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Triplet state studies of β-carbolines

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Dedicated to Professor Ralph S. Becker on the occasion of his 75th birthday

Abstract

Low temperature phosphorescence spectra, quantum yields and lifetimes, and room temperature triplet–triplet absorption spectra and lifetimes have been obtained for the neutral and cationic forms of the three β-carbolines: harmine (Hi), harmane (Ha) and norharmane (NorH). Based on the phosphorescence spectra, the energies of the lowest triplet states are 25 250 (Hi), 24 750 (Ha) and 24 400 cm^{-1} (NorH) for the neutral forms and 22 900 (Hi), 21 750 (Ha) and 21 850 cm^{-1} (NorH) for the corresponding cations. Phosphorescence quantum yields are in the range 0.4 and 0.6 for the neutral forms, and are found to decrease on protonation, while phosphorescence lifetimes vary from 3.15 s for the neutral form of Hi to 6.89 s for the cationic form of NorH. In all the cases, the lowest excited triplet state is suggested to have $^3(\pi, \pi^*)$ character. For several of the systems, the sum of emission yields is less than 1, indicating that internal conversion may also be an important deactivation route for the lowest excited singlet state. Microsecond flash photolysis studies show that the triplet states of all three carbolines undergo acid–base equilibria in water with $\mathrm{p}K_\mathrm{a}$ values in the region 4.4–5.0 at room temperature. In water and ethanolic solutions, the cationic forms show two absorption maxima at ca. 450 and 580 nm, while the neutral forms have only one absorption maximum in the visible spectral region at ca. 510 nm. In nitrogen purged solutions, triplet state lifetimes are 530 (Hi), 380 (Ha) and 600 μs (NorH), and the triplet states are quenched by β-carotene with rate constants $k(\mathrm{av}) = 3.3(\pm 0.9) \times 10^9\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$ close to but slightly lower than those expected for a diffusion controlled process. © 2001 Published by Elsevier Science B.V.

Keywords: β-Carbolines; Phosphorescence; Triplet–triplet absorption spectra; Acid–base behaviour

1. Introduction

The β-carbolines are a group of naturally occurring alkaloids with a tricyclic pyrido(3,4-b)indole ring system [1,2]. Much emphasis has been focussed on the spectroscopy and photophysical properties of these systems. In large part, this is associated with their known phototoxic effects towards various organisms [3–6]. They have been shown to act as photosensitisers in the presence of oxygen to produce both superoxide radical anion [7,8] and singlet oxygen [7]. Their phototoxicity also appears to be strongly associated with the diffusion of these molecules into lipophilic regions of cells or other biological targets [5]. Solubility and partitioning studies show that the neutral forms of the β-carbolines have strong affinities for hydrophobic domains [9–11]. We thus feel that one of the major phototoxic processes in vivo involves reactions of singlet oxygen, or other reactive oxygen species, with substrates following photosensitisation by the triplet state of the neutral form of the β-carboline.

Extensive investigations have been carried out on their fluorescence in aqueous [12–23,48] and organic [24–30] solvents. Because of their attractive emission properties, their use has also been proposed as fluorescence standards [16]. Their behaviour in water-in-oil microemulsions has also been studied [31,32] to try to understand partitioning in biological systems. The β-carbolines undergo acid–base equilibria, and because of large differences in charge density in the ground and lowest excited singlet states [26], there are marked differences in the effects of pH on their absorption and fluorescence spectra [12–15,18,20–23,48].

Although different interpretations have been given for the kinetics of excited state proton transfer [21,22,48], there is a general consensus on the equilibria involved. Depending upon pH and solvent, it is suggested that the compounds can exist in four forms: the cation, the neutral form, a zwitterion (or an alternative quinone-type canonical form), and an anion. In the pH range used here (0–12.5), the only ground state species present are the neutral and

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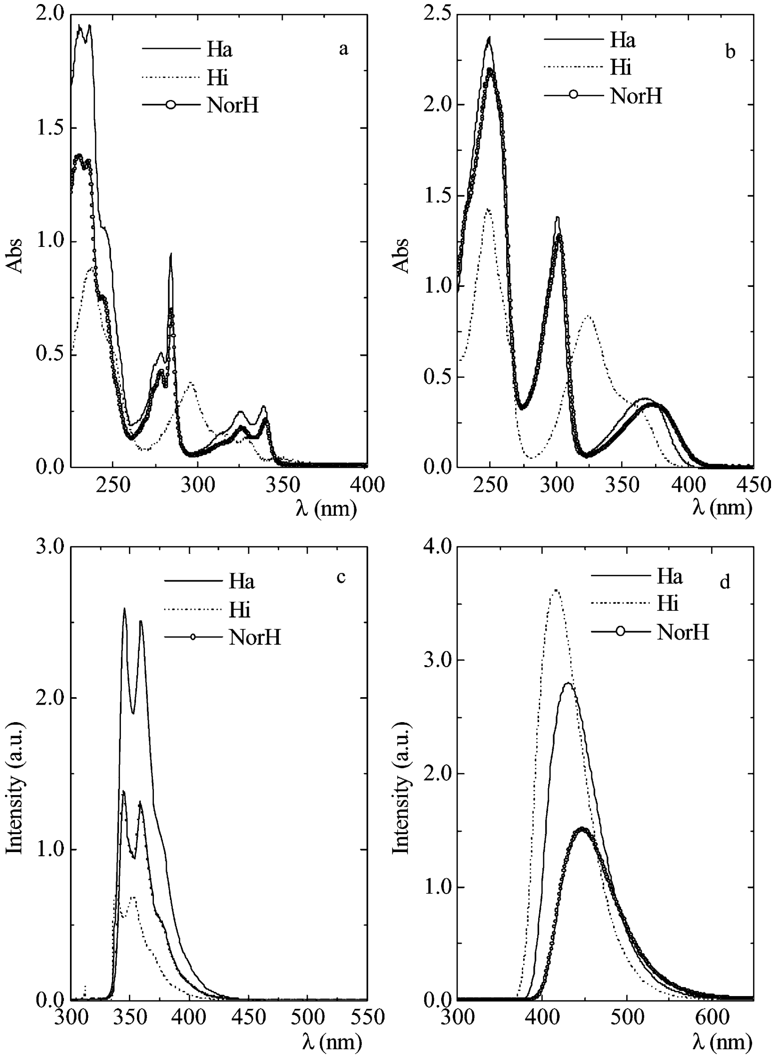


Fig. 1. Spectra of β-carbolines used in this study: (a) and (b) absorption spectra of neutral and cationic forms; (c) and (d) fluorescence spectra ($\lambda_{\mathrm{excitation}}$ 337 nm) of neutral and cationic forms, respectively.

cationic forms. In emission, fluorescence from cationic, neutral and zwitterionic forms can be observed over this pH range [22,23,26]. The anion is not observed in either absorption or emission at pH values less than 13.5 [14,23].

In contrast to the situation with the ground and lowest excited singlet state, relatively little has been published on the triplet states of β-carbolines [33–36]. This is a surprising omission, considering the possible role of singlet oxygen in the phototoxic action of these molecules [3–7,37]. In this work, we report results from studies of low temperature fluorescence and phosphorescence emission spectra, and room temperature microsecond flash photolysis of the three β-carbolines harmine, harmane and norharmane, with particular emphasis on the nature and dynamics of the lowest excited triplet state. Fig. 1 shows the absorption and room temperature emission spectra of each compound in the neutral and protonated forms.

2. Experimental

2.1. Materials

Harmine, harmane and norharmane were used as purchased (Aldrich). Solvents and other reagents were of the purest grade commercially available and were used without further purification. Solutions were prepared with typical concentrations 2×10^{-5}–10×10^{-5} M for both luminescence and flash photolysis. In some cases, these were limited by solubility problems. The following glasses, with compositions given in v/v ratios, were used for low temperature work: diethylether:ethanol:ammonia (28% solution in water) (10:9:1) (glass A), ethanol:hydrochloric acid (37% solution in water) (19:1) (glass B), ethanol:methanol (1:1) (glass C), methylcyclohexane:pentane (4:1) (glass D) [38].

2.2. Methods

Room temperature and 77 K emission spectra were obtained using a Jobin-Yvon JY3 D spectrofluorimeter equipped with a Hammamatsu R928 photomultiplier. Spectra were corrected using a Rhodamine B quantum counter and Kodak white reflectance standard. 77 K spectra were obtained using a custom-made quartz dewar with samples contained in borosilicate glass NMR tubes. Microsecond flash photolysis studies were made with a standard Applied Photophysics 200 J instrument (pulse duration 10 μs given as the flash half-peak height), with $\frac{1}{2}$ cm toluene (cut off wavelength $<$ 280 nm) filters to minimise photodegradation due to UV excitation. The photolysis solution (∼30 ml) was contained in a 10 cm path length cell. Solutions were purged with nitrogen for 30 min to remove oxygen before beginning the experiments and maintained under an atmosphere of nitrogen throughout the experiment. Data were collected on a Gould OS470 oscilloscope and analysed on a BBC Archimedes computer.

3. Results and discussion

3.1. Luminescence

Fig. 2a shows the total emission (fluorescence and phosphorescence) spectrum for all three sensitisers in the alkaline glass A where only the neutral ground state is present. Fig. 2b shows the phosphorescence spectra only, obtained by gating-out the short-lived fluorescence. Similarly, Fig. 3a shows the total emission spectra for all three compounds in the acidic glass B, where only the cationic ground state is present, while Fig. 3b shows the phosphorescence spectra. From the onset of the phosphorescence, energies of the lowest triplet states of Ha (neutral 24 750 cm^{-1}, cation 21 750 cm^{-1}), NorH (neutral 24 400 cm^{-1}, cation 21 850 cm^{-1}) and Hi (neutral 25 250 cm^{-1}, cation 22 900 cm^{-1}) were estimated. Other low temperature emission data for both glasses are collected in Table 1. Whilst Hadley et al. [33] was not sure whether the phosphorescence observed in their systems were due to neutral or cationic forms of the β-carbolines, it is clear both from the strong similarities between their spectra and those seen by us in alkaline glasses, and the fact that their phosphorescence lifetimes are in perfect agreement with our values for these glasses that they were looking at the emission from the neutral form of the β-carboline triplets. Our value for the phosphorescence lifetime of norharmane is also in reasonable agreement with the value given by Tomas et al. [34]. The long phosphorescence lifetimes observed for both the neutral and cationic forms indicate that in both cases the emission is from a triplet π–π* state.

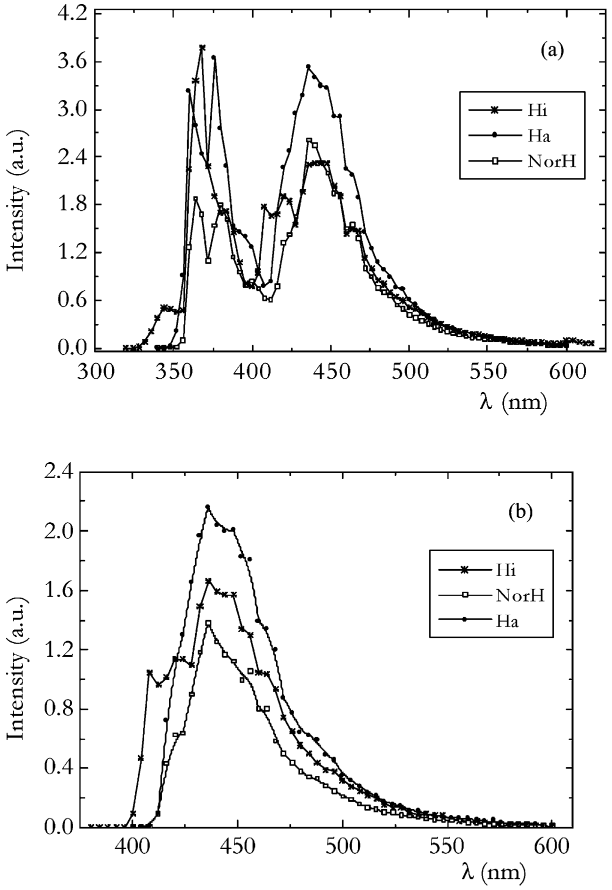


Fig. 2. (a) Total emission spectra (fluorescence and phosphorescence, $\lambda_{excitation}$ 337 nm) of harmine (1.7×10^{-5} M), harmane (3.8×10^{-5} M) and norharmane (3.0×10^{-5} M) in diethylether:ethanol:ammonia (glass A) at 77 K; (b) phosphorescence spectra of the above systems.

Emission is from a single species only, the cationic and neutral forms, respectively, in both the acidic and alkaline glasses. However, in ethanol/methanol the situation is more complicated. In this solvent system, for Ha both emission spectra and phosphorescence lifetime indicate that emission is predominantly from the neutral form, but for Hi and NorH the phosphorescence spectra show significant contributions from both neutral and cationic form. Phosphorescence lifetimes are wavelength independent indicating equilibration between cationic and neutral forms on the observation timescale. A comparison of the lifetime data indicates a cationic/neutral ratio of 1.26 for Hi, 1.06 for Ha and 1.61 for NorH.

3.2. Flash photolysis

3.2.1. General observations

Flash photolysis of these compounds in nitrogen purged solution in a variety of solvents generates a transient species which absorbs throughout the visible spectrum and which

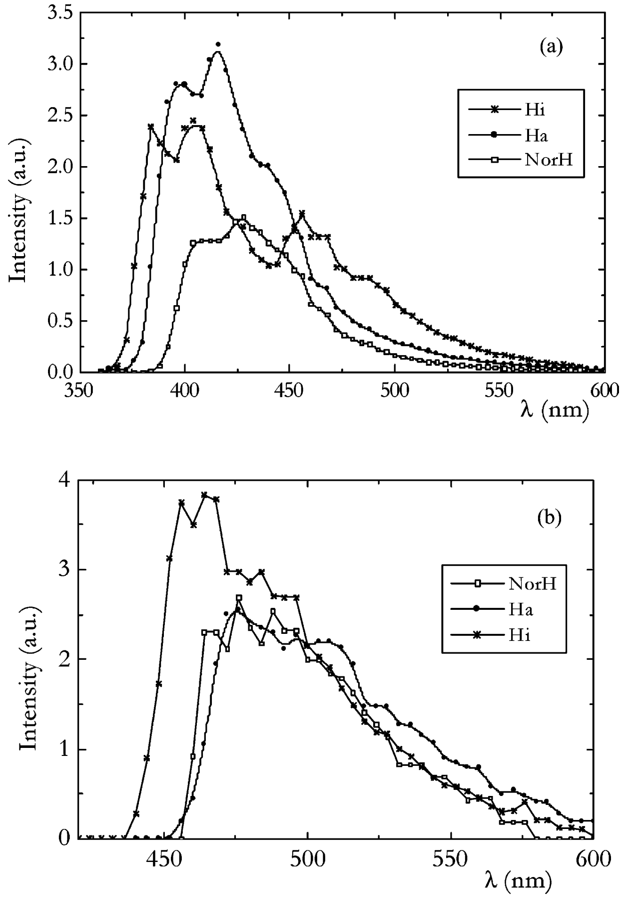


Fig. 3. (a) Total emission spectra (fluorescence and phosphorescence, $\lambda_{\text{excitation}}$ 337 nm) of harmine (1.3×10^{-5} M), harmane (4.0×10^{-5} M) and norharmane (5.2×10^{-5} M) in ethanol:hydrochloric acid (glass B) at 77 K; (b) phosphorescence spectra of the above systems, normalised with the relative intensities proportional to the quantum yields.

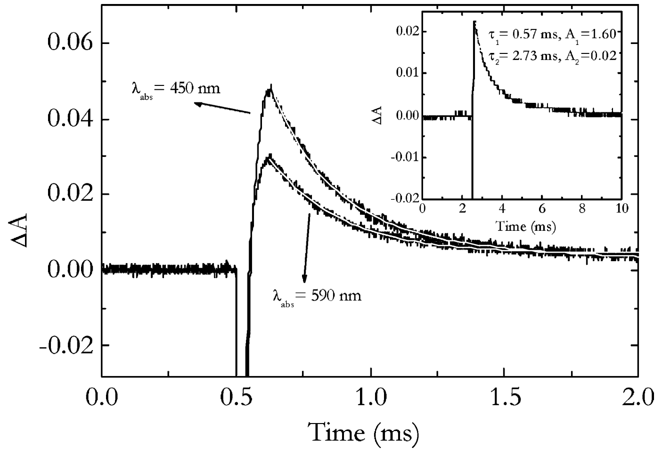


Fig. 4. Kinetic traces of decay of transient absorptions at 450 and 590 nm following flash photolysis of an aqueous solution of harmine (2.9×10^{-5} M) at pH 0. The main figure shows treatment as single exponentials, while the inset indicates analysis as double exponential decays.

decays within ca. 5 ms. The transient spectra are both pH and solvent dependent. In some studies, particularly those using DMSO as solvent, a second longer lived transient was also observed as a weak, broad structureless absorption throughout the visible spectrum and which decays over a much longer timescale. As will be discussed below, we believe that the short-lived transient is the sensitiser triplet. The nature of the long-lived transient is unclear, although it may be due to photodegradation since, under those conditions in which this transient was most prominent, a slight residual colouration of the solution could be observed after repeated exposure to the excitation flash. In aerated solution, no transients with lifetime in the microsecond to seconds timescale can be observed.

3.2.2. Kinetics

The main part of Fig. 4 shows typical decay curves of the fast component of harmine at two wavelengths, together with the exponential fit for the initial part of the decay, while the inset illustrates the fast and slow transients treated as two exponentials, with lifetimes 570 μs and ≥2.75 ms. Since we do not know whether the slow component follows strictly first-order decay, and the two lifetimes are rather different, we have chosen to treat these two species as kinetically distinct. For the case of harmine, the lifetime calculated for a single exponential decay (530 μs) is, within experimental error, identical to that obtained from the biexponential treatment, justifying this approach. Although the transient spectra depend upon pH and solvent for any given conditions,

Table 1
Low-temperature fluorescence and phosphorescence data for β-carbolines in organic glasses at 77 K

β-Carboline	Solvent species	L.T. fluorescence yield	ϕ_F/ϕ_P	L.T. phosphorescence yield[a]	$\phi_F + \phi_P$	$\tau_{\text{phosphorescence}}$ (s)
Hi	N[b]	0.46	0.74	0.62	1.08	3.15
Hi	C[c]	0.51	2.7	0.19	0.70	3.96
Ha	N[b]	0.32	0.81	0.42	0.74	5.70
Ha	C[c]	0.76	3.4	0.22	0.98	6.05
NorH	N[b]	0.30	0.70	0.43	0.73	4.27
NorH	C[c]	0.62	4.0	0.16	0.78	6.89

[a] Phosphorescence quantum yields have been obtained by measurement relative to fluorescence intensities and quantum yields making the assumption that the fluorescence quantum yields are independent of temperature.

[b] Neutral in diethylether:ethanol:ammonia (28% in water) matrix.

[c] Cation in ethanol:hydrochloric acid (37% in water) matrix.

Table 2
Triplet state lifetimes of β-carbolines and rate constants for quenching by β-carotene on flash photolysis of solutions at room temperature

β-Carboline	τ_T (μs)	k_Q ($M^{-1}\,s^{-1}$)
Hi	530	$3.3(\pm 1) \times 10^9$
Ha	380	$2.3(\pm 1) \times 10^9$
NorH	600	$4.0(\pm 1) \times 10^9$

the decay rate for the fast component is constant across the visible spectrum.

For all three compounds in ethanol, the fast transient was dynamically quenched by β-carotene with a rate constant close to but rather lower than that for a diffusion-controlled process (Table 2). We assign the short-lived species to the triplet state on the basis of the near diffusion controlled quenching by β-carotene, the lack of any transients under aerated conditions, and as will be discussed later, the similar acid/base behaviour observed using phosphorescence and transient absorption spectra in neutral alcohols. However, β-carotene was found to have no effect on either the magnitude or the lifetime of the long-lived species. Hence we can say that the long-lived species is not produced as a consequence of the decay of the triplet state. The fact that it is not produced in aerated solutions suggests that it cannot simply be a product of photoionisation of the β-carboline. Attempts to determine the nature of this long-lived species have, so far, proved elusive. Although there may have been a very weak residual absorption following decay of the long-lived species, studies of the effect of light intensity on this component proved inconclusive. However, since our primary goal has been to characterise the triplet state, and this species does not arise from decay of the triplet, we have not pursued it further.

3.2.3. pK_a transitions in water

Fig. 5 shows transient spectra at pH 0 and 10 for Hi recorded 0.2 ms after the flash; Fig. 6 shows spectra at intermediate pH values. As the pH is increased, the transient absorption changes in form from that in acid solution, which shows two bands at 450 and 580 nm, to a spectrum with only one band at 510 nm in alkali. We interpret these as being due to the presence of protonated and neutral triplet, respectively. There are no indications of formation of any excited triplet state of a zwitterion, although this is an important species in the lowest excited singlet state [23,26]. Fig. 7 shows the variation in absorption for harmine as a function of pH at 580 nm and the corresponding logarithmic plot, from which a pK_a value of 4.4(±0.1) can be obtained. The pH dependent behaviour of Ha and NorH are similar and indicate pK_a values of 4.5 ± 0.5 and 5.0 ± 0.5, respectively. These values can be compared with pK_a's of 7–8 for the ground state and ca. 13 for the lowest excited singlet state of these compounds (Scheme 1). These results suggest either that there are marked differences in the electron distribution in the S_1 and T_1 states, or that for kinetic reasons it is not possible to reach thermodynamic equilibrium in the S_1 state. In organic

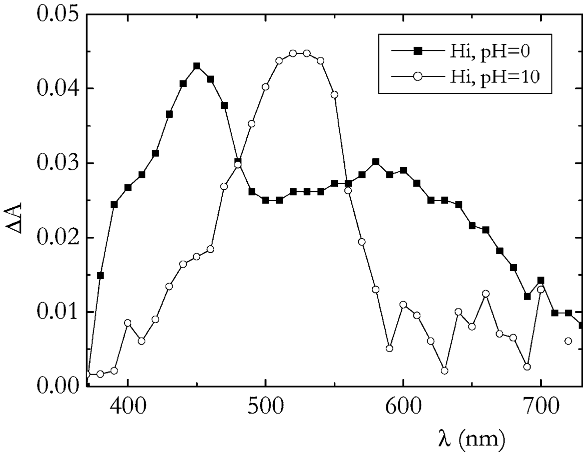


Fig. 5. Transient absorption spectra observed following flash photolysis of aqueous solutions of harmine (2.9×10^{-5} M) at pH 0 and 10.

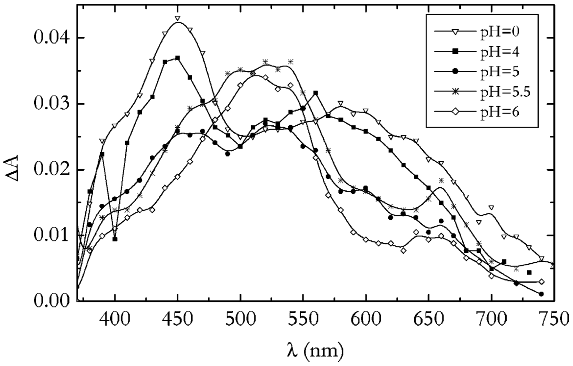


Fig. 6. Transient absorption spectra observed following flash photolysis of aqueous solutions of harmine (2.9×10^{-5} M) at various pH values between 0 and 6.

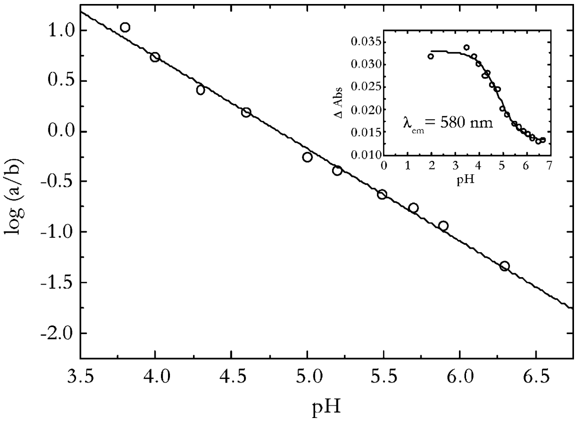
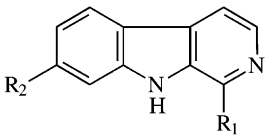


Fig. 7. Changes in absorbance at 580 nm with pH (inset) and corresponding logarithmic plot for pK_a determination following flash photolysis of aqueous solutions of harmine (2.9×10^{-5} M). $a = (\Delta Abs_C^* - \Delta Abs)$, $b = (\Delta Abs - \Delta Abs_N^*)$, where ΔAbs_C^* and ΔAbs_N^* correspond to the pure cationic (pH 1.0) and neutral (pH 7.0) forms, respectively, and ΔAbs is the absorbance change at intermediate pH values.



(1) Harmine	R_1= CH_3	R_2= OCH_3	pK_a= 8.0	$pK_a(S_1)$=12.9
(2) Harmane	R_1= CH_3	R_2= H	pK_a= 7.7	$pK_a(S_1)$=12.8
(3) Norharmane	R_1= H	R_2= H	pK_a= 7.2	$pK_a(S_1)$=13.0

Scheme 1.

solvents, it has been shown that the acid–base equilibria of a series of β-carbolines are not established within the lifetimes of their lowest excited singlet states [30] whilst the complex kinetic scheme observed with the excited singlet state of harmine in aqueous solutions also suggests that the fast relaxation processes preclude the establishment of the true thermodynamic equilibria [23]. However, calculations [30], using the Förster cycle [39] suggest that the true pK_a values for the excited singlet states of these compounds may be even higher than those observed experimentally. It would thus seem that the differences in acid–base behaviour between the S_1 and T_1 states must be related to differences in electron distribution. It is suggested from theoretical calculations and the magnitude of the molar absorption coefficient [26,33] that the lowest excited singlet state has π–π^* character in these compounds in polar solvents. The separation between the lowest $^1(\pi, \pi^*)$ and $^1(n, \pi^*)$ states for Ha and NorH is very small (≈1000 cm^{-1}), and as was first reported for chlorophyll [40] and has since been observed with closely related *N*-heterocyclic systems having more than one ring, such as quinoline [41] and indole [42], the relative energies for the $^1(n, \pi^*)$ and $^1(\pi, \pi^*)$ states are strongly solvent dependent. Indeed, in norharmane, a change of the fluorescent state from $^1(\pi, \pi^*)$ to $^1(n, \pi^*)$ has been reported on decreasing solvent polarity [25]. However, in all cases, in polar protic solvents the excited $^1(\pi, \pi^*)$ state always seems to be of lowest energy [25,40–42]. From the measured phosphorescence lifetimes, the lowest triplet states also appear to have (π, π^*) character. The difference in the acid–base character of the S_1 and T_1 states is, therefore, not due to the orbital origin of the states, but is associated with some other facet of the electron distribution. Studies on some other heterocyclic polyacenes have suggested that the singlet excited state has a more marked charge separation, while the triplet has predominantly biradical character [43]. It is possible that similar factors are involved with the β-carbolines.

The observed phosphorescence quantum yields for the neutral form show efficient intersystem crossing, and as discussed by El-Sayed [44,45], the $S_1 \rightsquigarrow T_1$ intersystem crossing is favoured by spin–orbit coupling. If the lowest excited singlet and triplet states of the β-carbolines in these solvents have predominantly (π, π^*) character, the most likely explanations for the efficient intersystem crossing are either that, due to the small energy separation, there is sufficient population of the $^1(n, \pi^*)$ state to allow this to become involved in these processes, or that there is extensive mixing of the $^1(\pi, \pi^*)$ and $^1(n, \pi^*)$ states. Lim [46] has discussed such mixing for close lying states in terms of vibronic coupling, and Olba et al. [25] have given good evidence that this type of interaction is involved in the "proximity effect" observed in the photophysical properties of norharmane.

3.2.4. *Time-dependent transient spectra in ethanol*

In further studies of the triplet properties of the neutral and cationic forms of these compounds, transient spectra have been obtained in alkaline ethanol, acidic ethanol, and neutral ethanol. Fig. 8 shows spectra obtained 0.2 ms after the excitation flash for all three sensitisers in alkaline ethanol. In all the cases, the spectra are similar to that of Hi in aqueous alkali, and show just one major band in the visible spectral region with λ_{max} at ca. 500 nm and weaker absorptions to either side. Spectra obtained in neutral ethanol are shown

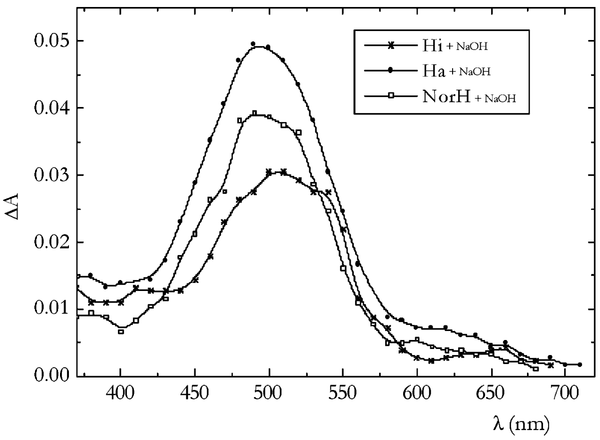


Fig. 8. Transient absorption spectra observed following flash photolysis of solutions in alkaline ethanol (pH 12.0) of harmine (5.4 × 10^{-5} M), harmane (5.6 × 10^{-5} M) and norharmane (8.0 × 10^{-5} M).

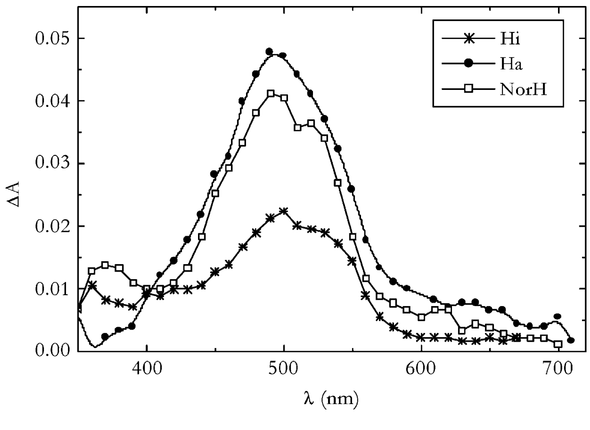


Fig. 9. Transient absorption spectra observed following flash photolysis of solutions in neutral ethanol of harmine (5.4 × 10^{-5} M), harmane (5.7 × 10^{-5} M) and norharmane (8.1 × 10^{-5} M).

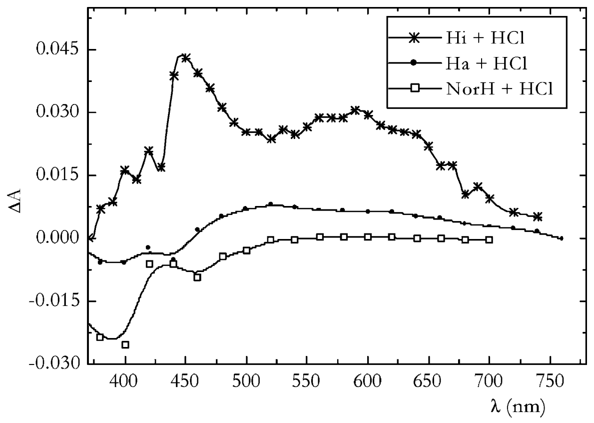


Fig. 10. Transient absorption spectra observed following flash photolysis of solutions in acidified ethanol of harmine (1.7×10^{-4} M), harmane (2.0×10^{-4} M) and norharmane (2.6×10^{-4} M).

in Fig. 9, while Fig. 10 shows spectra obtained 0.2 ms after the excitation flash for the compounds in acidified ethanol. For Hi, the spectrum in acidified ethanol is similar to that observed in water at pH 0. The spectra for Ha and NorH are very different but similar to each other, and consist of a bleaching below ca. 500 nm, where the ground state cation absorbs and fluoresces and a weak structureless absorption occurs at longer wavelengths. The reason for this difference is not clear.

4. Conclusions

Microsecond flash photolysis of each of the three compounds, in acidic, alkaline and neutral solutions, gives rise to transient species which can reasonably been assigned to triplet states which undergo acid/base equilibria in protic solvents. Triplet state pK_a values are ca. 2 pK_a units lower than the corresponding ground state but ca. 8 pK_a units lower than the first excited singlet states. Although both S_1 and T_1 have (π, π^*) character, we believe that the large basicity difference between these two excited states results from differences in charge distribution.

All three compounds phosphoresce in both alkaline and acidic glasses at 77 K. Phosphorescence quantum yields for the cationic forms of the dyes are ca. 0.2, while those of the neutral forms are markedly higher (0.4–0.6); differences between phosphorescence lifetimes of neutral and cationic forms are less marked. These results are consistent with $S_1 \rightsquigarrow T_1$ intersystem crossing in the neutral form being more efficient than in the cation, and with the proposed interaction between the $^1(\pi, \pi^*)$ and $^1(n, \pi^*)$ states in the neutral form and the removal of this interaction on protonation of the pyridine nitrogen atom. In many cases, the sum of the fluorescence and phosphorescence yields is less than 1 suggesting that internal conversion is also an important route for S_1 deactivation.

β-Carbolines are powerful natural photosensitisers, and although phototoxicity studies show an oxygen dependence, this does not correlate with the in vitro singlet oxygen or hydrogen peroxide photoproduction by the sensitisers in aqueous alcohol media [5]. In aqueous media, protonation of these molecules is shown to be important in both excited singlet [22] and triplet states (this work). Both triplet state formation and singlet oxygen photosensitisation [47] decrease on protonation of the β-carbolines. This work shows that in their neutral forms these compounds have significant triplet state yields and long-lived triplet states, which may play an important role in their photosensitisation reactions in vivo in the presence of oxygen.

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