# Competition between Protonation and Deprotonation in the First Excited Singlet State of 2-(3′-Hydroxy-2′-pyridyl)benzimidazole in Acidic Solutions

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The photoinduced proton-transfer processes of 2-(3′-hydroxy-2′-pyridyl)benzimidazole in acidic solutions of acetonitrile, ethanol, and water have been studied by means of UV−vis absorption and fluorescence spectroscopy. In all the solvents considered, the ground-state species under acidic conditions is the enol cation, protonated at the benzimidazole N(3). Upon excitation, both the acidity of the hydroxyl group and the basicity of the pyridyl nitrogen increase, the acid−base properties of the solvent determining if protonation or deprotonation processes will be observed. Thus, in acetonitrile, the excited cation does not suffer any transformation, its fluorescence being observed, whereas in ethanol, some molecules dissociate at the hydroxyl group to yield the neutral keto form, and emission from both this keto species and the cation is observed. Since water can act as both acid and base, the enol cation undergoes in this solvent a two-step tautomerization by two different routes to yield the keto cation protonated at the pyridyl nitrogen. One pathway involves the deprotonation of the enol cation at the hydroxyl group to yield the neutral keto tautomer, followed by protonation of this tautomer at the pyridyl nitrogen to afford the keto cation. A second pathway implies the protonation of the enol cation by $H_2O$ (and by $H^+$ at high acidity) at the pyridyl nitrogen to give a dication, which deprotonates at the hydroxyl group, yielding also the keto cation. Only emission from the neutral keto form and the keto cation has been observed, the ratio between them being pH dependent.

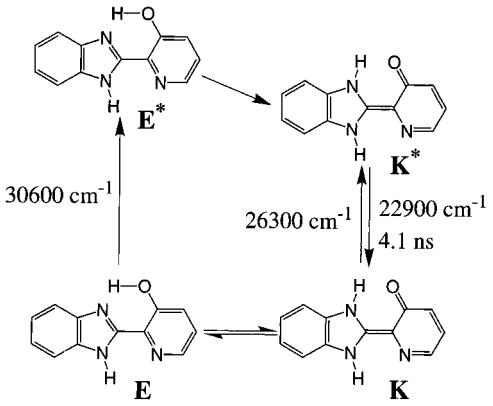
## Introduction

It is well-known that the molecules having acidic and basic groups can undergo photoinduced proton-transfer processes as a result of the change in acidity and basicity experienced by these groups in the excited state. A molecule containing both an acidic and a basic group in close proximity and with a suitable geometry may undergo an excited-state intramolecular proton transfer (ESIPT) from the acidic to the basic site. There has been a growing interest in these kind of processes in the last years,[1−3] owing to both the importance of understanding this ultrafast reaction at a molecular level[4−6] and the applications of the molecules that undergo those kind of processes, ranging from polymer UV stabilizers[7−10] and laser dyes[11−14] to photochromic materials.[7]

There are many ESIPT processes involving the transfer of a hydroxyl proton to an aromatic nitrogen.[2b,3a] Examples of ESIPT dyes containing these groups are 2-(2′-hydroxyphenyl)benzimidazole,[15−20] 2-(3′-hydroxy-2′-pyridyl)benzimidazole (HPyBI),[21,22] 2-(2′-hydroxyphenyl)benzoxazole,[23−27] and 2-(2′-hydroxyphenyl)benzothiazole.[23,28,29]

Even though these processes have been extensively investigated, most of the studies focus on proton transfer in nonaqueous solvents. The photophysics of the ESIPT dyes in water are in general more complicated than in other solvents. However, these studies are very important because of the interest of water as solvent and because of the fact that the special properties of water allow the observation of many processes that do not occur in other solvents. HPyBI is a striking example of a molecule showing different behavior in water than in other solvents. A first study in nonaqueous solvents showed[21] that this molecule exists in the ground state as enol form **E** (see Scheme 1). Upon excitation, this species undergoes an ultrafast intramolecular proton transfer to yield the keto tautomer **K***. We investigated the behavior of HPyBI in aqueous solution[22] and demonstrated that water plays an important role in the tautomerization processes, since ground-state HPyBI was shown to maintain in aqueous solution a tautomeric equilibrium between the enol form **E** and the keto form **K** (Scheme 1), both species being almost isoenergetic in water. **K** has been detected only in the ground state in water. Upon excitation of **E**, a fast ESIPT to yield the keto form **K*** takes place, only the fluorescence from **K*** being observed in all the solvents considered.

**SCHEME 1: Excitation and Deactivation of HPyBI in Neutral Aqueous Solution**




Our present goal is to investigate the behavior of ESIPT dyes in aqueous and nonaqueous acidic solution, as it has been scarcely studied.[15,20,29] In this work we attempt to get information on the photochemistry of HPyBI in acidic solution, which a priori might be very different depending on which cation is formed in the ground state upon protonation. HPyBI contains two basic nitrogen sites, the benzimidazole N(3) and the pyridyl nitrogen. As may be inferred from previous investigations on similar molecules,[20,30] the p$K_a$ could be similar for both groups. If HPyBI is first protonated at the benzimidazole N(3), the cation

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might undergo two different processes, (i) deprotonation of the hydroxyl group in the excited state as a result of the increase in acidity of this group upon excitation (as it was observed for 2-(2′-hydroxyphenyl)benzimidazole)[20] and (ii) protonation at the pyridyl nitrogen induced by the increase in basicity of this group in the excited state (the cation of 2-(2′-pyridyl)benzimidazole, which is protonated at the benzimidazole N(3) in the ground state, undergoes a photoinduced proton transfer to yield the cation protonated at the pyridyl nitrogen in the excited state).[31] On the other hand, if HPyBI is first protonated at the pyridyl nitrogen, the cation might suffer an ESIPT from the OH group to the benzimidazole N(3), as it was observed for the excited enol form in neutral media. The photochemistry of HPyBI will depend on which cation is formed in the ground state, but it will also be the result of the change in both acidity of the hydroxyl group and basicity of the pyridyl nitrogen induced by excitation. It is interesting to know the result of these competing protonation and deprotonation processes for HPyBI. In this work, we investigate the influence of acidity on the photoinduced proton-transfer processes of HPyBI by using UV−vis absorption spectroscopy and by means of steady-state and time-resolved fluorescence spectroscopy. We have studied the behavior of HPyBI in acidic aqueous solutions and in acidified ethanol and acetonitrile.

## Experimental Section

HPyBI was synthesized as described elsewhere.[22] Solutions were made up as previously described[22] in spectroscopy grade ethanol, acetonitrile, and double-distilled water; all aqueous solutions contained 1.8% (v/v) ethanol. Solutions were not degassed. Acidity was varied with $HClO_4$, NaOH or $NaH_2PO_4$/$Na_2HPO_4$ buffer (made up with Merck p.a. products). Ionic strength was maintained at 0.1 mol $dm^{-3}$ with Merck p.a. $NaClO_4$, except at high acidities, where the ionic strength was the concentration of the acid. All experiments were carried out at 25 °C.

Acidity constants were determined spectrophotometrically as described previously,[30] based on pH measurements or the acidity function $H_+$[32] for high-acid media. pH was measured with a Radiometer PHM 82 pH meter equipped with a Radiometer Type B combined electrode. UV−vis absorption spectra were recorded in Kontron Uvikon 810 and 930 spectrophotometers. Fluorescence excitation and emission spectra were recorded in a Spex Fluorolog-2 FL340 E1 T1 spectrofluorometer, with correction for instrumental factors by means of a Rhodamine B quantum counter and correction files supplied by the manufacturer. Fluorescence lifetimes were determined by single-photon timing in an Edinburgh Instruments CD-900 spectrometer equipped with a hydrogen-filled nanosecond flashlamp and the analysis software supplied by the manufacturer.

Theoretical equations were fitted to experimental data by means of a nonlinear weighted least-squares routine based on the Marquardt algorithm.

## Results

**1. Absorption Spectra in Aqueous Solution: Influence of pH.** Absorption spectra of HPyBI in aqueous solution of various acidities have been shown previously.[22] Two bands are observed in neutral media, band I with maximum at 30 600 $cm^{-1}$ and band II with maximum at 26 300 $cm^{-1}$. Band II disappears upon increasing acidity, a new band with maximum at 30 300 $cm^{-1}$ being observed. This band does not change with acidity between pH 3 and $H_+ = 0$ and is very similar to band I observed in neutral media. An increase of acidity for $H_+ < 0$ induces a shift to the red in the spectrum, a new band with maximum at 27 900 $cm^{-1}$ being obtained at $H_+ = -4$. A spectrum with maximum at around 28 200 $cm^{-1}$ is obtained when the pH increases from neutral media.

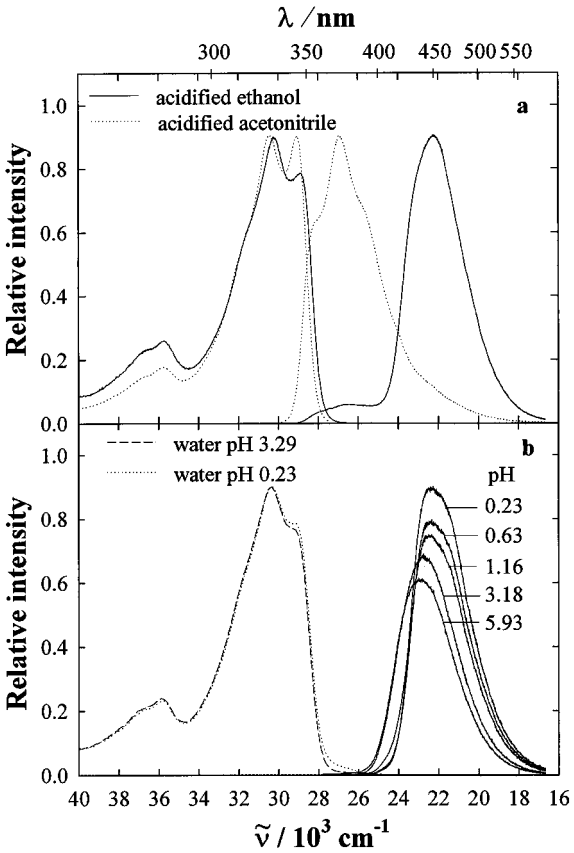



**Figure 1.** (a) Normalized fluorescence excitation and emission spectra of HPyBI in acidified acetonitrile ($[HClO_4] = 2.4 \times 10^{-4}$ mol $dm^{-3}$, $\tilde{\nu}_{em} = 27\,000$ $cm^{-1}$, $\tilde{\nu}_{exc} = 30\,500$ $cm^{-1}$, $[HPyBI] = 2.6 \times 10^{-6}$ mol $dm^{-3}$) and ethanol ($[HClO_4] = 1.1 \times 10^{-4}$ mol $dm^{-3}$, $\tilde{\nu}_{em} = 22\,500$ $cm^{-1}$, $\tilde{\nu}_{exc} = 30\,300$ $cm^{-1}$, $[HPyBI] = 1.6 \times 10^{-6}$ mol $dm^{-3}$). (b) Fluorescence excitation and emission spectra of HPyBI in water at various acidities ($\tilde{\nu}_{em} = 22\,700$ $cm^{-1}$, $\tilde{\nu}_{exc} = 28\,100$ $cm^{-1}$, $[HPyBI] = 1.8 \times 10^{-6}$ mol $dm^{-3}$).

**2. Fluorescence Spectra and Lifetimes.** Figure 1a shows the fluorescence excitation and emission spectra of HPyBI in acidified acetonitrile and ethanol. In these solutions, $HClO_4$ concentration was chosen to ensure that all HPyBI was in its monoprotonated form, judging by the changes in the absorption spectra. The fluorescence excitation spectrum in acetonitrile resembles the absorption spectrum observed in acidic aqueous solution of pH ≈ 3. The emission spectrum ($\tilde{\nu}_{max} = 27\,000$ $cm^{-1}$) overlaps the excitation spectrum showing a normal Stokes shift, and the emission decays monoexponentially with a lifetime of 1.5 ns. In acidified ethanol, HPyBI exhibits dual fluorescence (Figure 1a), emitting both a weak band at about 27 000 $cm^{-1}$ with a normal Stokes shift and a stronger lower energy band at 22 200 $cm^{-1}$ that coincides with the fluorescence band obtained in neutral ethanol.[22] A biexponential decay of the fluorescence was observed in acidified ethanol ($[HClO_4] = 4.6 \times 10^{-4}$ mol $dm^{-3}$) between 27 000 and 23 500 $cm^{-1}$ with lifetimes of 0.2 and 3.4 ns. The amplitude of the faster decay decreases with decreasing emission wavenumber, whereas that of the slower decay increases.

The fluorescence of HPyBI observed in acidic aqueous solution (Figure 1b) shows an anomalous Stokes shift (∼8000

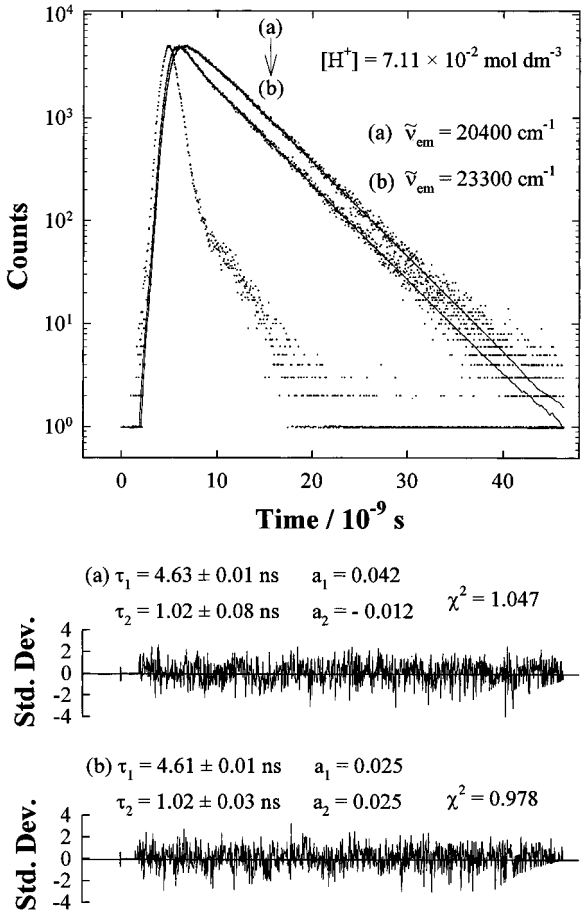



**Figure 2.** Fluorescence decay of HPyBI in aqueous solution of $[HClO_4] = 7.11 \times 10^{-2}$ mol dm$^{-3}$ at $\tilde{\nu}_{em} = 20\,400$ and 23 300 cm$^{-1}$ and lamp profile ($\tilde{\nu}_{exc} = 29\,800$ cm$^{-1}$). The decays were analyzed to fit a biexponential function. Weighted residuals for each emission wavenumber are also shown. [HPyBI] $= 1 \times 10^{-5}$ mol dm$^{-3}$.

cm$^{-1}$). The emission band resembles that obtained in neutral solution ($\tilde{\nu}_{max} = 22\,900$ cm$^{-1}$),[22] but it shifts to the red as the pH is decreased ($\tilde{\nu}_{max} = 22\,700$ cm$^{-1}$ at pH 3.29, 22 300 cm$^{-1}$ at pH 0.23). Although an isosbestic wavenumber between the neutral forms and the cation was used as excitation wavenumber, an increase of the fluorescence intensity was observed as the pH decreased. Furthermore, the excitation spectrum was independent of pH between pH $\approx$ 3 and pH $\approx$ 0 and coincident with the absorption spectrum obtained under the same conditions. Both excitation and emission spectra in acidic solution were independent of the monitoring emission and excitation wavenumbers.

The decay of HPyBI fluorescence in acidic aqueous solution was investigated at 23 300 and 20 400 cm$^{-1}$. Figure 2 shows the decays for $[HClO_4] = 7.11 \times 10^{-2}$ mol dm$^{-3}$. The decay was biexponential at both wavenumbers between pH 1 and pH 3. The slower decay showed a lifetime of 4.6 ns, independent of pH and with positive amplitude at both wavenumbers. The faster decay showed a pH-dependent lifetime with positive amplitude at 23 300 cm$^{-1}$ and negative amplitude at 20 400 cm$^{-1}$. A plot of the reciprocal of the two lifetimes against the acid concentration is depicted in Figure 3.

## Discussion

**1. Ground-State Acid−Base Equilibria.** The absorption spectra of aqueous HPyBI solutions of various acidities show the existence of three acid−base equilibria in the range from

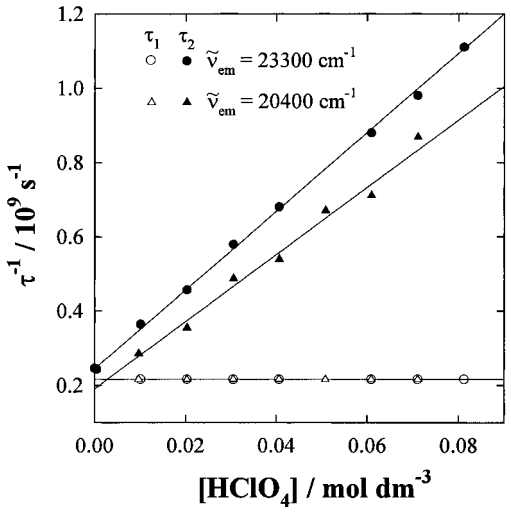



**Figure 3.** [H$^+$] dependence of the reciprocal of the lifetimes $\tau_1$ and $\tau_2$ obtained from the biexponential fit of the fluorescence decay of HPyBI in aqueous solution at $\tilde{\nu}_{em} = 23\,300$ and 20 400 cm$^{-1}$. The results are mean values of repeated measurements.

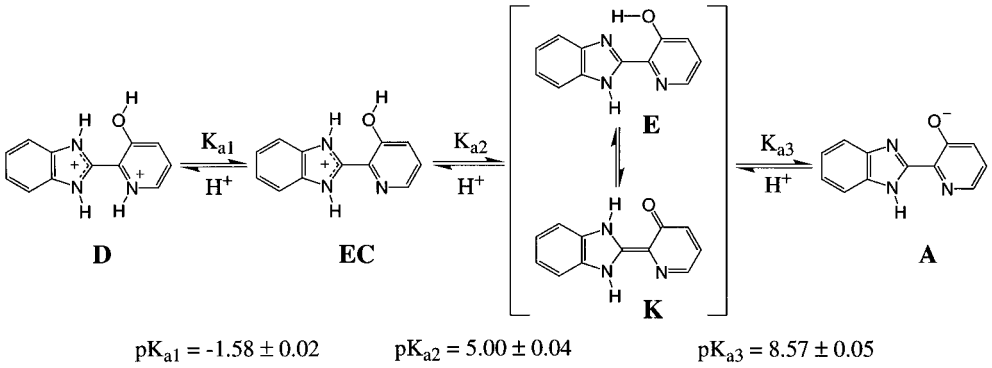
$H_+ = -4$ to pH $= 12$. Analysis[30] of the absorbance−pH (or $H_+$) data at fixed wavenumbers yields the following values of the acidity constants: p$K_{a1} = -1.58 \pm 0.02$, p$K_{a2} = 5.00 \pm 0.04$, and p$K_{a3} = 8.57 \pm 0.05$. According to these results, we suggest that five different forms of HPyBI are involved in ground-state acid−base equilibria: the anion **A**, the two neutral forms **E** and **K**, the monocation **EC**, and the dication **D** (Scheme 2). The existence in neutral aqueous solution of a tautomeric equilibrium between the enol form **E** and the keto form **K**, responsible for the absorption bands I and II, respectively, has been discussed in a previous paper.[22] Deprotonation of the neutral forms affords the anion **A**, similar to that formed by 2-(2′-hydroxyphenyl)benzimidazole (p$K_a = 8.83$).[20] It is experimentally found that the monocation absorption spectrum resembles that of the neutral enol form **E**, in keeping with the behavior of 2-(2′-hydroxyphenyl)benzimidazole[20] and 2-(2′-pyridyl)benzimidazole,[31] which are protonated at the benzimidazole N(3). Therefore, it seems likely that the monocation of HPyBI is the enol cation **EC** (Scheme 2). The second protonation takes place at the pyridyl nitrogen to afford the dication **D**, its spectrum being shifted to the red with regard to that of the monocation, as already observed for 2-(2′-pyridyl)-benzimidazole.[31]

In acidified acetonitrile and ethanol solutions, the absorption and fluorescence excitation spectra of HPyBI resemble that of aqueous solution of pH $\approx$ 3 (Figure 1). These spectra are therefore attributed to the enol cation **EC**.

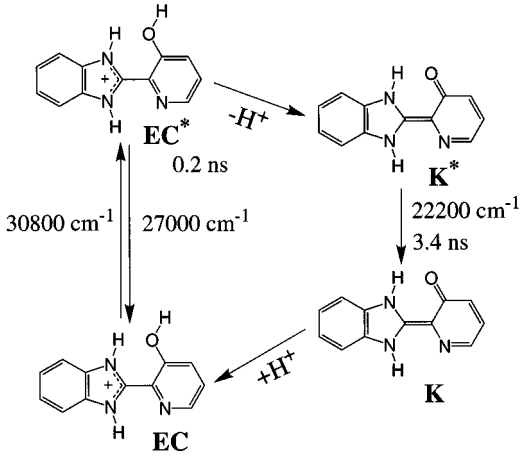
**2. Fluorescence in Acidic Media.** Excitation of the enol cation **EC** in acidified acetonitrile (Figure 1a) induces a fluorescence band that overlaps the excitation spectrum and decays monoexponentially; it is therefore attributed to the excited monocation **EC***, which decays with a lifetime of 1.5 ns.

Dual fluorescence is observed for HPyBI in acidified ethanol solution (Figure 1a). The weaker band is located at about the same position as the emission of **EC*** in acidified acetonitrile and overlaps the excitation band of **EC**. It must therefore be concluded that the band is due to the monocation **EC***, which decays with a lifetime of 0.2 ns. Furthermore, the strong band, with a lifetime of 3.4 ns, coincides perfectly with that of the keto tautomer **K***, which is known to be the fluorescent species in neutral ethanol.[22] In view of these results, we propose that in ethanol the enol cation undergoes an effective deprotonation of the hydroxyl group in the excited state to yield the neutral

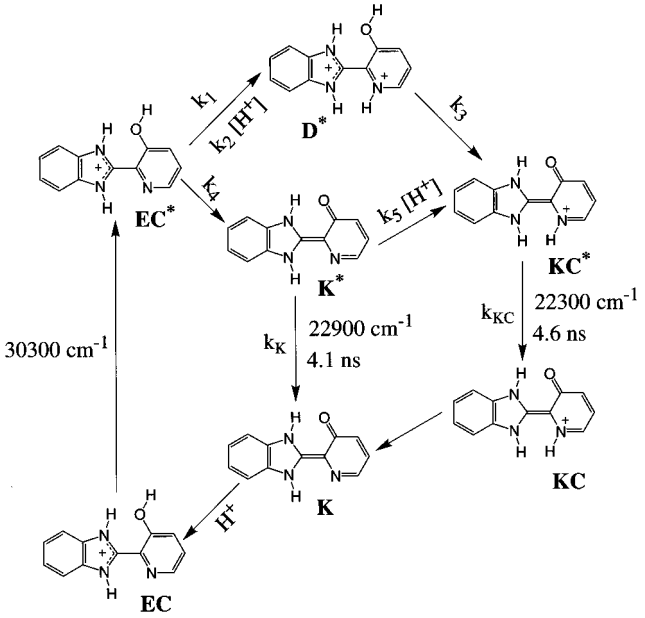
**SCHEME 2: Acid−Base and Tautomeric Equilibria of Ground-State HPyBI in Aqueous Solution**




**SCHEME 3: Excitation and Deactivation of HPyBI in Acidified Ethanolic Solution**




**SCHEME 4: Excitation and Deactivation of HPyBI in Acidic Aqueous Solution**




keto form (Scheme 3). The fact that this process does not take place in acetonitrile is probably due to the inability of this solvent to accept the proton. The decrease of the lifetime of **EC*** on going from acetonitrile to ethanol supports this interpretation.

In aqueous solution of pH 3, the fluorescence excitation spectrum of HPyBI (Figure 1b) coincides with the absorption spectrum in the same conditions, attributed to the enol cation **EC**. The fluorescence band shows a big Stokes shift, indicating that the emission is probably due to a species different from **EC***. It was also observed that, except for a small shift to the red, this fluorescence band is almost coincident with that obtained in neutral media,[22] which corresponds to the keto tautomer **K***. It seems probable therefore that the excited enol cation **EC*** undergoes a very efficient deprotonation of the hydroxyl group in water to afford the keto tautomer **K***. No fluorescence of **EC*** is observed, probably due to the great ability of water to accept the proton, deprotonation of **EC*** being faster in water than in ethanol. These results indicate that the acidity of the OH group increases upon excitation, the ability of the solvent to accept the proton determining if deprotonation of **EC*** (water), deprotonation competing with its fluorescence (ethanol), or just **EC*** fluorescence (acetonitrile) will be observed. A similar behavior was previously found for the cation of 2-(2′-hydroxyphenyl)benzimidazole[20] in water and ethanol.

If the keto tautomer **K*** were the only fluorescent species formed from **EC*** in aqueous solution, the emission spectrum should be independent of acidity from neutral medium to pH $\approx$ 0. However, it is observed (Figure 1b) that the fluorescence shifts to the red and its intensity increases as the pH decreases even though the excitation wavenumber corresponds to an isosbestic point between the neutral species and the cation. Furthermore, the fluorescence decay, which is monoexponential in neutral media, becomes biexponential as the pH decreases (Figures 2 and 3). From these results, it is clear that **K*** is not the only fluorescent species in acidic aqueous solution. However, it must be pointed out that the enol cation is the only species present in the ground state, since the fluorescence excitation spectrum remains the same from pH $\approx$ 3 to pH $\approx$ 0 (Figure 1b) and coincides perfectly with the absorption spectrum of the enol cation. These results indicate that the modifications of the fluorescence spectrum are caused by a process taking place in the excited state. Furthermore, the fluorescence decays at different pH values (Figure 3) show that one of the fluorescent species is quenched by protons. This suggests that the process taking place in the excited state involves the protonation of **K***, its lifetime decreasing from the value of 4.1 ns in neutral or slightly acidic media as the acidity increases, appearing at the same time a new lifetime of 4.6 ns. We propose that **K*** protonates in the excited state to yield a cation. Taking into account that this cation cannot be **EC*** and is formed by protonation of **K***, it is reasonable that this new species is the keto cation **KC*** protonated at the pyridyl nitrogen (Scheme 4). Additional evidence in favor of this interpretation is the fact that 2-(2′-pyridyl)benzimidazole[31] and similar species[33,34] are known to experience an increase in basicity of the pyridyl nitrogen upon excitation. For the related molecule 2-(2′-hydroxyphenyl)benzimidazole,[20] no quenching by protons of the

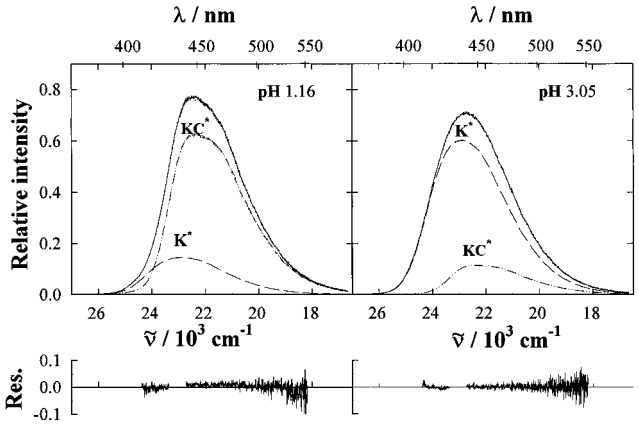



**Figure 4.** Experimental (—) fluorescence spectra of HPyBI in aqueous solutions of pH 1.16 and 3.05, and calculated spectra (···) obtained by fitting a linear combination of the fluorescence spectra of **K*** and **KC***, together with the individual contributions of these spectra. Relative values of the residuals are also shown, with a break in the region of solvent Raman bands. $\tilde{\nu}_{exc} = 28\ 100\ cm^{-1}$. [HPyBI] $= 1.8 \times 10^{-6}$ mol $dm^{-3}$.

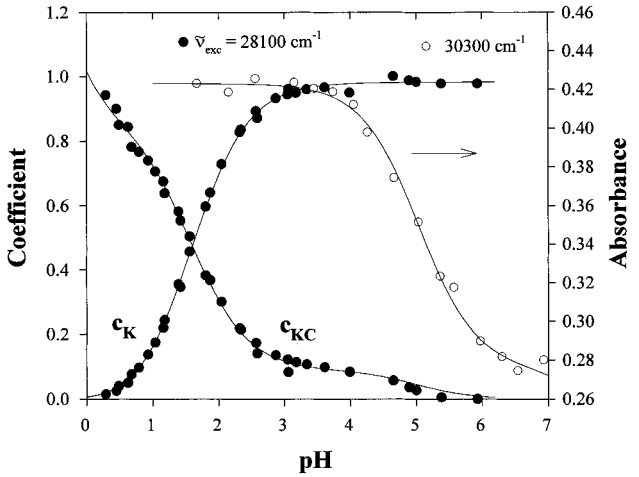



**Figure 5.** Plot of the pH dependence of the relative contributions of **K*** and **KC*** to the fluorescence spectrum of HPyBI under excitation at 28 100 $cm^{-1}$ (the solid curves are the result of fitting eqs 2 and 3 to the experimental data) and of the absorbance of HPyBI in aqueous solution at 30 300 $cm^{-1}$.

keto tautomer fluorescence under similar conditions has been observed, since it lacks the pyridyl nitrogen. From the above findings, we propose that in acidic aqueous solution there are two fluorescent species formed from **EC***, the neutral keto form **K*** and the keto cation **KC***, protonated at the pyridyl nitrogen. In the following a quantitative study will be done to test this hypothesis and to determine the nature of the processes taking place in the excited state.

Assuming that the fluorescence spectra obtained in acid media over the pH range 0−6 are due to the emission of **K*** and **KC***, any measured spectrum should be a linear combination of the pure spectra of **K*** and **KC***. Accordingly, the fluorescence intensity at each emission wavenumber $F(\tilde{\nu}_{em})$ may be expressed as

$$F(\tilde{\nu}_{em}) = C_K F_K(\tilde{\nu}_{em}) + C_{KC} F_{KC}(\tilde{\nu}_{em}) \qquad (1)$$

where $F_K(\tilde{\nu}_{em})$ and $F_{KC}(\tilde{\nu}_{em})$ are the fluorescence intensities at the same emission wavenumber of pure reference spectra for **K*** and **KC*** and the coefficients $C_K$ and $C_{KC}$ represent the contribution of each reference spectrum to the spectrum of the mixture. The pure fluorescence spectrum of **K*** is obtained by direct excitation of **K** in band II (26 300 $cm^{-1}$) at pH 6. We have taken the fluorescence spectrum obtained at pH 0.23 to be the pure spectrum of **KC***, since the pH dependence of the red shift observed in the fluorescence spectrum (Figure 1b) reaches a plateau in this region. A series of fluorescence spectra recorded at various acidities were satisfactorily decomposed as linear combinations of these two reference spectra (Figure 4). The pH dependence of the coefficients obtained are shown in Figure 5 together with the pH dependence of the absorbance in the same acidity range.

The above results support the hypothesis that **K*** and **KC*** are the fluorescent species. It remains to be determined the mechanism of their formation from **EC***, the primary excited species. It is clear that **K*** is formed by deprotonation of **EC***, water acting as a base in this process. As pH decreases, **K*** protonates to afford **KC*** (Scheme 4). Since the quenching by protons is probably a diffusion-controlled process ($k_5 \approx 10^{10}$ $mol^{-1}$ $dm^3$ $s^{-1}$), it can only compete with the deactivation of **K*** ($\tau_K = 4.1$ ns) at pH < 3. To explain the observed increase in the contribution of **KC*** emission to the experimental fluorescence as the pH decreases from pH 5 to pH 3 (Figure 5), it must be concluded that there is another process leading to the formation of **KC*** at pH > 3. This process cannot be the protonation of **K*** by water, since in this case the relative proportions of **K*** and **KC*** would be pH independent and the fluorescence spectrum would not shift to the red as acidity increases over the pH range 5−3, as experimentally observed (Figure 1b). Since the conversion **EC*** → **KC*** implies both protonation of the pyridyl nitrogen and deprotonation of the hydroxyl group, it seems likely that a two-step mechanism involving protonation of **EC*** at the pyridyl nitrogen to afford a dication followed by deprotonation of this dication at the hydroxyl group can take place (Scheme 4). Moreover, to explain effective protonation taking place at pH > 3, water must act as acid in that process (rate constant $k_1$). Similar processes involving excited-state protonations at the pyridyl nitrogen by $H_2O$ have been observed for 2-(2′-pyridyl)benzimidazole[31] and similar species.[2,33] Furthermore, **EC*** should also be protonated by $H^+$ when the acidity is high enough (rate constant $k_2$), as shown in Scheme 4. Once the dication is formed, it suffers a fast deprotonation at the hydroxyl group to yield **KC***, no fluorescence from **D*** being observed in the acidity range studied. Additional evidence in favor of this hypothesis comes from the fact that upon direct excitation of the dication at $H_+$ slightly lower than 0, the emission spectrum of **KC*** is obtained.

According to the mechanism proposed (Scheme 4), the biexponential fluorescence decay of HPyBI in acidic aqueous solution implies that both **EC*** and **D*** must have a very short lifetime, at least shorter than the time resolution of our single-photon counting equipment (0.1 ns). This would be in agreement with the fact that no fluorescence of **EC*** and **D*** could be observed, indicating that the deactivation of both species by other routes is very fast. Yet further evidence emerges from the fact that the decay of **EC*** becomes faster on going from acetonitrile (1.5 ns), where **EC*** is the only fluorescent species, to ethanol (0.2 ns), where a weak fluorescence from **EC*** is still observed although the emission from **K*** predominates. It seems then reasonable to suppose that in water, where no fluorescence from **EC*** was observed, the lifetime is even shorter. On the other hand, the rate constant for the deprotonation **D*** → **KC*** must have a very high rate constant, $k_3$, due to the increase in acidity of the OH group upon excitation, the same applying for the similar process **EC*** → **K*** with a rate constant $k_4$ (Scheme 4). This is in keeping with the observed

deprotonation rate constants for other aromatic alcohols, being as high as $5.8 \times 10^{10}$ $s^{-1}$.[2] If the formation and deactivation of **EC*** and **D*** are faster than the time resolution of our apparatus, we will just be able to follow both the deactivation of **KC*** and **K*** and the formation of **KC*** from **K***. It seems reasonable therefore that at 23 300 $cm^{-1}$, where **K*** contributes more than **KC*** to the fluorescence, two lifetimes $\tau_1$ and $\tau_2$ with positive amplitudes are obtained, whereas at 20 400 $cm^{-1}$, where the emission from **KC*** predominates, the amplitude of $\tau_2$ becomes negative, corresponding to the rise time of formation of **KC*** from **K***. According to this interpretation, the lifetime $\tau_1 = 4.6$ ns (independent of pH) corresponds to **KC***, while $\tau_2$ (pH dependent) corresponds to **K*** and must verify $1/\tau_2 = k_K + k_5$ [$H^+$]. The reciprocal of $\tau_2$ showed a linear dependence on the acid concentration (Figure 3), in keeping with our interpretation. The values obtained for $\tau_1$ at the two wavelengths investigated are the same, whereas the values of $\tau_2$ are slightly different (Figure 3). This difference could be due to a transient dynamic quenching by $H^+$ (not considered in the biexponential fit) or even to a small contribution of **EC*** and **D*** lifetimes to the observed decay. Fitting straight lines to the experimental data of $\tau_2^{-1}$ versus acid concentration shown in Figure 3, a mean value of $k_5 = 1 \times 10^{10}$ $mol^{-1}$ $dm^3$ $s^{-1}$ is obtained, which as expected is on the same order as the diffusion-controlled limit.

To test the adequacy of the mechanism put forward to explain the behavior of excited HPyBI in acidic aqueous solution (Scheme 4), a quantitative analysis of the pH dependence of the fluorescence intensity was performed. We have deduced expressions for the acidity dependence (pH range 0−6) of the coefficients $C_K$ and $C_{KC}$, representing the contribution of **K*** and **KC*** to the fluorescence (eqs 2 and 3).

$$C_K = \frac{\chi_K\left[K_{a1}K_{a2} + \dfrac{\delta K_{a1}[H^+]}{1+\beta+\alpha[H^+]}\right]}{(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2)(1+\gamma[H^+])} \quad (2)$$

$$C_{KC} = \chi_{KC} \times \frac{\left[K_{a1}\gamma[H^+]\left\{[H^+] + \dfrac{K_{a2}}{\delta}\right\} + \dfrac{K_{a1}[H^+]\{\beta+\alpha[H^+]\}}{1+\beta+\alpha[H^+]} + \left(\dfrac{\epsilon_D}{\epsilon_{EC}}\right)^{28100}[H^+]^2(1+\gamma[H^+])\right]}{(K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2)(1+\gamma[H^+])} \quad (3)$$

$$\delta = \frac{\epsilon_{EC}^{28100}(K_{EK}+1)}{\epsilon_E^{28100} + K_{EK}\epsilon_K^{28100}} \quad (4)$$

In these equations, $\chi_K$ and $\chi_{KC}$ represent empirical instrumental parameters, $K_{a1}$ and $K_{a2}$ are the previously defined ground-state acidity constants, $\epsilon_K^{28100}$, $\epsilon_E^{28100}$, $\epsilon_{EC}^{28100}$, and $\epsilon_D^{28100}$ are the molar absorption coefficients of **K**, **E**, **EC**, and **D**, respectively, at the excitation wavenumber 28 100 $cm^{-1}$, $K_{EK}$ is the concentration ratio in the ground-state tautomerization equilibrium, $K_{EK}$ = [K]/[E], and $\alpha$, $\beta$, and $\gamma$ are rate constant ratios: $\alpha = k_2/k_4$, $\beta = k_1/k_4$, and $\gamma = k_5/k_K$.

Equations 2 and 3 were globally fitted to the experimental values of $C_K$ and $C_{KC}$ obtained from the spectral decomposition at each pH (see Figure 5). Taking into account that 28 100 $cm^{-1}$ is an isosbestic point for the cation **EC** and the neutral forms **E** and **K**, the constant $\delta$ has a value of 1. Furthermore, the values of $K_{a1}$, $K_{a2}$, and $(\epsilon_D/\epsilon_{EC})^{28100}$ are also known from the absorption measurements. Therefore, the values of these parameters were fixed in the fit. Solid curves in Figure 5 represent the calculated functions. The goodness of the fit supports the proposed mechanism. The corresponding optimized values of $\alpha$, $\beta$, and $\gamma$ are

$$\alpha = \frac{k_2}{k_4} = 2.9 \pm 0.8 \text{ mol}^{-1} \text{ dm}^3 \quad (5)$$

$$\beta = \frac{k_1}{k_4} = 0.121 \pm 0.007 \quad (6)$$

$$\gamma = \frac{k_5}{k_K} = 37 \pm 2 \text{ mol}^{-1} \text{ dm}^3 \quad (7)$$

The parameters $\alpha$ and $\beta$ show the relative efficiency of the competing processes undergone by **EC***. A comparison between the rates of protonation and deprotonation of **EC*** can be established: $(k_1 + k_2[H^+])/k_4 = \beta + \alpha$ [$H^+$]. This ratio has a value of 1 at [$H^+$] = 0.3 mol $dm^{-3}$, indicating that deprotonation predominates at lower concentrations of protons, whereas at higher concentrations protonation becomes faster. At low concentration of protons (pH > 3), $\beta \gg \alpha$ [$H^+$], indicating that protonation and deprotonation by $H_2O$ take place. Moreover, the value of $\beta$ shows that the deprotonation process is 8 times faster than the protonation process. On the other hand, the rates of the two competing protonation processes of **EC***, by $H_2O$ and by $H^+$, can also be compared: $(k_2[H^+]/k_1) = (\alpha\ [H^+]/\beta) = 24[H^+]$. From this expression it can be derived that at [$H^+$] = $4 \times 10^{-2}$ mol $dm^{-3}$ (pH = 1.4) both rates are the same. At pH > 1.4 protonation by $H_2O$ predominates, while at pH < 1.4 protonation by $H^+$ is more important. Furthermore, the quenching rate constant $k_5$ can be obtained from $\gamma$ if the value of $k_K$, the deactivation rate constant of **K***, is known. From the fluorescence lifetime of **K*** in neutral medium ($\tau_K = 4.1$ ns), a value for $k_K$ of $2.4 \times 10^8$ $s^{-1}$ is found. Thus, the rate constant $k_5$ was calculated to be $9 \times 10^9$ $mol^{-1}$ $dm^3$ $s^{-1}$. This value is close to the diffusion-controlled limit and agrees well with that previously obtained from fluorescence lifetime measurements.

Assuming that the protonation of **EC*** by $H^+$ is also a diffusion-controlled process, $k_2 \approx 1 \times 10^{10}$ $mol^{-1}$ $dm^3$ $s^{-1}$, estimated values of $k_1 \approx 4 \times 10^8$ $s^{-1}$ and $k_4 \approx 3 \times 10^9$ $s^{-1}$ are obtained from $\alpha$ and $\beta$, which are typical values for this kind of process. For example, the rate constant for the protonation of the pyridyl nitrogen by water in excited 2-(2′-pyridyl)benzimidazole is $3.1 \times 10^8$ $s^{-1}$,[31] and similar values have been found for other aromatic amines.[2,33] On the other hand, the deprotonation rate constant in excited aromatic alcohols is about $10^9$ $s^{-1}$.[2]

## Conclusions

HPyBI is protonated in acid media at the benzimidazole N(3) to afford the enol cation **EC**. Both the acidity of the hydroxyl group and the basicity of the pyridyl nitrogen increase in the first excited singlet state. The properties of the solvent determine if protonation or deprotonation are observed:

1. In acetonitrile, **EC*** just deactivates to the ground state, showing fluorescence.

2. In ethanol, some **EC*** molecules deprotonate at the hydroxyl group to yield the keto tautomer **K***, dual fluorescence from **EC*** and **K*** being observed.

3. In water, **EC*** experiences two competing processes: deprotonation at the hydroxyl group and protonation at the pyridyl nitrogen by $H_2O$ and by $H^+$, the ratio between those processes being pH dependent. The keto tautomer **K***, formed by deprotonation of **EC***, suffers a diffusion-controlled quench-

ing by $H^+$ to afford the keto cation **KC***. On the other hand, the dication resulting from protonation of **EC*** undergoes a fast deprotonation at the hydroxyl group to give also **KC***. In acidic aqueous solution, the observed fluorescence is composed of the emission of **K*** and **KC***, both spectra being very similar. The ratio between the contributions of **KC*** and **K*** to the fluorescence increases as the pH decreases. Emission from **EC*** was not detected at any pH because of the fast deprotonation and protonation processes undergone by **EC***. The rate constants for most of the processes involved in the mechanism have been estimated.

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