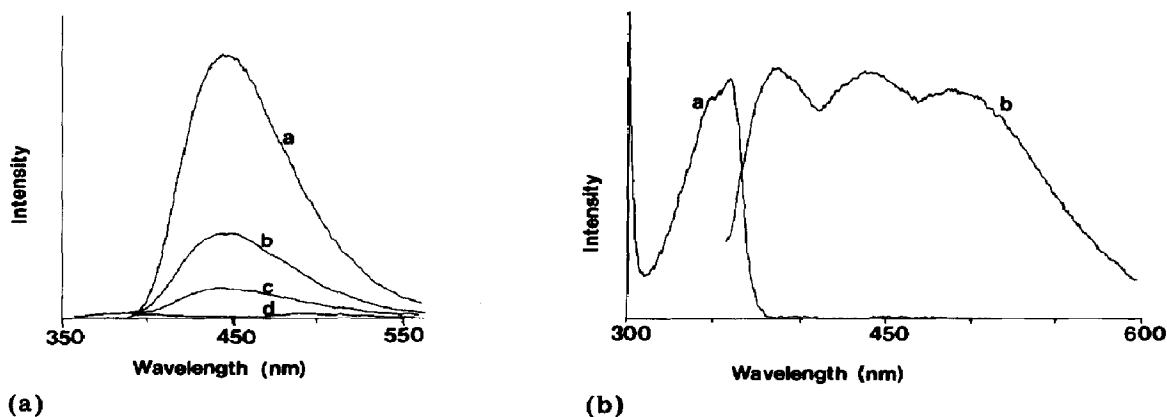

(a)

(b)

Fig. 3. (a) Emission spectra of β-carboline solutions for an excitation wavelength of 354 nm: curve a, pH 3; curve b, pH 10.1; curve c, pH 11.2; curve d, pH 13. (b) The fluorescence excitation spectrum (curve a) and the emission spectrum (curve b) of β-carboline solution at pH 12.3 (sensitivity approximately 50 times greater than that in (a)).

sion maxima at 380 and 510 nm are both present with excitation into the neutral species absorption band. However, excitation solely of the ground state anion (λ_{exc} = 390 nm) yields only the emission band at 510 nm (Fig. 4). Fluorescence lifetime and spectral data for β-carboline solutions under various pH conditions are summarized in Table 1.
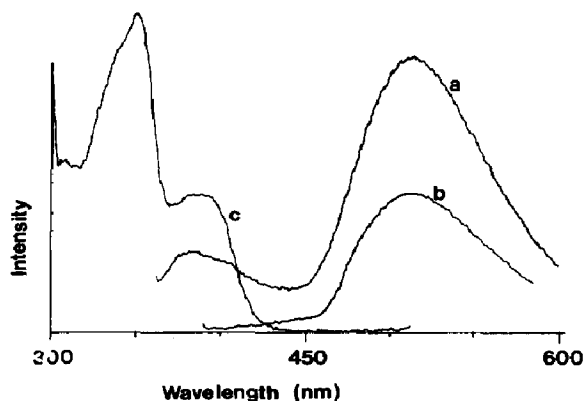

Fig. 4. Fluorescence spectra of β-carboline at pH 14: curve a, λ_{exc} = 350 nm; curve b, λ_{exc} = 390 nm; curve c, fluorescence excitation spectrum for an emission wavelength of 510 nm.

TABLE 1

Fluorescence lifetimes and OH^- ion quenching data for β-carboline at 25 °C

pH	τ_F (ns)	k_{OH} ($\times$ 10^{-10} M^{-1} s^{-1})
3.6	22.0 ± 0.1[a]	—
11.2	5.68 ± 0.03[a]	8.3
11.4	4.1 ± 0.1[a]	7.9
11.8	1.62 ± 0.03[a]	9.0
14	1.60 ± 0.03[b]	—

[a] Cation emission (λ_{max} = 450 nm).
[b] Zwitterion emission (λ_{max} = 510 nm).

We believe that these observations may be explained by the excited state acid–base equilibria scheme illustrated in Fig. 5.

The emission band at 380 nm observed in alkaline solutions may be assigned to fluorescence from the neutral species since its spectral character is the "mirror image" of the excitation spectrum and it is similar to the fluorescence observed from β-carboline in diethyl ether. The low intensity of this emission in aqueous solution and the strong fluorescence from the cation suggest that protonation of the neutral species in the excited state is very rapid. The fluorescence lifetime of the 380 nm emission was, in fact, found to be below the resolution of our photon counting equipment (less than 300 ps) and could not be observed on the laser system owing to the flint glass optics of our streak camera.
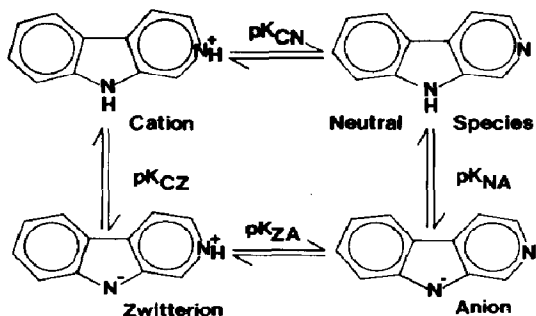
Fig. 5. An acid–base equilibria scheme for β-carboline.

At pH 10 the concentration of H_3O^+ ions is insufficient to explain the rapid protonation of β-carboline by a diffusion-controlled process [6, 12]. Thus, in the excited state β-carboline must abstract protons from water to form the cation. The proposal that the pyridine ring nitrogen is a stronger base than the OH^- ion in the excited β-carboline molecule is supported by the observed constant intensity of the neutral species emission between pH 10 and pH 13. A large change in the fluorescence intensity at 380 nm with pH would be expected if either H_3O^+ or OH^- ions were involved in the proton transfer mechanism.

Two approaches may be used to obtain information concerning the magnitude of the excited state pK of the cation–neutral species equilibrium (pK^*_{CN} in Fig. 5). For two species A and B involved in the acid–base equilibrium

$$\text{AH} + \text{B} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \text{A} + \text{HB}$$
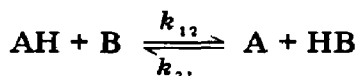
the following relationships apply [12]:

$$K = \frac{k_{12}}{k_{21}} = \frac{[\text{A}][\text{HB}]}{[\text{HA}][\text{B}]} \tag{1}$$

$$pK_{\text{AH}} = -\log\left(\frac{[\text{A}][\text{H}_3\text{O}^+]}{[\text{AH}]}\right) \tag{2}$$

$$\Delta(pK) = \log k_{21} - \log k_{12} \tag{3}$$

where

$$\Delta(pK) = pK_{\text{AH}} - pK_{\text{HB}}$$

If $\Delta(pK)$ is positive and proton transfer is diffusion controlled, then k_{21} becomes independent of $\Delta(pK)$ and thus there will be a linear relationship between $\log k_{12}$ and $\Delta(pK)$. An estimate of the rate of protonation of the excited neutral molecule was obtained by observing the rise time of emission from the cation using the picosecond laser–streak camera. This rise time was less than 100 ps, providing a lower limit estimate of 10^{10} s^{-1} for k_{12},

which is consistent with a diffusion-controlled process [12]. Using eqn. (3), a value of 14 for pK^*_{CN} was obtained. A further method for calculating pK^*_{CN} is to use the well-known Forster relationship [6]

$$pK - pK^* = \frac{\Delta E_H - \Delta E}{2.3RT} \tag{4}$$

where ΔE_H and ΔE are the excited state energies of the protonated species and unprotonated species respectively. These values were determined from the emission and absorption spectra [6] to give $pK^*_{CN} = 14.7$, which is in reasonable agreement with the previous calculation. The conclusion that the excited state pyridine ring nitrogen is more basic than the OH^- ion supports the results of molecular orbital calculations by Deumié *et al.* [8].

The absence of any long-lifetime component in the decay curves of the emission at 380 nm suggests that there is no pH-dependent back reaction to re-form excited neutral species from the cation. The pH-dependent quenching of the β-carboline cation fluorescence must therefore lead to the formation of another species, possibly the zwitterion proposed in Fig. 5.

To confirm this proposal, the fluorescence properties of 2-methyl-β-carboline were studied. This compound may only exist as a cation except in alkali when it loses a proton from the pyrrole ring nitrogen ($pK = 10.9$) to form a zwitterionic species. The fluorescence lifetime and spectral data for this derivative under various conditions of pH are summarized in Table 2. In acid solutions the observed fluorescence is quite similar to that of the β-carboline cation. However, as the pH increases, only one new emission band appears, with a maximum at 510 nm (Fig. 6). The fluorescence spectrum and lifetime of this zwitterion species closely resemble the emission band of β-carboline at 510 nm (*cf.* Tables 1 and 2). The rate constant k_{OH} for quenching of the cation in alkaline solutions may also be readily calculated for β-carboline and its 2-methyl derivative from

$$k_{TOT} = k_{NR} + k_R + k_{OH}[OH^-] \tag{5}$$

where k_R and k_{NR} are respectively the radiative rate constant and the non-radiative rate constant of the cation and k_{TOT} is the observed quenching rate. The k_{OH} values obtained for both compounds are very similar and diffusion controlled (Tables 1 and 2). These observations confirm that the emission at 510 nm observed from β-carboline in alkaline solutions arises from an excited state zwitterion (Fig. 5).

A value $pK^*_{CZ} = 4.3$ for the 2-methyl derivative may be obtained using the Forster relationship (eqn. (4)). This value of the excited state pK for the cation–zwitterion equilibrium is much less than the calculated value of pK^*_{CN} (14.7), indicating that the excited state zwitterion should predominate in alkaline solutions. It is apparent from eqn. (3) that the OH^- ion must be the proton acceptor involved in the formation of the zwitterion and only at a pH greater than 10 will significant diffusion-controlled quenching of the cation occur.

In β-carboline solutions at pH 14 there is a new band in the absorption spectrum owing to the presence of some anion species. Excitation of the

TABLE 2

Fluorescence lifetimes and OH⁻ ion quenching data for 2-methyl-β-carboline at 25 °C

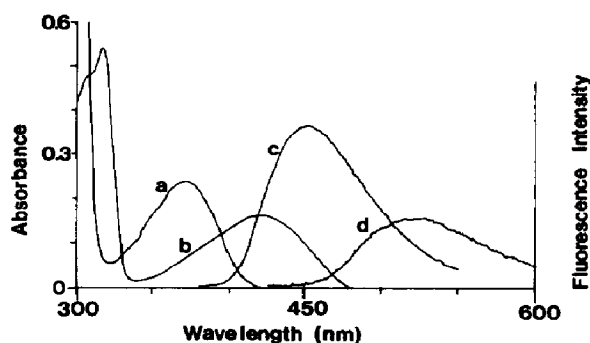| pH	τ_F (ns)	k_{OH} ($\times 10^{-10}$ M^{-1} s^{-1})
2.0	22.9 ± 0.2[a]	—
10.3	16.3 ± 0.2[a]	8.9
10.5	14.2 ± 0.06[a]	8.5
10.8	10.0 ± 0.1[a]	9.0
11.2	6.0 ± 0.1[a]	7.8
13	1.32 ± 0.05[b]	—

[a] Cation emission (λ_{max} = 455 nm).
[b] Zwitterion emission (λ_{max} = 510 nm).

Fig. 6. Absorption spectra and fluorescence spectra for 2-methyl-β-carboline: curves a and c, at pH 2; curves b and d, at pH 13 (the sensitivity for curve d was 30 times greater than that for curve c).

anion produces only 510 nm emission characteristic of the zwitterion species, suggesting that rapid protonation of the excited state anion occurs. Since no anion emission can be detected, only estimates for pK^*_{ZA} and pK^*_{NA} may be calculated from the Forster equation (eqn. (4)), using the energy differences in the absorption spectra for the respective species. It has been claimed that consideration of absorption energies alone in the Forster equation may give pK^* values that err significantly towards the ground state pK value [12]. The pK^*_{ZA} value obtained (Table 3) indicates that the excited state anion most probably abstracts a proton from water to form the zwitterion.

4. Conclusions

The values for the ground state and excited state pKs for the acid–base equilibria present in aqueous solutions of β-carboline are summarized in Table 3. It is apparent that these molecules exhibit quite unusual excited state acid–base behaviour. In particular, the formation of a cation and a

TABLE 3

Ground and excited state pK values for β-carboline acid–base equilibria (cf. Fig. 5)

	pK (ground state)	pK* (excited state)
pK_{CN}	7.9	14.7
pK_{CZ}	10.9	4.1
pK_{NA}	14.5 [a]	8 [a]
pK_{ZA}	11.5 [b]	15 [a]

[a] Estimates (see text).
[b] Obtained by difference, pK_{CN} + pK_{NA} − pK_{CZ}.

previously unreported zwitterion species via proton transfer with the solvent is a major process in alkaline solutions after light absorption. It might be expected that other carbolines will display similar excited state properties.

Acknowledgments

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