FLUORESCENCE AND PHOSPHORESCENCE OF AROMATIC β-CARBOLINES ON DISPERSED SOLID PHASE AT 77 K AND ROOM TEMPERATURES

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Summary

The fluorescence and phosphorescence spectra of the alkaloids norharman, harman and harmine and their hydrochlorides were studied at 77 K. The spectra of the basic molecules dissolved in ether–isopentane–ethanol show a well-resolved vibrational structure, whereas the spectra of hydrochlorides dissolved in an H_2SO_4–ethanol mixture are poorly resolved and in the case of fluorescence spectra some blue shift is noticed for these acid molecules. Fluorescence and phosphorescence spectra have also been recorded at room temperature for acid molecules adsorbed on cellulose, but they have a poorer intensity and worse resolution than those recorded at 77 K.

The pK_a values for the acid–base equilibrium on the first excited triplet T_1 were derived using the Förster–Weller relation. They were found to lie near the values corresponding to the first excited singlet S_1 rather than the values found for the ground state S_0.

1. Introduction

The fluorescence of some aromatic β-carbolines in aqueous solutions at 25 °C has been studied for a wide range of pH values [1]. Similar studies have been carried out for partially hydrogenated β-carbolines [2]. The pK_a values for the acid–base equilibrium of Fig. 1 on the first excited singlet state have been derived from these studies. It is well known that the fluorescence spectrum is strongly dependent on both the temperature and the phase of the medium in which the molecules are dissolved or dispersed. In this sense we are now concerned with the investigation of the emission spectra of aromatic β-carbolines in both the acid (BH^+) and basic (B) forms in solid phases at 77 K.

The pK_a values for the acid–base equilibria on the first triplet state can be found from the phosphorescence spectra of the molecular species B
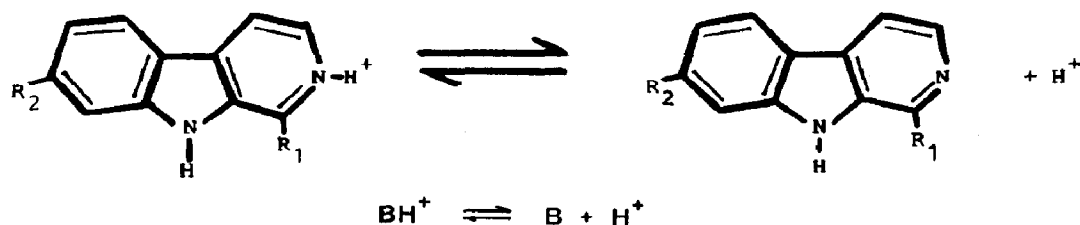
Fig. 1. Acid–base equilibrium for β-carbolines: $R_1 = R_2 = H$, norharman; $R_1 = CH_3$, $R_2 = H$, harman; $R_1 = CH_3$, $R_2 = CH_3O$, harmine.

and BH^+ by using the Förster–Weller method [3]. The results obtained in this way can only be considered as an estimate if the phosphorescence spectra are recorded in a solid matrix at 77 K and the acid–base equilibrium can be achieved in liquid protic media. However, the approximate values of pK_a derived for the first triplet (T_1) by means of the Förster–Weller method can indicate whether the basicity of the aromatic β-carbolines in this state lies near the basicity of the ground state (S_0) or that of the first excited singlet (S_1).

Fluorescence and phosphorescence studies were performed for norharman (β-carboline, i.e. $9H$-pyrido[3,4-b]indole), harman (1-methyl-β-carboline) and harmine (1-methyl-7-methoxy-β-carboline).

2. Experimental details

2.1. Materials

Norharman and harmine hydrochlorides and basic norharman were purchased from the Sigma Chemical Co., and harman hydrochloride, harmine and harman were obtained from Fluka A.G. Buchs S.S.

The basic species of these molecules were dissolved on 5:5:2 ether–isopentane–ethanol (EPA). The hydrochloride solutions were prepared using a 1:100 (by volume) H_2SO_4–ethanol mixture. Analytical grade sulphuric acid obtained from Merck was used and all the solvents were Uvasol grade from Merck. The solute concentration for all the solutions was 10^{-4} M.

At room temperature the H_2SO_4–ethanol hydrochloride solutions show absorption spectra very similar to those recorded on aqueous media at pH values near pH 1 [1] with small hypsochromic displacements. This behaviour shows that the aromatic β-carbolines are in the BH^+ form in H_2SO_4–ethanol solutions.

The spectra recorded for EPA solutions of the B species appear very similar to the spectra obtained for aqueous solutions at pH > 10 [1]. We therefore conclude that the molecular species present in EPA solutions is the basic form B of the aromatic β-carbolines.

2.2. *Apparatus*

Uncorrected excitation, fluorescence and phosphorescence spectra were recorded using a Perkin–Elmer MPF-44A spectrofluorometer equipped with a phosphorescence accessory. The spectra at 77 K were recorded in transparent matrices produced by freezing the system to liquid nitrogen temperature. The absorption spectra at room temperature were recorded using a Cary 219 spectrophotometer.

2.3. *Emission from adsorbed phase*

Ionic molecular species are adsorbed well by cellulose surfaces. The surfaces on which the β-carboline hydrochlorides were adsorbed were prepared as follows. A solution containing 0.5×10^{-3} M β-carboline hydrochloride is adjusted to pH < 1. A strip of clean filter paper is immersed in this solution for a few minutes and is then dried over a dessicant. We found that increasing the ionic strength of the solution, by adding NaCl for example, enhanced the adsorption.

The dried strip of paper was placed in the spectrofluorometer cell and the fluorescence spectrum was recorded. The phosphorescence and delayed fluorescence, if any, can be detected by adding a chopper to the cell compartment.

3. Results and discussion

3.1. *Fluorescence at 77 K*

The excitation and fluorescence spectra of norharman are shown in Figs. 2 and 3 as an example. The maxima corresponding to 0–0 transitions for all the molecules studied are summarized in Table 1 together with those reported for the spectra at 25 °C.

The fluorescence spectra of all three BH^{+} species show a poorly resolved structure of two maxima and one shoulder at lower energies. It can also be seen from Table 1 that in all cases the spectra are blue shifted with respect to the fluorescence at room temperature. However, we have shown that the excitation spectrum at 77 K is the same as that recorded at room

TABLE 1

Frequencies of the lowest fluorescence maxima

	$\bar{\nu}_{0-0}(\mathrm{BH}^{+})$ (cm^{-1})		$\bar{\nu}_{0-0}(\mathrm{B})$ (cm^{-1})	
	77 K	*298 K*	*77 K*	*298 K*
Norharman	24750	22727	27930	27700
Harman	25380	23255	28090	27932
Harmine	26040	24096	29000	28409
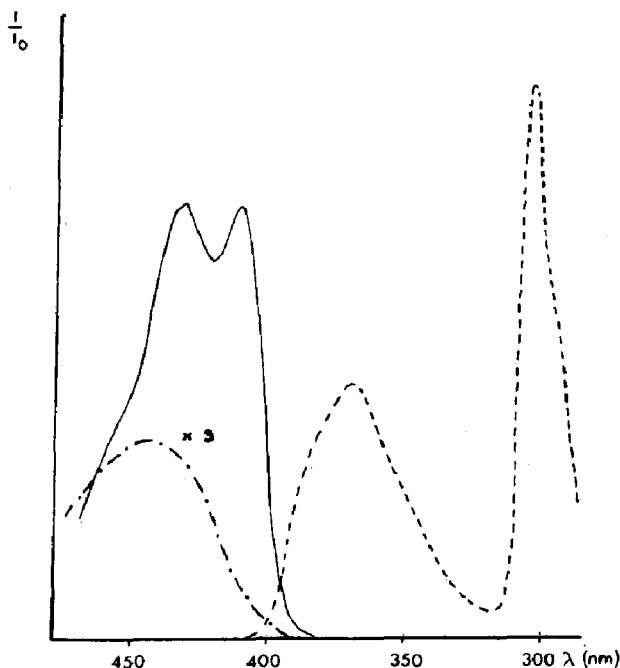
Fig. 2. Fluorescence spectrum (———) (λ_{ex} = 305 nm) and excitation spectrum (— — —) (λ_{em} = 425 nm) of norharman hydrochloride in ethanol–H_2SO_4 at 77 K; fluorescence spectrum (— · —) of norharman hydrochloride in the dispersed phase at room temperature (λ_{ex} = 305 nm).

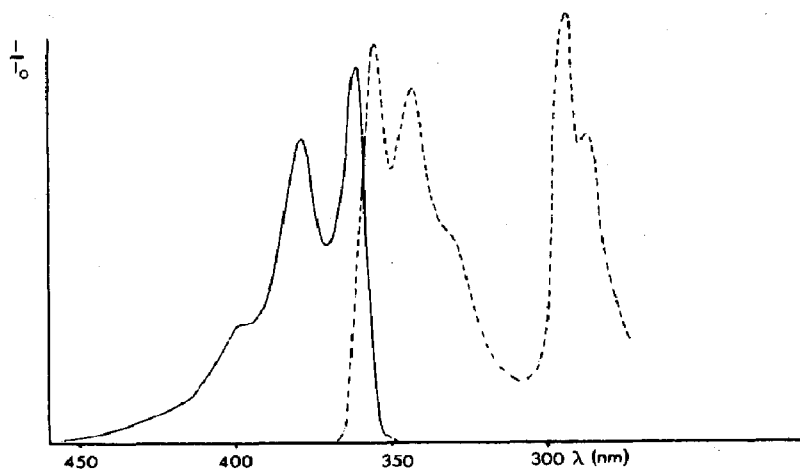Fig. 3. Fluorescence spectrum (———) (λ_{ex} = 287 nm) and excitation spectrum (— — —) (λ_{em} = 377 nm) of norharman (B) in EPA at 77 K.

temperature for all three BH^+ species with improved resolution owing to the low temperature.

The resolution of the spectra of the B species is better at 77 K than at room temperature, although the emission maxima lie very close to the

maxima recorded at room temperature in the liquid phase. It can also be seen that the symmetry between the fluorescence and excitation spectra is quite good. In the case of harmine the 0–0 excitation and fluorescence bands almost coincide.

The most noticeable differences between the fluorescence spectra at 77 K and at room temperature are those reported for the acid forms, *i.e.* the blue shift and the resolution of several peaks. This type of modification of the emission behaviour has been assigned to excimer formation [4], as initially identified by Förster [5] and Förster and Kasper [6], and is exhibited by a large number of aromatic compounds [7, 8]. However, the dimer bands are only observed at moderately high concentrations of the solute, and are not detected at concentrations below 10^{-5} M. The energy difference between the maxima of the monomer and the dimer is quite high, of the order of 4000 - 5000 cm^{-1}. In our work we have observed that the fluorescence behaviour of the BH^+ species is independent of the concentration of the solution, because the spectra are unchanged at concentrations as low as 10^{-6} M. In addition, the excitation spectra are the same for all the fluorescence maxima.

Another possible reason for these differences is the existence of two electronic states of the same multiplicity with almost equal energy. When such a situation occurs at room temperature in the liquid phase, internal conversion to the lower state occurs immediately after Franck–Condon excitation, with consequent fluorescence emission from that state. Internal conversion in the solid phase at low temperatures will be restricted by the difficulty of molecular relaxation, and fluorescence from the Franck–Condon excited state will occur so that two emission transitions will be observed. Therefore there will be a shift in the fluorescence spectrum towards shorter wavelengths on reducing the temperature, whereas the excitation spectrum will remain constant as is observed in this work.

However, this explanation is unlikely to be correct in our case because the pyridinic nitrogen atom is attached to a hydrogen atom in the BH^+ species, and thus the formation of the low n, π^* excited states that generate the situation described above is impeded. In addition molecular orbital calculations for the BH^+ species [9] have shown that the energy gap between the first and second excited singlet states is significant, and this also makes the behaviour described above unlikely.

We believe that the modifications of the molecular environment in the solid phase at 77 K relative to that in the liquid phase at room temperature are the origin of the differences in the fluorescence spectra as has also been suggested elsewhere [10]. Thus the rigidity of the vitreous phase retards the free relaxation of the molecular environment. Since the BH^+ species is charged, the solute–solvent interactions are strong and influence the spectroscopic behaviour. Because the relaxation is restricted the solvated form at 77 K is more energetic than that at room temperature in the liquid phase where the solvent rearrangement is free, and consequently the fluorescence at 77 K should occur at shorter wavelengths. The improved resolution of

the fluorescence spectrum is due to the phase rigidity and the low temperature.

The fluorescence behaviour of the basic species at 77 K does not differ significantly from that observed at room temperature. Obviously the resolution is improved but the displacements of the maxima are insignificant. We conclude that neither the rigidity of the vitreous EPA phase nor the temperature have a significant effect on the solvated basic molecules.

The differences observed in the fluorescence behaviour at 77 K and room temperature must be kept in mind during the discussion of the phosphorescence emission of these molecules.

3.2. Phosphorescence at 77 K

The phosphorescence spectra of the acid and basic forms of norharman are shown in Figs. 4 and 5 as an example. The spectra of the acid forms are less well resolved than those of the basic forms and consequently the location of the 0–0 band is not clearly established. The phosphorescence of the acid species occurs at lower frequencies than that of the basic species. The 0–0 band can be located more easily in the basic species. A summary of the wavelengths of these maxima is shown in Table 2.

It is well known that the better resolution of the spectra of the basic species is due to the fact that, since it is neutral, its lower electronic states are similar and the positions of nuclear equilibrium do not change significantly on excitation. Consequently the overlap between the lower vibrational levels of the excited and ground states will be important, thus implying that 0–0, 0–1 and 0–2 will be the most intense components. The acid species are electrically charged and will show marked differences between the electronic states as a result of solvation. This produces changes in the equilibrium geometry for the various electronic states, leading to less effective overlapping between the lower vibrational levels. This in turn leads to low intensity bands for the less energetic transitions, and consequently the phosphorescence spectrum is observed as a broad poorly resolved band.
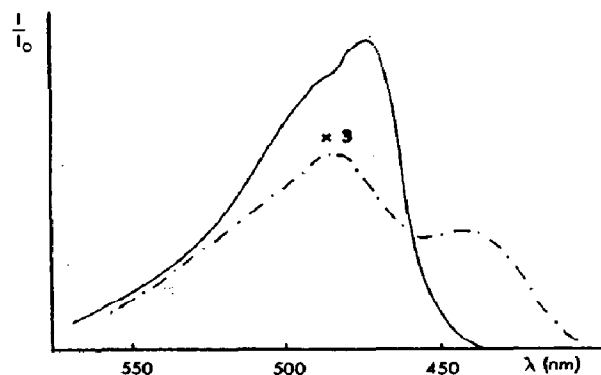

Fig. 4. Phosphorescence spectra of norharman hydrochloride (λ_{ex} = 370 nm) in ethanol–H_2SO_4 at 77 K (———) and in the dispersed phase at room temperature (— · —).
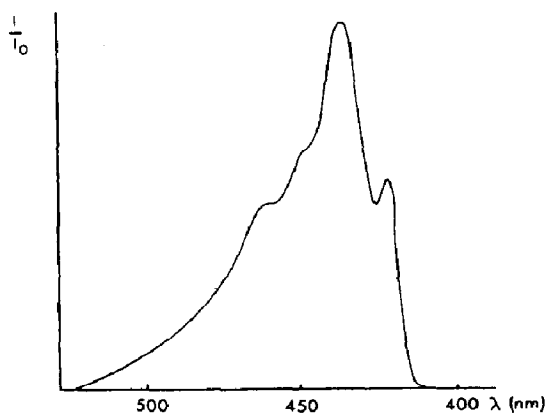
Fig. 5. Phosphorescence spectrum of norharman (B) in EPA at 77 K (λ_{ex} = 350 nm).

TABLE 2

Frequencies of the lowest phosphorescence maxima at 77 K

	$\bar{\nu}_{0\rightarrow0}(BH^+)$ (nm)	$\bar{\nu}_{0\rightarrow0}(B)$ (nm)	$\Delta\bar{\nu}_{0\rightarrow0}$ (nm)
Norharman	21097	23697	2600
Harman	21008	23585	2577
Harmine	21600	24500	2900

3.3. Phosphorescence at room temperature

As has been stated by Schulman and Walling [11], the phosphorescence of some compounds can be recorded at room temperature if they can be adsorbed on a dry inert surface. The non-radiative transitions are restricted in the adsorbed microcrystals and the radiative processes can be observed. However, not all substances can be adsorbed satisfactorily. More precisely we have observed that, whereas ionic substances, *i.e.* the acid β-carboline species BH⁺, are adsorbed well by cellulose, the basic species B is not adsorbed significantly or the adsorbed molecules, if any, do not exhibit phosphorescence.

The intensity of the phosphorescence registered is very small in each case, and it is necessary to increase the slit width of the spectrofluorometer with a corresponding loss of resolution. The emission spectrum of norharman hydrochloride is shown by the chain curve in Fig. 4. The spectra were recorded using the chopper in order to eliminate the fluorescence emission. The fluorescence spectra for the dispersed phase at room temperature were also recorded (see Fig. 2). A low intensity band at wavelengths corresponding to the fluorescence emission can be seen in the phosphorescence spectra.

Since the spectra were recorded with a time delay with respect to the excitation by using the chopper these bands are indeed fluorescence bands, *i.e.* the delayed fluorescence phenomenon is present in the adsorbed phase. The fluorescence observed in the adsorbed phase for the acid species is in good agreement with that observed in acid aqueous solutions. In addition the phosphorescence band is red shifted with respect to the band recorded at 77 K. This shift must be taken into account if the wavelength of the bands is used in the calculation of any properties.

Despite the poor resolution of the spectra, the advantage of this technique is that the phosphorescence spectra of ionic species can be recorded at room temperature, thus avoiding the problem of finding a suitable solvent for low temperature measurements. This is because the adsorbed molecules cannot achieve acid–base equilibrium in the excited state and hence we can be sure that the radiating molecule is the same as that which has been excited.

3.4. Basicity of the first triplet T_1

The energy differences between the first excited states are shown in Table 3. The values given were derived from the 0–0 transitions of the fluorescence and phosphorescence spectra at 77 K and must be considered only as estimates because of the inaccuracies in locating the maxima. It can be seen that the S_1 states are about $10 - 12$ kcal mol^{-1} more energetic than the T_1 states, and these differences can explain the delayed fluorescence observed in the experiments with adsorbed species at room temperature.

The pK_a values for T_1 can be found by using the Förster expression [3]

$$pK_{T_1} = pK_G + \frac{h}{RT \ln 10} \Delta \bar{\nu}_{0-0}$$

where $\Delta \bar{\nu}_{0-0}$ is the difference between the 0–0 band energies of the phosphorescence spectra of the basic species B and the protonated species BH$^+$ and T is the absolute temperature. The results obtained are affected by the inaccuracy of location of the 0–0 transitions and by the small variations in these transitions with temperature for the acid species BH$^+$.

TABLE 3

Energy differences between the lowest electronic states

| | $\Delta E(S_0-S_1)$ (kcal mol^{-1}) | | $\Delta E(S_0-T_1)$ (kcal mol^{-1}) | | $\Delta E(S_1-T_1)$ (kcal mol^{-1}) | |
	BH$^+$	B	BH$^+$	B	BH$^+$	B
Norharman	71.0	79.6	60.6	68.1	10.4	11.5
Harman	72.7	80.2	60.2	67.8	12.5	12.4
Harmine	74.8	83.0	62.1	70.4	12.7	12.6

TABLE 4

pK_a values estimated from emission data at 77 K

	pK_G[a]	pK_{S_1}	pK_{T_1}
Norharman	7.2	13.9	12.7
Harman	7.7	13.6	13.1
Harmine	8.0	14.2	14.1

[a] See ref. 1.

The estimated values for p$K_a(T_1)$ are given in Table 4. It can be seen that the basicity of the T_1 states is stronger than that of the corresponding ground states and is very near the basicity of the first excited singlets. The basicities of the triplet states of the β-carbolines are very similar to those of the first singlet states but are quite different from those of the ground states, in contrast with reports in the literature [12 - 14] establishing that the basicities of the T_1 states are nearer to those of the ground state than to those of the first singlet state.

Acknowledgment

References

1 F. Tomás Vert, I. Zabala Sánchez and A. Olba Torrent, *J. Photochem.*, *23* (1983) 355 - 368.
2 F. Tomás Vert, I. Zabala Sánchez and A. Olba Torrent, *J. Photochem.*, *26* (1984) 285.
3 T. Förster, *Z. Electrochem.*, *54* (1950) 42.
4 R. W. Chambers, T. Tajiwara and D. R. Kearns, *J. Phys. Chem.*, *78* (1974) 380.
5 T. Förster, *Z. Phys. Chem. N.F.*, *1* (1954) 275.
6 T. Förster and K. Kasper, *Z. Electrochem.*, *59* (1955) 976.
7 J. B. Birks and L. G. Christophorou, *Proc. R. Soc. London, Ser. A*, *274* (1963) 552 - 564.
8 J. B. Birks, *Prog. React. Kinet.*, *5* (1970) 273.
9 J. M. Aulló, *Doctoral Thesis*, University of Valencia, 1976.
10 S. Suzuki, T. Fujii and K. Sato, *Bull. Chem. Soc. Jpn.*, *45* (1972) 1937.
11 M. E. Schulman and C. Walling, *J. Phys. Chem.*, *77* (1973) 902.
12 A. Weller, *Prog. React. Kinet.*, *1* (1961) 199.
13 G. Gennari, G. Galiazzo and G. Cauzzo, *Gazz. Chim. Ital.*, *104* (1974) 535.
14 I. Janic, P. Ristic and A. Kawski, *Bull. Acad. Pol. Sci., Ser. Sci., Math., Astron. Phys.*, *22* (1974) 1857.