IONIZATION EQUILIBRIA AND ELECTRONIC SPECTROSCOPY OF 5-HYDROXYINDOLE-2-CARBOXYLIC ACID

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Summary

The pH and Hammett acidity dependences of the electronic absorption and fluorescence spectra of 5-hydroxyindole-2-carboxylic acid and its methyl ester have been studied. The results indicate that the prototropic species formed in the ground and excited singlet states are different, except for the trianion and the dication. The protonation and deprotonation constants have been evaluated and discussed.

1. Introduction

The combination of absorption and fluorescence spectra of aromatic acids and bases has revealed many facets of their protonation–deprotonation reactions in the ground and first electronically excited singlet states [1 - 5]. For example, the carboxylic group becomes more basic if $\pi \rightarrow \pi^*$ is the lowest energy transition and thus leads to red and blue shifts in the spectral characteristics on protonation and deprotonation respectively. Similarly, the —OH group, $>$NH group and $-NH_2$ group become stronger acids on excitation and thus lead to red shifts and blue shifts on deprotonation and protonation. Although the spectral behaviour of the molecules containing both electron donating and electron withdrawing groups remains qualitatively the same in the ground and the excited states, there are certain molecules that contain the above kind of functional groups where the excited state reactions are quite different from those in the ground state [6 - 10]. The gain in acidity of the electron donating group and the gain in basicity of the electron withdrawing group in the same electronically excited molecule is so great that the order of dissociation of the two groups is reversed with respect to the normal order observed in the ground state.

5-Hydroxyindole-2-carboxylic acid (HIC) is a multifunctional molecule. It contains three deprotonation centres (—OH, —COOH and $>$NH groups) and two protonation sites (the carbonyl group and the carbon atom of the ring at position 3). The possible protonation and deprotonation reactions of HIC have been discussed and the pK_a values in both the S_0 and the S_1 states

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have been calculated. These results are further supplemented by the similar reactions of the methyl ester of HIC. The schemes representing the various proton transfer reactions are given in Figs. 1 and 2.

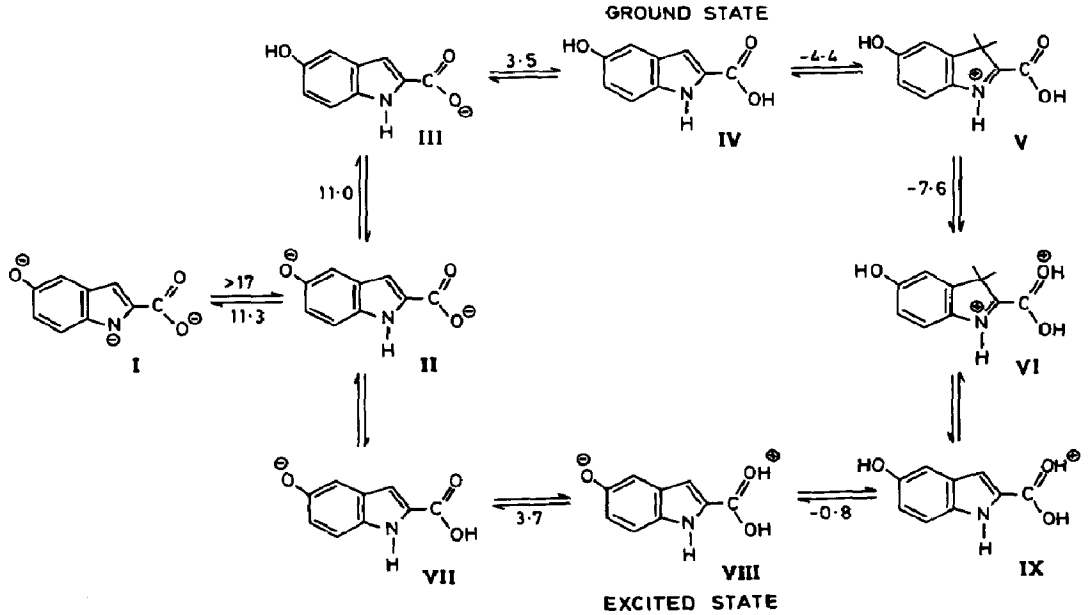

Fig. 1. Scheme representing the various proton transfer reactions of HIC.

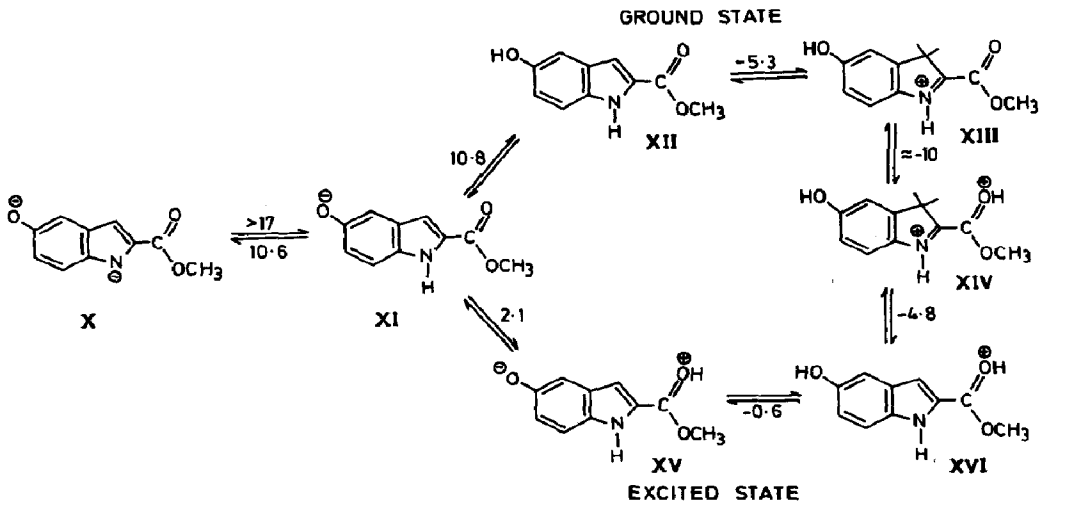

Fig. 2. Scheme representing the various proton transfer reactions of the methyl ester of HIC.

2. Method and materials

HIC was obtained from Aldrich Chemical Company and was further purified from ethanol. The methyl ester of HIC was prepared according to the procedure described in the literature [11]. The purity of the above compounds was checked by their melting points as well as the observation of the same fluorescence maxima when excited with different wavelengths. Analytical grade sulphuric acid, sodium hydroxide and Spectrograde methanol were used as received. Analytical grade cyclohexane, ether, acetonitrile and ethanol were further purified according to the procedure described in the literature [12]. Triply distilled water was used for aqueous solutions. The modified Hammett acidity scale [13] (below pH 1) and Yagil's basicity scale [14] (above pH 13) were used for the H_2SO_4–H_2O and the NaOH–H_2O mixtures respectively.

The absorption spectral measurements were carried out using a Shimadzu 190 spectrophotometer with a U135 recorder. The fluorescence measurements were carried out using a recording spectrofluorometer, fabricated in our laboratory; details are available elsewhere [15]. The quantum yields were calculated using quinine sulphate in 0.1 M H_2SO_4 as a standard [16] and the wavelength for excitation was 330 nm. Fluorometric titrations were carried out by exciting at the isosbestic wavelengths of the species involved in the various equilibria. The concentrations used were in the range 10^{-4} - 10^{-5} M except for low temperatures where the concentration was 10^{-3} M. The pH values of the solutions in the range 1 - 13 were measured using a model CL44A Toshniwal pH meter.

3. Results and discussion

3.1. Absorption spectra

The absorption maxima and log ϵ_{max} in various solvents and at different pH values of HIC and its methyl ester are listed in Tables 1 and 2. The absorption spectra of the various prototropic species of HIC are shown in Fig. 3. The scheme representing the various protonation–deprotonation reactions occurring in the ground and excited singlet states are shown in Figs. 1 and 2 for HIC and its methyl ester respectively. At H_- 16, the long wavelength band (about 350 nm) is very broad and the other band systems appear at 294, 286 and 244 nm. At pH 11 or below the above band systems are blue shifted (312 (shoulder (sh)), 290 and 212 nm), particularly the 350 and 244 nm bands. At pH 3 or below, a red shift is observed in the above maxima with values at 337 (sh), 295 and 213 nm. At below $H_0 - 3$ there is a pronounced red shift in the bands at 295 and 213 nm and the long wavelength shoulder at 337 nm becomes red shifted but no definite shoulder or maximum is observed, although the absorbance is increased at $\lambda \geqslant 337$ nm. Furthermore, the intensity of the 213 nm band system in particular decreases sharply and develops into two bands at 225 nm (sh) and 196 nm.

TABLE 1

Absorption maxima and log ϵ_{max} of 5-hydroxyindole-2-carboxylic acid in different solvents and at various pH values

Solvent or species	λ_{max} (nm) (log ϵ_{max})			
Ether	225 (4.39)	283 (4.07)	295 (4.05)	325 (3.36)
Acetonitrile	210 (4.69)	283 (4.36)	295 (4.37)	325 (3.79)
Methanol	208 (4.67)	283 (4.31)	292 (4.32)	312 —
Ethanol	215 (4.55)	288 (4.33)	294 (4.33)	325 (3.72)
Neutral (pH 1)	204 (4.38)	212.5 (4.35)	295 (4.26)	337 sh —
Monocation (H_0 — 5)	196 (4.54)	225	318 (4.10)	
Dication (H_0 —10)	212 (4.32)	232 (4.20)	331 (4.3)	350 sh
Monoanion (pH 7)	212 (4.45)	282	290 (4.19)	312
Dianion (pH 13)	224 (4.32)	286 (4.20)	294 (4.17)	350 (4.32)

Temperature, 298 K.
sh, shoulder.

TABLE 2

Absorption maxima and log ϵ_{max} of the methyl ester of 5-hydroxyindole-2-carboxylic acid in different solvents and at various pH values

Solvent or species	λ_{max} (nm) (log ϵ_{max})					
Cyclohexane	190	256	267	287	302	317
Ether	202	260	271		307.5	
Acetonitrile	209 (4.35)	215 (4.34)	287 (4.24)	295 (4.28)		329 (3.78)
Methanol	208 (4.28)	215 (4.25)	287 —	296 (4.21)		329 (3.59)
Neutral (pH 1)	202 (4.83)	212 (4.81)	—	295 (4.75)		325
Monocation (H_0 —5)	192 (4.7)	200	262 (3.92)	—		330
Dication (H_0 —10)	200 (4.75)	236 (4.33)	—	331 (4.55)		362 —
Monoanion (pH 7)	212 (4.97)		287 (4.78)	294		348 (4.21)

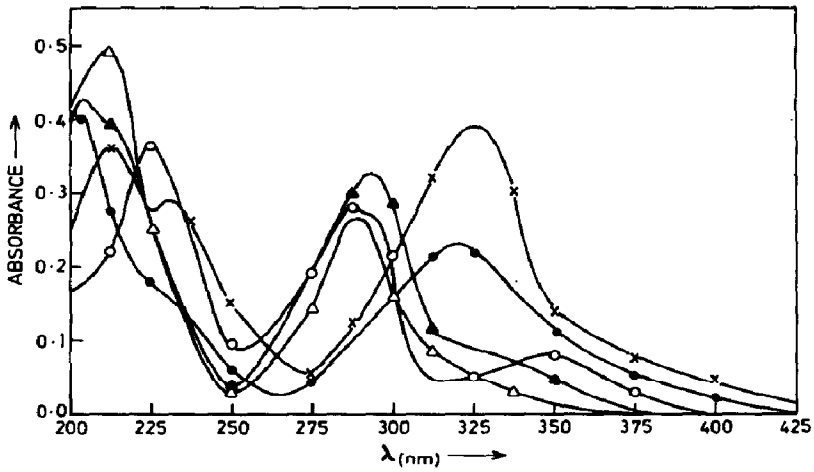

Fig. 3. Absorption spectra of the various prototropic species of HIC at 298 K: —○—, dianion; —△—, monoanion; —▲—, neutral; —●—, monocation; —×—, dication.

At below $H_0 - 6$, a further red shift in all the bands is observed, *i.e.* 350 (sh), 331, 232 and 212 nm. Similar changes are also observed for the methyl ester.

Yagil has found that the pK_a for the deprotonation of the imino group in the indole-2-carboxylate ion is 17.13 [14]. The presence of the phenolate ion at the 5-position can further decrease the acidity of the imino group in HIC and thus it can be concluded that the deprotonation of the imino group in HIC and its methyl ester may not be complete even at the highest H_- value (16) used in this experiment. Between H_- 16 and pH 11, the HIC species is the dianion (II), deprotonated from the hydroxyl and the carboxylic groups. The blue shift in the absorption spectra below pH 9 confirms that protonation has taken place at the hydroxyl oxygen atom; thus, between pH 4 and pH 9, the HIC species is the monoanion (III) deprotonated from the carboxyl group. The red shift in the pH range 3 - 0 leads to the formation of the neutral species (IV). Both the above results are consistent with earlier results of protonation at the phenolate [5] and the carboxylate ion [5]. Protonation at the carbon atom at position 3 of the indole molecule causes bands to develop on the higher and the lower side of 213 nm, with a decrease in the intensity of the 213 nm band, thus indicating that the monocation (V) is formed by protonating the carbon centre of the indole ring at position 3. This is also consistent with earlier results [17]. Lastly, the large red shift in all the bands confirms the formation of the dication on protonation of the carbonyl group. A similar trend is observed in the methyl ester, except for the formation of one species less, confirming the assignment of the various species and their equilibrium reactions under different hydrogen ion concentrations.

The pK_a values for the various equilibria in both HIC and its methyl ester were calculated spectrophotometrically and the values are given on the arrows of Figs. 1 and 2 respectively.

3.2. Fluorescence spectra

The fluorescence maxima and quantum yields in various solvents and at different pH values of HIC and its methyl ester are given in Table 3. The fluorescence spectra of the various prototropic species of HIC and its methyl ester are shown in Figs. 4 and 5 respectively. At above pH 11, the fluorescence band appears at 500 nm; between pH 2 and pH 13, the fluorescence maximum is at 405 nm. Between pH 4 and H_0-2 the band maximum is at 464 nm; there is no fluorescence between H_0-2 and H_0-5 and a broad band appears at 500 nm below H_0-6. For the methyl ester, at pH 10 or above, λ_{max}(fluo) = 500 nm; between pH 3 and pH 0, λ_{max}(fluo) = 410 nm; from $H_0 = -1$ to pH 4, λ_{max}(fluo) = 464 nm; λ_{max}(fluo) = 370 nm at H_0-1 to H_0-5 and, at 510 nm, below H_0-4.

From the above data it is clear that the trianion of HIC (I) is the species above pH 11 because it is observed that the imino group becomes a stronger acid in the S_1 state compared with the S_0 state [18 - 21].

The blue shift in the fluorescence spectrum (405 nm) below pH 11 indicates that the protonation occurs at the nitrogen atom (species II) although it can also take place at the oxygen atom of the phenolate group. The latter can be rejected on the grounds that the hydroxyl group becomes a stronger acid on excitation, whereas the pK_a^* calculated from fluorometric titrations (Fig. 6 (pK_a^* 11.3)) shows it to be more basic than the ground state value (about pK_a 10) [5]. Below pH 4, the red shift indicates that

TABLE 3

Fluorescence maxima and quantum yields of 5-hydroxyindole-2-carboxylic acid and its methyl ester in different solvents and at various pH values

Solvent or species	*HIC*		*Methyl ester of HIC*
	λ_{max}(fluo)	ϕ_f	λ_{max}
Cyclohexane	—	—	353, 367, 388
Ether	382	0.112	380
Acetonitrile	404	0.147	400
Methanol	403	0.164	440
Ethanol	418	0.127	—
Water (pH 14) (trianion)	500	0.049	500[a]
Water (pH 11) (dianion)	405	0.145	410[b]
Water (pH 7)	405	—	410
Water (pH 2) (zwitterion)	460	—	464[c]
Monocation (H_0-4)	—	—	370
Dication (H_0-10)	500	0.002	520

[a] Dianion.
[b] Monoanion.
[c] Zwitterion.

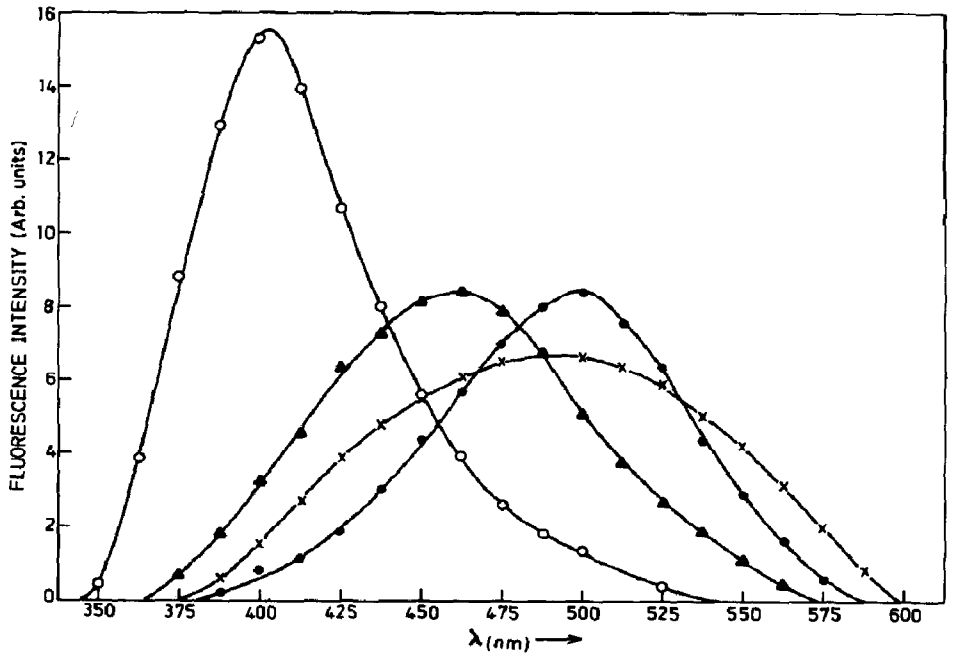

Fig. 4. Fluorescence spectra of the various prototropic species of HIC at 298 K: —●—, trianion; —○—, dianion; —▲—, zwitterion (scale multiplied by 3); —x—, dication (scale multiplied by 3).

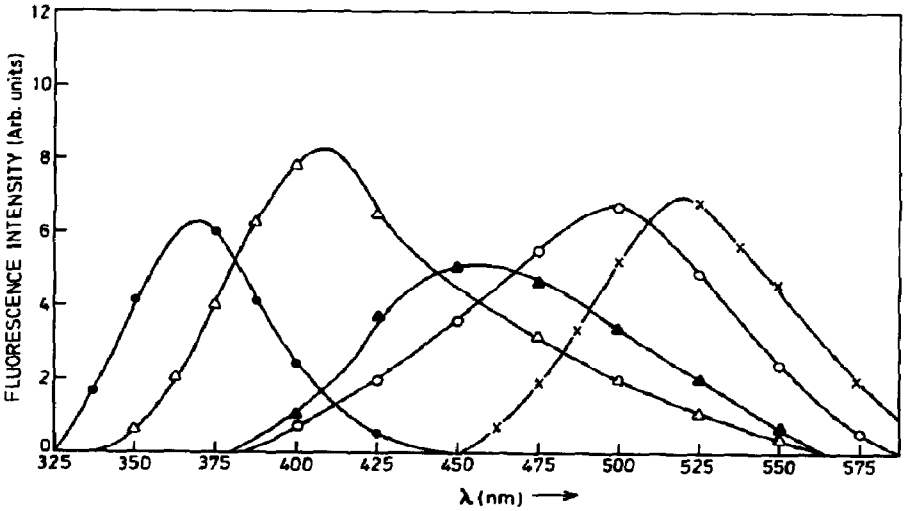

Fig. 5. Fluorescence spectra of the various prototropic species of the methyl ester of HIC at 298 K: —○—, dianion; —△—, monoanion; —▲—, zwitterion; —●—, monocation; —x—, dication.

protonation should occur at the carboxylate oxygen because protonation at the phenolate ion leads to a blue shift. This red shift is very large (from 405 to 465 nm) and the intensity of the 405 nm band remains constant over a large pH range, whereas for the methyl ester the 410 nm band exists between

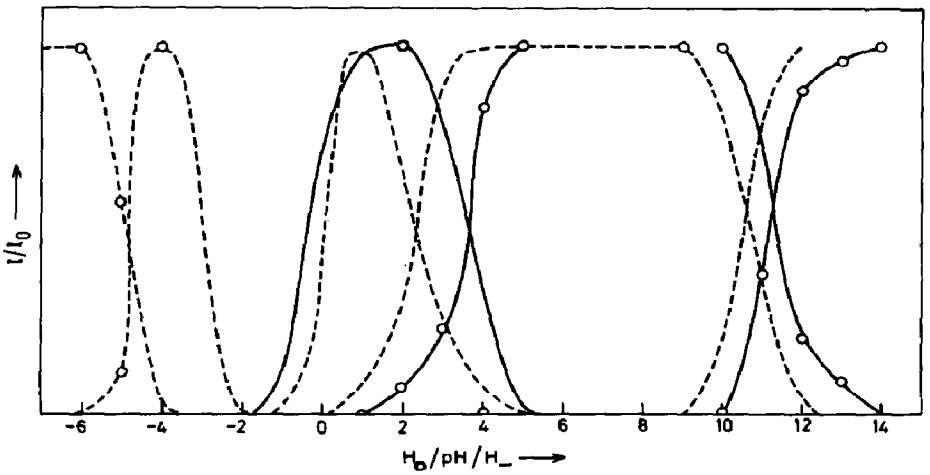

Fig. 6. Plot of I/I_0 *vs.* H_0/pH/H_ for HIC and its methyl ester at 298 K: —○—, HIC; - -○- -, the methyl ester.

pH 0 and pH 13 and the 464 nm band exists between H_0-1 and pH 4. In the latter compound, there is no replaceable hydrogen atom of the —COOH group and thus the fluorescence maxima of species XI should be either equal to or at longer wavelength than that of species II. Furthermore, in between pH 9 and pH 0 there should be another species (probably VII) of HIC similar to XI. There may be two reasons why we do not observe this species. Firstly, the increase in the acidity of the imino group and the increase in the basicity of the carboxylic group are so great that species II exists over a very narrow range of pH. Secondly, the difference in the fluorescence maxima of species II and VII may not be large and thus we are not able to separate out the two maxima. The latter argument seems to be more probable as the fluorescence band broadens in this pH range.

The 464 nm fluorescence band belongs to the zwitterion (VIII and XV) of HIC and its methyl ester as a red shift is commonly observed in the carbonyl protonation [17]. Below pH 0, the 464 nm band is completely quenched in HIC, whereas in the methyl ester no fluorescence is observed in the H_0-1 - H_0-2 range, but a weak blue-shifted emission is noticed at 370 nm below H_0-2. The non-observance of fluorescence emission at H_0-1 and H_0-2 from the cation (XVI) of the methyl ester could be due to the very low fluorescence quantum yield of the methyl ester, which is difficult to detect. The 370 nm band can be assigned to the monocation species XVI and not to species XIII, although it is present in the ground state. This is because (i) λ_{max}(fluo) of the zwitterion is always larger than that of its cation or anion and (ii) the cations formed by protonating position 3 of 5-hydroxy derivatives always fluoresce at about 500 nm [22]. On the same grounds we can conclude that it is the non-fluorescent species IX of HIC which is formed between H_0-1 and H_0-6 in the S_1 state and not species V. Lastly, in the H_0-5 - H_0-10 range, the 510 nm band in both HIC and its methyl ester is due to the dication (VI and XIV) formed

by protonating the carbon 3-position of the indole ring. This is a characteristic feature of 5-hydroxyindole derivatives [22], as stated earlier.

Fluorometric titration curves are shown in Fig. 6 and the pK_a^* values calculated are given on the arrows of Figs. 1 and 2. The pK_a^* value between species XVI and XV is calculated from the decrease in the relative fluorescence intensity of species XV and not from the formation curve of XVI. This is because (i) species XVI is very weakly fluorescent compared with species XV and (ii) the pK_a^* value is close to that obtained for the similar species of HIC, *i.e.* VIII and IX. In this study the Förster cycle method cannot be applied to calculate the pK_a^* values of the various equilibria because the species involved in the ground state and the first excited singlet state are quite different.

In conclusion it can be mentioned that the proton transfer reactions in the first excited singlet state depend on the change in acidity or basicity of the substituents attached to the parent molecule.

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