Ionization Equilibria of Harmol and Harmalol in Concentrated Hydroxide Solutions

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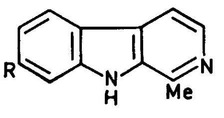
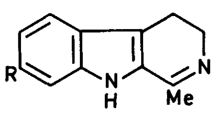
The deprotonation equilibria of the β-carboline alkaloids harmol (1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol) and harmalol (4,9-dihydro-1-methyl-3*H*-pyrido[3,4-*b*]indol-7-ol) in concentrated potassium hydroxide have been examined by u.v. spectrophotometry at 25 °C. The pK_a values obtained using the Hammett and the Excess Acidity Functions are reported and compared with those of related compounds.

The chemistry of the β-carboline alkaloids has aroused much interest in recent years, owing to the wide range of biological activity displayed by these compounds.[1–3] Recently, we have investigated the acid dissociation equilibria of several β-carboline alkaloids in concentrated hydroxide solutions with the principal aim of studying the influence of aromaticity on the acidity of the β-carboline ring.[4,5] In these media, ionization of β-carbolines involves the feebly acidic NH group of the indole ring on the β-carboline skeleton; therefore the pK_a values must be expressed in terms of an acidity function. Previous work had established that the deprotonation of β-carbolines follows closely the *H*-acidity function, defined originally by Yagil for the deprotonation of indole derivatives.[6]

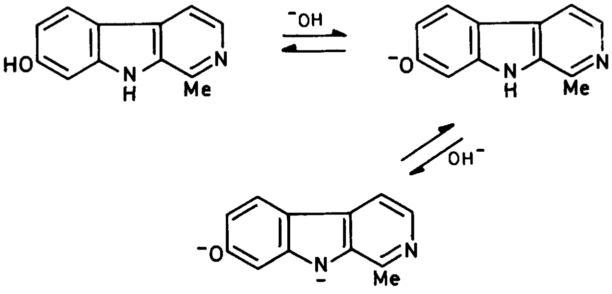
We have now extended our study to the deprotonation of the indole NH group of the naturally occurring β-carboline alkaloids harmol (1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol) (**1**) and harmalol (4,9-dihydro-1-methyl-3*H*-pyrido[3,4-*b*]indol-7-ol) (**2**). We thought it was worthwhile to study the acid dissociation of these polyfunctional compounds, because the existence of a phenolic group in their structures, which deprotonates in the pH region,[7–9] provides the possibility of investigating the effect of a negative charge on the acidity of the β-carbolines. Also, it would be of interest to test the validity of the H_2^- acidity function established by Yagil[6] for monoanion–dianion equilibria of indole derivatives which has not been extensively studied.



(**1**) R = OH
(**3**) R = OMe



(**2**) R = OH
(**4**) R = OMe



Scheme.

Experimental

Harmol and harmalol hydrochloride monohydrates were purchased from Aldrich and used as received. Stock solutions of these compounds were prepared in methanol. The aqueous hydroxide solutions were prepared from Merck reagent grade potassium hydroxide as described by Yagil[6] and were standardized against standard sulphuric acid solutions.

The ionization constants were determined spectrophotometrically by the experimental method employed in previous work.[5] U.v. spectra and absorbance measurements were obtained with a Lambda-5 spectrophotometer.

Results and Discussion

As an example, the Scheme shows the two-step deprotonation equilibria of harmol (**1**). Since the phenolic deprotonation steps of harmol and harmalol take place in dilute basic media and their pK_a values are well established,[7–9] they have not been studied here.

The second acid dissociation equilibrium of these compounds has been examined by u.v. spectrophotometry at 25 °C. The u.v. spectra of harmol and harmalol in KOH at concentrations selected to ensure exclusive formation of monoanions (HA^-) and dianions (A^{2-}) are depicted in Figures 1 and 2. The shifts caused by the second deprotonation in harmol and harmalol are different. After deprotonation the peaks at 331 and 365 nm of harmol and harmalol monoanions, respectively, are reduced in intensity and a new peak at 345 nm (harmol) or a shoulder at 310 nm (harmalol) appears. In both cases a band around 278 nm is present in the dianion spectrum.

The ionization ratios, $I = [HA^-]/[A^{2-}]$, were determined from equation (1) at two different wavelengths; d_{HA^-}, $d_{A^{2-}}$, and

$$I = (d_{HA^-} - d)/(d - d_{A^{2-}}) \qquad (1)$$

d are the absorbances at the monitored wavelength for monoanions, dianions, and various intermediate hydroxide concentrations, respectively.

Owing to the structural characteristics of the compounds under study it seemed reasonable to consider both the H_- and H_{2-} acidity functions as established by Yagil for neutral and monoanionic indole derivatives[6] respectively. As can be seen from the data in Table 1 plots of log I *vs.* H_- and H_{2-} give good straight lines for both compounds. However, it is clear from the values of the slopes that the H_- function describes the ionization of harmol and harmalol more accurately than the H_{2-} function. Indeed, the slope of log I *vs.* H_- for harmalol is sufficiently close to unity for us to assume that its ionization follows this acidity function. Moreover, the value of the slope of log I *vs.* [OH] plot for this compound (0.31) is similar to the

Table 1. Ionization data for harmol and harmalol in KOH at 25 °C

	λ/nm^a	d(log I)/dH_-^{b}	r^c	d(log I)/dH_{2-}^{b}	r^c
Harmol (**1**)	331	0.84	0.992(8)	0.66	0.991(8)
	279	0.83	0.999(8)	0.65	0.999(8)
Harmalol (**2**)	363	1.09	0.994(4)	0.86	0.993(4)
	308	1.08	0.994(5)	0.85	0.994(5)

^{a} Wavelengths employed for measurements. ^{b} Slopes of log I *vs.* H_- or H_{2-}. ^{c} Correlation coefficients; the figures in parenthesis are the numbers of points taken.

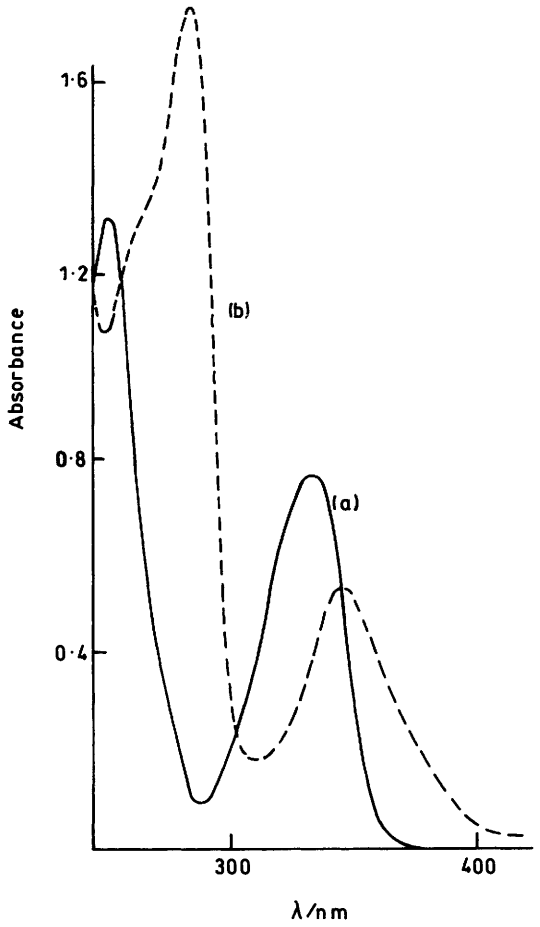


Figure 1. Absorption spectra of (a) the monoanion and (b) the dianion from harmol in 0.1M-KOH and 14M-KOH, respectively

corresponding value for most of the compounds used by Yagil to establish the H_- scale (0.29).[6]

The observation that the H_- acidity function describes the acid dissociation of a monoanion better than the H_{2-} function is not new; such behaviour is shown by tryptophan[6] and substituted sulphamides.[10] Although, in the case of tryptophan, Yagil[6] argued that the negative charge on the tryptophan monoanion is not conjugated with the indole ring, it should be noted that the indolic ionizations of 5-hydroxytryptophan and the 5-hydroxytryptamine (serotonin) are also better described by the H_- than by the H_{2-} acidity function.[11] It seems that the utility of the H_{2-} acidity function is restricted to describing the ionization of indole carboxylate anions.

The problem of estimating the acidity of a group of substances with various values of d(log I)/dH (where H represents some acidity function) may be approached in two fundamentally different ways which have been discussed recently.[12]

Table 2. Values of $(H)_{1/2}$ and pK_a obtained from HAFM and EAFM for the deprotonation equilibria of harmol and harmalol and related compounds in concentrated KOH solutions at 25 °C^a

	HAFM		EAFM	
	$(H_-)_{1/2}$	$(H_{2-})_{1/2}$	pK_a	r
Harmol (**1**)	15.91	16.70	15.75	0.994
Harmalol (**2**)	15.92	16.56	16.01	0.979
Harmine (**3**)	14.43^b		14.35	0.952
Harmaline (**4**)	15.34^b		15.39	0.985
2,3-Dimethylindole	15.43^b		15.57	0.988

^{a} Ionization data for compounds in Table 2 have been deposited as Supplementary Publication No. SUP56713 (6pp.). For details see Instructions for Authors (January issue). ^{b} Values taken from refs. 4 and 5.

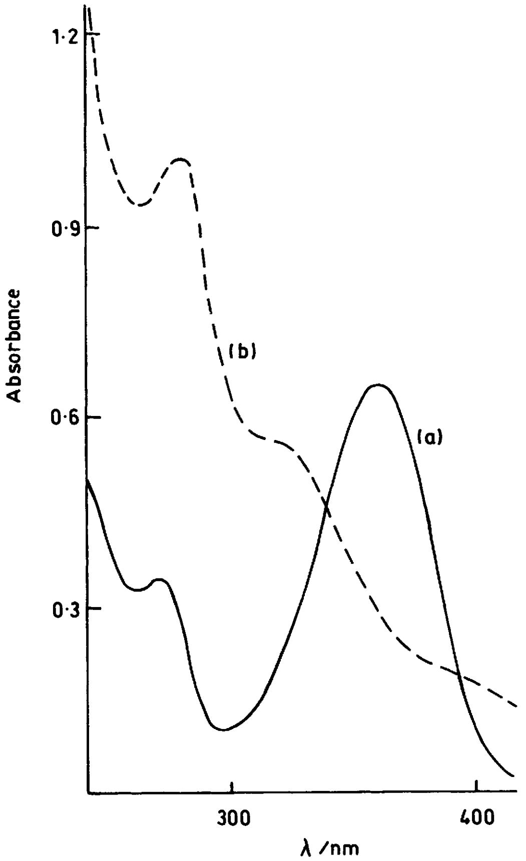


Figure 2. Absorption spectra of (a) the monoanion and (b) the dianion from harmalol in 0.1M-KOH and 14M-KOH, respectively

The first, the Hammett Acidity Function Method (HAFM), uses the value of H at half-ionization, $(H)_{1/2}$ (log I = 0). The parameters can be calculated by using equation (2).

$$\log I = \mathrm{m}(H) + \mathrm{p}K_a \qquad (2)$$

The second approach, the Excess Acidity Function Method (EAFM), does not involve the use of any acidity function, although it does make use of the indicator overlap principle to determine the quantity $X = \log (f_{A_*^-}/f_{HA_*}f_{OH^-})$ for a reference compound. In this method pK_a values may be obtained from equation[13] (3).

$$pK_w + \log [OH^-] - \log a_{H_2O} + \log I = m^* \log (f_{A_*^-}/f_{HA_*}f_{OH^-}) + pK_a \quad (3)$$

Plots of ($pK_w + \log [OH^-] - \log a_{H_2O} + \log I$) *vs.* $\log (f_{A_*^-}/f_{HA_*}f_{OH^-})$ give pK_a values as the intercept.

In order to obtain accurate pK_a values for the deprotonation equilibria of harmol and harmalol we have used HAFM with both H_- and H_{2-} acidity functions and EAFM. The activities of water in potassium hydroxide solutions utilized in the latter method were calculated by the procedure used by Hannigan and Spillane,[10] and the X values were taken from the data reported by these authors for benzimidazole as reference compound. The results of both treatments are collected in Table 2. For comparison, this table also includes some published data[4,5] for related compounds.

As can be seen from Table 2, the agreement between the pK_a values calculated by EAFM and the $(H_-)_{1/2}$ values obtained by HAFM is satisfactory, but there are divergences with the $(H_{2-})_{1/2}$ values. The validity of EAFM can be verified by comparison of the pK_a values obtained by both methods for 2,3-dimethylindole, harmine (**3**), and harmaline (**4**), which are known to behave as H_- acids. Accordingly we suggest that the pK_a values obtained from EAFM are an accurate measure of the acid strength of all these compounds.

Because of the extrapolation procedure used in the calculations, the error in the pK_a values of harmol and harmalol obtained from EAFM could be large and is difficult to evaluate. However, it is possible to draw some reasonable conclusions. First, these pK_a values indicate that harmol and harmalol are less acidic, with respect to deprotonation of the indole NH group, than harmine and harmaline. A similar decrease of acidity is observed for the indolic ionization of the 5-hydroxy-subsititued derivatives of tryptophan and tryptamine, which are about 1 pK_a unit less acidic than the parent compound. Secondly, the decrease in acidity in going from harmine to harmol is greater than from harmaline to harmalol. In fact, in spite of the difference of *ca.* 1 pK_a unit between harmine and harmaline, the pK_a values of harmol and harmalol are very close.

These results can be interpreted in terms of the influence of the aromaticity of the β-carboline ring. The greater the aromaticity, the greater will be the delocalization of a negative charge. Stabilization of the charge on the phenolic oxygen will give rise to an increase in pK_a value, but stabilization of the charge on the indole nitrogen will produce the opposite effect. Thus the pK_a values of harmol and harmalol reflect the influence of these two factors, the first being the dominant one.

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