FLUORESCENCE AND PHOSPHORESCENCE OF HARMOL AND HARMALOL AT 77 K

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Summary

We have investigated the fluorescence and phosphorescence spectra of harmol and harmalol in the solid phase at 77 K. Three molecular species, namely, the cation, the neutral molecule and the molecular anion are characterized for harmol in both the fluorescence and phosphorescence spectra. We have also identified three molecular species, namely, the cation, the zwitterion and the molecular anion in the fluorescence spectra of harmalol but only the cation and the molecular anion in the phosphorescence spectra.

The excited state pK_a values in the first excited triplet state T_1 have been estimated from the Förster equation. It is found that in the first excited triplet state the ring nitrogen atom is more basic and the hydroxy group is more acidic than in the ground state.

1. Introduction

The acid–base behaviour of harmol (1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol) and of harmalol (4,9-dihydro-1-methyl-3*H*-pyrido[3,4-*b*]indol-7-ol) (Fig. 1) in aqueous solution at 25 °C has been previously studied in the ground and the first excited singlet states [1, 2]. These compounds are markedly fluorescent alkaloids, whose physiological and pharmacological properties, and particularly their hallucinogenic character, have been previously described [3 - 6].

The subject of this paper is to study the acid–base equilibria in the first excited triplet state through the phosphorescence properties of harmol and harmalol at 77 K. As an example the equilibria for harmol are shown in Fig. 1.

For these studies we used ethanolic solvents which allow suitable glasses to be obtained at 77 K. These solvents are especially suitable because

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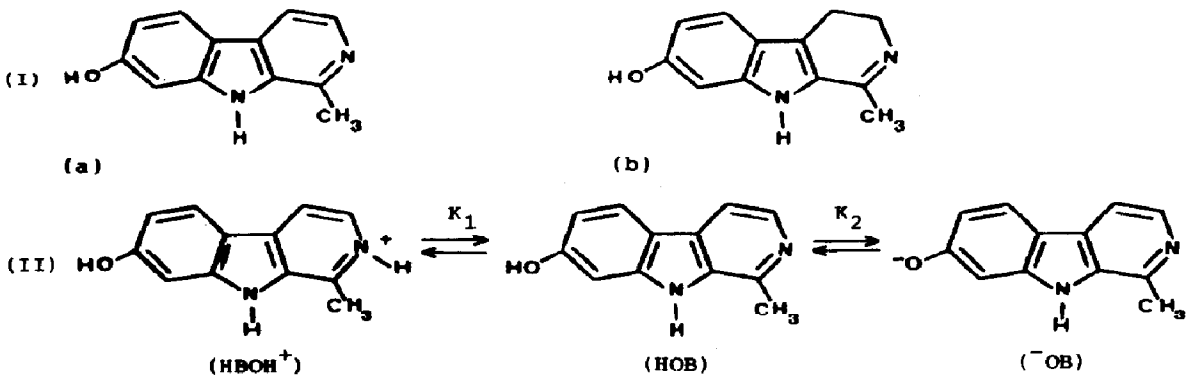


Fig. 1. (I) The molecular structures of (a) harmol and (b) harmalol. (II) The acid–base equilibria of harmol.

they allow the existence of all the molecular species which participate in the acid–base equilibria in the systems under study.

To identify the molecular species which are dominant in the solvent used, we first recorded the absorption spectra. To study the effect of phase rigidity and low temperature on the fluorescence spectra, we recorded the fluorescence spectra in the liquid phase at 25 °C and then the same spectra at 77 K. Finally, we obtained the phosphorescence spectra at 77 K in the ethanolic solvents.

By application of the Förster method [7], we obtained pK_a values in the first excited triplet state and compared them with the pK_a values in the first excited singlet state.

We found that the ring nitrogen atom is more basic and the hydroxy group is more acidic for the molecules studied in the first excited triplet state than in the ground state and this behaviour shows more resemblance to that of the first excited singlet state than to that of the ground state.

2. Experimental details

Harmol and the hydrochlorides of harmalol and harmol were purchased from the Sigma Chemical Company. The acidic solutions were prepared in an H_2SO_4–ethanol (1:100 by volume) mixture. The basic solutions were obtained in NaOH–ethanol mixtures.

Ethanol (Uvasol grade), H_2SO_4 and NaOH (both analytical grade) were obtained from Merck.

The concentrations of the samples were 10^{-4} M before freezing.

The absorption spectra at 25 °C were recorded using a Cary 219 spectrophotometer.

Uncorrected excitation, fluorescence and phosphorescence spectra were recorded using a Perkin–Elmer MPF-44A spectrofluorometer with low temperature and phosphorescence accessories. The spectra at 77 K were

recorded in transparent matrices produced by freezing the system to liquid nitrogen temperature.

The absorption and fluorescence spectra at 25 ± 0.1 °C were measured using thermostatted solutions.

3. Results and discussion

3.1. Electronic absorption spectra

The H_2SO_4–ethanol solutions of the hydrochlorides of harmol and harmalol show absorption spectra very similar to those recorded in aqueous media at pH values near pH 1 [1, 2] with small red shifts, indicating that the molecules studied are in the acid form $HOBH^+$ where HOB represents the neutral molecules of harmol and harmalol.

The absorption spectra of harmol and harmalol in NaOH–ethanol, with an NaOH concentration of 0.17 M or larger, are very similar to those of the anionic species of both molecules in aqueous solutions, providing evidence that in these solutions the ^-OB species is present.

The NaOH–ethanol solutions with an NaOH concentration lower than 0.17 M show absorptions corresponding to the neutral molecules of harmol and harmalol. These assignments are derived from the observation of the corresponding absorption spectra of the two molecules in aqueous solutions and the absorption spectra of harmol in ethanol and tetrahydrofuran solutions.

In contrast with the results in aqueous solution, we have not detected the absorption corresponding to the zwitterionic species in the molecules studied.

The first absorption band maxima for solutions of each species are listed in Table 1.

TABLE 1

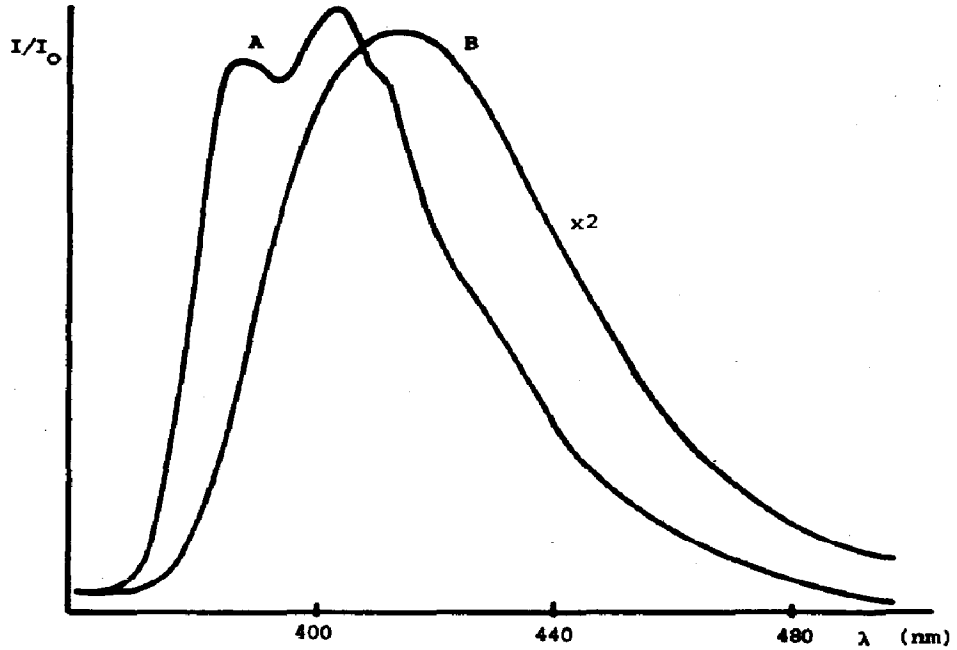
Frequencies of the first absorption band maxima

	$\bar{\nu}_{max}$ (cm^{-1})		
	$HOBH^+$	*HOB*	*^-OB*
Harmol	27777	29673	29411
Harmalol	26178	29154	27027

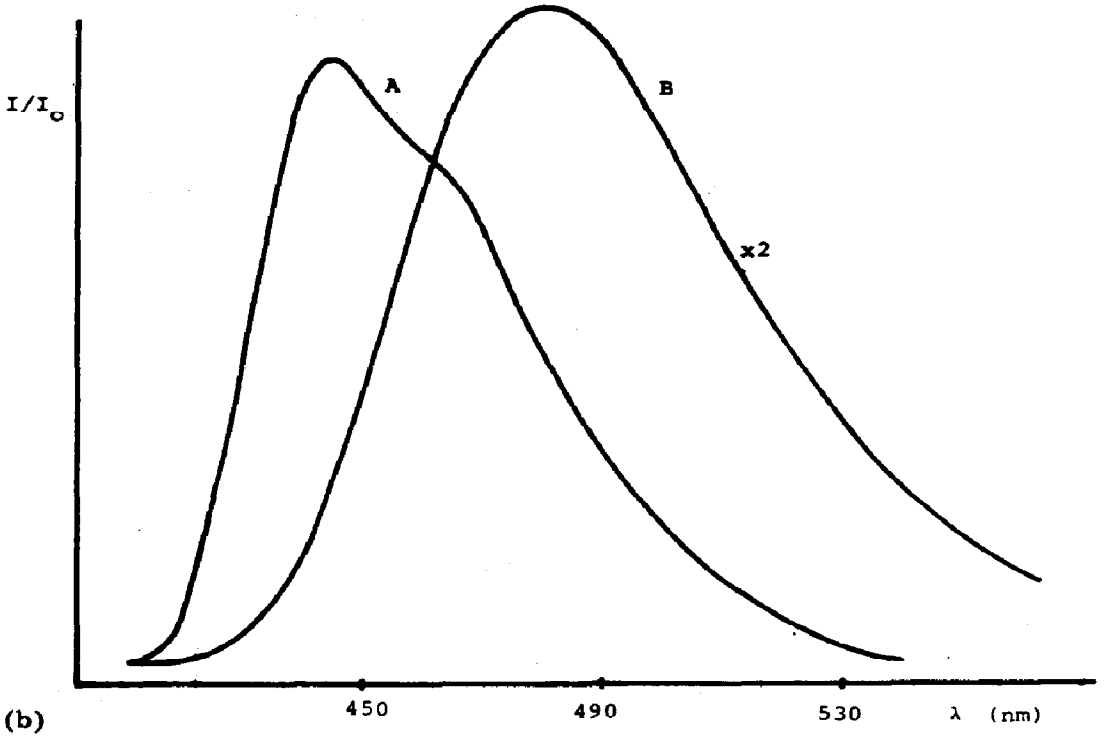
3.2. Fluorescence spectra

The fluorescence spectra of harmol and harmalol in H_2SO_4–ethanol mixtures show the emission corresponding to the cationic $HOBH^+$ species in both compounds (Fig. 2).

At 25 °C the fluorescence spectra are similar to those recorded in aqueous solution [1, 2]. The modification of the molecular environment at



(a)



(b)

Fig. 2. Fluorescence spectra in H_2SO_4–ethanol solutions for (a) harmol (λ_{ex} = 320 nm) and (b) harmalol (λ_{ex} = 380 nm): curves A, at 77 K; curves B, at 298 K.

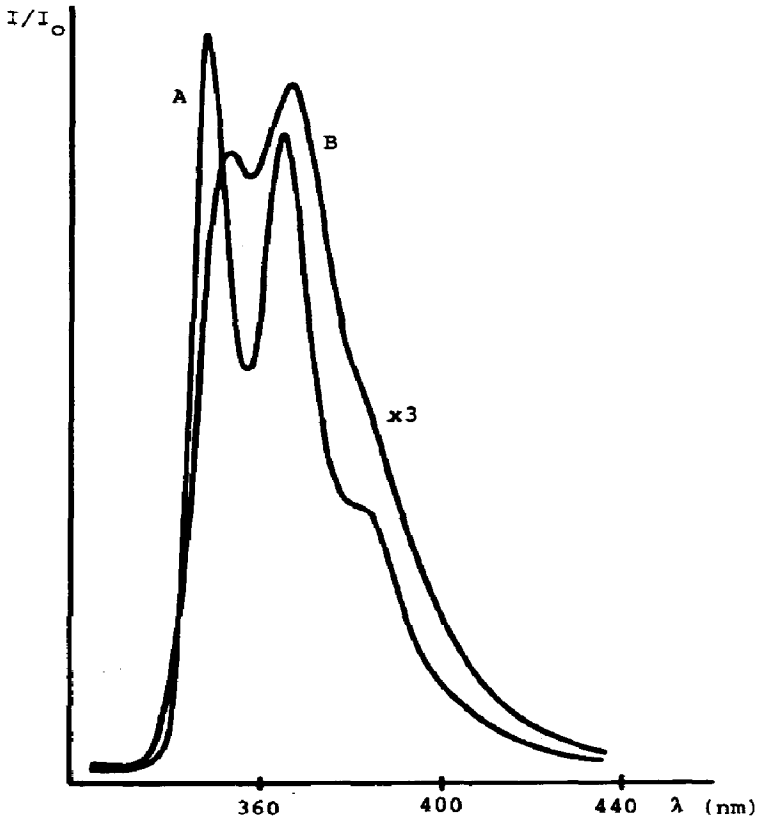


Fig. 3. Fluorescence spectrum of harmol in ethanol (λ_{ex} = 303 nm): curve A, at 77 K; curve B, at 298 K.

77 K is the origin of the enhanced resolution and the blue shift in the solid phase at 77 K relative to that in the liquid phase at 25 °C [8]. However, the excitation spectra are found to be the same at both temperatures.

The fluorescence spectrum of harmol in ethanol shows the emission corresponding to the neutral form HOB. The excitation spectrum is similar to the absorption spectrum of this species. An improved resolution is found at 77 K together with a slight blue shift (Fig. 3).

Harmol in NaOH–ethanol solutions with an NaOH concentration of 0.17 M and larger shows emission spectra of the anionic $^-$OB form. These spectra show a blue shift and a higher resolution at 77 K than at 25 °C. At both temperatures the excitation spectra are the same, indicating that the emitting species is the same. To confirm that the emitting species is $^-$OB, we carried out experiments in the adsorbed phase following the procedure described earlier [9] which takes advantage of the well-known fact that ionic molecular species adsorb strongly on cellulose surfaces. The fluorescence spectra recorded under these conditions are similar to the emission spectra observed at 25 °C. Hence, they can correspond to fluorescence from a charged species. The basicity of the medium ensures that this species is the $^-$OB form (Fig. 4).

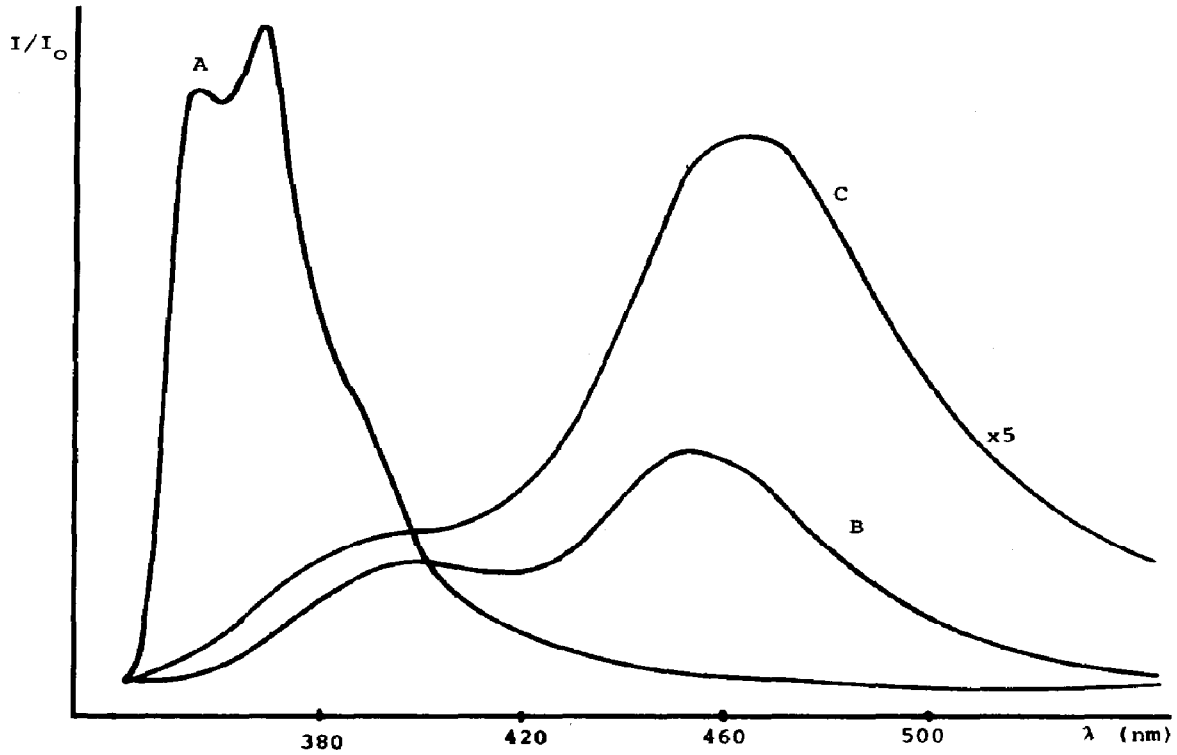


Fig. 4. Fluorescence spectra of harmol in NaOH–ethanol solutions ([NaOH] = 0.34 M; λ_{ex} = 320 nm): curve A, at 77 K; curve B, at 298 K; curve C, in dispersed phase at 298 K.

Harmalol in NaOH–ethanol solutions with a concentration lower than 0.17 M at 25 °C shows two emission bands (Fig. 5). By increasing the basicity, it is found that the intensity of the more energetic band increases in relation to the less energetic emission band. By comparing them with the fluorescence spectra in aqueous solution [2], we conclude that these emissions correspond to the anionic form $^-$OB and the zwitterionic form $^-$OBH$^+$ respectively. The same behaviour is found in the adsorbed phase. Therefore the emitting systems must be these ionic molecular species. However, the increase in the intensity of the more energetic emission band with increasing basicity of the solvent is in good agreement with the increasing concentration of the more basic $^-$OB species. Moreover, using λ_{ex} = 400 nm, only the less energetic emission corresponding to $^-$OBH$^+$, which is the only species that can be excited, is found (in aqueous solution, $\lambda_{ab}(^-OBH^+)$ = 431 nm).

It should be noted that we have not obtained any solution where the only emitting species is neutral harmalol HOB, as it was in the case of harmol in ethanol solution.

At 77 K the behaviour observed for harmalol in NaOH–ethanol solutions is the same as that at 25 °C, but the emission bands of the $^-$OB and $^-$OBH$^+$ species show better resolution and blue shifts relative to those in the liquid phase at 25 °C.

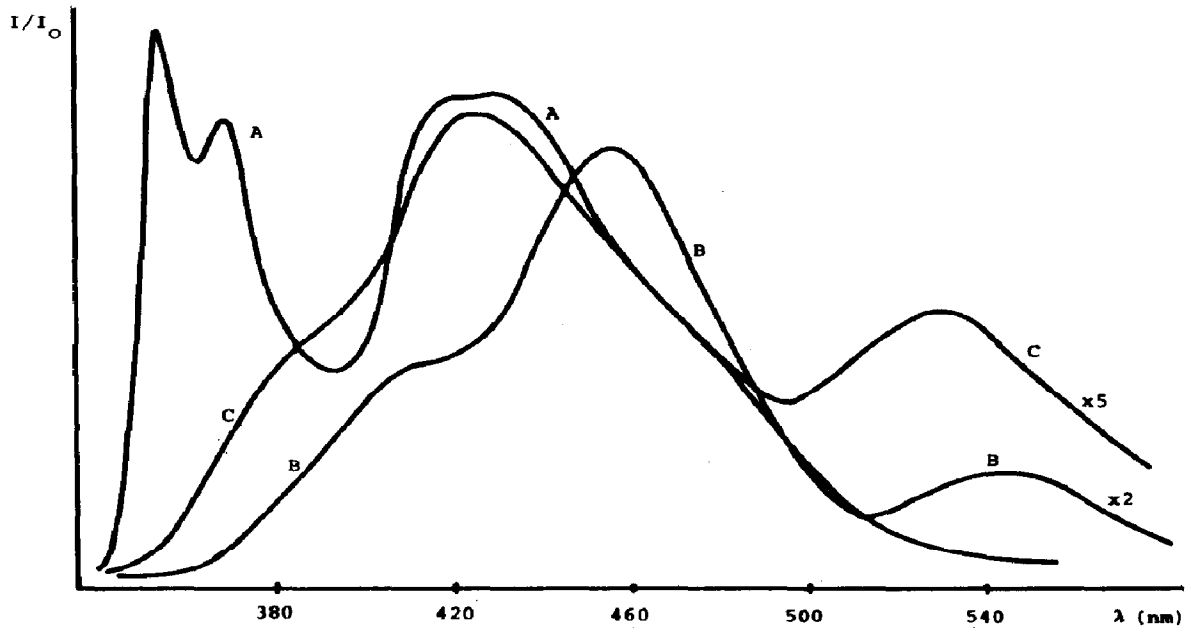


Fig. 5. Fluorescence spectra of harmalol in NaOH–ethanol solutions ([NaOH] = 0.26 M; λ_{ex} = 330 nm): curve A, at 77 K; curve B, at 298 K; curve C, in dispersed phase at 298 K.

TABLE 2

Frequencies of the lowest fluorescence maxima

	$\bar{\nu}_{0-0}$ (cm^{-1})							
	HBOH$^+$		*HOB*		*$^-$OBH$^+$*		*$^-$OB*	
	77 K	*298 K*	*77K*	*298 K*	*77 K*	*298 K*	*77 K*	*298 K*
Harmol	25773	23980	28818	28409.0			28089	25000
Harmalol	22573	20920	28735[a]	26666[a]	23255	18281	28328	24390

[a]By analogy with harmaline.

As has been suggested elsewhere [8, 9], the modification of the molecular environment in the solid phase at 77 K relative to that in the liquid phase at 25 °C causes the differences in the fluorescence spectra.

The solute–solvent interactions of charged species can be strong and will result in a greater modification of their emissive behaviour compared with that of the neutral species with changes in temperature and phase rigidity.

The maxima corresponding to 0–0 transitions for the molecules studied are listed in Table 2 together with those reported for the spectra at 25 °C.

3.3. Phosphorescence spectra

The phosphorescence spectra in the H_2SO_4–ethanol mixture at 77 K show an emission corresponding to the cationic $HOBH^+$ species for both

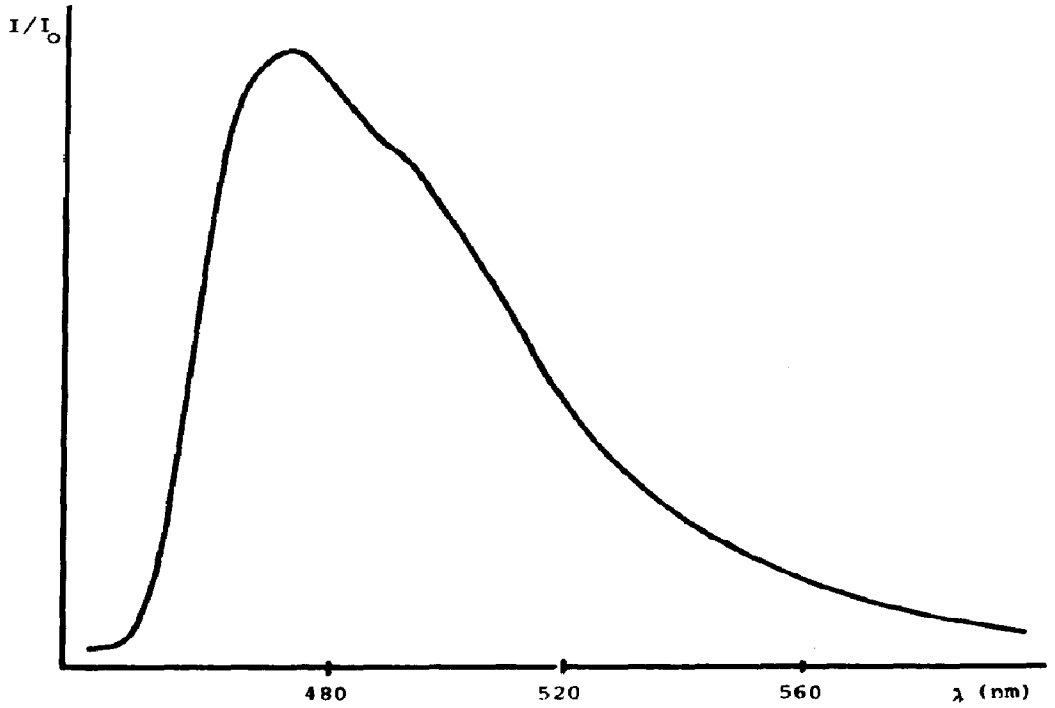


Fig. 6. Phosphorescence spectrum of harmalol in H_2SO_4–ethanol solution at 77 K (λ_{ex} = 380 nm).

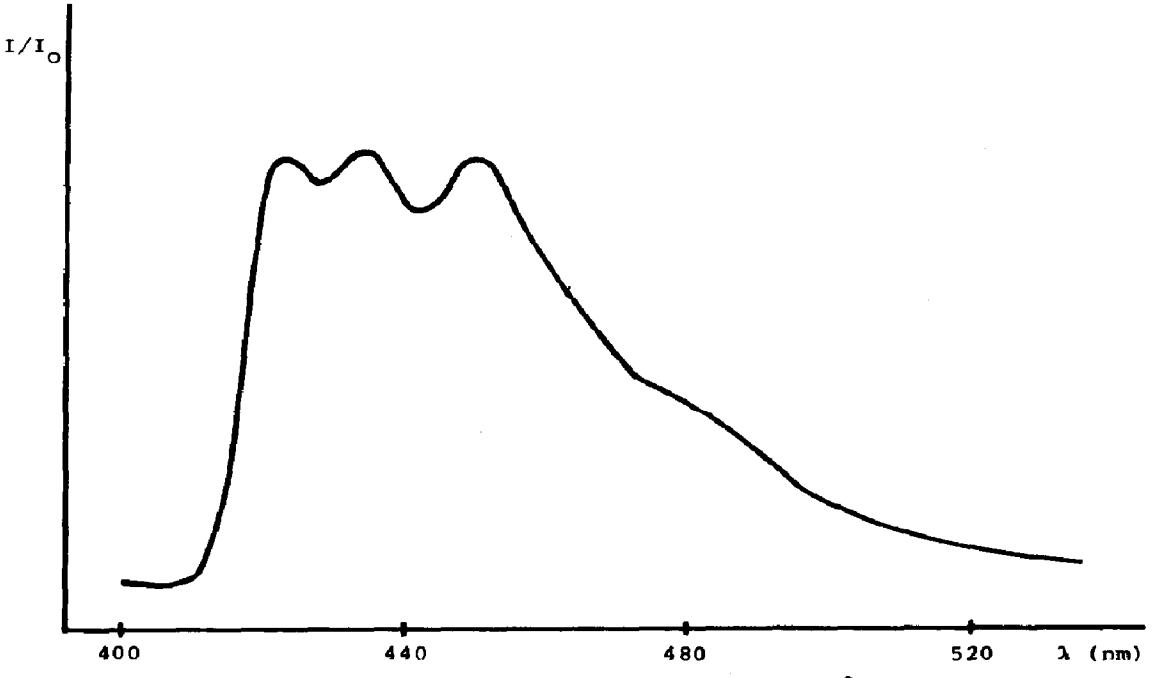


Fig. 7. Phosphorescence spectrum of harmol in ethanol at 77 K (λ_{ex} = 303 nm).

harmol and harmalol. The phosphorescence spectrum of harmalol is shown in Fig. 6 as an example.

The phosphorescence spectrum recorded for harmol in ethanol solution corresponds to emission from the neutral species (Fig. 7). The excitation spectrum is similar to the absorption spectrum of this molecular species.

In NaOH–ethanol mixtures, with an NaOH concentration of 0.17 M and larger, the phosphorescence spectra recorded correspond to emission from the $^{-}$OB species for harmol and harmalol. As an example, the phosphorescence spectrum of harmol is shown in Fig. 8. The excitation spectra are similar to those recorded in fluorescence for the anionic species.

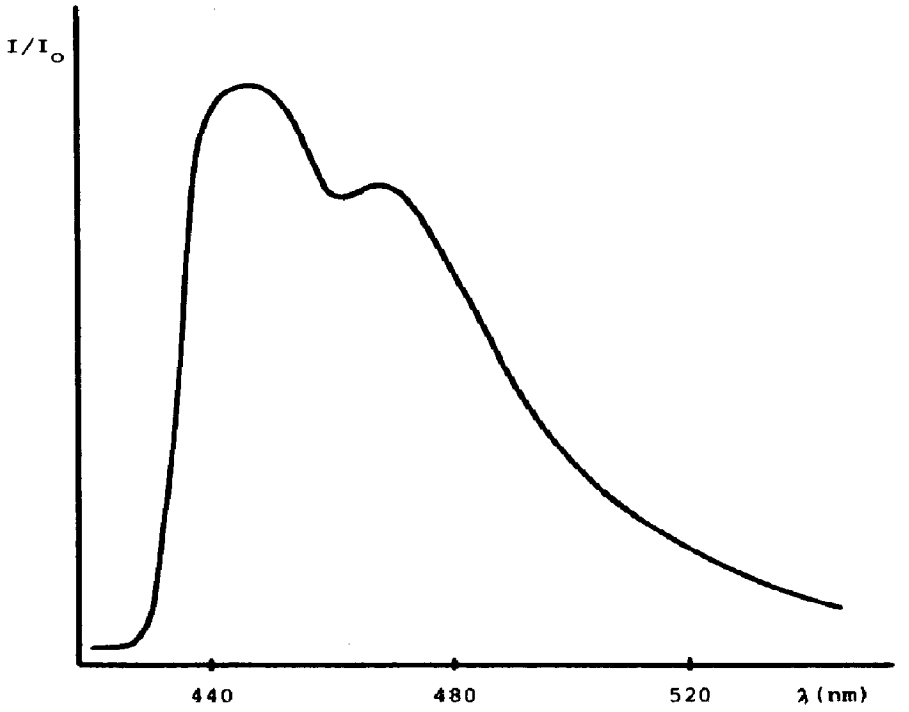


Fig. 8. Phosphorescence spectrum of harmol in NaOH–ethanol solution ([NaOH] = 0.34 M; λ_{ex} = 320 nm).

The main difference in relation to the fluorescence spectra is that harmalol does not show the phosphorescent emission corresponding to the zwitterionic species. However, neutral harmalol was not available, and therefore we could not obtain the emission corresponding to HOB. As in aqueous solution [2], we may assume that the emission of the neutral species of harmalol will be similar to that of the neutral harmaline which has an OCH_3 group in place of the OH group of the harmalol.

The frequencies of the 0–0 transitions for the molecules studied are summarized in Table 3.

TABLE 3

Frequencies of the lowest phosphorescence maxima at 77 K

	$\bar{\nu}_{max}$ (cm^{-1})		
	HBOH$^+$	*HOB*	$^-$*OB*
Harmol	21008	23640	22371
Harmalol	21141	24390[a]	22522

[a]By analogy with harmaline.

3.4. Basicity of the first excited triplet state T_1

The pK_a values for the first excited triplet state T_1 were estimated by applying the Förster equation:

$$pK_a(T_1) = pK_a(S_0) + \frac{0.625}{T}\Delta\bar{\nu}_{0-0}$$

where T is the absolute temperature and $\Delta\bar{\nu}_{0-0}$ (cm^{-1}) is the difference between the 0–0 frequencies of the phosphorescence spectra of the basic and acidic conjugated species of the acid–base equilibria.

The energy differences between the first excited states derived from the 0–0 transitions of the fluorescence and phosphorescence spectra at 77 K are summarized in Table 4. It is found that the T_1 states are about 17 - 69 kJ mol^{-1} less energetic than the S_1 states.

TABLE 4

Energy differences between the lowest electronic states

	$\Delta E(S_0–S_1)$ (kJ mol^{-1})			$\Delta E(S_0–T_1)$ (kJ mol^{-1})			$\Delta E(S_1–T_1)$ (kJ mol^{-1})		
	HOBH$^+$	*HOB*	$^-$*OB*	*HOBH*$^+$	*HOB*	$^-$*OB*	*HOBH*$^+$	*HOB*	$^-$*OB*
Harmol	308.4	344.8	336.1	251.4	282.9	267.7	57.0	61.9	68.4
Harmalol	270.1	343.8	338.9	252.9	291.8	269.5	17.1	52.0	69.4

The disparity in the $S_0–S_1$ energy differences for the $HOBH^+$ species of harmol and harmalol compared with the other comparative data given may reflect the involvement of the extra double bond in harmol, as compared wih harmalol, in delocalizing the positive charge.

Estimated $pK_a(T_1)$ and $pK_a(S_1)$ values are listed in Table 5. It can be seen that the ring nitrogen atom is more basic and the hydroxy group is more acidic for harmol and harmalol in the first excited triplet state than in the ground state, and that the basicities of T_1 are nearer to those of the S_1 state than to those of the ground state. These results are in contrast with other results reported in the literature [10 - 12] for similarly related compounds, for which the estimated basicities of the first excited triplet states

TABLE 5

Excited state pK_a values estimated from the emission data at 77 K

	$pK_a(S_0)$[a]	$pK_a(S_1)$	$pK_a(T_1)$
Harmol			
pK_1	8.0	14.4	13.5
pK_2	9.4	7.9	6.7
Harmalol			
pK_1	10.0	22.6	16.8
pK_2	10.1	9.0	6.2

[a]See refs. 1 and 2.

are nearer to those of the ground state than to those of the first excited singlet states. However, our results agree with those found for other β-carbolines [9].

References

1 F. Tomás, I. Zabala and A. Olba, *J. Photochem.*, *31* (1985) 253.
2 F. Tomás, I. Zabala and A. Olba, *J. Photochem.*, *26* (1984) 285.
3 T. Robinson, *The Biochemistry of Alkaloids*, Springer, Berlin, 1968, p. 132.
4 N. S. Buchholtz and W. O. Boggan, *Biochem. Pharmacol.*, *26* (1977) 1991.
5 C. Mitra and S. R. Guha, *Biochem. Pharmacol.*, *28* (1979) 1137.
6 S. G. Hadley and A. S. Muraki, *J. Forensic Sci.*, *19* (1974) 657.
7 T. Förster, *Z. Electrochem.*, *54* (1950) 42.
8 S. Suzuki, T. Fujii and K. Sato, *Bull. Chem. Soc. Jpn.*, *45* (1972) 1937.
9 J. Romero, I. Zabala, J. M. Aullό, F. Tomás and A. Olba, *J. Photochem.*, *29* (1985) 405.
10 A. Weller, *Prog. React. Kinet.*, *1* (1961) 199.
11 I. Janic, P. Ristic and A. Kawski, *Bull. Acad. Pol. Sci., Ser. Sci., Math., Astron. Phys.*, *22* (1974) 1857.
12 G. Gennari, G. Galiazzo and G. Cauzzo, *Gazz. Chim. Ital.*, *104* (1974) 535.