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Ground and excited state interactions between tetrahydroharmane and pyridine

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Abstract

The interactions between 1-methyl-9H-1,2,3,4-tetrahydropyrido[3,4b]indole (tetrahydroharmane) and pyridine in 1% v/v MeOH–buffer medium were studied by UV–visible absorption and steady state and time-resolved fluorescence measurements. Ground state association and fluorescence quenching constants were calculated using the generalized Stern–Volmer plot. The results show that the dynamic contribution predominates over static binding. Indole, structurally related to tetrahydrobetacarboline, shows a similar behaviour, indicating that the piperidine ring of tetrahydrobetacarboline does not play an important role in the interaction. The methylation of the nitrogen atoms of both compounds demonstrates that ground state binding does not occur through hydrogen bonding, indicating a weak stacking interaction.

Keywords: Interactions; Tetrahydroharmane; Pyridine

1. Introduction

Betacarboline (9H-pyrido[3,4b]indole) belongs to an important class of markedly fluorescent molecules which are widely used in biological and biophysical studies. The betacarboline nucleus, in different oxidation states, has attracted interest for some time due to its cytotoxic and neuroregulatory agent properties [1–4]. Although the formation of molecular complexes with endogenous receptors has been suggested as the mechanism of action of these drugs, little is known about the nature of the complexes.

Tetrahydrobetacarbolines, the less aromatic members of the betacarboline series, are structurally related to indole, and therefore it is expected that they will interact with different receptors in a similar manner to indole. These compounds, due to their π-donor character and interesting fluorescent properties, have been extensively used as model compounds for numerous studies on the binding to biologically important acceptors [5–8].

In a general programme aimed at understanding the interaction between betacarbolines and biologically important molecules, we have selected pyridine as the basic heteroaromatic nitrogen ring with the ability to interact with the betacarboline nucleus. To our knowledge, no information is available on the complexes of indoles with neutral pyridine, except in non-polar solvents where, as in the case of carbazole derivatives, the interaction occurs via hydrogen bonding [9,10]. In addition, for analytical purposes, the quenching of the fluorescence of skatole by pyridine in water has been reported [11].

Thus due to the lack of information on the nature of the complexes between betacarbolines and pyridine in aqueous solutions, we have decided to carry out a systematic study on the formation of these complexes. To begin, we have selected the non- aromatic derivative of betacarbolines, tetrahydroharmane. In addition, for comparison, the interaction of the model compound, indole, with pyridine has been analysed.

2. Experimental details

Pyridine (PY), indole (IND) and *N*-methylindole (NMIND) were commercial products of the best available quality (better than 98%, Aldrich) and were used as received. Tetrahydroharmane (THN) was synthesized by a method given in the literature based on the condensation of piruvic acid and tryptamine [12]. N_2,N_9-dimethyltetrahydroharmane (DMTHN) was prepared by the methylation of its N_9-methyl derivative [13] with dimethylsulphate.

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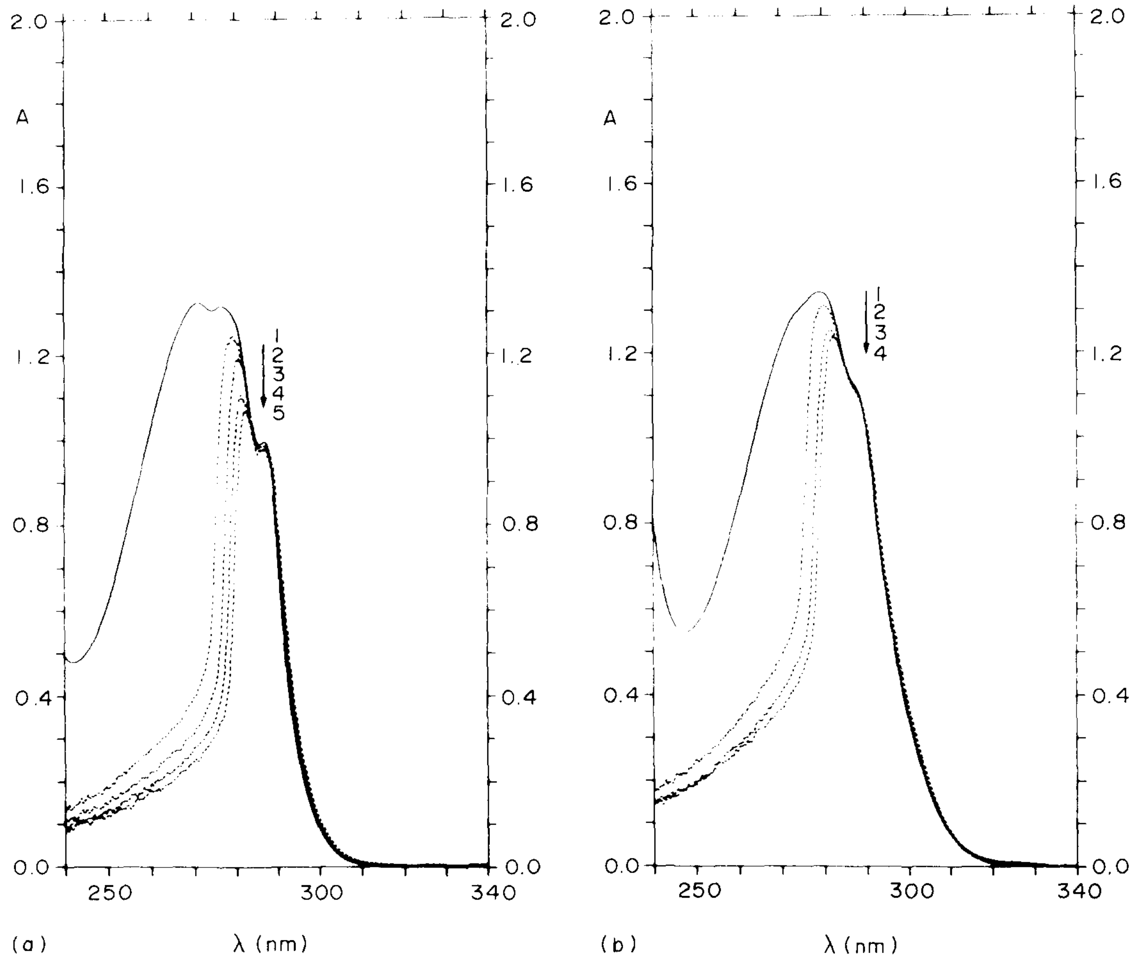
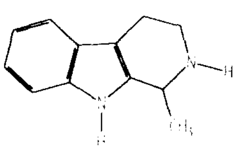
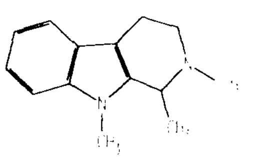


Fig. 1. Effect of the addition of PY on the absorption spectrum of THN in aqueous buffer solutions. [THN] = 2×10^{-4} M. (a) pH 8; [PY]: (1) 0 M; (2) 0.2 M; (3) 0.4 M; (4) 0.6 M; (5) 0.8 M; (b) pH 10; [PY]: (1) 0 M; (2) 0.2 M; (3) 0.4 M; (4) 0.6 M.



THN



DMTHN

Stock solutions of indoles or alkaloids were prepared daily in 10% v/v MeOH–H_2O and kept in the dark. PY was dissolved in NH_3–NH_4Cl (0.2 M) buffer of pH 8 or 10 (the ground state pK_a value of THN, calculated by a distribution method, was 8.8 ± 0.1) and was always added in at least 100-fold excess over the fluorophore.

Absorption spectra were obtained using a Perkin Elmer Lambda-5 spectrophotometer. For steady state fluorescence studies, a Perkin Elmer 650-40 spectrofluorometer was used, equipped with a data processor 650-0178 to obtain corrected spectra and interfaced to a PC for handling of the spectra. The fluorescence decays were measured using the time-correlated, single-photon counting method (Edinburgh FL 900 CD fluorometer). This instrument operates with a flash lamp excitation source filled with 0.4 bar H_2 (pulse width, 0.8 ns) and grating monochromators with a spectral range of 200–850 nm and a reproducibility of ±0.05 nm. The temperature was maintained to within ±0.2 °C.

3. Results and discussion

With increasing PY concentration, the absorption spectrum of THN in buffered 1% v/v methanol–water solutions (pH 8 and 10) shows slight but systematic changes (Fig. 1). The hypochromic effects indicate the formation of weak ground state complexes. Unfortunately, the pronounced overlap of the PY and THN absorption spectra precludes the direct calculation of the association constants K_S.

Fluorescence spectra were obtained by exciting the system at 291 nm, where the absorbance of the mixture does not change appreciably with the addition of PY (see Fig. 1) and the absorbance of PY itself is negligible.

The fluorescence bands of the cationic and neutral forms of THN are observed at 354 and 370 nm respectively. At these wavelengths, the fluorescence titration curves (Φ/Φ_0 vs. pH) intersect at pH 8.8 and show that the cationic and neutral forms are the only emitting species at pH 8 and 10 respectively. Experiments at both pH values were carried out.

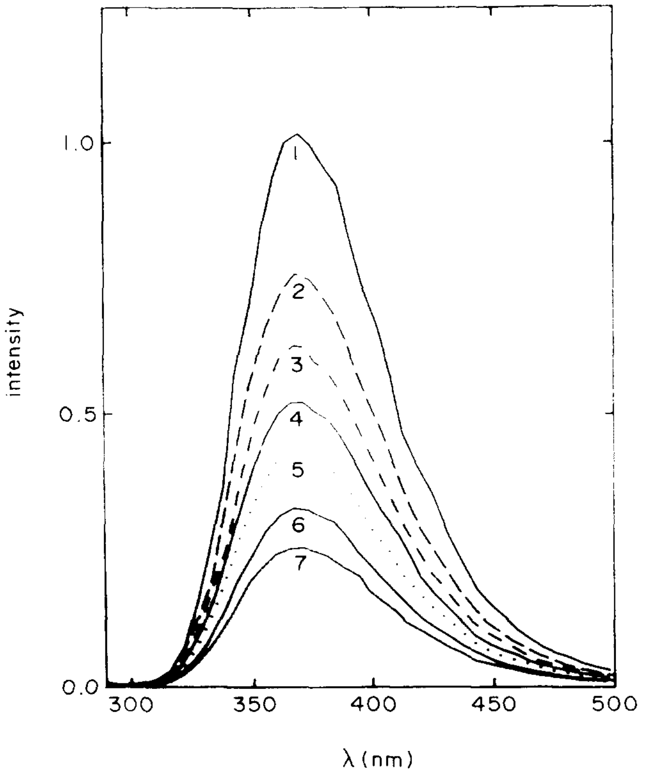


Fig. 2. Effect of the addition of PY on the corrected fluorescence spectrum of THN. [THN] = 2×10^{-4} M; λ_{exc} = 291nm; slit widths = 5/5 nm; pH 10; [PY]: (1) 0 M; (2) 1×10^{-2} M; (3) 2×10^{-2} M; (4) 3×10^{-2} M; (5) 4×10^{-2} M; (6) 6×10^{-2} M; (7) 8×10^{-2} M.

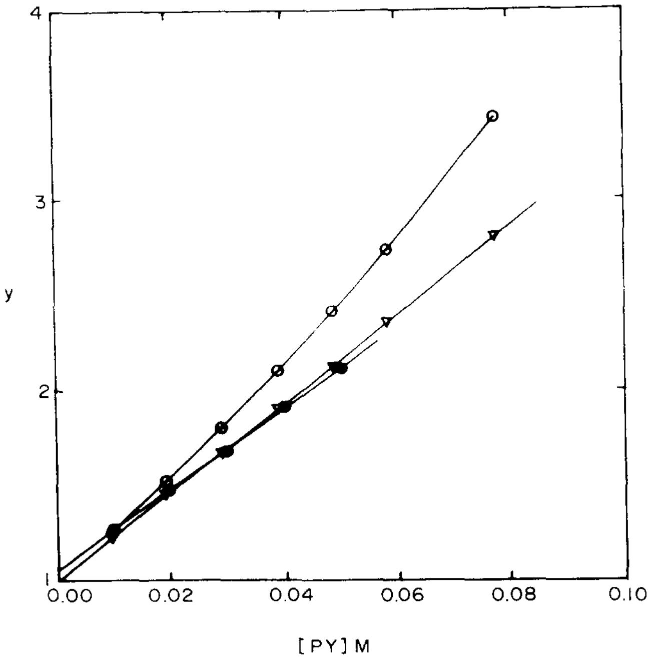


Fig. 3. Stern–Volmer plots for the quenching of THN by PY at pH 10. T = 25 °C; [THN] = 2×10^{-4} M; ○, I_0/I; ●, τ_0/τ; ▽, $(I_0/I)\ e^{-2.63[\mathrm{Py}]}$.

In both cases, a similar quenching effect was observed and, as shown in Fig. 2, the spectral shapes do not change on addition of PY.

Lifetimes were measured in aerated solutions, since quenching by oxygen appears to be unimportant [14]. On addition of PY, the fits of the decay time curves are clearly monoexponential, thus confirming the lack of any fluorescent species apart from THN. The calculated τ_0 values at 25 °C are 5.1 and 3.8 for cationic and neutral forms respectively.

Stern–Volmer plots of the steady state fluorescence intensities and lifetime ratios vs. the PY concentration are shown in Fig. 3. Whereas the plots of τ_0/τ vs. [PY] are linear, those of I_0/I deviate upwards. These results demonstrate the presence of static in addition to dynamic quenching. Indeed, an excellent fit of the generalized Stern–Volmer equation

$$\frac{(I_0/I)-1}{[\mathrm{PY}]} = (K_S + K_D) + (K_S K_D)[\mathrm{PY}] \qquad (1)$$

is found, where K_S and K_D correspond to the static and dynamic constants respectively. From the slopes of τ_0/τ vs. [PY], we can identify the dynamic quenching constant K_D ($K_D = k_q\tau_0$). The values of K_S, K_D and k_q are reported in Table 1 for both cationic and neutral species. As can be seen, protonation on the piperidine nitrogen atom has a small influence on the values of these parameters.

Moreover, the ratios of the fluorescence quantum yield, obtained from the integrated areas of the fluorescence spectra, also fit Eq. (1). As solutions of the quadratic equation, we obtain 28.0 and 4.2 M^{-1} and 23.4 and 4.6 M^{-1} for K_D and K_S at pH 8 and 10 respectively, which agree reasonably well with the results obtained from the fluorescence intensities.

The temperature dependence of the quenching constants was analysed using an Arrhenius plot, and the results are shown in Table 2. The variation of K_S with temperature is very small, with $\Delta H = -1.4 \pm 0.3$ kcal mol^{-1}. As can be appreciated from the data in Table 2, the lifetime of THN in the absence of PY is temperature dependent. From the cal-

Table 1
Static and dynamic quenching parameters at 25 °C

Compound	K_S (M^{-1})	K_D (M^{-1})	τ_0 (ns)	k_q ($M^{-1}s^{-1}$)$\times10^{-9}$	K_{SV} (M^{-1}) [a]
THN (pH 8)	4.5 ± 0.7	25.8 ± 0.9	5.1	5.1	26.9 ± 0.5
THN (pH 10)	3.2 ± 0.2	22.4 ± 0.3	3.8	5.9	21.3 ± 0.7
DMTHN	3.0 ± 0.4	46.0 ± 0.8	9.4	4.9	50 ± 4
IND	4.4 ± 0.3	24.8 ± 0.4	3.8	6.5	26 ± 1
NMIND			7.9	6.2	49.1 ± 0.7

[a] Obtained from the slope of τ_0/τ vs. [PY].

Table 2
Dependence on temperature of the static and dynamic quenching constants of the THN–PY system at pH 8

T (°C)	K_S (M^{-1})	K_D (M^{-1})	τ_0 (ns)	k_q ($M^{-1}s^{-1}$)$\times10^{-9}$	T/η
15	4.9 ± 0.9	23 ± 1	6.12	3.77	253
25	4.5 ± 0.7	25.8 ± 0.9	5.09	5.07	335
34	4.2 ± 0.3	28.3 ± 0.7	4.34	6.52	418
39	4.1 ± 0.5	31.6 ± 0.7	3.92	8.06	469

culated bimolecular quenching constant k_q at each temperature, we obtain a ΔH value of 5.5 ± 0.8 kcal mol^{-1} at pH 8.

As observed in Tables 1 and 2, the k_q values and their temperature dependence are of the order of magnitude expected for a diffusion-controlled reaction. Thus we determined the theoretical bimolecular rate constant k_0 using the Smoluchowski and Stokes–Einstein equations

$$k_0 = \frac{4\pi N}{1000}(R_{\mathrm{THN}} + R_{\mathrm{PY}})(D_{\mathrm{THN}} + D_{\mathrm{PY}}) \qquad (2)$$

$$D = \frac{kT}{6\pi\eta R} \qquad (3)$$

where R and D are the molecular radii and diffusion coefficients respectively, η is the solvent viscosity and N is Avogadro's number.

With the reported molar volumes for harmane [15] and PY [16], values of 3.9 and 3.1 Å have been estimated for R_{THN} and R_{PY} respectively. With these data, the calculated constant k_0 is 7.5×10^9 M^{-1} s^{-1}, i.e. only somewhat higher than the experimental values. On the other hand, the experimental quenching constants are also linear with T/η, which provides evidence for the diffusive nature of the quenching.

At this point, it should be noted that, when the quencher concentrations are large, positive deviations of the Stern–Volmer equation can be observed due to the so-called "sphere of action" effect [17]. Indeed, it is known that, if the quencher molecules are adjacent to the fluorophore at the moment of excitation, within which the probability of quenching is unity, the origin of the apparent small static component K_S is not due to complex formation. This model can be described by a modified Stern–Volmer equation

$$\frac{I_0}{I} = (1 + K_D[\mathrm{PY}]) \exp\left(\frac{[\mathrm{PY}]VN}{1000}\right) \qquad (4)$$

where V is the volume of the active sphere.

The THN–PY quenching data fit the above equation excellently, with $K_D = 25.2 \pm 0.5$ and 23.1 ± 0.1 M^{-1} and $(VN/1000) = 3.1 \pm 0.1$ and 2.63 ± 0.05 M^{-1} for cations and neutral species respectively at 25 °C. As can be seen in Fig. 3, the dynamic portions of the quenching, obtained by plotting $I_0/I\ e^{-3.1}$ or $I_0/I\ e^{-2.63[\mathrm{Py}]}$ vs. [PY], are almost coincident with those obtained from lifetime measurements. From the exponential term of Eq. (4), we obtain an experimental radius of around 10 Å for the active sphere. Thus, although these results do not enable the existence of the sphere of action phenomenon to be eliminated, this cannot be the only effect because modifications in the absorption spectra would not be expected. Moreover, we have obtained similar, but more pronounced, spectral variations in cyclohexane (Fig. 4) compared with those observed in aqueous solutions; a specific interaction in the ground state would be clearer in this medium.

With respect to the nature of this specific interaction, it is possible that THN forms hydrogen bonds with PY. Indeed, carbazole, which is structurally related to THN, is known to form hydrogen-bonded complexes with PY in non-polar aprotic solvents; the absorption spectra show clear red shifts and isosbestic points [18]. However, as noted before, the spectrum for the THN–PY system in cyclohexane is similar to that obtained in aqueous medium. In contrast with the behaviour of carbazole, a hydrogen-bonded complex band is not observed.

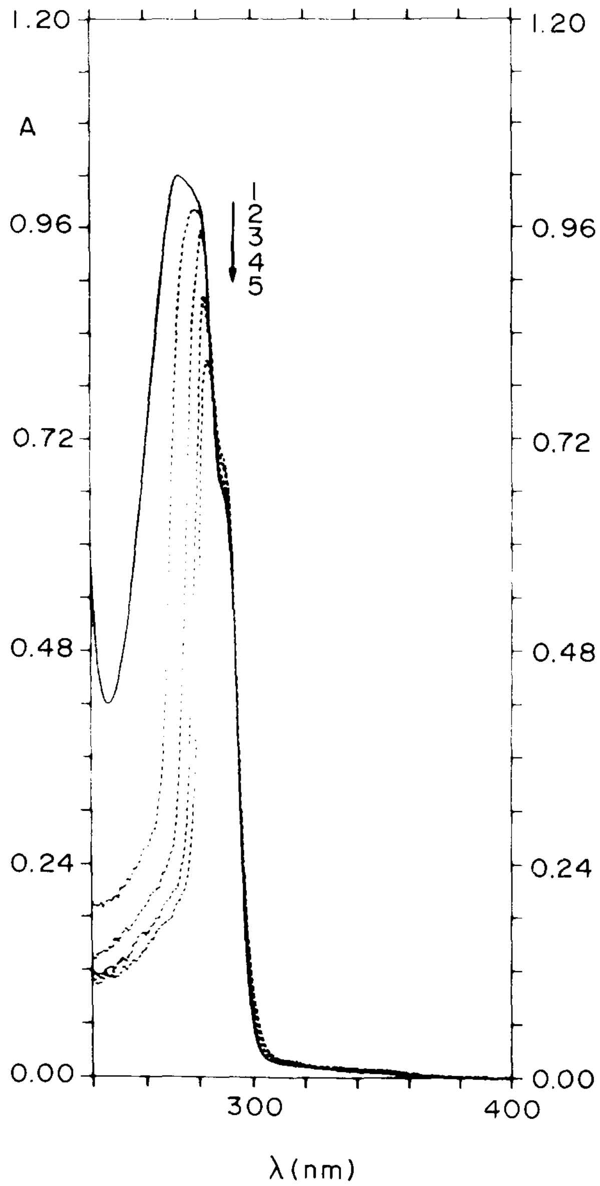


Fig. 4. Effect of the addition of PY on the absorption spectrum of THN in cyclohexane solutions; [THN] $= 2 \times 10^{-4}$ M; [PY]: (1) 0 M; (2) 4×10^{-3} M; (3) 8×10^{-3} M; (4) 1.2×10^{-2} M; (5) 1.6×10^{-2} M.

The absence of this type of interaction in the THN–PY system can be confirmed using DMTHN. In spite of the blocking of the N atoms, this compound also fulfils the generalized Stern–Volmer equation (Table 1). In this case, K_D is greater than the value for the non-methylated compound, but this is due to the higher τ_0 value of the methylated derivative (9.4 ns in pH 8 buffer). The calculated constant k_q is the same as that of THN within experimental error.

Finally, the interaction of IND and NMIND with PY has also been studied (see Table 1). It can be seen that the behaviour of the IND–PY system is similar to that observed for THN. In the case of NMIND, although the data do not fit the generalized Stern–Volmer equation, and therefore the static contribution to the quenching cannot be determined, the I_0/I vs. [PY] plot deviates upwards, as in the case of IND. Moreover, the k_q value obtained from the τ_0/τ data is similar to that calculated for IND.

From the above results, we can conclude that a common mechanism operates in both IND and THN systems, and the piperidine ring has a very small influence in these processes.

Although our results do not provide direct evidence for the formation of molecular complexes in the ground state, indole derivatives are known to form π–π stacked charge transfer complexes with pyridinium ions [19,20], which quench the indole fluorescence. In the present study, similar but weaker interactions could be responsible for the static quenching observed.

Ishida et al. [20], using an indole–pyridinium model compound, observed almost complete quenching of the indole fluorescence, and ascribed this effect to the prominent existence of an intramolecular stacked form in the excited state. In our case, the unmodified shape of the fluorescence bands does not enable the existence of non-fluorescent stacked complexes in the excited state to be eliminated; nevertheless, the temperature dependence indicates that collisional quenching seems to be the main mechanism operating in such interactions.

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