Ionization of β-Carbolines in Concentrated Hydroxide Solutions

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The ionization of several β-carbolines (norharman or β-carboline, harman, harmine, harmaline, and tetrahydroharmine) in concentrated potassium hydroxide solutions have been examined. The pK_a values, calculated using the H_- acidity function, indicate that these compounds are stronger acids than indole and their acidities increase with the degree of aromaticity of the compounds in the order tetrahydro < dihydro < fully aromatic β-carbolines. This sequence can be interpreted in terms of resonance stabilization of the indolate anion.

Because of their pharmacological properties, β-carbolines (**I**)—(**III**) have been the subject of extensive chemical and biochemical investigation.[1–6,†] Interest in β-carboline chemistry has recently been increased by reports of their inhibitory influence toward monoamine oxidase (MAO),[7] serotonin uptake by synaptosomas,[8] and the sodium pump.[9]

The acid–base behaviour of β-carbolines has long been of interest and much recent work has focussed on this subject.[10–15] Owing to the acidic indole NH group and the basic non-indole nitrogen atom on the β-carboline skeleton, these compounds can exist in aqueous solutions, at least, as three differently charged species, whose equilibria, exemplified for β-carboline (**Ia**) as the model compound, are depicted in the Scheme.

Whereas the protonation equilibria on the non-indole nitrogen atom have been extensively investigated and pK_a values are available,[10–15] information about the dissociation of the indole NH group is very scanty. It must be noted that this group is feebly acidic[16,17] and dissociates only in concentrated hydroxide solution and, therefore, an acidity function must be used to provide the pK_a values.

In view of the paucity of data, it was of interest to examine the dissociation equilibria of β-carbolines in concentrated hydroxide solution. This paper presents pK_a data on the H_- acidity scale of several β-carboline derivatives of biochemical and pharmacological interest. In particular the influence of the degree of aromaticity was investigated to provide information of this effect on acidity.

Experimental

β-Carboline (**Ia**), harman (**Ib**), harmine hydrochloride hydrate (**Ic**), and harmaline hydrochloride dihydrate (**II**), were purchased from EGA Chemie and were used as received. These compounds were pure by t.l.c. Tetrahydroharmine (**III**) was prepared from harmaline by reduction with sodium borohydride and purified by recrystallization from methanol. Stock solutions of these compounds were prepared in methanol and stored in the dark to avoid photo-oxidation. The hydroxide solutions were prepared from Merck A.R. potassium hydroxide as described by Yagil[16] and their concentrations were determined by titration with standardized sulphuric acid solutions.

The ionization constants were determined by the spectrophotometric method.[18] The necessary solutions were prepared by pipetting portions of each alkaloid solution (50—100 μl) into hydroxide solution (5 cm³) in sufficient amounts to give accurately measurable absorbances (final concentrations *ca.* 1×10^{-4}M). After thorough mixing, the final solutions were kept in a constant temperature bath at 25.0 ± 0.1 °C. In some cases where turbid solutions were formed, warming in hot water was necessary to bring about complete dissolution.

Absorption spectra and absorbance measurements were made with a Lambda-5 spectrophotometer whose cell compartment was also thermostatted at 25.0 ± 0.1 °C. Usually, a hydroxide solution of the same concentration to the test solution was used as reference.

Results and Discussion

As expected, deprotonation of β-carbolines significantly affects their electronic absorption spectra, since the indole NH group is part of a conjugated system. The first two columns in the Table show the position of the absorption maxima, $\lambda_{max.}$, of the neutral species and their conjugated bases.

As can be seen in going from dilute to concentrated hydroxide solution, the intensities of the bands of the acid forms of fully aromatic β-carbolines decreased and new absorption bands or a shoulder (harmine) appeared at *ca.* 360—380 nm where the acid forms did not absorb. These systems showed good isosbestic points.

Partially reduced β-carbolines behaved differently to the other members of the series. Thus, the intense absorption band of harmaline in its acid form at 330 nm decreased upon protonation but no new band appeared. On the other hand, the positions of the absorption maxima of tetrahydroharmine were unaffected by changes in hydroxide concentration although a considerable increase in absorbance occurs at 300—340 nm upon conversion into base. However, it should be noted that the spectrum of the base form of this latter compound could not be obtained because of the turbidity of its more concentrated hydroxide solution.

The ionization ratios for all the other compounds were calculated from equation (1) where d_{B^-} and d_{BH} are the absorbances at the chosen wavelengths of the fully ionized species and the non-ionized species, respectively and d is the absorbance at the wavelength monitored for various intermediate hydroxide concentrations. In obtaining these data we used points corresponding to log I values within ±0.75, because clear deviations from parallel behaviour were observed beyond these limits.

$$I = \frac{[BH]}{[B^-]} = \frac{d_{B^-} - d}{d - d_{BH}} \qquad (1)$$

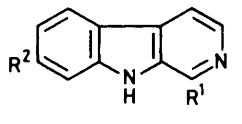
The effect of varying the wavelength at which ionization ratio measurements were taken was checked in the case of harman. In other cases, the calculation of I was performed at only one wavelength, as listed in the Table.

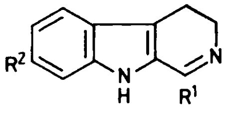
[†] β-Carboline is named systematically as pyrido[3,4-*b*]indole; trivial names are used throughout.

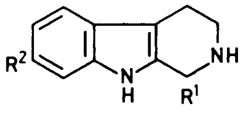
Table. Absorption characteristics and ionization data for β-carbolines in KOH at 25 °C

Compound	BH, $\lambda_{max.}$/nm	B^-, $\lambda_{max.}$/nm	λ/nm[a]	$-d \log I/d\ H_-$[b]	r[c]	pK_a
(Ia)	338, 348	380	380	1.00 ± 0.02	0.991(7)	14.49 ± 0.07
(Ib)	334, 346	375	334	0.93 ± 0.16	0.992(6)	14.50 ± 0.07
			375	0.98 ± 0.03	0.986(7)	14.45 ± 0.09
(Ic)	298	298, 362 (sh)	362	1.02 ± 0.06	0.997(9)	14.43 ± 0.02
(II)	330	330	330	1.02 ± 0.2	0.996(5)	15.34 ± 0.03
(III)	270, 296	270, 296	276, 304			15.6 ± 0.2

[a] Wavelength employed for measurement. [b] *I.e.* slopes of plots of log I against H_-. [c] Correlation coefficients of these plots. The figures in parentheses are the number of points taken.







(I)

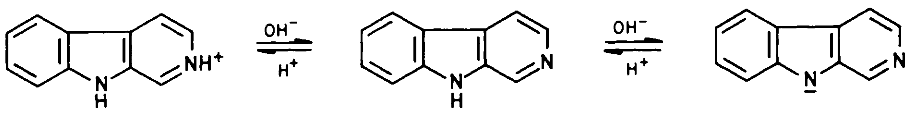
a; $R^1 = R^2 = H$

b; $R^1 = CH_3$, $R^2 = H$

c; $R^1 = CH_3$, $R^2 = OCH_3$

(II) $R^1 = CH_3$, $R^2 = OCH_3$

(III) $R^1 = CH_3$, $R^2 = OCH_3$



(Ia)

Scheme. Ionization equilibria of β-carboline

In examining the ionization behaviour of β-carbolines it seemed appropriate to consider the H_- acidity function stablished by Yagil[16] to describe the ionization of indoles in concentrated hydroxide solution. Plots of log I against H_- gave straight lines whose slopes and correlation coefficients are reported in the Table. It can be noted that the values of these slopes were sufficiently close to unity to permit the conclusion that the ionization of these compounds follows the H_- acidity function. Hence, the pK_a values were calculated using equation (2) and the data are reported in the Table.

$$pK_a = H_- + \log I \qquad (2)$$

As can be seen, the pK_a value of harman is fairly independent of the wavelength used for the ionization ratio determination. On the other hand, it is in very good agreement with the value of 14.6 previously reported.[11]

In the case of tetrahydroharmine, since a spectrophotometric titration curve was impossible to obtain because of the aforementioned solubility problems, we have employed the method of Marioni and Calmon[19] to estimate its pK_a value. According to this method, which only requires a part of the titration curve, the pK_a value can be found, by using equation (3), from the

$$d = d_{B^-} + (d_{BH} - d)\, h_-/K_a \qquad (3)$$

slope of the plot of d against $(d_{BH} - d)\, h_-$, where h_- = antilog $(-H_-)$. Since the error in this method may be important, absorbance measurements were carried out at two different wavelengths, the pK_a of tetrahydroharmine reported in the Table being the average of these results.

Inspection of the pK_a data in the Table reveals that β-carbolines are stronger acids than indole (pK_a 16.97).[17] Thus, annelation of the pyridine ring to indole to give β-carboline causes an increase of the acidity by 2.5 pK_a units. The higher acid strength of β-carboline compared with indole follows the trend of the five-membered heterocycles pyrrole, imidazole, and pyrazole and their benzologues indole, benzimidazole, and indazole; the extension of conjugation raises the acidity due to resonance stabilization of the anions.[17]

However, the increase of acidity in going from indole to β-carboline compared with the increase of 0.5 pK_a units observed between pyrrole and indole seems exceptionally high. Unfortunately, the different effects of benzene and pyridine annelation cannot be discussed because the acid strength of the parent compound 6-azaindole is unknown. By supposing that annelation effects of benzene and pyridine rings are additive, a pK_a value of *ca.* 15 could be predicted for 6-azaindole.

It should be also noted that the acidities of β-carbolines increase with the degree of aromaticity of the β-carboline skeleton in the order tetrahydro < dihydro < fully aromatic β-carbolines as would be expected for the resonance stabilization of the indolate anions.

Finally, we can briefly comment on the rather small influence of methoxy and methyl substitution at C-7 and C-1 of the β-carboline ring, because these substituents do not make possible additional resonance forms for the anion. In contrast, an exceptionally high increase in acidity occurred with the substitution of a methyl group on the pyridine nitrogen atom, as illustrated by 2-methyl-β-carboline, whose pK_a is 10.9.[10] In this case it is evident that the positively charged quaternary nitrogen atom is an additional factor for stabilization of the anions.

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