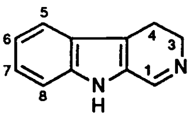
Acid–Base and Spectral Properties of β-Carbolines. Part 2. Dehydro and Fully Aromatic β-Carbolines

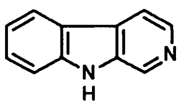
Manuel Balón,* José Hidalgo, Pilar Guardado, María A. Muñoz and Carmen Carmona
Departamento de Química Física, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain

The absorption and fluorescence spectra of a set of dehydro and fully aromatic β-carboline (9*H*-pyrido[3,4-*b*]indole) derivatives have been investigated in aqueous solutions of varying acidity. Within the pH-range, cationic (nitrogen protonation) and neutral species are evidenced from both absorption and fluorescence spectra. Additional zwitterionic species can also be detected from the fluorescence spectra. In highly basic media, outside the pH-range, anionic species (pyrrolic nitrogen deprotonation) are formed. Ground state ionization constants for the different acid–base equilibria involving these species have been determined spectrophotometrically at 25 °C. Lowest excited singlet state pK_as have been estimated from the Förster–Weller cycle. The influence of structural variations on the spectra and on the acid–base properties of these compounds is discussed.

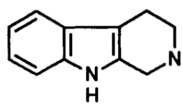
As a continuation of the preceding paper,[1] we report now the results of our investigations on the acid–base properties and spectral characteristics of dehydro- (DB, **1**) and fully aromatic (BC, **2**) β-carbolines (9*H*-pyrido[3,4-*b*]indoles). Because of their extended π-electron systems, the acid–base and spectral properties of these compounds differ markedly from those of the less aromatic tetrahydro-β-carbolines (THBC **3**), previously studied. The β-carboline ring **2** is composed of a π-deficient pyridinic ring, fused to a π-excessive indole ring, and therefore, it retains, although modified, many properties of these rings.[2] Thus, the pyridinic nitrogen atom of the BC and DBC derivatives is protonated in aqueous solutions within the pH-range, whereas the pyrrolic nitrogen atom deprotonates in basic media outside the pH-range. BCs are widespread in nature[3] and their striking fluorescences, recognized long ago,[4] have been used for analytical purposes.[5,6] The pH-dependence of the absorption and fluorescence spectra of several naturally occurring BC derivatives has been thoroughly investigated.[7–14] Conversely, possibly owing to the rarity in nature of the DBC derivatives, the spectra of these partially aromatic compounds have not been so extensively studied. There are, however, detailed studies on the pH-dependence of the absorption and fluorescence spectra of the two more representative and almost unique naturally occurring DBCs; harmaline (**10**) and harmalol (**11**).[9,10,14–16]



1

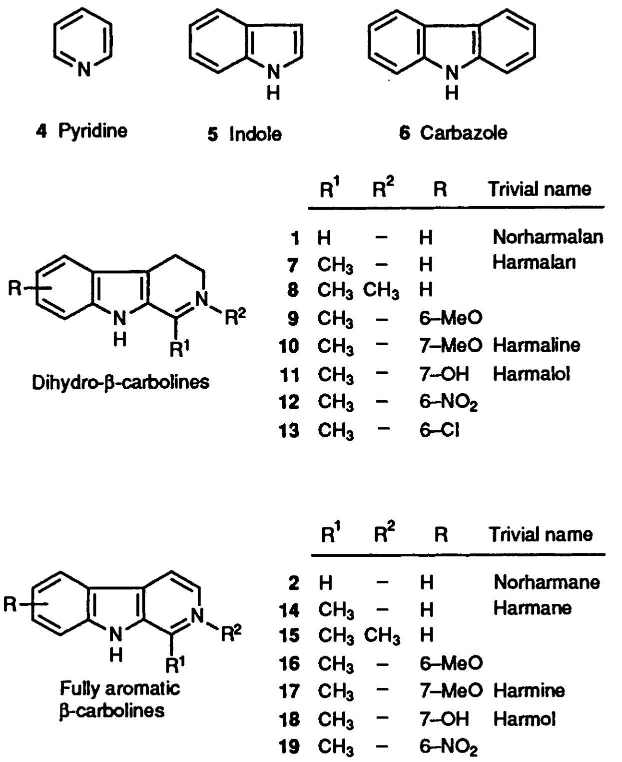


2



3

On the other hand, there are also some studies on the spectra of the DBC and BC derivatives in acid and basic media outside the pH-range. Thus, we have previously studied the absorption and fluorescence spectra of several of these compounds in highly basic media[17,18] and a report on their spectral properties in concentrated sulfuric acid solutions has also recently appeared.[19] However, the spectral changes attributed by the latter authors to the prototropic processes are possibly masked by the sulfonation reactions experienced by most of the DBC and BC derivatives in those media.[20,21]



4 Pyridine **5** Indole **6** Carbazole

Dihydro-β-carbolines

	R¹	R²	R	Trivial name
1	H	–	H	Norharmalan
7	CH_3	–	H	Harmalan
8	CH_3	CH_3	H	
9	CH_3	–	6-MeO	
10	CH_3	–	7-MeO	Harmaline
11	CH_3	–	7-OH	Harmalol
12	CH_3	–	6-NO_2	
13	CH_3	–	6-Cl	

Fully aromatic β-carbolines

	R¹	R²	R	Trivial name
2	H	–	H	Norharmane
14	CH_3	–	H	Harmane
15	CH_3	CH_3	H	
16	CH_3	–	6-MeO	
17	CH_3	–	7-MeO	Harmine
18	CH_3	–	7-OH	Harmol
19	CH_3	–	6-NO_2	

Fig. 1 Structural formulae of dehydro and fully aromatic β-carbolines and related compounds

The aim of this paper is to present a more systematic and extensive study on the acid–base properties and spectral characteristics of the DBC and BC derivatives. This study, and the preceding one, may help to gain a better comprehension of the electronic effects brought about by structural modifications in the β-carboline ring. The DBC and BC derivatives and selected model compounds are shown in Fig. 1.

Experimental

Compounds **2**, **4**, **5**, **6**, **10**, **11**, **14**, **17** and **18** were commercial products of the best available quality (>98%, Aldrich Química, Sigma, Lancaster) and were used without further purification. The DBC derivatives **7**, **9**, **12** and **13** were prepared by oxidation of their corresponding THBC derivatives with $KMnO_4$ in THF

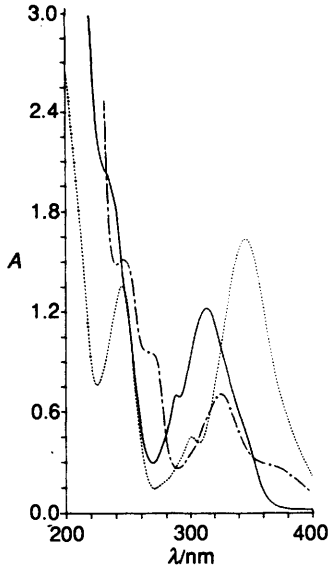


Fig. 2 Absorption spectra of the species involved in the prototropic equilibria of **7**. **C** (· · · ·), **N** (——) and **A** (— — —). Spectra obtained in 0.1 mol dm^{-3} HCl, 0.1 mol dm^{-3} KOH and 14 mol dm^{-3} KOH, respectively.

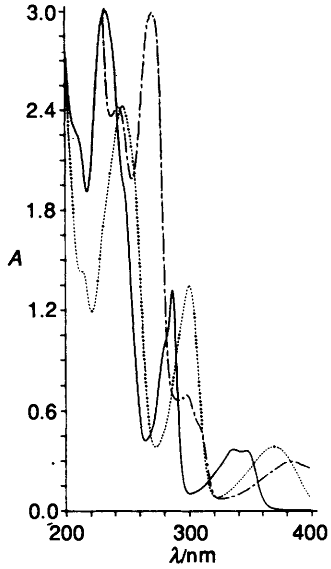


Fig. 3 Absorption spectra of the species involved in the prototropic equilibria of **2**. **C** (· · · ·), **N** (——) and **A** (— — —). Spectra obtained in 0.1 mol dm^{-3} HCl, 0.1 mol dm^{-3} KOH and 14 mol dm^{-3} KOH.

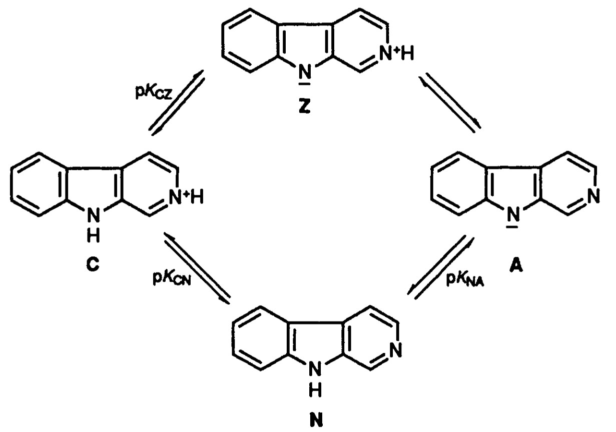
at 0 °C.[22] Compound **19** was prepared by nitration of **14** with concentrated nitric acid in sulfuric acid.[23] The compound **16** was prepared by dehydrogenation of its THBC derivative with Pd/charcoal under reflux.[23] The *N*-pyrido methylated compounds **8** and **15** were obtained by refluxing **7** and **14**, with dimethyl sulfate in benzene.[24]

Stock solutions of the DBC and BC derivatives were prepared in methanol as in the preceding paper.[1] Buffer solutions for UV–VIS spectrophotometry (I = 0.100 mol dm^{-3}) were prepared as described in the bibliography[25] and the pHs of the solutions were measured on a pH-meter Radiometer Copenhagen Mod. PHM82.

Absorption and fluorescence measurements were carried out using the apparatus and procedures described in the preceding paper.[1] Ground state ionization constants for the pyridinic nitrogen atom protonation, were calculated from ionization data obtained spectrophotometrically by using the Henderson–Hasselbach equation. As in the previous work, ionization data for the pyrrolic nitrogen atom deprotonation were comparatively analysed by the Hammett acidity function (HAF) and the excess acidity (EA) methods.[26]

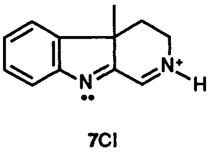
Results and Discussion

Absorption and Fluorescence Spectra.—Excluding indole ring protonation DBC and BC derivatives experience similar prototropic equilibria to those observed for the less aromatic THBCs. Conversely, at least for most of the DBC and BC derivatives in concentrated sulfuric acid media, sulfonation[20,21] predominates over the protonation process. On the other hand, zwitterionic species are also presumed to be formed in the excited states of these compounds. Therefore, the spectral modifications induced by the change of the acidity and basicity of the media can be explained by the prototropic equilibria shown in Scheme 1.



Scheme 1 Prototropic equilibria of the BC ring system

The electronic absorption spectra of cationic (**C**), neutral (**N**), and anionic (**A**) species of the parent compounds **7** and **2** are depicted in Figs. 2 and 3, respectively. The spectrum of the DBC derivative **7N** consists of two main absorption bands centred at 215 and 313 nm which are flanked by other overlapping and less intense bands. In the ionic species **7A** and **7C** the long wavelength bands are bathochromically shifted by about 10 and 30 nm, respectively. The essential features of these spectra are maintained for other DBC derivatives, the more important modifications being those induced by substituents at the benzene positions of the DBC ring (Table 1).



7CI

The strong red shift experienced by the long wavelength band upon protonation, is in agreement with the increase of the resonance interaction between the electronic indolic π-system and the exocyclic double bond predicted by theoretical calculations. Savory and Turnbull[10] have suggested the possibility of an indoleninium structure **7CI** for DBC cations. Thus, it must be pointed out that the absorption spectra of the related 3-*H*-2-phenylindoleninium cation,[27] exhibit a band of similar intensity and position (345 nm).

The absorption spectrum of **2N** consists of five bands (215, 233, 286, 337 and 347 nm) and two shoulders (250 and 280 nm). Aullo and Tomas Vert[28] have used the semiempirical CNDO method to carry out a theoretical study of the absorption

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

	C (Cation)		Z (Zwitterion)	N (Neutral)		A (Anion)	
Compound	λ_m^{abs} (log ε)	λ_m^{fl} (φ)	λ_m^{fl}(φ)	λ_m^{abs}(log ε)	λ_m^{fl}(φ)	λ_m^{abs}(log ε)	λ_m^{fl}(φ)
1	246 (4.04)[b] 359 (4.34)			235 (4.20)[b] 242 (4.20) 318 (4.16)			
2	214 (4.18) 247 (4.41) 300 (4.16) 369 (3.36)	445 (0.52)	511	215 (4.50) 233 (4.56) 250s (4.41) 280s (4.02) 286 (4.15) 337 (3.60) 347 (3.60)	385 (0.16)		450 (0.06)
3	219 (4.40) 270s (3.80) 276 (3.79) 286 (3.65)	353 (0.06)	—	220 (4.39) 271 (3.76) 278 (3.81) 286 (3.64)	368 (0.21)	315 (3.68)	412 (<0.01)
4	251s (3.63)[b] 256 (3.70) 261s (3.53)	n.f.	—	240s (3.05)[b] 246s (3.22) 251 (3.37) 257 (3.42) 263 (3.25)	360 (<0.01)	—	—
5	—	—	—	216 (4.54)[b] 266s (3.76) 270 (3.77) 278 (3.76) 287 (3.68)	346 (0.25)	288 (4.31)	408 (0.03)
6	—	—	—	211 (4.43)[b] 227s (4.53) 233 (4.57) 243 (4.38) 253 (4.38) 282s (3.99) 291 (4.44) 322 (3.52) 334 (3.44)	360	*ca.* 342 (2.8)	419
7	243 (4.01) 301 (3.52) 344 (4.06)	434 (0.49)	494	215 (4.48) 232s (4.29) 288 (3.83) 313 (4.09)	380 (0.10)	246 (4.18) 268 (3.96) 326 (3.84) 370s (3.48)	444 (0.02)
8	246 (4.12) 305 (3.75) 350 (4.17)	436	500	274 (4.04)[b] 316 (3.97) 365 (3.84)	—	—	
9	208 (4.29) 245s (3.78) 303 (3.56) 358 (3.84)	486		214 (4.46) 245s (3.78) 294 (3.74) 320 (3.77)	414	316 (3.71) 335 (3.78)	465
10	204 (4.37) 215 (4.35) 259 (3.88) 373 (4.36)	480 (0.46)		219 (4.43) 258 (3.99) 330 (4.21)	*ca.* 375	266s (3.95) 330 (4.01)	435 (<0.001)
12	236 (3.85) 300s (3.65) 354 (4.05) 400 (3.61)	n.f.	n.f.	209 (4.45) 344 (3.81) *ca.* 400 (3.74)	n.f.	289 (3.92) 320s (3.64) 399 (3.68) 494 (3.74)	n.f.
13	288 (3.60) 350 (4.18)			212 (4.38) 292 (3.80) 322 (3.61)			
14	245 (3.66) 299 (3.64) 364 (3.59)	434 (0.76)	491	240 (3.66) 288 (3.62) 300 (3.61) 334 (3.57) 348 (3.57)	380 (0.17)	298 (3.83) 310s (3.65) 383 (3.48)	440 (0.06)
15	248 (4.40) 303 (4.17) 366 (3.35)	437	511	272 (4.20)[c] 321 (3.82) 357 (3.25)			
16	231 260 304 398		505	227 (4.10) 294 (3.82) 357 (3.25)	420	276 (4.12) 314 (3.72) 399 (3.24)	483
17	247 (4.53) 320 (4.27) 360s (3.88)	420 (0.32)	482	240 (4.61) 298 (4.22) 326 (3.75) 388s (3.69)	372	300	435 (0.03)

Table 1 (*continued*)

	C (Cation)		Z (Zwitterion)	N (Neutral)		A (Anion)	
Compound	λ_m^{abs} (log ε)	λ_m^{fl} (φ)	λ_m^{fl}(φ)	λ_m^{abs}(log ε)	λ_m^{fl}(φ)	λ_m^{abs}(log ε)	λ_m^{fl}(φ)
19	236 (4.20) 280 (4.17) 350 (3.70)	n.f.		230 (4.27) 272 (4.01) 296 (3.84) 344 (3.64) 368 (3.64)	n.f.	242 (4.19) 288 (3.96) 328 (3.85) 344 (3.64) 550 (3.71)	n.f.

[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] Data taken from ref. 29. [c] Zwitterionic species.

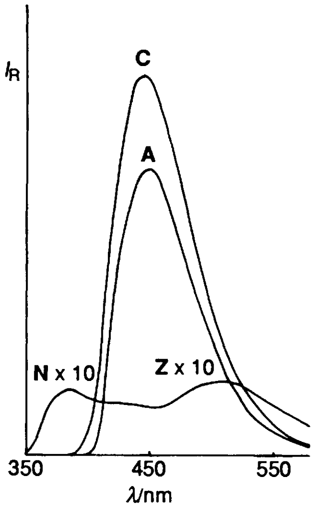


Fig. 4 Corrected fluorescence emission spectra of the species involved in the prototropic equilibria of **2**. **C** (0.1 mol dm^{-1} HCl), **N** and **Z** (0.1 mol dm^{-3} KOH) and **A** (14 mol dm^{-3} KOH).

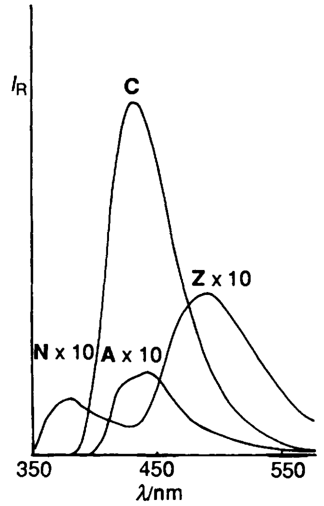


Fig. 5 Corrected fluorescence emission spectra of the species involved in the prototropic equilibria of **7**. **C** (0.1 mol dm^{-3} HCl) **N** and **Z** (0.1 mol dm^{-3} KOH) and **A** (14 mol dm^{-3} KOH).

spectra of several BC derivatives. They have assigned the bands of the **2N** spectrum to π–π* transitions, except for the band at 215 nm and the shoulder at 280 nm which are due to n–π* transitions and disappear upon protonation. Moreover, the bands at 337 and 347 nm of **2N** are replaced by only one broad band at 369 nm in **2C**. Deprotonation of **2N** produces similar spectral changes, but the red shifts are more pronounced. The spectral characteristics of other BC derivatives are collected in Table 1.

The fluorescence spectra of **2** and **7** are shown in Figs. 4 and 5 and the main features of the spectra of other DBC and BC derivatives are reported in Table 1. These data show that cations are the more fluorescent species. Their fluorescences can be observed over most of the pH-region (*ca.* 0–9). In more basic media (pH 10–14) the spectra of DBC and BC compounds show a double fluorescence band, the zwitterions and neutral molecules being the emitting species. Zwitterions fluoresce more intensely than the neutral molecules, the fluorescence of the latter species being rather weak and their maxima only clearly apparent in media of pH 13–14. In more concentrated basic media ($H_0 > 15$) the emissions of the anions can be observed. The wavelengths of the emission maxima of all these species follow the sequence **N** < **C** < **A** < **Z**.

As a result of the easy ionization of the phenolic groups,[30] the dependence of the absorption spectra of **11** and **18** with KOH concentration is more complicated. Additional zwitterionic, mono- and di-anionic species are formed in moderate and highly concentrated basic media. Since the particular characteristics of these spectra have been previously discussed in enough detail we will not examine them here. It must also be mentioned that, whereas the 6-NO$_2$ THBC derivative is weakly fluorescent, neither the nitro dehydro (**12**) nor the aromatic (**19**) derivatives are fluorescent. This is possibly due to the complementary deactivating influence of the electron-withdrawing character of the NO$_2$ group and charge migration from the indolic towards the pyridinic moiety of these compounds.

Acid–Base Properties and Ionization Constants.—The ionization constants for the pyridinic nitrogen atom protonation of the DBC and BC derivatives are collected in Table 2. These data show that the BC **2** is a stronger base than **4**[31] and isoquinoline (pK_a = 5.40)[31] but less basic than 6-azaindole (pK_a = 7.95).[31] This sequence of basicity indicates that the annellation process does not always contribute to stabilize the cations more than the neutral molecules. The DBC **7** is even more basic than the BC **2**, but only slightly less basic than the related 2-methyl-2,3-didehydropyridine (pK_a = 9.55).[31]

It is interesting to note that, although far from the pyridinic nitrogen atom, the substituents on the benzene ring of DBC and BC derivatives are able to transmit their electronic effects to this centre.[34,35] The influence of those substituents on the acidity of this atom seems to operate mainly through conjugative interactions. Thus, these effects can be roughly related with their +R or −R character. However, inductive effects also seem to be operative, as judged by the different influence of the -OMe group on the pK_as of the pair of compounds **9–10** and **16–17**. It seems as if for the transmission of the electronic effects 7-MeO groups were in *para* position (+R > −I) and 6-MeO in *meta* position (+R < −I)[32], with respect to the pyridinic nitrogen atom. Also, noteworthy is the great acid weakening effect of the phenolic OH groups of **11** and **18**. This fact is in agreement with the strong electron-donating character of the O$^-$ group, since the phenolic groups of these compounds deprotonate at lower pHs than their pyridinic nitrogen atoms.[30]

The results of the ionization data analysis for the ground state pyrrolic deprotonation equilibria of DBC and BC derivatives are summarized in Table 3. As can be seen, plots of log *I* *vs* H_- are linear, the slopes being very close to unity for most of the DBC derivatives and somewhat greater for the BC derivatives. Thus, whereas DBCs behave as indole, BCs are more similar to carbazole. Data in Table 3 also show the existence of a satisfactory correspondence between $(H_-)_{\frac{1}{2}}$ and pK_a (EA) values. Again, the pK_a (HAF) values in this table evidence the divergences pointed out in the preceding paper.[1]

A perusal of the ionization constants reported in Table 3 shows that **2** is a stronger acid than **5**. This sequence follows the trend of the five-membered heterocycles: pyrrole, imidazole and pyrazole and their benzoanalogues indole, benzimidazole and indazole;[36] the extension of conjugation raises the acidity owing to the resonance stabilization of the anions. On the other hand, the acidity order **2** > **6** points to an extra stabilization for the annellation of a pyridine ring instead of a benzene ring. However, the exceptional increase of acidity from **5** to **2** (*ca.* 2 pK_a units) compared with the increase of < 1 pK_a unit observed between pyrrole and indole and between **2** and **6** is really noteworthy and it has been theoretically analysed with enough detail in a previous paper.[37]

It should also be noted that the acidities of β-carbolines increase with the degree of aromaticity of the β-carboline ring in the order THBC < DBC < BC as would be expected for the resonance stabilization of the indolate anions. Because this acidity sequence is also maintained in the gas phase,[37] the solvation contribution must be almost constant among the β-carboline series.[38]

Table 2 Ionization constants for the protonation equilibria of the pyridil nitrogen atom of DBC and BC derivatives at 25 °C

Compound	pK_a
2	6.85 ± 0.03
3	*ca.* 8.6[a]
4	5.16[b]
7	8.99 ± 0.02
9	9.21 ± 0.05
10	9.54 ± 0.03
11	11.30 ± 0.04
12	7.63 ± 0.04
13	9.39 ± 0.25
14	7.34 ± 0.03
16	6.91 ± 0.08
17	7.73 ± 0.02
18	9.51 ± 0.03
19	6.24 ± 0.03

[a] Estimated value (see preceding paper). [b] Taken from ref. 31.

Methylation at the 1-position of the BC ring has no appreciable effect on the acidity. In contrast, an exceptionally high increase in acidity occurs when the methyl group is substituted on the pyridine nitrogen atom. In this case, it is evident that the positively charged quarternary nitrogen atom is an additional factor for the stabilization of the anions. On the other hand, the influence of substituents at the benzene ring of DBC and BC rings follows the expected trend; electron-donating substituents, such as O^-, decrease the acidity of the pyrrolic nitrogen atom, whereas electron accepting groups, such as NO_2, produce the opposite effect on the acidity. It must be noted that, as theoretically predicted, substituent effects are quite independent of the degree of aromaticity of the β-carboline skeleton.[38]

Finally, we want to make a brief comment on the changes in acidity or basicity experienced by DBC and BC derivatives upon excitation to the lowest singlet excited states. These changes can be analysed from the ΔpK_a data (ΔpK_a = pK_a^G − pK_a^S) reported in Table 4, which have been estimated from the Förster–Weller cycle[39] as in the preceding paper. These data show that the acidity of cationic and neutral species of DBC and BC derivatives follows the opposite trend upon excitation.

Cations are less acidic in the excited than in the ground state, whereas neutral species become stronger acids upon excitation.

The changes in acidity experienced by these β-carboline derivatives upon excitation are as expected as compared with those of some related compounds. Thus, with respect to the pyridinic nitrogen atom, the DBC and BC derivatives behave as pyridinium and isoquinolinium cations,[39,40] whereas for pyrrolic deprotonation they behave as indole[41] or carbazole.[42] Therefore, both the pyridinic and the indolic moieties retain their individual characteristics after the annellation to form the β-carboline skeleton.

The different sign for the shifts in acidity experienced by these nitrogen atoms reflects their different involvement in the aromatic π-electron system of the β-carboline ring. Whereas the lone electron pair of the pyridinic nitrogen atom is nonbonded and perpendicular to the π-system, the lone electron pair of the pyrrolic nitrogen is conjugated with the β-carboline π-system.

Table 3 Ionization data analysis for the pyrrolic deprotonation equilibria of DBC, BC and model compounds at 25 °C

		HAF			EA	
Compound	d log *I*/d[KOH]	*m*	pK_a	$(H_-)_{\frac{1}{2}}$	*m**	pK_a
2	0.36 ± 0.05 (0.994)	1.24 ± 0.17 (0.994)	18.04 ± 2.5	14.55	0.98 ± 0.11 (0.996)	14.53 ± 0.04
3[a]	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
5[b]	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
6[b]	0.39 ± 0.04 (0.996)	1.36 ± 0.13 (0.996)	20.40 ± 2	15.00	1.48 ± 0.17 (0.994)	15.18 ± 0.08
7	0.31 ± 0.03 (0.997)	1.08 ± 0.11 (0.997)	16.03 ± 1.5	14.84	1.03 ± 0.21 (0.987)	14.88 ± 0.1
9	0.27 ± 0.02 (0.998)	0.95 ± 0.06 (0.998)	13.71 ± 0.96	14.43	0.75 ± 0.12 (0.989)	14.48 ± 0.06
10	0.29 ± 0.04 (0.996)	1.01 ± 0.14 (0.996)	15.63 ± 2	15.48	0.99 ± 0.31 (0.981)	15.40 ± 0.18
11	0.31 ± 0.06 (0.994)	1.08 ± 0.19 (0.994)	17.12 ± 3	15.85	1.18 ± 0.37 (0.981)	16.03 ± 0.28
12	—	—	—	—	—	12.55 ± 0.05
14	0.39 ± 0.03 (0.999)	1.37 ± 0.08 (0.999)	19.85 ± 1	14.49	1.25 ± 0.25 (0.992)	14.47 ± 0.07
15	—	—	—	—	—	11.20[b]
16	0.60 ± 0.13 (0.991)	2.07 ± 0.44 (0.991)	29.96 ± 6.5	14.47	1.49 ± 0.06 (0.999)	14.43 ± 0.06
17	0.43 ± 0.02 (0.999)	1.45 ± 0.05 (0.999)	21.04 ± 1	14.51	1.04 ± 0.18 (0.994)	14.43 ± 0.03
18	0.25 ± 0.02 (0.999)	0.85 ± 0.04 (0.999)	13.69 ± 0.64	16.10	0.78 ± 0.06 (0.999)	15.83 ± 0.06
19	—	—	—	—	—	12.04 ± 0.05

[a] Taken from preceding paper. [b] Taken from ref. 33.

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

	ΔpK_a	
Compound	C	N
1	−5.5	+6.5
5	—	+4.5
6	—	+3.1
7	−6.4	+5.3
9	−7.2	+4.2
10	−9.6	+3.5
14	−4.8	+5.9
16	—	+6.3
17	−5.4	+0.4

Acknowledgements

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

References

1 Part 1, preceding paper.
2 For a review on β-carboline chemistry see: R. A. Abramovitch and I. D. Spencer, *Adv. Heterocycl. Chem.*, 1964, **3**, 79.
3 J. R. F. Allen and B. R. Holmstedt, *Phytochem.*, 1980, **19**, 1573.
4 W. H. Perkin and R. Robinson, *J. Chem. Soc.*, 1919, **115**, 933.
5 W. D. Denckla and H. K. Dewey, *J. Lab. Clin. Med.*, 1967, **69**, 160.
6 S. G. Hadley, A. S. Muraki and K. Spitzer, *J. Forensic. Sci.*, 1974, **19**, 657.
7 R. Sakurovs and K. P. Ghiggino, *J. Photochem.*, 1982, **18**, 1.
8 O. S. Wolfbeis, E. Fürlinger and R. Wintersteiger, *Monatsh Chem.*, 1982, **113**, 509.
9 O. S. Wolfbeis and E. Fürlinger, *Z. Phys. Chem. Neue Folge.*, 1982, **129**, 171.
10 B. Savory and J. H. Turnbull, *J. Photochem.*, 1984, **24**, 355.
11 F. Tomas Vert, I. Zabala Sanchez and A. Olba Torrent, *J. Photochem.*, 1983, **23**, 355.
12 J. Romero Berto, I. Zabala Sanchez, J. M. Aullo Reverte, F. Tomas Vert and A. Olba Torrent, *J. Photochem.*, 1985, **29**, 405.
13 F. Tomas, I. Zabala and A. Olba, *J. Photochem.*, 1985, **31**, 253.
14 A. Olba Torrent, F. Tomas Vert, I. Zabala Sanchez and F. Medina Casamayor, *J. Photochem.*, 1987, **37**, 109.
15 F. Tomas Vert, I. Zabala Sanchez and A. Olba Torrent, *J. Photochem.*, 1984, **26**, 285.
16 M. Krishnamurthy and S. K. Droga, *Photochem. Photobiol.*, 1986, **44**, 571.
17 M. Balón, M. A. Muñoz, J. Hidalgo, M. C. Carmona and M. Sánchez, *J. Photochem.*, 1987, **36**, 193
18 J. Hidalgo, C. Carmona, M. Balón and M. A. Muñoz, *Pharm. Weekblad.*, 1990, **12**, 142.
19 M. Krishnamurthy and S. K. Droga, *J. Chem. Soc., Perkin Trans 2*, 1986, 1247.
20 M. A. Muñoz, M. Balón, C. Carmona, J. Hidalgo and M. López Poveda, *Heterocycles*, 1988, **27**, 2067.
21 M. C. Carmona, M. Balón, J. Hidalgo, M. Muñoz and M. López Poveda, *Can. J. Chem.*, 1989, **67**, 720.
22 S. Misztal and M. Cegla, *Synthesis*, 1985, 1134.
23 B. T. Ho, K. Li, K. E. Walker, L. W. Tansey, P. M. Kralih and W. H. McIsaac, *J. Pharm. Sci.*, 1970, **59**, 1445.
24 A. P. Gray, E. E. Spinner, D. C. Schlieper and C. J. Cavallito, *J. Am. Chem. Soc.*, 1955, **77**, 3533.
25 D. D. Perrin and B. Dempsey, in *Buffers for pH and Metal Ion Control*, Chapman and Hall, London, 1974.
26 R. A. Cox and K. Yates, *Can. J. Chem.*, 1983, **61**, 2225.
27 M. J. Kamlet and J. C. Dacons, *J. Org. Chem.*, 1961, **26**, 220.
28 J. M. Aulló and F. Tomas Vert, *An. Quim.*, 1979, **75**, 11.
29 W. L. F. Armarego, in *Physical Methods in Heterocyclic Chemistry*, Ed. A. R. Katritzky, Academic Press, London, New York, 1971, vol. III, ch. 4.
30 K. T. Douglas, R. K. Sharma, J. F. Walmsley and R. C. Hider, *Mol. Pharmacol.*, 1983, **23**, 614.
31 A. Albert, in *Physical Methods in Heterocyclic Chemistry*, Ed. A. R. Katritzky, Academic Press, London, New York, 1963, vol. I.
32 N. S. Isaacs, in *Physical Organic Chemistry*, Longman, London, 1987.
33 M. Balón, M. C. Carmona, M. A. Muñoz and J. Hidalgo, *Tetrahedron*, 1989, **45**, 7501.
34 M. Balón, M. A. Muñoz, M. C. Carmona and M. Sánchez, *J. Chem. Soc., Perkin Trans 2*, 1985, 1683.
35 M. C. Carmona, M. Balón, D. González, J. Maraver and M. Sánchez, *J. Chem. Soc., Perkin Trans 2*, 1986, 409.
36 G. Yagil, *Tetrahedron*, 1967, **23**, 2855.
37 C. Carmona, J. Hidalgo, E. Sánchez Marcos, R. R. Pappalardo, M. Muñoz and M. Balón, *J. Chem. Soc., Perkin Trans 2*, 1990, 1881.
38 M. Muñoz, M. Balón, J. Hidalgo, C. Carmona, R. R. Pappalardo and E. Sánchez Marcos, *J. Chem. Soc., Perkin Trans 2*, 1991, 1729.
39 S. G. Schulman, in *Modern Fluorescence Spectroscopy*, Ed. E. L. Wehry, Heyden, London, 1976, vol. 2, ch. 6.
40 E. Vander Donckt, *Progr. React. Kinet.*, 1970, **5**, 273.
41 E. Vander Donckt, *Bull. Soc. Chim. Belg.*, 1969, **78**, 69.
42 J. Hidalgo, C. Carmona, M. A. Muñoz and M. Balón, *J. Chim. Phys.*, 1990, **87**, 555.
43 A. P. Gray, *J. Am. Chem. Soc.*, 1955, **77**, 5930.

Paper 2/03476J
Received 26*th June* 1992
Accepted 5*th October* 1992