ACIDITY CONSTANTS OF HARMALINE AND HARMALOL IN THE GROUND AND EXCITED SINGLET STATES

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(Received November 28, 1983; in revised form January 23, 1984)

Summary

The pH dependence of the absorption and fluorescence spectra of the alkaloids harmaline and harmalol was investigated. Two species, the cation and the neutral molecule, are characterized for harmaline in both the absorption and the fluorescence spectra. Harmalol, however, forms four different species in the ground electronic state, the cation, the neutral molecule, the zwitterion and the anion molecule, depending on the pH of the solution, and only three species, the cation, the zwitterion and the anion, are detected in the first excited singlet state. By comparison with model compounds, it is shown that harmalol exists as a mixture of uncharged and zwitterionic molecules at pH 10.

The pK_a values associated with the ground state equilibria were determined spectrophotometrically. The excited state pK_a values were estimated by applying the Förster equation. It is found that the hydroxy group of harmalol is more acidic and the ring nitrogen atom more basic (in both harmaline and harmalol) in the first excited singlet than in the ground states.

1. Introduction

Like norharman [1], harmaline (4,9-dihydro-7-methoxy-1-methyl-3*H*-pyrido(3,4-*b*)indole) [2, 3] (Fig. 1(a)) and harmalol (4,9-dihydro-1-methyl-3*H*-pyrido(3,4-*b*)indol-7-ol) [4 - 6] (Fig. 1(b)) are markedly fluorescent alkaloids which are of considerable pharmacological interest at present, being hallucinogens [7].

Fig. 1. Molecular structures of (a) harmaline and (b) harmalol.

0047-2670/84/$3.00

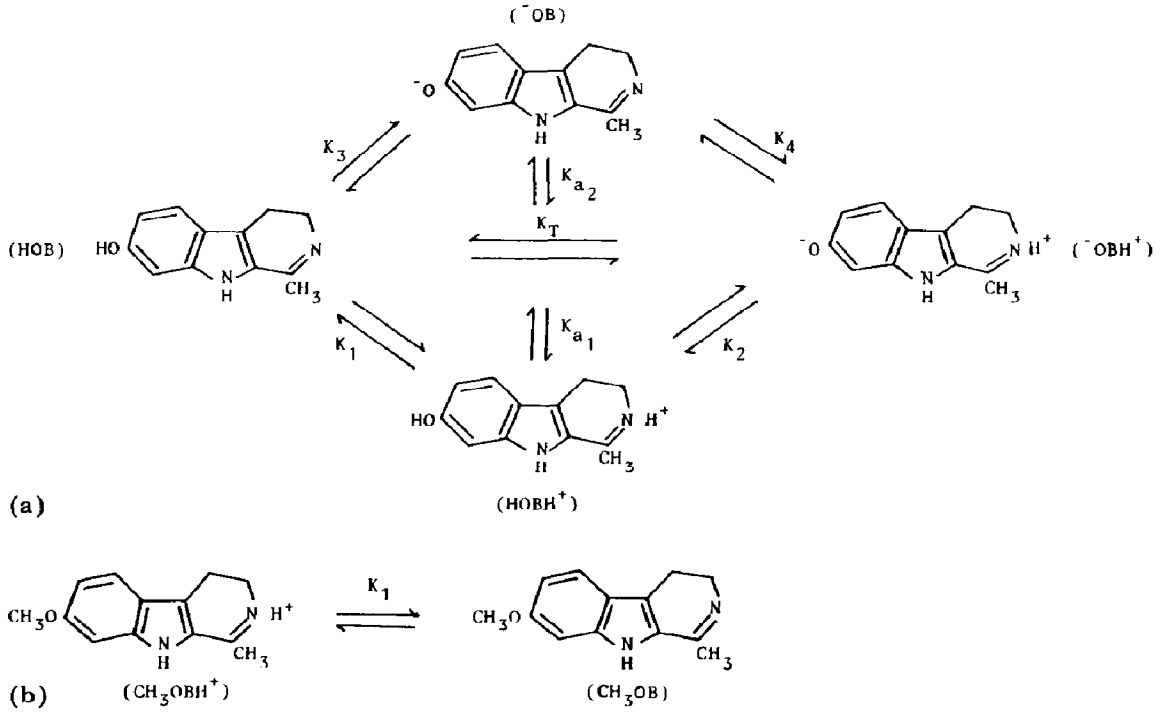

Fig. 2. Acid–base equilibria scheme for (a) harmalol and (b) harmaline.

Because of the hydroxy function and the basic nitrogen atom in its pyridinedihydro nucleus, harmalol can exist in four different molecular forms in the ground electronic state, their respective equilibria being governed by seven pK_a values. In this work we have considered the possibility of a tautomeric equilibrium between the neutral harmalol molecule HOB and the zwitterion $^-$OBH$^+$ structures (Fig. 2(a)) by analogy with harmol [8].

K_{a_1} and K_{a_2} are the first and second apparent acid dissociation constants of the harmalol cation HOBH$^+$. These constants can be evaluated experimentally by spectrophotometric methods [9], by using the following formulae:

$$pK_{a_1}(S_0) = pH - \log\left(\frac{A_{HOBH^+} - A}{A - A_{(HOB + {}^-OBH^+)}}\right) \quad (1)$$

$$pK_{a_2}(S_0) = pH - \log\left(\frac{A_{(HOB + {}^-OBH^+)} - A}{A - A_{{}^-OB}}\right) \quad (2)$$

where A_{HOBH^+}, $A_{^-OB}$, $A_{(HOB + {}^-OBH^+)}$ and A are the absorbance values of a solution of pure HOBH$^+$, a solution of pure anion $^-$OB, a solution of an equilibrium mixture of HOB and $^-$OBH$^+$ species, and the solution under investigation respectively, all with the same overall concentration of the alkaloid.

From the relation outlined on Fig. 2, the following expressions can be derived:

$$K_{a_1} = K_1(K_T + 1) = \frac{K_2(K_T + 1)}{K_T} \tag{3}$$

$$K_{a_2} = \frac{K_3}{K_T + 1} = \frac{K_4 K_T}{K_T + 1} \tag{4}$$

K_1, K_2, K_3, K_4 and K_T can be determined from the experimental spectroscopic data by making some additional assumptions. The simplest approach to the determination of K_T is purely spectroscopic. If it is assumed that the change on going from HOB to a reference compound, say CH_3OB (Fig. 1(a)), does not affect the basicity of the pyridinic nitrogen, the value of K_a for the equilibrium

$$CH_3OBH^+ \rightleftharpoons CH_3OB + H^+$$

can be considered as a good approximation to the value of K_1. Then, the value of K_T can be derived from eqn. (3) by using this value for K_1 and the value of K_{a_1} derived experimentally.

As reported elsewhere [9], when the K_T value is in the range $0.01 < K_T < 100$, the observed absorption spectrum exhibits the characteristic bands of both HOB and $^-OBH^+$ species, as observed for harmalol.

The pK_a values for the first singlet excited state, $pK_a(S_1)$, were estimated by means of the Förster cycle [10] by using

$$pK_a(S_1) - pK_a(S_0) = \frac{0.625}{T} \Delta\bar{\nu} \tag{5}$$

where $\Delta\bar{\nu}$ (cm^{-1}) is the difference between the energies of the 0–0 electronic transitions in the basic and the protonated species or, in a more general case, between the molecular species on each side of the equilibria and T is the absolute temperature.

The determination of the energy of the 0–0 transition was made by averaging the energies corresponding to the less energetic maximum of the absorption electronic spectrum and that corresponding to the most energetic maximum of the fluorescence spectrum.

2. Experimental details

2.1. Materials

Harmaline and harmalol hydrochlorides were obtained from Sigma Chemical Company. The buffer solutions used were 0.1 M HAc–NaAc (Ac ≡ CH_3COO^-) mixtures for the pH range $3 < pH < 4$, 1/15 M Na_2HPO_4–KH_2PO_4 mixtures for the pH range $5 < pH < 8$ and 0.1 M glycine–NaOH mixtures for the pH range $9 < pH < 13$.

2.2. Apparatus

The UV absorption spectra were recorded on a Cary 219 spectrophotometer. Uncorrected fluorescence emission and excitation spectra were recorded on a Perkin–Elmer MPF-44A spectrofluorometer.

The pH values were measured directly by means of a radiometer 26 pH meter, to within 0.01 pH unit.

All the experiments were carried out on thermostatted solutions at a temperature of 25.0 ± 0.1 °C.

3. Results and discussion

3.1. Electronic absorption spectra

In aqueous solutions harmaline can exist in two different forms; the equilibrium is governed by the pK_a value according to Fig. 2(b). Consequently the absorption spectra are strongly dependent on the pH of the solution, as shown in Fig. 3. The corresponding data are compiled in detail in Table 1.

The values of $A_{CH_3OBH^+}$ and A_{CH_3OB} were taken from the spectra of solutions at pH 1 and 1 M NaOH respectively. These absorbance values were recorded at λ = 370 nm.

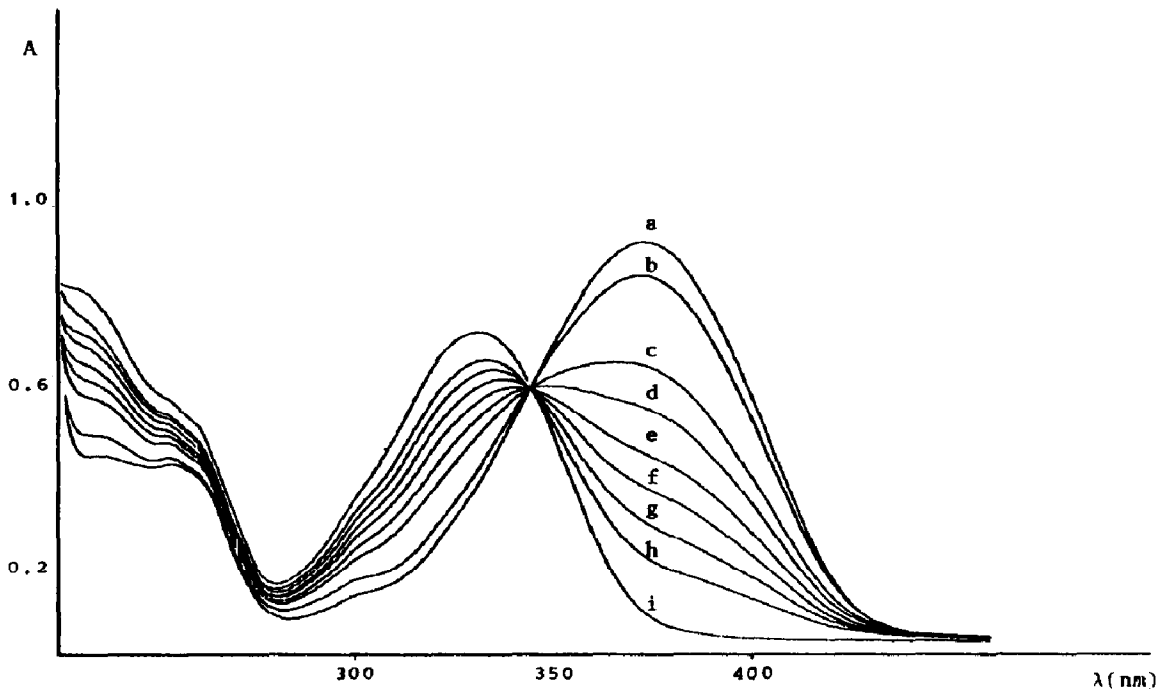

Fig. 3. Absorption spectra of harmaline solutions (concentration $C = 5 \times 10^{-5}$ M; ionic strength $I = 0.1$): spectrum a, pH 1.05; spectrum b, pH 9.16; spectrum c, pH 9.76; spectrum d, pH 9.97; spectrum e, pH 10.19; spectrum f, pH 10.36; spectrum g, 10.55; spectrum h, pH 10.81; spectrum i, pH 12.90.

TABLE 1

Wavelengths λ_{max} of the maxima in the absorption and fluorescence bands

	λ_{max} (nm) *for the following species*			
	$HOBH^+$	*HOB*	*$^-OBH^+$*	*^-OB*
Harmaline				
Absorption	372	330	—	—
Fluorescence	480	377	—	—
Harmalol				
Absorption	370	340	431	363
Fluorescence	475	377[a]	530	450

[a]By analogy with harmaline.

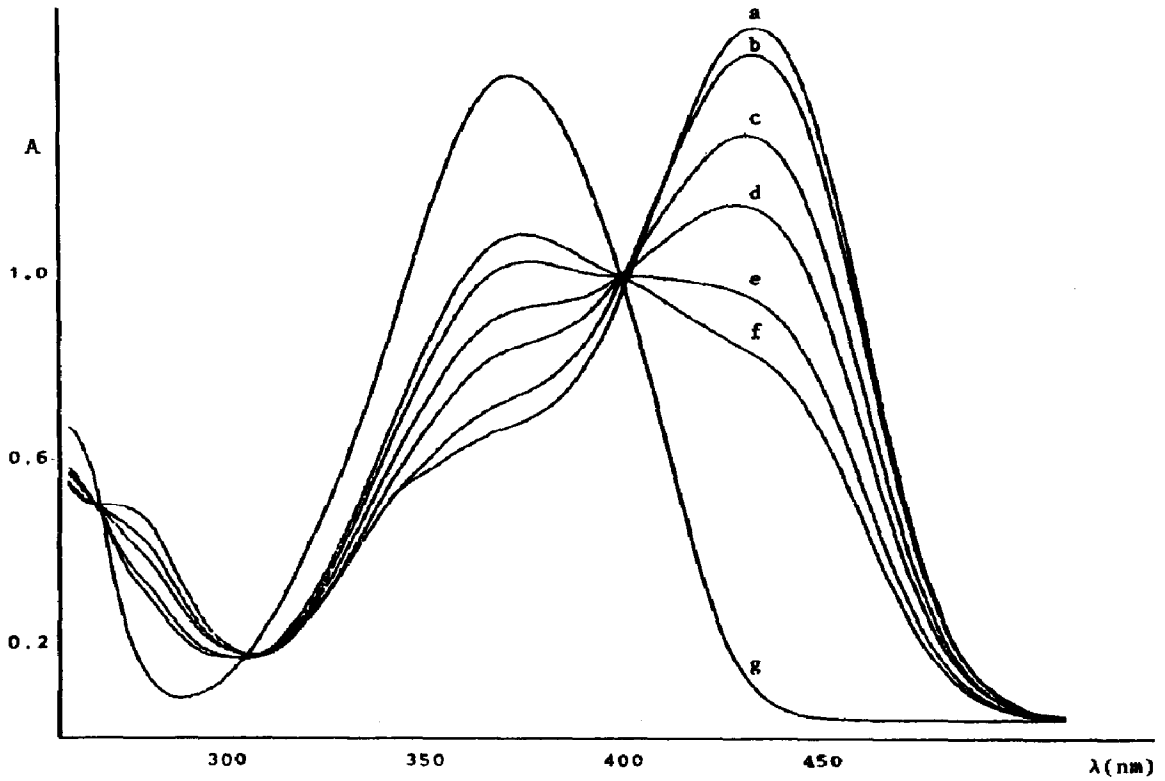

Fig. 4. Absorption spectra of harmalol solutions (concentration $C = 5 \times 10^{-5}$ M; ionic strength $I = 0.1$): spectrum a, pH 10.28; spectrum b, pH 9.63; spectrum c, pH 9.24; spectrum d, pH 9.01; spectrum e, pH 8.77; spectrum f, pH 8.59; spectrum g, pH 3.98.

Harmalol has different UV absorption spectra in dilute acid and in neutral and alkaline solutions (Figs. 4 and 5). Very little change was found in the absorption spectrum over the pH range from 1 to 7 and from pH 13 to 1 M NaOH, and it was concluded that the spectrum at pH 1 corresponds to

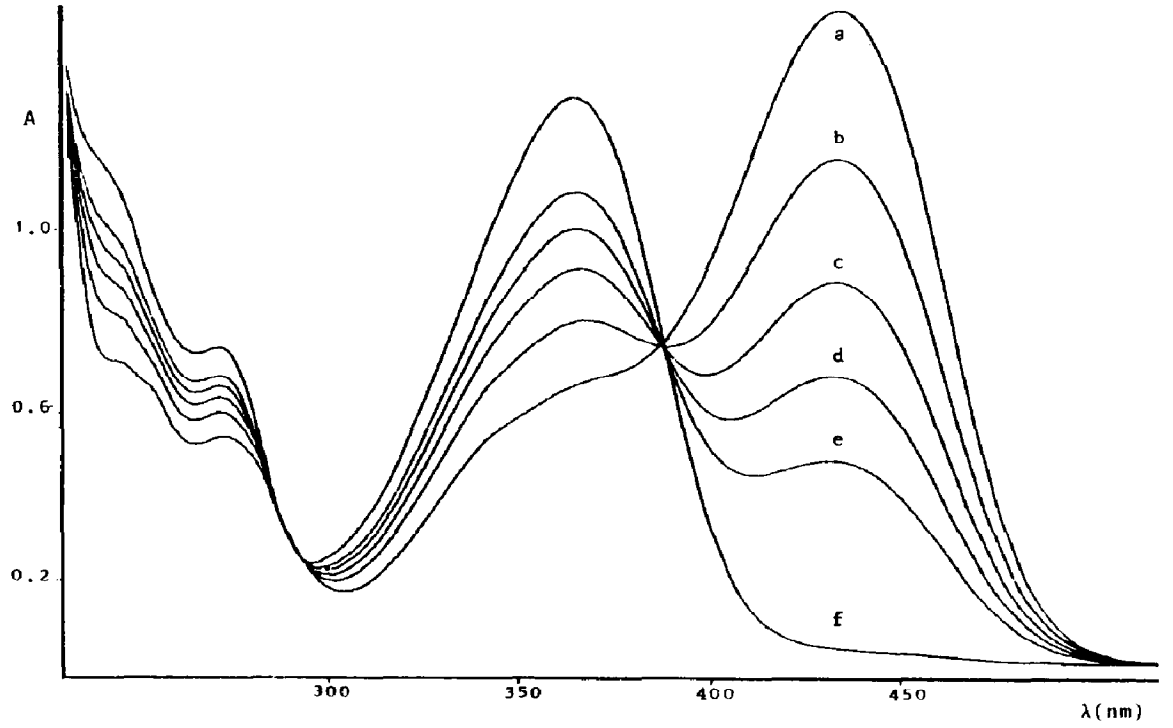

Fig. 5. Absorption spectra of harmalol solutions (concentration $C = 5 \times 10^{-5}$ M; ionic strength $I = 0.1$): spectrum a, pH 10.44; spectrum b, pH 11.16; spectrum c, pH 11.48; spectrum d, pH 11.72; spectrum e, pH 11.95; spectrum f, pH 12.92.

the species $HOBH^+$ (λ_{max} = 370 nm), whereas the spectrum at 1 M NaOH corresponds to the anionic ^-OB species (λ_{max} = 363 nm).

However, the separated spectra of the two tautomeric species (HOB and $^-OBH^+$) are less easily obtained, since from pH 7 to pH 13 the spectrum changes continuously and no pH range is found where the spectrum is constant.

The spectrum recorded at pH 10.2 can be considered as a good approximation to the spectrum of a mixture of the free base HOB (λ_{max} = 340 nm) and the zwitterionic species $^-OBH^+$ (λ_{max} = 431 nm). It is likely that ionized forms $HOBH^+$ and/or ^-OB are also present.

The 431 nm band was assigned to the zwitterionic species $^-OBH^+$ because the strong red shift is due to the ionic character of this species and also because the 340 nm band must be assigned to the neutral base HOB by analogy with harmaline which shows an absorption band at 330 nm.

The absorption maxima for solutions of each species are summarized in Table 1. The values of A_{HOBH^+} and $A_{^-OB}$ were taken from the spectra of solutions at pH 1 and 1 M NaOH respectively. The values of $A_{(HOB + {}^-OBH^+)}$ (eqns. (1) and (2)) are not readily evaluated experimentally since, at pH 10.2, where the tautomeric species are dominant, small concentrations of

$HOBH^+$ and ^-OB species are present, provided that the pK_{a_1} and pK_{a_2} values are similar.

The value of $A_{(HOB+{}^-OBH^+)}$ was estimated from the spectra of two solutions at higher pH values (11.6 and 11.5) where the concentration of $HOBH^+$ must be insignificant, but the concentration of ^-OB can be appreciable. By applying eqn. (2) to both solutions and assuming that pK_a does not change over this small pH range, the value of $A_{(HOB+{}^-OBH^+)}$ is found. All the absorbance values for the different harmalol solutions were recorded at λ = 440 nm.

The pK_a values for the equilibria between the different species of harmaline and harmalol in aqueous solutions at 25 °C in the electronic ground state are listed in Table 2.

TABLE 2

pK_a values for the ground and first singlet excited states of harmaline and harmalol (*cf.* Fig. 2)

	$pK(S_0)$	$pK(S_1)$
Harmaline		
pK_1	10.0	19.5
Harmalol		
pK_{a_1}	8.6	—
pK_{a_2}	11.5	—
pK_1	10.0	16.2
pK_2	8.6	2.3
pK_3	10.1	2.9
pK_4	11.5	19.5
pK_T	−1.4	−7.5

3.2. Fluorescence spectra

The existence of two forms of harmaline in the S_1 excited state is evident from its fluorescence spectra (Fig. 6) with maxima at 480 nm (CH_3OBH^+ cation) and 377 nm (neutral CH_3OB). The fluorescence spectra of harmalol over a wide range of pH (1 - 13) are shown in Figs. 7 and 8. λ_{ex} was chosen at the second isosbestic point to guarantee the absorption of every molecular species.

From the fluorescence spectra in aqueous solutions it can be inferred that only three species are present, namely the cation ($HOBH^+$), the zwitterion ($^-OBH^+$) and the anion (^-OB) in alkaline solutions. The intense cation fluorescence with a peak at 475 nm can be observed in the pH range 1 - 9 and the zwitterion fluorescence (λ_{max} = 530 nm) is observed from pH 6 to pH 12. In more alkaline solutions the fluorescence emission from the zwitterion is strongly reduced and another band appears at 450 nm correspond-

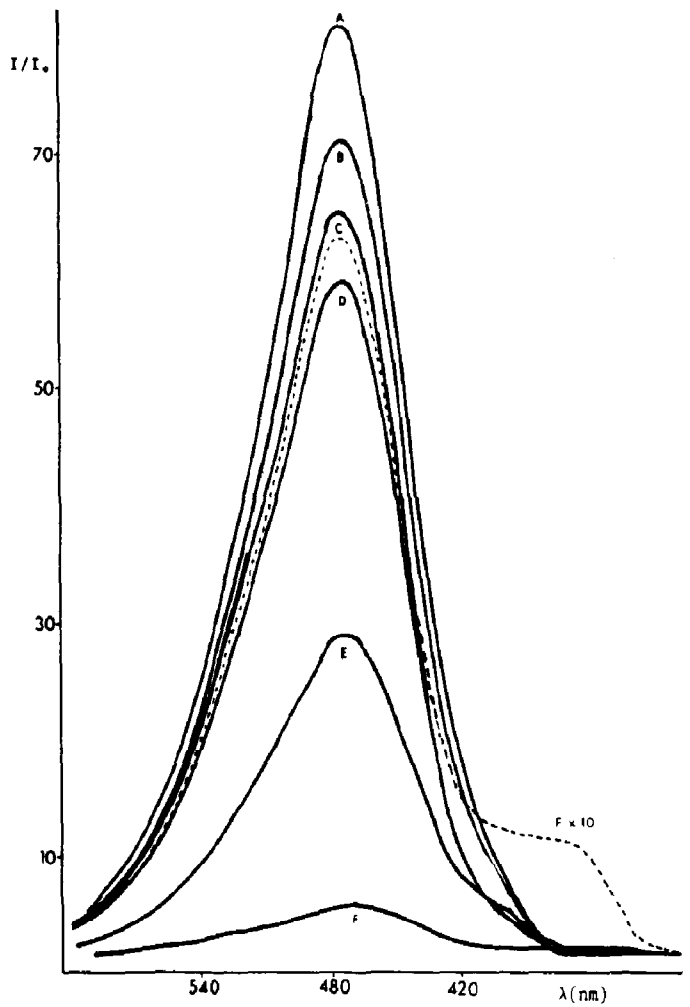

Fig. 6. Fluorescence spectra of harmaline solutions (concentration $C = 5 \times 10^{-5}$ M; $\lambda_{ex} = 270$ nm): spectrum A, pH 3.95; spectrum B, pH 5.88; spectrum C, pH 7.04; spectrum D, pH 8.13; spectrum E, pH 10.25; spectrum F, pH 13.10.

Fig. 7. Fluorescence spectra of harmalol solutions (concentration $C = 5 \times 10^{-5}$ M; $\lambda_{ex} = 270$ nm): spectrum A, pH 1.02; spectrum B, pH 6.42; spectrum C, pH 7.03; spectrum D, pH 8.12.

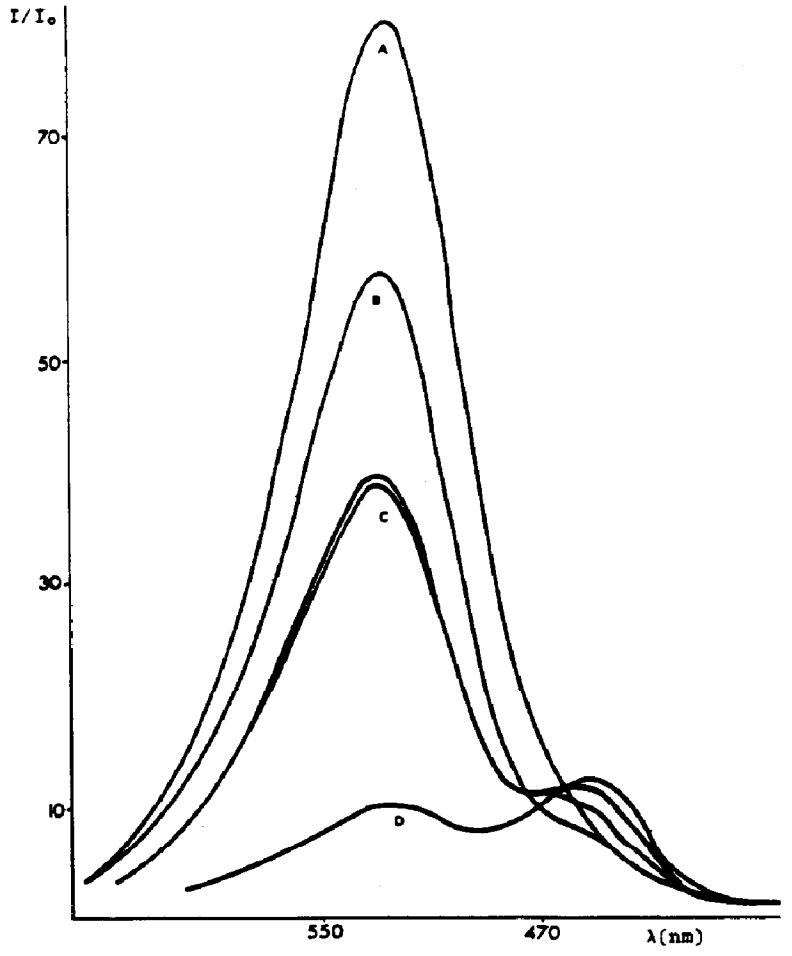

Fig. 8. Fluorescence spectra of harmalol solutions (concentration $C = 5 \times 10^{-5}$ M; λ_{ex} = 270 nm): spectrum A, pH 10.08; spectrum B, pH 11.12; spectrum C, pH 12.04; spectrum D, pH 13.00.

ing to the anion. No fluorescence of neutral harmalol could be detected in aqueous solutions. The corresponding peak would appear at about 377 nm by analogy with harmaline.

The fluorescence maxima for solutions of each species are summarized in Table 1. The pK_a values for the equilibria in aqueous solutions at 25 °C between the various species of harmaline and harmalol in the lowest excited electronic singlet state ($pK_a(S_1)$) are listed in Table 2.

4. Conclusion

From the pK_a data compiled in Table 2 it can be concluded that the hydroxy group of harmalol is more acidic and the pyridinic nitrogen atom

is more basic in the excited singlet S_1 than in the ground states. This behaviour is similar to that observed for other nitrogen heterocycles [11 - 14].

It can also be noted that the equilibrium constants undergo marked changes on excitation. Thus, the equilibrium between the HOB and the $^{-}OBH^{+}$ forms of harmalol, where both forms are present in measurable quantities ($K_T(S_0) = 0.58$), shifts excitation in the direction of the ion dipolar species ($K_T(S_1) = 10^{7.54}$).

The absence of emission from the HOB species suggests that the tautomeric equilibrium is established in the excited state.

Acknowledgment

This work was supported by the Comision Asesora de Investigación Cientifica y Tecnica, Spain.

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