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Excited-state prototropic reactions of tetrahydroharmane

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Steady-state and time-resolved fluorescence measurements of THHN (9*H*-1-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole), have been carried out as a function of the acidity or basicity of the medium. In these media, four different THHN species have been detected, namely: dication (DC), cation (C), neutral (N) and anion (A). Attempts have been made to determine the excited state pK_a^* values for the three prototropic processes: N–A, C–N and DC–C reactions. The dicationic and anionic forms, observed outside the 0–14 pH range, are extremely weakly fluorescent species and only the second-order rate constants for the protonation and deprotonation processes, respectively, could be determined. In the case of the C–N process, studied in near-neutral media, excited-state proton exchange is much slower than fluorescence emission and the inflection points in the fluorimetric titration curves correspond quite closely to the ground state pK_a^G.

The physicochemical properties of a large family of compounds with the betacarboline nucleus (9*H*-pyrido[3,4-*b*]-indole) are of great interest because this ring constitutes, in some of its different aromaticity degrees, the basic structural unit of several alkaloids of biological and pharmacological importance.[1–6] Although the precise biochemical basis responsible for these properties still remains speculative, it seems that betacarbolines can interact with molecules such as enzymes, proteins and DNA.

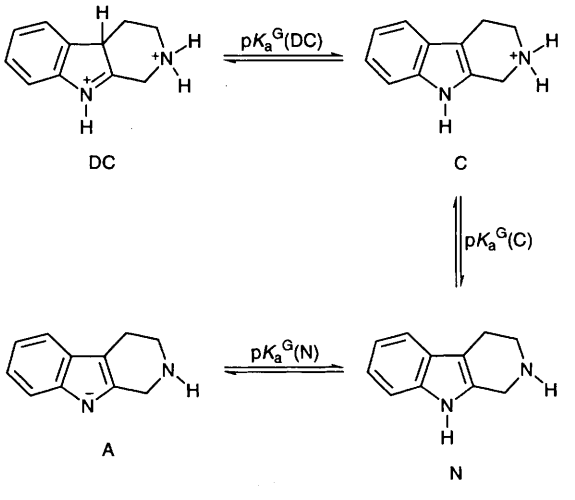
Because of the presence of several polyfunctional groups in these tricyclic rings, one important physicochemical property of these molecules is the influence of the acidity or basicity of the media on the ground and singlet excited states. In fact, it is known that the electronic absorption and emission fluorescence spectra, as well as other properties of betacarbolines, can be influenced greatly by changes in the acidity of the medium.[7] Moreover, the protonation state of these compounds can affect strongly the strength and nature of the aforementioned interactions between betacarbolines and biochemical molecules.[8]

Therefore, a complete study of the prototropic properties of these compounds in both the ground and singlet excited states will be of great theoretical and practical utility in order to understand the chemical and biochemical behaviour of these molecules. In this sense, and in relation to the acid–base properties of the ground state, we have already reported a systematic theoretical and experimental study on the influence of aromaticity and substituents in the betacarboline ring.[9–12]

However, as yet there is little information on the excited-state acid–base equilibria and no consensus on the nature and lifetimes of the different prototropic species involved in such equilibria.[13,14] Thus, we have decided to carry out a systematic study on the photophysical behaviour of these derivatives when the acidity of the media is changed. For this purpose, we have used steady-state and time-resolved techniques. To start with, we have elected to study THHN, the 1-methyl derivative of the less aromatic parent compound of the betacarboline series, 9*H*-1,2,3,4-tetrahydropyrido[3,4-*b*]indole (THBC). For THBC derivatives, we have previously reported steady-state fluorescence spectra in the H_I/pH/H_- range of −11 to +18. Four molecular species, dication (DC), cation (C), neutral (N) and anion (A), (see Scheme 1), were characterized and the corresponding pK_a^* values were estimated by the Förster–Weller cycle.[12,15]

Time-resolved fluorescence data for some derivatives of THBC have also been published. In this respect, Medina *et al.*[16] measured the lifetimes and quantum yields of THBC in different pure solvents. The remaining published measurements were carried out with THBC and its 3-carboxy derivative, and they were restricted to aqueous solutions in near-neutral media. In these media, depending on the derivative under study, THBC or its 3-carboxy derivative, cationic (or neutral) and zwitterionic (or anionic) forms exist, respectively. Thus, Tilstra and co-workers[17,18] used the 3-carboxy derivative as a rotationally constrained model to test the conformer hypothesis for the fluorescence decay of tryptophan, reporting fluorescence decay results in the pH range 5.5–10.5. This compound, as well as THBC itself, has also been studied by McMahon *et al.*[19] and more recently by Eftink *et al.*[20] In the last paper, the authors studied the pH dependences of the steady-state fluorescence of both compounds and also the lifetimes of the zwitterionic (or anionic) and cationic (or neutral) species. None of these papers reported a detailed analysis of the steady-state and time-resolved fluorescence properties of THBC in this pH range.

Finally, it has also been reported that in strong alkaline and acid media, the quantum yields of THBC derivatives show marked decreases characteristic of indole derivatives.[21,22] These quenching mechanisms have been postulated to involve diffusional quenching by H_3O^+ and OH^-, or, at least partially, to be due to protonation of the pyrrolic moiety of the THBC ring or deprotonation of the indole NH group. Again, no systematic studies were carried out at these extremes of pH.



This paper presents detailed steady-state and time-resolved fluorescence studies of one derivative of THBC, *i.e.* THHN, under the different acidity or basicity conditions where the four prototropic species, DC, C, N and A, can exist.

Experimental

Chemicals

Tetrahydroharmane (THHN) was prepared by decarboxylation of its CO_2H derivative which was obtained from the Pictet–Spengler reaction of tryptamine and pyruvic acid,[23] [UV–VIS spectrum of THHN in cyclohexane has an absorption maximum at 272 nm with log (ε/dm^3 mol^{-1} cm^{-1}) = 3.707]. Stock solutions were prepared daily in water and stored in the dark.

Perchloric acid solutions were prepared by dilution with distilled water of perchloric acid (Fluka Chemika; 70% by mass), and base solutions from potassium hydroxide (Merck Reagent Analysis). Appropriate mixtures of boric acid (0.1 mol dm^{-3}) and sodium hydroxide (0.1 mol dm^{-3}) were used as buffered solutions in the pH range 8–10. The values of the pHs of the solutions were measured in a Radiometer Copenhagen pHM82 standard pH-meter.

Absorbance measurements

Absorbances were measured on a Perkin-Elmer Lambda-5 spectrophotometer equipped with thermostatted cell holders. Sample absorbance values were adjusted to <0.1 at the corresponding excitation wavelengths for both steady-state and time-resolved fluorescence measurements. The ground state pK_a^G for the cation–neutral equilibrium was determined spectrophotometrically. Owing to the strong overlap of the absorption spectra of both species (Fig. 1), the first derivative spectra were used to obtain significant absorbance changes at 290 nm. A clear isoclinic point can be observed in the spectra. A mean value of 8.68 $\pm$ 0.07 was obtained for the pK_a^G of this equilibrium. This value is in good agreement with that obtained in our laboratory from octanol–water partition coefficients (8.73 $\pm$ 0.03).

Steady-state fluorescence measurements

Fluorescence measurements were made in a Perkin-Elmer 650-40 spectrophotofluorimeter equipped with a Perkin-Elmer 650-0178 data processor and interfaced to a Multitech Acer 500 computer for the handling of the spectra. All the spectra were obtained in 1 cm quartz cells at 25.0 $\pm$ 0.1 °C. Absolute fluorescence quantum yields, ϕ, were measured at an excitation wavelength of 280 nm by comparison of the corrected emission spectra with the spectrum of tryptophan. Sample quantum yields were calculated by using a value of 0.14 for tryptophan[24] in phosphate-buffered solutions of pH 7, and by means of eqn. (1), where the subscripts r and x refer to the reference and unknown solutions, respectively, ϕ is the quantum yield, $A(\lambda)$ is the absorbance per cm of the solution at the excitation wavelength and D is the integrated area under the corrected spectra.

$$\phi_x = \frac{D_x}{D_r}\frac{A_r(\lambda_r)}{A_x(\lambda_x)}\phi_r \qquad (1)$$

Time-resolved fluorescence measurements

Fluorescence decays were collected by time-correlated single photon counting on a FL-900CD Edinburgh Analytical instrument. The FL-900CD spectrometer was controlled by an application program operating from within the GEM/3 environment. The excitation source was a nanosecond (nF900) flash lamp filled with 0.4 bar H_2 operated at 40 kHz with *ca.* 6 kV applied across a 1 mm electrode gap. Fluorescence decays from the samples were acquired to (1–1.5) $\times$ 10^4 counts in the peak. Fluorescence decay data were fitted by reference deconvolution to a sum of exponentials with amplitudes, α_i, and lifetimes, τ_i. Goodness of the fit was judged by the magnitude of the reduced χ_r^2 and the shape of the autocorrelation function of the weighted residuals.[25]

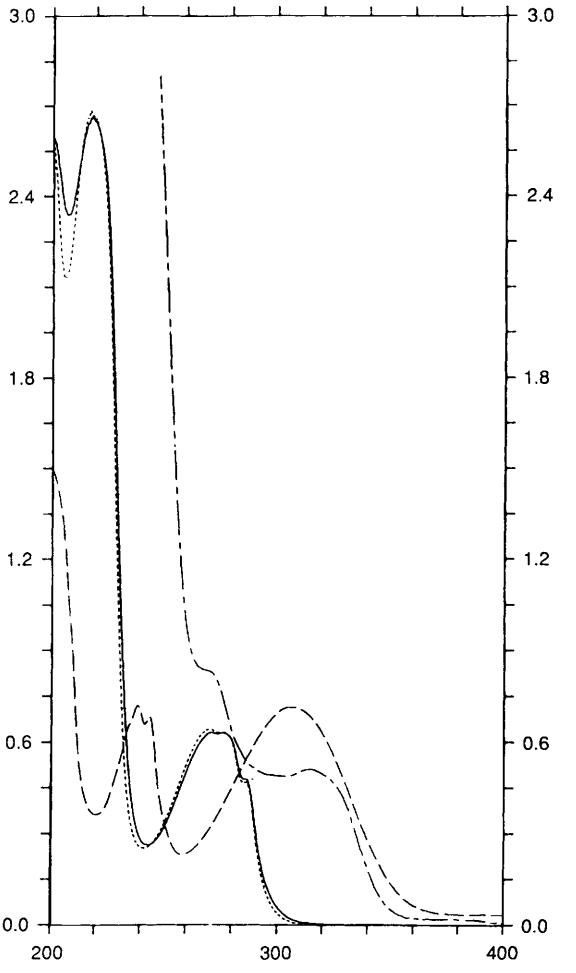


Fig. 1 Absorption spectra of the species involved in the prototropic equilibria of THHN: DC (– – –), C (· · · · ·), N (——) and A (— · — · —)

Results and Discussion

The absorption and fluorescence spectra of the different prototropic forms of THHN (DC, C, N and A), have been reported elsewhere.[15] The dication and anion are very weakly fluorescent species whose emissions are obtained outside the 0–14 pH range, while the cationic and neutral species have been observed within this pH range. Balón *et al.*[15] reported the ground-state pK_a^G values for the DC–C and N–A equilibria together with the singlet excited-state pK_a^* values calculated by the Förster–Weller cycle. It is known that in applying this cycle, the wavelength for the 0–0 transition should be used.[26] However, because the 280 nm broad band is the result of overlapping π–π^* transitions, the calculated ΔpK_a values should be considered only as estimated values. Data from ref. 15, as well as those obtained in the present work, are recorded in Table 1.

On the basis of the data in Table 1, we have carried out steady-state and time-resolved fluorescence studies on the different acidity regions [DC–C (perchloric acid solutions), C–N

Table 1 Spectral characteristics, fluorescence lifetimes and ionization constants of the different molecular species of THHN

	λ_m^{abs}/nm [log(ε/dm^3 mol^{-1} cm^{-1})[a]]	λ_m^{fl}/nm (λ_{exc})[b]	τ/ns	pK_a^G	pK_a^*
DC	237 [3.62] 243 [3.59] 303 [3.80]	443 (303)		−7.80 ± 0.46	1.5
C	218 [4.41] 270 [3.81] 277 [3.80] 286 [3.65]	353 (286)	6.4	8.68 ± 0.07	6.2
N	219 [4.39] 274_s[c] [3.77] 279 [3.84] 286 [3.66]	369 (286)	4.9	16.14 ± 0.17	9.4
A	318 [3.74]	400 (318)			

[a] Estimated error in log(ε/dm^3 mol^{-1} cm^{-1}) is ±0.008. [b] λ_{exc} is the excitation wavelength. [c] Subscript s refers to a shoulder.

(buffered solutions, pH 7–10) and N–A (potassium hydroxide solutions)] where the reactions should occur in the first excited singlet state. Here we describe the results obtained for the different prototropic processes.

Neutral–anion reaction

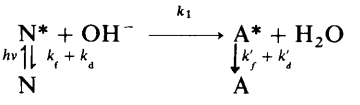
The variation in steady-state fluorescence of neutral THHN with the increase of potassium hydroxide concentration from 10^{-3} to 0.1 mol dm^{-3} is shown in Fig. 2(*a*). Throughout this concentration range, the absorption and excitation spectra corresponding to the neutral form remain unchanged. This behaviour is identical to that observed for similar compounds. Thus, the fluorescence of simple indoles[21] and some indole derivatives such as tryptophan,[20] is quenched with increasing pH in the alkaline region (pH 11–14), while no modification of the absorption spectra is observed in the same pH region. Also, the excited state pK_a^* for the deprotonation of the pyrrolic NH group of THHN is probably *ca.* 11 (see Table 1), which is similar to the values of 11.6–12.6 reported for indoles and their derivatives.[21,27]

On the other hand, in spite of the weakly fluorescent properties of the anion, the spectra, which are normalized to a constant intensity of the bands [Fig. 2(*b*)], emphasize the fact that the fluorescence band maxima at the highest base concentrations is shifted by *ca.* 40 nm. From the integrated area of these spectra, we have calculated the relative quantum yields (ϕ_0/ϕ) at the different potassium hydroxide concentrations. A Stern–Volmer plot of ϕ_0/ϕ *vs.* [OH$^-$] gives a linear relationship, with a gradient of 42 ± 2 dm^3 mol^{-1} and an intercept at the origin 1.15 ± 0.08 (r = 0.998).

We have also measured the lifetimes of the lowest singlet excited states of THHN in the KOH concentration range 10^{-3}–0.1 mol dm^{-3}. Measurements at higher base concentrations were not performed because the intensities were very weak and the fluorescence lifetimes were found to be in the limit of resolution of our photon counting equipment. Decay curves, acquired at λ_{exc} = 286 nm and at different emission wavelengths between 369 and 400 nm, always gave good single exponential fits, with the calculated lifetime (4.9 ns at [KOH] ⩽ 10^{-3} mol dm^{-3}) decreasing with increasing KOH concentrations. Attempts to fit the data to biexponential functions were unsuccessful because one amplitude was near zero and neither the χ_r^2 value nor the shape of the autocorrelation function improved. The plot of τ_0/τ *vs.* KOH concentration is linear with a gradient of 45 ± 2 dm^3 mol^{-1} and an intercept at the origin of 0.97 ± 0.09 (r = 0.999). These parameters are the same (within experimental error) as those previously calculated from the ϕ_0/ϕ *vs.* [OH$^-$] plot.

As mentioned in the Introduction, the observed alkaline quenching may be attributed to a diffusional quenching by OH$^-$ ions or may be, at least partially, due to deprotonation of the indole NH group. Also (as suggested by a referee), the addition of KOH could give rise to a fluorescent quenching due to aggregation produced by the increased ionic strength. Although we have not measured the static fluorescence in the presence of an inert salt, the lifetime of neutral THHN (4.9 ns at pH = 10.1) adjusted with a diluted KOH solution (10^{-3} mol dm^{-3}) is the same as that obtained in a buffered solution of Na_2HPO_4–NaOH at pH = 10.6 and constant ionic strength (0.1). Note that *N*-methylindole derivatives, which lack the NH group, do not show quenching at high pH values.[20,21] This fact, together with the mentioned red-shift of fluorescence band maxima at the highest KOH concentration, lead us to suggest the deprotonation process as the mechanism responsible for the observed quenching.

Therefore, all the experimental results can be accounted for by the reactions in Scheme 2



Scheme 2

where k_f or k_f', k_d or k_d' and k_1 are the rate constants for fluorescence, non-radiative deactivation and the deprotonation process, respectively. In Scheme 2, we have neglected back transfer from A* owing to the very weak fluorescent nature of the anion, *i.e.* the short lifetime of the anion and the consequent absence of back transfer.

According to Scheme 2, under photostationary conditions and when N* is excited exclusively, we obtain the following relationship:

$$\frac{\phi_0}{\phi} \text{ or } \frac{\tau_0}{\tau} = 1 + k_1\tau_0[OH^-] \quad (2)$$

With the values of τ_0 for the neutral form and the slopes of the ϕ_0/ϕ and τ_0/τ plots, an average value of 8.9 × 10^9 dm^3 mol^{-1} s^{-1} can be calculated for the second-order rate constant of the deprotonation process. This value is similar to those reported by Chattopadhyay *et al.*[28] for the deprotonation reaction of indole, diphenylamine and carbazole. As suggested by these authors, diffusion probably also controls the deprotonation process of the pyrrolic NH group of THHN.

Cation–neutral reaction

The changes in the steady-state fluorescence of cationic THHN with increasing pH, from 7 to 10, are shown in Fig. 3. The emission maximum occurs at 353 nm in the cation, shifting to 369 nm in the neutral form. As mentioned before, in the same pH range, the absorption spectrum is also modified slightly (pK_a^G = 8.68 ± 0.07).

Because the fluorescence spectra of the acid–base pair

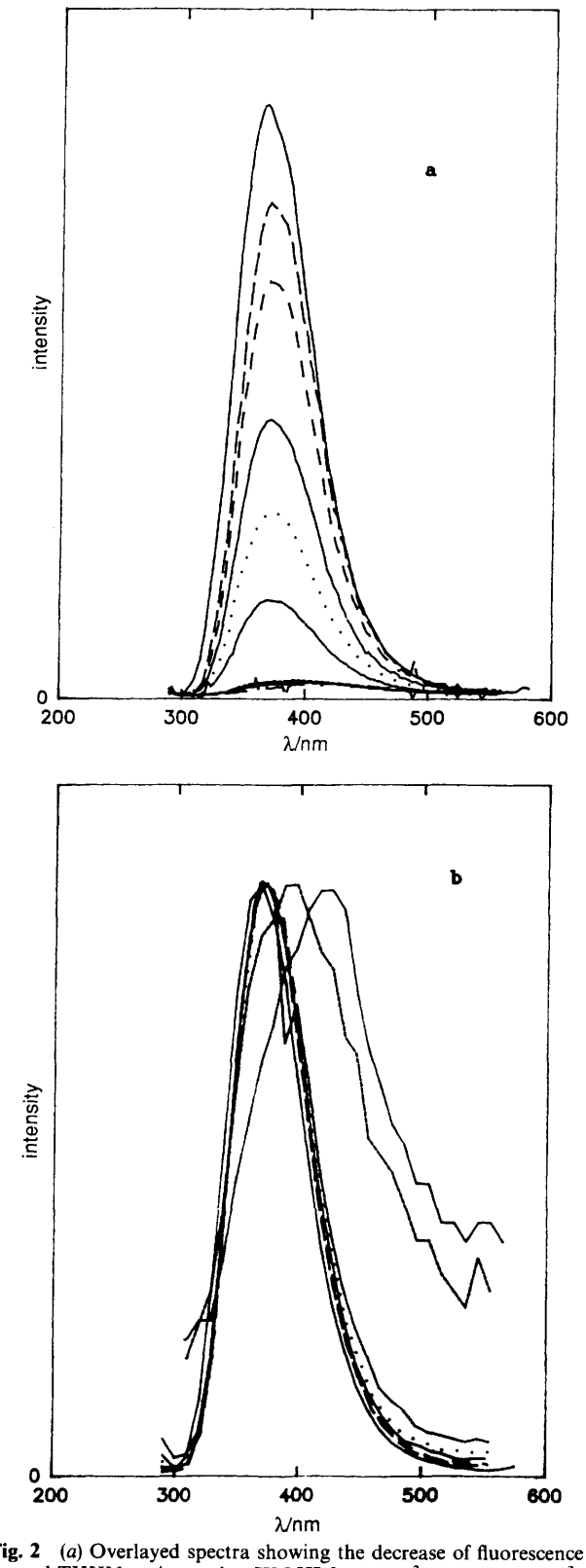


Fig. 2 (*a*) Overlayed spectra showing the decrease of fluorescence of neutral THHN on increasing [KOH] from 10^{-3} to 1.6 mol dm^{-3}. (*b*) Superimposed spectra under the same conditions.

overlap strongly, the measured fluorescence intensities of the cationic and neutral forms must each be corrected for the intensity component due to the other form. The true fluorescence intensities (I_c and I'_c) are related to the measured intensities (I at 353 nm and I' at 369 nm) according to:[26]

$$I = I_c + k'I'_c$$
$$I' = I'_c + kI_c \qquad (3)$$

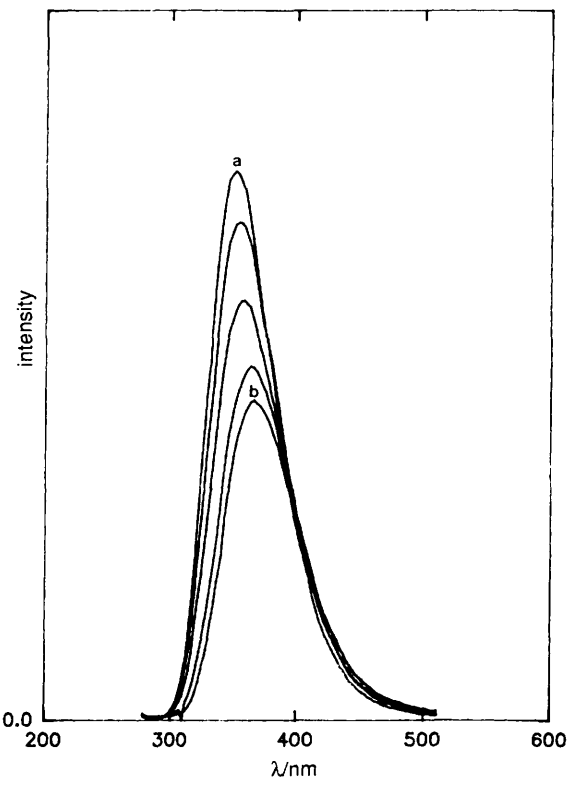


Fig. 3 Changes in fluorescence spectra of cationic THHN on increasing pH from 7 (a) to 10 (b)

where k and k', the overlap ratios of the cation and neutral forms, respectively, can be obtained by making measurements on solutions containing only one species in the excited state. By rearranging these equations, we can obtain the true fluorescence intensities in terms of I and I', k and k':

$$I_c = \frac{I - k'I'}{1 - kk'}$$
$$I'_c = \frac{I' - kI}{1 - kk'} \qquad (4)$$

The plot of the normalized intensities *vs.* the pH of the medium is shown in Fig. 4. The two curves intercept at pH = 8.8, which is very near to the pK_a^G value. The excited state pK_a^* of the amino group in THHN, estimated from the Förster–Weller cycle, is 6.2. As in the case of tryptophan and other derivatives, this estimated pK_a^* is between *ca.* 1.5 and 2.5 pH units smaller than the ground state pK_a^G.

The fluorescence lifetime data for THHN at 25 °C and several pH values have also been measured. At pH 5–7, fluorescence decay curves collected at an emission wavelength of 353 nm gave good fits to a single exponential with an average lifetime of 6.4 ns. The autocorrelation functions always fluctuated randomly about zero and neither the χ_r^2 value nor the shape of the autocorrelation function improved for double exponential fits. Also, the calculated lifetime was independent of the emission wavelength. This value is similar to that reported by Eftink *et al.*[20] for the cation of THBC (6.3 ns), but is larger than the 5.6 ns reported by McMahon *et al.*[19] At pH 10, the decay was also monoexponential and independent of the emission wavelength. The lifetime of the neutral form at this pH (4.9 ns) is also similar to that reported by McMahon *et al.*[19] (4.5 ns).

This behaviour is identical to that observed by Tilstra *et al.* for the 3-carboxy derivative of THBC.[17] For this compound, whose pK_a^G at 25 °C is 8.8, the zwitterion at pH 7 and the anion at pH 10.5 are the observed species with lifetimes of 6.2 and 4.9 ns, respectively. At this point, the following outstand-

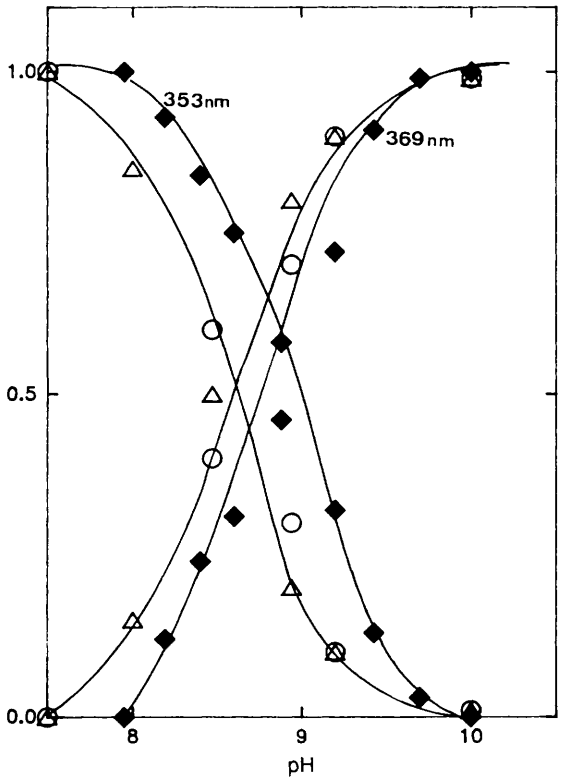


Fig. 4 pH dependence of: (◆) normalized intensities (at 353 and 369 nm), (○) measured relative amplitudes and (△) relative amplitudes calculated using eqn. (5)

ing fact should be cleared up. Colluci *et al.*,[18] and more recently Eftink *et al.*,[20] have reported double exponential decays for the zwitterion (6.3 and 3.6/2.1 ns) and for the anion (5 and *ca.* 2.8 ns) of the 3-carboxy THBC, respectively. This behaviour was explained by these authors as reflecting two stable conformations in this compound. According to Eftink *et al.*, the indole ring, particularly the pyrrole portion that is nearest to the piperidinyl ring, develops an increased positive charge in the excited state. Thus, the excited indole ring has a large dipole with its positive pole pointing through the C(2) atom of the pyrrole ring. The two half-chair conformers of the non-aromatic ring will both have the NH_2^+ group at the same distance from the indole C(2) position. However, the CO_2^- group is nearer to the C(2) position in its axial (T) conformer, and is further from the C(2) position in its equatorial (T′) conformer. Since upon excitation, the newly created large dipole will interact with both the NH_2^+ and CO_2^- groups, the authors explain the biexponential decay to arise from the excited state T′ → T relaxation process occurring on the fluorescence timescale. Assuming this model for the compound under study, which lacks the CO_2^- group, both the cationic and neutral forms should, as observed, decay monoexponentially.

However, as can be seen in Fig. 5, when the fluorescence decays of THHN are measured at pHs near the pK_a^G value, they are clearly biexponentials with lifetimes around the values obtained previously for the cationic and neutral forms (6 and 5, respectively). This behaviour, as already observed for the 3-carboxy derivative of THBC,[17] indicates that the excited-state proton transfer rates are probably slow relative to fluorescence emission, so the biexponential decays only reflect ground state heterogeneity. In such a case, the lifetimes should be independent of pH, but the amplitudes, α_i, should depend on the relative concentrations of cationic and neutral forms.

Decay curves at pH 7–10 were collected at different emission wavelengths. Owing to the difficulty of resolving two closely spaced decays, the data were fitted by curve analysis with one lifetime fixed at 6.4 ns. The average value obtained for the second lifetime was 4.9 ns, in excellent agreement with the lifetime of neutral THHN. The relative amplitudes obtained at the different pHs are plotted in Fig. 4. As can be seen, the two curves intercept at pH = 8.5, which is close to the pK_a^G. However, to check these results, we have also calculated the amplitude ratio at the different pHs by an independent procedure. As is known, the amplitudes will also be proportional to the molar absorption coefficient, ε, the fluorescence intensities, I, and the radiative rates, k_r, of the cationic and neutral forms according to the following equation:

$$\frac{\alpha_C}{\alpha_N} = \frac{\varepsilon_C I_C k_{r,C}[C]}{\varepsilon_N I_N k_{r,N}[N]} \quad (5)$$

In computing this amplitude ratio, the relative absorption coefficient was determined from the ratio of the absorbance of the solutions at pH 7 and 10 containing equal concentrations of THHN. The relative intensity at 353 nm was calculated from the corrected emission spectra of the same solutions. The ratio of the radiative rates was estimated from the quantum yields and the lifetimes. The absolute quantum yields were calculated (as mentioned in the Experimental) using the quantum

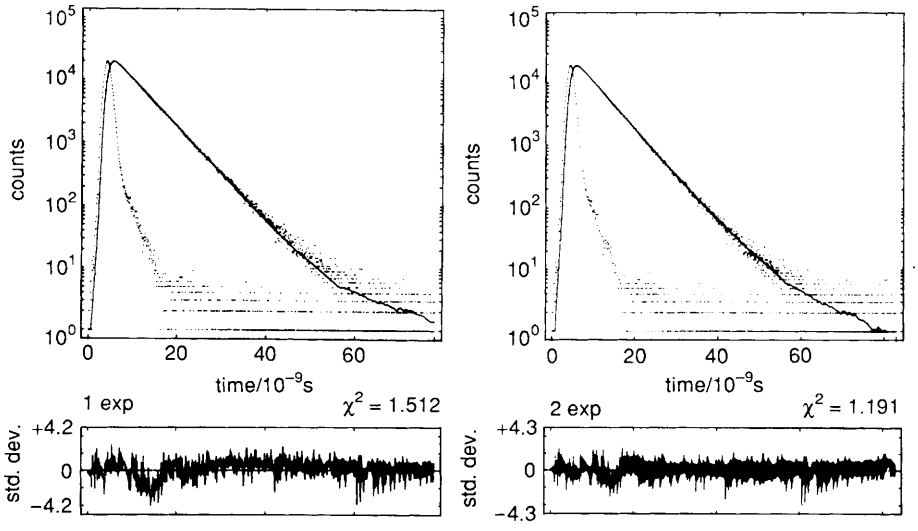


Fig. 5 Fluorescence decay of THHN at pH = 8.7, 25 °C and weighted residuals and autocorrelation functions for single (left) and double (right) exponential analysis of the decays. Ex = 286 nm, Expol = No, Em = 353 nm, Empol = No, temperature = 25.4 °C. $T_1 = 5.66 \times 10^{-9}$ s (left). $T_1 = 6.43 \times 10^{-9}$ s and $T_2 = 4.97 \times 10^{-9}$ s (right).

yield of tryptophan as the reference. Finally, the value of [C]/[N] at a given pH was estimated from the Henderson–Hasselbalch equation:

$$\mathrm{pH} - \mathrm{p}K = \log \frac{[\mathrm{C}]}{[\mathrm{N}]} \qquad (6)$$

The calculated relative amplitudes at different pHs are also plotted in Fig. 4. As can be seen, the measured amplitudes are in good agreement with the theoretical values. Thus, only the ground-state pK_a^G is obtained from the fluorimetric titrations for the C–N equilibrium. It is, therefore, concluded that the prototropic equilibrium is not established within the lifetimes of the lowest excited singlet states of these molecules and, thus, the fluorescence intensities reflect the relative ground state concentrations of the cationic and neutral forms.

Finally, the apparent ΔpK_a ($pK_a^G - pK_a^*$) value of THHN, *ca.* 2.5 pH units, can be explained as a consequence of the mentioned development of a large dipole in the excited indole ring. This dipole produces a strong destabilization of the positively charged NH_2^+ and, therefore, an increase of the acidity constant of this group in the excited state.

Dication–cation reaction

The fluorescence intensity of the cationic form of THHN is quenched appreciably with the increasing $HClO_4$ concentration in the range 5×10^{-4}–0.1 mol dm^{-3}. We could not work at higher acid concentrations owing to the extremely weak fluorescence of the samples. Throughout this acidity range, the absorption and excitation spectra remain unchanged. Moreover, the fluorescence spectrum is not affected when the inorganic counter-ion concentration is changed by the addition of up to 2 mol dm^{-3} $NaClO_4$. This behaviour, observed previously for other indole derivatives,[20,21,29] has been considered generally to be due to an acid-catalysed ring protonation or a collisional quenching by hydrogen ions in the excited state.

Unfortunately, there is no evidence for the formation of the dication in the range of acid concentrations used in the present work. We have reported the fluorescence band maxima of this species (*ca.* 440 nm) at a sulfuric acid concentration of 18 mol dm^{-3}. Thus, if the dicationic species were formed, a red shift of the emission spectra should be expected. However, the spectra normalized to constant intensity of the bands do not show such a shift. It is probable that the very weak fluorescent nature of the dication, even lower than that of the anion, precludes this observation.

From the integrated area of the fluorescence spectra we have calculated the relative quantum yields, ϕ_0/ϕ, at the different $HClO_4$ concentrations. A plot of ϕ_0/ϕ *vs.* $[H^+]$ is linear with a gradient of 37.9 ± 0.9 dm^3 mol^{-1} and an intercept at the origin of 0.99 ± 0.07 ($r = 0.999$).

We have also calculated the lifetimes of the lowest singlet excited state of THHN in the same range of acid concentration. Decay curves, acquired at $\lambda_{exc} = 273$ nm and at different emission wavelengths between 353 and 390 nm, always gave good single exponential fits. The lifetime of the cation (6.4 ns) decreases with the increasing H^+ concentration up to the limit of resolution of our photon-counting equipment. Again, no influence of ClO_4^- anion concentration is observed. The plot of τ_0/τ *vs.* $[HClO_4]$ is linear with a gradient of 38 ± 2 dm^3 mol^{-1} and an intercept 1.04 ± 0.1 ($r = 0.999$).

Therefore, both steady-state and direct-lifetime measurements show that quenching of the cationic form of THHN by protons is a dynamic process. From the values of τ_0 for the cation and the ϕ_0/ϕ and τ_0/τ *vs.* $[H^+]$ plots, an average value of 5.9×10^9 dm^3 mol^{-1} s^{-1} can be calculated for that quenching rate constant. This value is similar to that reported by Lasser and Feitelson[29] for the quenching of tryptamine by protons (5.6×10^9 dm^3 mol^{-1} s^{-1}), but it is smaller than the corresponding value for other indole derivatives such as, *e.g.* 3-β-hydroxyethylindole, 14×10^9 dm^3 mol^{-1} s^{-1}. According to the authors, this discrepancy can be attributed to the fact that in the first case, it is the protonated substrate which reacts with the protons, while the neutral derivative is the reactant in the second case.

THHN is also already protonated at the exocyclic nitrogen atom in the acidity range used in this work. Therefore, we would expect a behaviour identical to that observed for tryptamine, *i.e.* protonation at the C(3) of the indole ring is probably the main mechanism for the quenching process.

We gratefully acknowledge the financial assistance of the Dirección General de Investigación Científica y Técnica (PB92-0668) and Junta de Andalucía.

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Paper 5/07864D; *Received 4th December*, 1995