Acid–base and Spectral Properties of β-Carbolines. Part 1. Tetrahydro-β-carbolines

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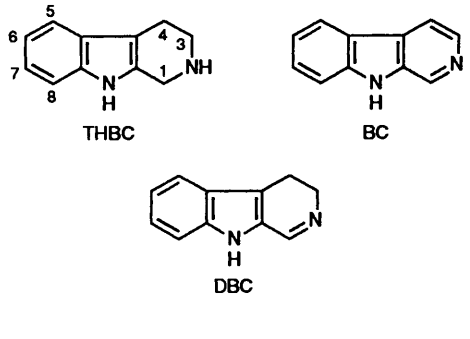
The absorption and fluorescence spectra of a set of tetrahydro-β-carboline(9*H*-1,2,3,4-tetrahydropyrido[3,4-*b*]indole) derivatives in the H_i/pH/H_- range of −11 to +18 have shown the presence of four different molecular species, namely: dication, cation, neutral and anion. Ionization data for the prototropic equilibria involving these species have been obtained spectrophotometrically at 25 °C and comparatively analysed by the Hammett acidity function and the excess acidity methods. The changes of acidity or basicity experienced by those species upon excitation to their lowest singlet excited states have been estimated from the Förster–Weller cycle. The influence of structural variations on the spectral and acid–base properties of these compounds is discussed.

The β-carboline ring (9*H*-pyrido[3,4-*b*]indole) constitutes, in some of its different aromaticity degrees, the basic structural unit of several alkaloids of biological and pharmacological interest.[1,2] Most of the naturally occurring compounds containing the β-carboline ring are either fully aromatic (BC) or they contain the 1,2,3,4-tetrahydro structure (THBC). Conversely, only a limited number of 3,4-dehydro-β-carbolines (DBC) have hitherto been found in nature.[2] Due to the wide occurrence of these compounds in plants and to the many biochemical properties exhibited by them, β-carbolines have been the subject of intensive chemical[3] and biochemical[4–8] research.



Much of the interesting chemistry of β-carbolines arises from their acid–base properties. Owing to the presence of several polyfunctional groups in this tricyclic ring, β-carbolines can experience different ionization equilibria inside and outside the pH-range. Thus, the non-pyrrolic nitrogen atom in the β-carboline skeleton, protonates in the pH-range, the basicity of this atom being dependent on the hydrogenation state of the pyridyl moiety of the β-carboline ring. On the other hand, the pyrrolic ring in these compounds gives them weakly acid and basic properties,[9] which can only be manifested in highly concentrated acid and basic media outside the pH-range.

Although some of these acid–base equilibria do not take place in biological systems, the understanding of the acid–base properties of β-carbolines is of fundamental interest to obtain information on many aspects of the chemistry of these compounds. Thus, the electronic absorption and fluoroescence spectra and other physical properties of β-carbolines are known to be greatly influenced by changes in the acidity or basicity of the media.[10] Also acid–base equilibria have a profound influence on the reactivity of these compounds.[3] Apart from the practical importance, these properties are also of theoretical interest since knowledge of them should afford information for the determination of the electronic effects brought about by structural variations in these molecules. Therefore, in the context of our current research programme on β-carboline chemistry, we have devoted preferential attention to investigate theoretically and experimentally the acid–base properties of these compounds.[11–19]

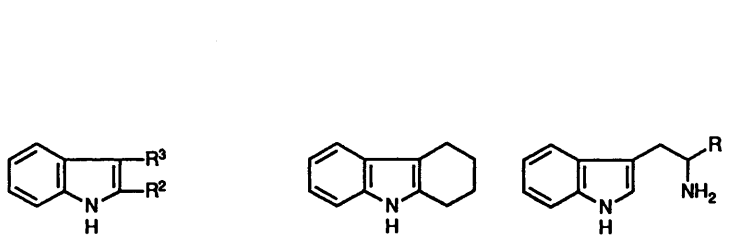
In previous papers we have dealt with some partial aspects of these equilibria and only moderate attention was paid to the influence of substituents. In this, and the following paper of this series, we aim at presenting a more systematic and extensive study of the influence of aromaticity and substitution on the acid–base and spectral properties of β-carbolines. In order to get a better comprehension, THBCs will be considered separately in this first paper. The other more aromatic derivatives, DBCs and BCs, will be the subject of the following paper.

In spite of the widespread occurrence of THBC derivatives in nature, studies of their acid–base properties and spectral characteristics are comparatively scarce.[20,21] THBCs are usually considered as typical indole derivatives. However, the presence of the exocyclic piperidinic ring, confers on these compounds distinctive physical properties and reactivity patterns,[3] which have received only limited attention. For the present study, we have selected the series of THBC derivatives and model compounds shown in Fig. 1. We have also included several naturally occurring compounds (Rauwolfia alkaloids) containing the THBC tricyclic system.

Experimental

Chemicals and Solutions.—Compounds **7**, **9**, **10** and **11** were prepared by decarboxylation of their 1-CO_2H derivatives, which were obtained from the Pictet–Spengler reaction of the corresponding tryptamines and pyruvic acid.[22] Compound **12** was prepared by nitration of **7** with nitric acid in concentrated sulfuric acid solution. All other THBCs and model compounds were commercial products of the best available quality (>98%, Aldrich, Sigma, Lancaster) and were used without further purification. Rauwolfia alkaloids **16** and **17** were the generous gift of Boehringer and Sohn.

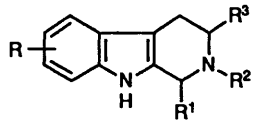
Stock solutions were prepared in the dark to avoid photodecomposition. Final solutions obtained by suitable dilution of the stocks with sulfuric acid or potassium hydroxide solutions were in the 5×10^{-4}–5×10^{-3} mol dm^{-3} range of concentration and they did not contain more than 5% v/v of methanol. Although spectral modifications with time were observed for some THBCs (particularly in highly concentrated sulfuric acid



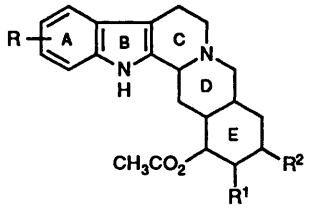
1 Indole ($R^2 = R^3 = H$)
2 2,3-Dimethylindole ($R^2 = R^3 = CH_3$)

3 Tetrahydrocarbazole

4 Tryptamine (R = H)
5 Tryptophan (R = CO_2H)

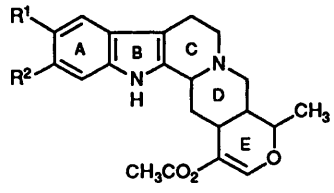


	R^1	R^2	R^3	R	Trivial name
6	H	H	H	H	Noreleagnine Tryptoline Tetrahydronorharmane
7	Me	H	H	H	Tetrahydroharmane
8	Me	Pr	H	H	
9	Me	H	H	6-MeO	
10	Me	H	H	7-MeO	Tetrahydroharmine
11	Me	H	H	6-Cl	
12	Me	H	H	6-NO_2	
13	Pr	H	CO_2H	H	



	R^1	R^2	R	C-1	C/D	D/E	Trivial name
14	OH	H	H	β	*trans*	*trans*	Yohimbine
15	OMe	TMB*	7-OMe	β	*cis*	*cis*	Reserpine

*TMB = trimethoxybenzoate



	R^1	R^2	C-1	C/D	D/E	Trivial name
16	H	H	α	*trans*	*cis*	Ajmalicine
17	OMe	OMe	β	*cis*	*cis*	Reserpiline

Fig. 1 Structural formulae of THBC derivatives studied

media) most of them were sufficiently stable for spectrophotometric measurements, their spectra being reversible during the initial times (15–20 min). In some instances, warming the solutions in the most concentrated basic media was necessary to bring about complete solution.

Sulfuric acid solutions were prepared by dilution with distilled water of sulfuric acid Reagent Analysis (Merck 96% w/w) and hydroxide solutions from Merck Reagent Analysis potassium hydroxide as described elsewhere.[23] Sulfuric acid and potassium hydroxide solutions were standardized against appropriate basic and acid solutions, respectively.

Procedure and Apparatus.—Absorption spectra were determined in a Perkin-Elmer Lambda-5 spectrophotometer equipped with thermostatted cell-holders. Fluorescence measurements were made in a Perkin-Elmer spectrophotofluorimeter equipped with a Perkin-Elmer Data Processor 650-0178. The sensitivity and stability of the apparatus were checked by using the Raman band of distilled water; likewise, the wavelengths of excitation and emission were checked by using the lines at 450.1 and 467.1 nm of the xenon lamp. All the absorption and fluorescence spectra were obtained in 1 cm quartz-cells at 25.0 ± 0.1 °C with solutions of similar concentrations and similar instrument settings.

The fluorescence quantum yields at 25 °C were determined by comparison of the corrected emission spectra with the spectrum of quinine bisulfate as standard using optically diluted solutions by means of eqn. (1) where the subscripts r and x refer to the

$$Q_x = Q_r \frac{A_r(\lambda_r)}{A_x(\lambda_x)} \frac{I(\lambda_r)}{I(\lambda_x)} \cdot \frac{D_x}{D_r} \cdot \frac{n_x^2}{n_r^2} \qquad (1)$$

reference and the unknown solution respectively, Q is the quantum yield, $A(\lambda)$ is the absorbance per centimetre of the solution at the excited wavelength, $I(\lambda)$ is the relative intensity of the exciting light at the wavelength λ, D is the integrated area under the corrected emission spectrum and n is the average refractive index of the solution at the maximum luminescence. Spectral data are summarized in Table 1.

Determination of Ionization Constants.—Ionization data I (I = [Acid]/[Base]), were obtained spectrophotometrically by the usual procedure, *i.e.*, from absorbance measurements at selected wavelengths of the free acid, the conjugate base and some of their mixtures. To obtain these data, we have carefully selected at least five points corresponding to log I values within ±0.75, because clear deviations from parallel behaviour were usually observed outside this range. For the sake of brevity, the ionization data are reported in Tables 2 and 3 only in mathematical form.

We have not attempted to measure the pK_a^G values for the piperidinic nitrogen atom protonation of THBC derivatives. The small spectral changes produced by these equilibria and the very low solubility of these compounds in water make them unsuitable for spectrophotometric or potentiometric determinations. Since up to now, there is not a unique and reliable method to obtain thermodynamic ionization constants[24] outside the pH-range, the ionization data were comparatively analysed by two different approaches: the Hammett acidity function (HAF) and the excess acidity (EA) methods.[25–28]

The Yates–McClelland eqn. (2) was employed in the HAF

$$\log I = -mH + pK_a^G(\text{HAF}) \qquad (2)$$

method, where H represents the H_1 or the H_- acidity function previously established for protonation[29] and deprotonation[23] equilibria of indole derivatives, respectively. It is known from

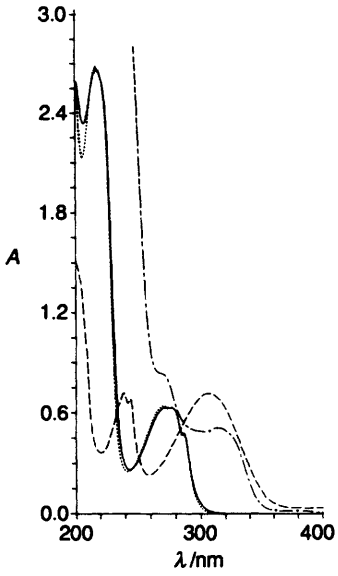


Fig. 2 Absorption spectra of the species involved in the prototropic equilibria of **6**. DC (– – – –), C (· · · ·), N (——) and A (— – —). Spectra obtained in 18 mol dm^{-3} H_2SO_4, 0.1 mol dm^{-3} HCl, 0.1 mol dm^{-3} KOH and 14 mol dm^{-3} KOH, respectively.

previous studies that β-carboline derivatives usually show good adherence to these acidity functions.[12–14]

The EA method is based on free energy relationships and it does not make use of any acidity function. In this method the pK_a^G values and the m^* solvation parameters for protonation and deprotonation equilibria can be obtained from eqns. (3) and (4), respectively, where water activites, A_w, and X functions

$$\log I - \log C_{H^+} = m_p^* X_p + pK_{ap}^G(EA) \quad (3)$$

$$pK_w + \log C_{OH^-} - \log A_w + \log I = m_d^* X_d + pK_{ad}^G(EA) \quad (4)$$

were calculated as elsewhere or taken from the literature.[15,28]

The differences between ground-state pK_a^G and singlet excited state pK_a^S, ΔpK_a, were estimated from the Förster–Weller cycle[30] by using eqn. (5) where ν_A and ν_b are the frequencies

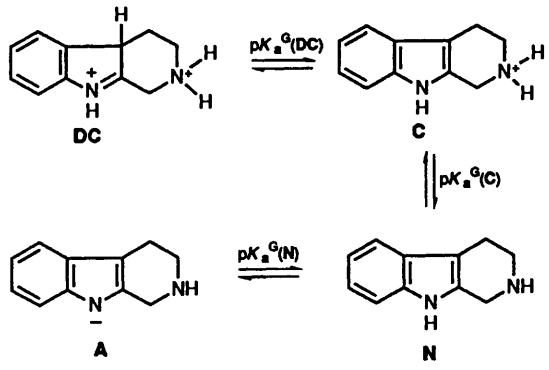
$$\Delta pK_a = pK_a^G - pK_a^S = \frac{N.h.c.}{2.303\ RT}(\nu_A - \nu_B) \quad (5)$$

involved in the transitions between the ground and the excited states of the conjugate acid–base species and the other symbols have the usual meanings.

Results and Discussion

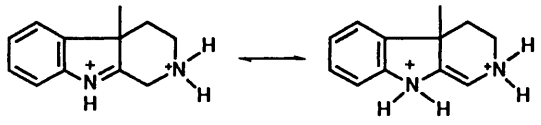
Absorption and Fluorescence Spectra.—THBC derivatives can experience the prototropic equilibria shown in Scheme 1. Protonation and deprotonation of the indole moiety of these compounds, pK_a^G(DC) and pK_a^G(N), take place outside the pH-range. In contrast, the piperidinic nitrogen atom protonates in the 6–9 pH-region.

The absorption spectra of the species involved in these equilibria for the parent compound **6** are shown in Fig. 2. The spectrum of the neutral species **6N** closely resembles that of **1N**.[31] Therefore, the bands in the **6N** spectrum can be attributed to the transitions from the ground state to the ^1L_b state (286 nm), the ^1L_a state (271 nm) and to the ^1B state (220 nm). On protonation at the piperidinic nitrogen atom, the spectrum of **6C** does not appreciably change up to $H_1 \sim -6$. In solutions of higher acidity dicationic species **6DC** are formed[32] and the spectra experience profound modifications.



Scheme 1 Prototropic equilibria of the THBC ring

Dications **6DC** possess the typical spectrum of 3*H*-indolium (or indoleninium) cations.[32,33] The peak at shorter wavelengths in the spectra of **6C** and **6N**, is replaced by two peaks of much lower intensity at 238 and 243 nm, respectively, whereas the band at longer wavelength is structureless and red shifted. This bathochromic shift of 20–30 nm is distinctive of tetrahydro-β-carbolinium cations, because this band in other simple indoles is practically coincident in position with that of neutral or cationic species. The contribution of enaminic structures (Scheme 2) may possibly be responsible for this difference. Tautomeric enamines have been repeatedly postulated to explain the more characteristic reactivity patterns of THBC.[3] Furthermore, ^{13}C NMR spectra of some THBC derivatives give evidence for the formation of such enaminic cations in highly concentrated sulfuric acid solutions.[32]



Scheme 2 Iminium–enaminium tautomeric equilibria of tetrahydro-β-carbolinium dications

In highly concentrated basic media ($H_- > 15$) the spectrum of **6N** is again modified. Deprotonation of the pyrrolic NH group is accompanied by a slight decrease in intensity and by a shift to the red of the long wavelength band of **6N**. Other details of the spectrum of **6A** could not be recorded, because of the very strong absorption of the potassium hydroxide solutions in the ultraviolet region.

Table 1 lists the absorption maxima of a set of THBC derivatives. It should be realized that the spectra of substituted THBCs usually retain the characteristic absorption bands of the parent compound, although they are modified in position and intensity. However, the effect exerted by some substituents on the absorption spectra of the dications is noteworthy, the two short wavelength bands of **6DC** being replaced by only one moderately intense band centred around 240–260 nm.

The fluorescence spectra of **6** experience changes at approximately the same acidic and basic regions, as the absorption spectra do. From these spectra (Fig. 3) four different emission bands can be observed. Therefore, it can be assumed that **DC**, **C**, **N** and **A** species in Scheme 1 are the ground state precursors of these fluorescences. As can be seen from Fig. 3, **6DC** is a very weakly fluorescent structure and its broad and red-shifted emission band can only be detected in highly concentrated sulfuric acid solutions ($H_1 < -4$). At pH *ca.* 0 the emission from **6C** begins to appear and it reaches its maximum intensity between pH = 4 and 9. At higher pH the intensity of the **6C** band at 353 nm progressively diminishes and simultaneously

Table 1 Electronic absorption and fluorescence emission spectra of the prototropic forms of THBCs and model compounds at 25 °C[a]

	DC (Dication)		C (Cation)		N (Neutral)		A (Anion)	
Compound	λ_m^{abs} (log ε)	$\lambda_m^{fl}(\varphi)$	λ_m^{abs} (log ε)	$\lambda_m^{fl}(\varphi)$	λ_m^{abs}(log ε)	$\lambda_m^{fl}(\varphi)$	λ_m^{abs} (log ε)	$\lambda_m^{fl}(\varphi)$
1	233 (3.59)[b,c]	n.f.	—	—	216 (4.54)[c]	346 (0.25)	288 (4.31)	408 (0.03)
	238 (3.58)				266s (3.76)			
	ca. 280 (3.68)				270 (3.77)			
					278 (3.76)			
					287 (3.68)			
3	230 (3.56)[b]	n.f.	—	—	227 (4.72)[c]	378	290 (3.89)	400
	238 (3.55)				282 (3.97)			
	276 (3.70)				290 (3.92)			
4	234 (3.64)[d]	508	218 (4.53)	358	220 (4.56)	361	303 (3.67)	424
	239 (3.62)		272s (3.71)		272s (3.66)			
	288 (3.68)		277 (3.73)		278 (3.77)			
			286 (3.65)		286 (3.63)			
6	238 (3.70)	424	219 (4.40)	353 (0.26)	220 (4.39)	368 (0.11)	320 (3.68)	412 (<0.01)
	243 (3.67)		270 (3.80)		271 (3.76)			
	304 (3.84)		276 (3.79)		278 (3.81)			
			286 (3.65)		286 (3.64)			
7	237 (3.62)	443	218 (4.41)	353	219 (4.39)	369	318 (3.74)	400
	243 (3.59)		270 (3.81)		274s (3.77)			
	303 (3.80)		277 (3.80)		279 (3.84)			
			286 (3.65)		286 (3.66)			
8	232 (4.06)	419	217 (4.43)	349	218 (4.46)	367		
	304 (3.84)		270 (3.86)		272s (3.83)			
			278 (3.84)		278 (3.87)			
			286 (3.70)		287s (3.78)			
9	255 (3.75)	490	218 (4.36)	334	220 (4.37)	373	303 (3.82)	391
	339 (3.86)		272 (3.84)		281 (3.88)		325s (3.65)	
			310s (3.72)		290 (3.86)			
			320s (3.75)					
10	218 (4.29)	392	220 (4.49)	356	220 (4.50)	377	304 (3.79)	393
	250 (3.70)	521	265 (3.62)		270 (3.66)			
	298s (4.07)		288 (3.72)		296 (3.75)			
	304 (4.20)							
	315 (4.08)							
11	240 (3.74)	437	224 (4.42)	347	216 (4.45)	363	292 (3.68)	424
	248 (3.69)		280 (3.72)		280 (3.66)		330s (3.30)	
	306 (3.79)		287 (3.73)		288 (3.67)			
			297 (3.60)		297 (3.59)			
12	211 (4.16)	371	211 (4.38)	335	410 (3.22)	372	552 (3.71)	n.f.
	255 (3.92)		240 (3.87)					
	410 (3.30)		387 (3.56)					
13	232 (4.12)		219 (4.39)					
	250s (3.95)		270 (3.81)					
	233 (4.29)		278 (3.80)					
			286 (3.66)					
14	235 (4.0)[f]	357	219 (4.40)	351 (0.04)	220 (4.41)[e]	330 (0.29)	295 (3.88)	365
	308 (3.8)		272 (3.92)		273 (3.93)		312s (2.10)	
			279 (3.92)		279 (3.93)			
			288 (3.84)		289 (3.85)			
15	269 (4.2)[f]	520 (0.01)	216 (4.62)	366 (0.04)	218 (4.60)[e]	353 (0.08)	303 (3.86)	375
	305 (4.3)		267 (4.15)		269 (4.16)		316 (2.06)	
			295 (3.98)		297 (3.98)			
16	269 (4.1)[f]	355	222 (4.41)	350 (0.03)	223 (4.40)[e]	347 (0.06)	310 (3.82)	368
	310 (3.8)		282 (3.96)		283 (3.98)			
			290 (3.87)		291 (3.90)			
17	267 (4.2)[f]	530 (0.07)	229 (4.64)	331	230 (4.63)[e]		320 (2.12)	370
	315 (4.2)		299 (4.02)		286 (4.01)			
			302 (3.96)		299 (4.01)			
					301 (3.97)			

[a] Absorption (λ_m^{abs}) and fluorescence (λ_m^{fl}) maxima are in nm; molar absorptivity (ε) is in $dm^3\ mol^{-1}\ cm^{-1}$; excitation wavelengths are underlined; φ = quantum yield. [b] These spectra correspond to indoleninium monocationic species. [c] Data taken from ref. 34. [d] Data taken from ref. 14. [e] Data taken from ref. 35. [f] Data taken from ref. 36.

the fluorescence band of **6N** at 368 nm increases. The fluorescence emission of **6N** reaches its maximum value at pH 10–11 and diminishes to almost zero just below H_- 14–15. In more basic media ($H_- > 15$) the fluorescence emission of **6A** at 412 nm can be observed.

The maxima and quantum yields of the fluorescence emissions of a set of THBC derivatives are listed in Table 1. As data in this table show, only the influence of methoxy substituents on the spectra of the dicationic species is noteworthy. Thus, the spectra of **9DC**, **10DC**, **15DC** and **17DC** show very large Stokes shifts. Furthermore, the spectrum of **10DC** shows a double fluorescence emission with bands at 392 and 521 nm.

It is well known that 5-OR indole derivatives fluoresce at *ca.* 520 nm in moderately concentrated acid solutions.[37] The origin of this emission band is still not fully elucidated. Thus, while there is a general agreement that the emitting species is

Table 2 Ionization data analysis for the indole deprotonation equilibria of THBCs and model compounds at 25 °C

Compound	d log *I*/d[KOH]	HAF			EA	
		m	pK_a	$(H_-)_{\frac{1}{2}}$	*m**	pK_a
1	0.29 ± 0.02 (0.999)	1.02 ± 0.06 (0.999)	16.90 ± 1	16.57	1.07 ± 0.13 (0.996)	16.72 ± 0.16
2	0.32 ± 0.02 (0.996)	1.11 ± 0.09 (0.996)	17.05 ± 1	15.36	1.17 ± 0.17 (0.989)	15.59 ± 0.10
3	0.33 ± 0.03 (0.998)	1.13 ± 0.12 (0.998)	17.17 ± 1	15.19	1.11 ± 0.10 (0.998)	15.20 ± 0.04
4	0.29 ± 0.01 (0.999)	1.01 ± 0.03 (0.998)	16.68 ± 0.4	16.51	1.06 ± 0.06 (0.999)	16.68 ± 0.08
6	0.31 ± 0.04 (0.998)	1.06 ± 0.12 (0.998)	17.61 ± 2	16.61	1.16 ± 0.25 (0.995)	16.84 ± 0.33
7	0.30 ± 0.03 (0.998)	1.05 ± 0.10 (0.998)	16.75 ± 2	15.95	1.14 ± 0.20 (0.994)	16.14 ± 0.17
9	0.18 ± 0.04 (0.987)	0.62 ± 0.16 (0.987)	9.80 ± 3	15.80	0.32 ± 0.30 (0.861)	15.33 ± 0.24
10	—	—	—	—	—	15.6 ± 0.2[a]
11	0.29 ± 0.04 (0.996)	1.22 ± 0.10 (0.994)	17.70 ± 3	14.51	1.04 ± 0.28 (0.991)	14.42 ± 0.04
12	0.45 ± 0.09 (0.992)	1.58 ± 0.33 (0.992)	22.48 ± 5	14.22	1.07 ± 0.20 (0.993)	14.07 ± 0.03
14	0.30 ± 0.03 (0.996)	1.06 ± 0.11 (0.996)	17.01 ± 2	16.05	1.16 ± 0.20 (0.987)	16.31 ± 0.20
16	0.32 ± 0.03 (0.997)	1.06 ± 0.08 (0.998)	17.09 ± 1	16.16	1.17 ± 0.15 (0.996)	16.21 ± 0.14
17	0.34 ± 0.07 (0.987)	1.18 ± 0.25 (0.986)	18.19 ± 4	15.41	1.27 ± 0.30 (0.947)	15.62 ± 0.30

[a] Estimated value, see ref. 14.

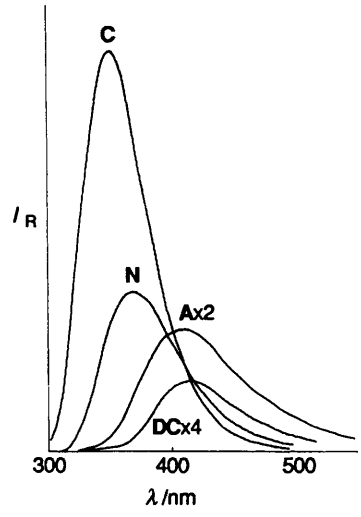


Fig. 3 Corrected fluorescence emission spectra of the species involved in the prototropic equilibria of **6**. DC (18 mol dm^{-3} H_2SO_4), C (0.1 mol dm^{-3} HCl), N (0.1 mol dm^{-3} KOH) and A (14 mol dm^{-3} KOH).

formed by a protonation process in the singlet excited state, the protonation site is still a matter of controversy. Protonations at the phenolic oxygen,[37] pyrrolic nitrogen[38] and 4- or 6-carbon[39,40] atoms of the indole ring have been proposed. Dual fluorescence emissions have also been observed in the spectra of some indoles with electron-donating groups substituents at the 5-position.[40] These emissions have been attributed to the existence of two different polarized excited states. Therefore, it is conceivable that the red-shifted emission bands of the methoxy-substituted dications of THBCs and the dual fluorescence emission of **10DC** could be related to similar phenomena.

Acid–Base Properties. Ionization Constants.—Although the piperidinic nitrogen atom protonation of THBCs has not been investigated, the magnitude of the pK_a^G(C) can be estimated from the sparse data existing in the literature.[21,41] Thus, a pK_a^G(C) of 8.6 can be obtained for the parent compound **6C** from the pK_as of 8.8, 9.4 and 9.6 reported for **13C**,[21] **4**[21] and **5**,[37] respectively. This pK_a value is about 1 and 2.5 units smaller than those of tetrahydroisoquinoline (9.41)[42] and piperidine (11.22)[42] respectively. Data from the literature also permit one to estimate a pK_a^G(C) value of *ca.* 7 for **7C**.[41] Unfortunately, there are no data for benzene-substituted THBC derivatives. However, since these substituents are far from the protonation site and resonance cannot contribute to the transmission of the electronic effects, no great influence of these substituents on pK_a^G(C) is expected.

The results for the ionization data analysis of the pyrrolic deprotonation equilibria of THBC derivatives are collected in Table 2. As can be seen, plots of log *I vs.* H_- are linear, and usually their slopes are very close to unity. Therefore, most of these THBCs behave as H_- indicators. On the other hand, as for simple indole derivatives, the agreement between EA and HAF methods is better when p$K_a^G(N)_{EA}$ data are compared with $(H_-)_{\frac{1}{2}}$ (H_- value at half ionization) values instead of with p$K_a^G(N)_{HAF}$ values.[43] It is due to the anchoring procedure used to construct the H_- acidity function. The differences between p$K_a^G(N)_{EA}$ and $(H_-)_{\frac{1}{2}}$ are not usually greater than ±0.2, a range within the error inherent to the determination of these parameters.

A perusal of the ionization constants in Table 2 shows that **6N** is a weaker acid than **2** and **3**, but similar to **4N**. Therefore, the piperidinic nitrogen atom of THBC has an acid weakening effect on the acidity of the pyrrolic NH group. Alkylation at the 1-position of the THBC ring increases the acidity of this group, but no appreciable effect is observed upon the subsequent substitution at the piperidinic nitrogen atom. On the other hand, the presence of a nitro group on the benzene ring of THBC has, as expected, a marked acid strengthening effect. Methoxy groups also produce a similar effect, but of rather smaller magnitude. This fact is noticeable, since the latter substituents exert a contrary effect on the acidity of the NH pyrrolic group of **1**.[43] Possibly, the decrease of the electronic density at the indolic β-carbon atom induced by the methoxy substituents is enhanced by the assistance of the piperidinic exocyclic ring in **6N**.

The analysis of the ionization data for the indolic ring protonation equilibria of THBC derivatives is reported in Table 3. As can be seen from these data, most of the THBC dications behave as H_1 indicators (slopes of log *I vs.* H_1 plots close to unity). The greatest deviations are observed, as in the case of indoles, for the benzene-substituted THBC derivatives.[43] The adherence of THBCs to the H_1 acidity function is noteworthy, since it indicates that H_1 is also suitable as a monocation–dication acidity function. In this sense, H_1 behaves as the H_- acidity function, which also satisfactorily describes a variety of monoanionic–dianionic ionization equilibria.[44] These facts reveal that electronic factors are more important than purely electrostatic factors to describe the ionization equilibria of very weak acids and bases. It must also be mentioned, that **13C** is, to our knowledge, the weakest base showing adherence to the H_1

Table 3 Ionization data analysis for the indole ring protonation equilibria of THBCs and model compounds at 25 °C

		HAF			EA	
Compound	d log I/d[H_2SO_4]	m	pK_a	$(H_I)_{\frac{1}{2}}$	m^*	pK_a
1	0.46 ± 0.07 (0.978)[a]	0.67 ± 0.10 (0.978)	−2.38 ± 0.30	−3.55	0.95 ± 0.17 (0.967)	−2.43 ± 0.3
2	0.70 ± 0.06 (0.996)	1.01 ± 0.09 (0.996)	−1.50 ± 0.13	−1.49	—	—[b]
3	0.70 ± 0.07 (0.996)	1.01 ± 0.14 (0.996)	−1.05 ± 0.11	−1.04	—	—[b]
4	0.77 ± 0.03 (0.998)[a]	1.11 ± 0.05 (0.998)	−7.06 ± 0.32	−6.36	1.47 ± 0.07 (0.998)	−6.30 ± 0.28
6	0.58 ± 0.03 (0.999)	0.84 ± 0.05 (0.999)	−6.02 ± 0.32	−7.17	1.08 ± 0.07 (0.989)	−5.59 ± 0.2
7	0.84 ± 0.05 (0.999)	1.22 ± 0.07 (0.999)	−8.82 ± 0.52	−7.23	1.60 ± 0.11 (0.998)	−7.80 ± 0.46
8	0.69 ± 0.04 (0.999)	1.00 ± 0.05 (0.999)	−7.74 ± 0.41	−7.74	1.28 ± 0.08 (0.998)	−6.93 ± 0.35
9	0.94 ± 0.03 (0.999)	1.37 ± 0.05 (0.999)	−9.35 ± 0.31	−6.82	1.81 ± 0.05 (0.999)	−8.17 ± 0.20
10	0.66 ± 0.02 (0.999)	0.96 ± 0.03 (0.999)	−7.58 ± 0.25	−7.89	1.21 ± 0.04 (0.999)	−6.82 ± 0.19
11	0.61 ± 0.05 (0.998)	0.89 ± 0.07 (0.998)	−6.37 ± 0.50	−6.70	1.13 ± 0.09 (0.998)	−5.78 ± 0.40
12	1.23 ± 0.03 (0.999)	1.78 ± 0.05 (0.999)	−17.82 ± 0.05	−10.01	2.00 ± 0.06 (0.999)	−14.01 ± 0.40
13	0.65 ± 0.03 (0.999)	0.94 ± 0.04 (0.999)	−8.98 ± 0.42	−9.55	1.07 ± 0.04 (0.999)	−7.62 ± 0.25
14	0.67 ± 0.03 (0.999)	0.97 ± 0.05 (0.999)	−8.10 ± 0.37	−8.35	1.21 ± 0.07 (0.998)	−7.23 ± 0.35
15	0.73 ± 0.03 (0.999)	1.06 ± 0.04 (0.999)	−9.76 ± 0.35	−9.21	1.28 ± 0.06 (0.999)	−8.54 ± 0.35
16	0.74 ± 0.03 (0.999)	1.07 ± 0.04 (0.999)	−8.83 ± 0.35	−8.25	1.34 ± 0.06 (0.999)	−7.81 ± 0.30
17	0.73 ± 0.02 (0.999)	1.05 ± 0.03 (0.999)	−7.66 ± 0.24	−7.30	1.36 ± 0.05 (0.999)	−6.83 ± 0.24

[a] Ionization data taken from ref. 29. [b] EA yields a curve.

Table 4 Acid–base properties of THBCs and model compounds in their lowest singlet excited states

	ΔpK_a[a]		
Compound	DC	C	N
1	—	—	+4.5
3	—	—	+4.9
4	−4.9	+0.7	+4.4
6	−9.2	+2.3	+6.0
7	−10.2	+2.5	+6.7
8	−9.3	+2.6	—
9	−9.3	+4.5	+4.0
10	−4.1	+2.0	+2.1
11	−8.5	+1.4	+4.6

[a] Estimated value from the Förster–Weller cycle, eqn. (5), by using the average of the absorption and emission maxima.

acidity function. This compound, therefore, allows us to expand this acidity scale up to *ca.* −10.

On the other hand, data in Table 3 show a great divergence between HAF and EA methods. Thus, for most of the THBC derivatives, differences of about 1 pK_a unit, and even greater, are usually observed. This is not unexpected, in view of the criticisms recently made of the universal validity of the EA approach.[45–48] The HAF and EA methods closely agree for moderately weak bases (pK_a values up to −5), but they diverge significantly for weaker bases. The present results also show that the differences between the pK_a values are not random; the HAF method systematically gives greater pK_a values than EA method. Furthermore, the divergence does not have its origin, as has been suggested, in the extrapolative nature of these methods, since the statistical errors affecting both methods are quite similar. Therefore, our results support the suggestion put forward by Johnson and Stratton[46] that the excess acidity scale X_p is not well behaved in the highest region of acidity.

In such a situation where all the members of a series of related compounds do not follow the same acidity function and the EA method clearly fails, we will adopt Cox's opinion[46] that, in spite of its empirical significance, $(H_I)_{\frac{1}{2}}$ values are the simplest estimation of the relative basicities of these compounds. Although comparisons between these parameters should be made with caution, fortunately most of the THBCs roughly follow the H_I acidity function and therefore their $(H_I)_{\frac{1}{2}}$ values are expected to be very close to the true thermodynamic pK_as.

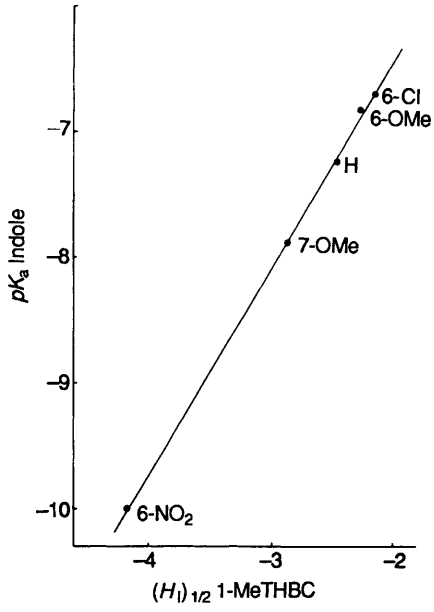


Fig. 4 Correlation between p$K_{a(EA)}$ values of substituted indoles (taken from ref. 43) and $(H_I)_{\frac{1}{2}}$ values of 1-methyl-1,2,3,4-tetrahydro-β-carboline derivatives (1-MeTHBC)

Data in Table 3 show that **6C** is a much weaker base than the related compounds **2** and **3**. It is even almost 1 pK_a unit less basic than **4**, which also yields dicationic species in concentrated acid media.[29,37] The differences in basicity between **6C** and typical indoles such as **2** or **3** must clearly be imputed to the strong destabilizing effect of the positive charge on the piperidinic nitrogen atom of **6C**. As would be expected, this effect is greater for **6DC**, than for the less sterically constrained dications of **4**. Alkylation at the 1-position has no appreciable effect on the basicity of the THBC ring, but alkylation at the piperidinic nitrogen atom decreases it. On the other hand, the effects of substituents on the benzene ring are as expected from their electron accepting or donating properties. As Fig. 4 shows, there is a close parallelism between the effects of these substituents on the basicities of the THBC and indole rings.[43]

Finally, data in Table 4 give an estimation of the changes in acidity and basicity experienced by the different species involved in the prototropic equilibria of THBC, upon excitation from the

ground to the lowest singlet excited states. The acidities of cationic and neutral molecules are enhanced upon excitation. Thus, THBC derivatives behave as typical indoles[49] with respect to pyrrolic deprotonation. Conversely, dicationic species are weaker acids in the excited than in the ground state, behaving therefore as anilinium cations.

Acknowledgements

We gratefully acknowledge financial support from the *Dirección General de Investigación Científica y Técnica* (PB89-0643) and *Junta de Andalucía.*

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Paper 2/03386K
Received 26*th June* 1992
Accepted 5*th October* 1992