Solvent-Dependent Ground- and Excited-State Tautomerism in 2-(6′-Hydroxy-2′-pyridyl)benzimidazole

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The ground- and excited-state tautomerism of 2-(6′-hydroxy-2′-pyridyl)benzimidazole (**1**) and 1-methyl-2-(6′-hydroxy-2′-pyridyl)benzimidazole (**2**) in various solvents was investigated by means of UV−vis absorption spectroscopy, steady-state and time-resolved fluorescence spectroscopy, and quantum-mechanical ab initio calculations. A solvent-dependent tautomeric equilibrium was observed for both compounds in the ground state between the lactim or normal form and the lactam tautomer resulting from a proton translocation between the hydroxyl group and the pyridine nitrogen. Here, we report evidences for a solvent-dependent switching in the nature of the excited-state proton transfer (ESPT) reactions undergone by **1** and **2**. In the aprotic solvent acetonitrile, no significant ESPT takes place. The protic solvents ethanol and water facilitate the proton transfer through bridges of solvent molecules, but each solvent catalyzes specifically a different ESPT process. In aqueous solution, the excited lactim species of **1** and **2** undergo a proton transfer from the hydroxyl group to the pyridine nitrogen, favoring the lactam tautomer in the first excited singlet state. In ethanol the ESPT takes place for **1** from the benzimidazole NH to the pyridine nitrogen, originating a new tautomer not observed in the ground state. Compound **2**, without a NH group, does not tautomerize in the excited state in ethanol.

Introduction

It is well-known that the excited-state acid−base properties of molecules may differ markedly from those in the ground state, leading to proton-transfer processes in the excited state. Molecules with acid and basic functionalities may undergo an excited-state intramolecular proton transfer (ESIPT) from the acidic to the basic site, yielding a phototautomer.[1−4] Many bifunctional molecules without the geometrical requirements for an ESIPT can still undergo an excited-state proton transfer (ESPT) assisted by species with hydrogen bond accepting and donating abilities, these acting as bridges between the acid and the basic site.[4−6] The understanding of these processes is of importance in chemistry and biology, as they could help to unravel complex problems as the mechanism of proton-relay processes in biological systems,[7] the transport of protons in water,[8−10] the mutagenesis mechanisms,[11−14] and molecular recognition.[15]

A group of bifunctional molecules experiencing assisted ESPT is constituted by molecules which undergo a proton transfer from a hydroxyl or a NH group to an aromatic nitrogen. Examples of this class of molecules are, e.g., 7-azaindoles,[4,5,16−20] hydroxyquinolines,[5,21−28] carbazole and 2-(2′-pyridyl)indole derivatives,[5,6,29−35] and 2-(2′-pyridyl) benzimidazole.[36−38]

Hydroxypyridines are known to show an equilibrium in the ground state between the hydroxypyridine or lactim form and the pyridone or lactam tautomer.[12,13,39−46] The influence of the solvent on this tautomerism has been studied, mainly for 2-hydroxypyridine, both experimentally[39−43] and theoretically,[44,45] these investigations concluding that the lactim form is more stable than its lactam tautomer in the gas phase, whereas the amount of lactam form increases with solvent polarity, and predominates in protic solvents. Upon electronic excitation of the lactim form, the acidity at the hydroxyl group and the basicity at the pyridine nitrogen increase, making possible an ESPT assisted by protic species between these two groups.

We have investigated the excited-state proton-transfer processes of several hydroxypyridines. Thus, we have shown[47] that upon excitation, the lactim monocations of 2-(6′-hydroxy-2′-pyridyl)benzimidazole (**1**) and 1-methyl-2-(6′-hydroxy-2′-pyridyl)benzimidazole (**2**) (see Chart 1) protonated at the benzimidazole N(3), experience in acetonitrile an alcohol-assisted ESPT from the hydroxyl group to the pyridine nitrogen as a result of the increase of basicity at the pyridine nitrogen and acidity at the OH group upon excitation. Similar changes in the acid−base properties have been observed for the related compounds 2-(3′-hydroxy-2′-pyridyl)benzimidazole[48] and its derivative methylated at the benzimidazole N(1).[49] The monocations of these compounds, protonated at the benzimidazole N(3), undergo in aqueous solution an excited-state proton transfer from the hydroxyl group to the pyridine nitrogen in a two-step process involving protonation at the pyridine nitrogen and deprotonation at the hydroxyl group. Moreover, the derivative 2-(2′-pyridyl)benzimidazole, without a hydroxyl group, experiences an increase in basicity of the pyridyl nitrogen in the electronic excited state, which triggers an alcohol-assisted ESPT from the benzimidazole NH to the pyridine nitrogen,[36,37] the protonation at the pyridyl nitrogen in neutral aqueous solution,[38] and a water-assisted ESPT from the benzimidazole

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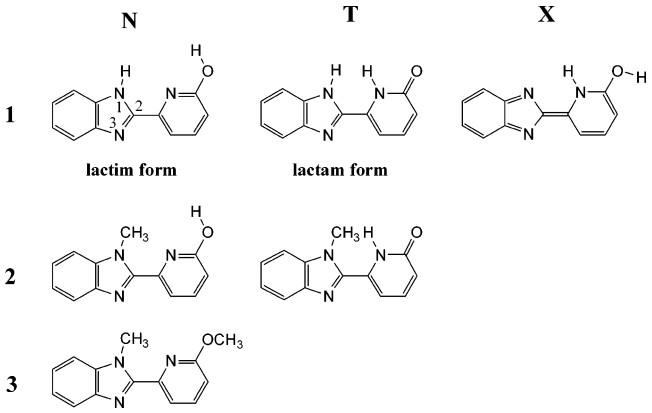
CHART 1: Molecular Structures of 1, 2, and 3 and Their Possible Tautomers N, T, and X



NH to the pyridine nitrogen in the protonated form in acidic aqueous solution.[38]

We have also recently shown[50] that the lactim monoanion of **1** deprotonated at the hydroxyl group experiences in aqueous and ethanolic solutions a solvent-catalyzed ESPT from the benzimidazole NH to the pyridine nitrogen to yield a lactam-type anionic tautomer, deprotonated at the benzimidazole NH. We found that in ethanol at low temperature this process is controlled by the solvent reorganization to give a cyclical complex with the needed geometry to yield the tautomer. Moreover, equilibrium between the lactim anion and its tautomer was already observed for **1** in the ground state in protic solvents, this ground-state tautomerism not taking place for the monoanion of the similar compound 2-(3′-hydroxy-2′-pyridyl)benzimidazole.[49]

Here, we report the first fluorescence studies in neutral media of **1** in several solvents. Because of the presence of different functional groups that can act as hydrogen donors or acceptors in both ground and excited state, we can anticipate this compound as a very useful photoprobe, not only for ESPT studies but also in DNA environments, as has been recently reported for 2-(2′-hydroxyphenyl)benzoxazole.[51,52] The excited lactim tautomer **N*** of **1** (Chart 1) may undergo in protic media a solvent-assisted ESPT either from the hydroxyl group or from the benzimidazole NH to the pyridine nitrogen, leading to tautomer **T*** (lactam) or **X***, respectively. Therefore, the main objectives of this work are as follows: (1) to investigate if **1** tautomerizes in the ground state in several solvents, (2) to find out whether upon excitation a phototautomerization takes place between the different acid/basic sites, and, in that case, (3) to determine the groups involved and the role of solvent in the global behavior. To throw light on these problems, we have also studied compound **2**, which can only give a phototautomerization from the hydroxyl group to the pyridine nitrogen, and 1-methyl-2-(6′-methoxy-2′-pyridyl)benzimidazole (**3**) which cannot tautomerize. Finally, the results are discussed in terms of a lactim−lactam equilibrium in the ground state and a solvent-controlled switching of the excited-state proton-transfer pathway followed by the lactim form.

Experimental Section

Compounds **1**, **2**, and **3** were prepared and purified as described elsewhere.[47] ^{1}H NMR data of these compounds in DMSO-d_6 are shown in the Appendix. Solutions were made up in double-distilled water and spectroscopy grade solvents and were not degassed. Acidity was varied with NaH_2PO_4/Na_2HPO_4 buffer (made up with Merck p.a. products). All experiments were carried out at 25 °C except otherwise stated.

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 a Varian Cary 3E spectrophotometer. 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. UV−vis absorption measurements at low temperature were performed using an Oxford Instruments liquid nitrogen cryostat model 1704 with an ITC 503 control unit and the spectra obtained were corrected for the temperature dependence of the solution volume. Fluorescence quantum yields were measured using quinine sulfate ($<3 \times 10^{-5}$ mol dm^{-3}) in aqueous H_2SO_4 (0.5 mol dm^{-3}) as standard ($\phi = 0.546$).[53,54] Fluorescence lifetimes were determined by single-photon timing in an Edinburgh Instruments FL-900 spectrometer equipped with a hydrogen-filled nanosecond flashlamp and the reconvolution analysis software supplied by the manufacturer.

Quantum-mechanical RHF ab initio calculations were performed using the GAUSSIAN 94 software package.[55]

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 Various Solvents. The absorption spectra of **1**, **2** and **3** in acetonitrile, ethanol, and neutral aqueous solutions are shown in Figure 1. It can be observed in this figure that the spectra of **1** and **2** were red shifted and much broader than that of **3**, showing intense absorption at 28000 cm^{-1} where **3** did not absorb.

The absorption spectra of **1** were measured in ethanol within the temperature range 160−290 K (Figure 2). On lowering the temperature, a drastic increase in the absorption at 30000−32000 cm^{-1} (band I) and decrease at 28000 cm^{-1} (band II) was observed. A clear isosbestic point at 29590 cm^{-1} was detected.

2. Quantum-Mechanical ab Initio Calculations. Ab initio calculations were performed for the lactim and lactam tautomers of **1** in gas phase in the ground state at the RHF/3-21G, RHF/6-31G, RHF/6-31G* and RHF/6-31+G* levels. The relative energies at each calculation level, and dipole moments at the RHF/6-31+G* level, are compiled in Table 1.

3. Fluorescence Spectra and Lifetimes. *Acetonitrile*. The fluorescence spectra of compounds **1** and **2** depended on the excitation wavenumber (Figure 3, parts a and b). Under excitation at band I of the absorption spectrum (30770 cm^{-1}), an emission spectrum overlapping its excitation spectrum was obtained with maximum at ∼27000 cm^{-1} and quantum yield values of 0.21 (**1**) and 0.22 (**2**) (Table 2). This band is similar to the fluorescence spectrum obtained for **3** under the same experimental conditions (Figure 3c). For the three compounds, the excitation spectrum recorded at the maximum of this emission band matched the position of band I in the absorption spectrum.

Under excitation at band I, the fluorescence decay at the maximum, 27000 cm^{-1}, was monoexponential with lifetimes of 1.67 ns for compound **1** (Table 3), 1.65 ns for compound **2** (Table 4), and 1.73 ns for compound **3**. When the fluorescence decay of **1** was recorded at lower wavenumbers, a more complicated behavior was observed. A lifetime of 1.6 ns remained as the main contribution to the decay (>70%), but we also detected a component with a lifetime between 3.2 and

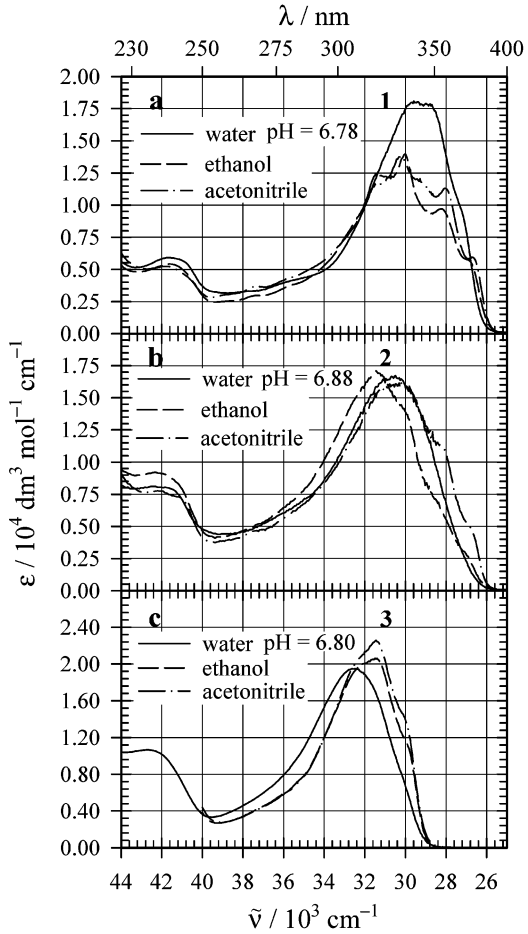


Figure 1. Absorption spectra of **1** (a), **2** (b), and **3** (c) in neutral aqueous solution, ethanol, and acetonitrile.

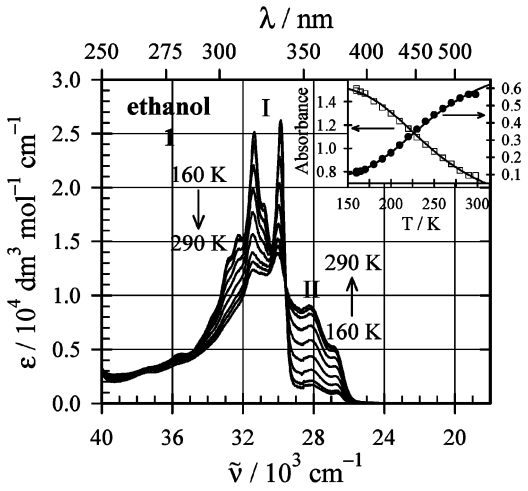


Figure 2. Absorption spectra of **1** in ethanol in the temperature range 160−290 K. The inset shows the temperature dependence of the absorbance for **1** at 28250 (●) and 31450 cm^{-1} (□) and the fitted functions.

4.6 ns (≈10%) and a third one with a very short lifetime (<0.1 ns) and increasing contribution as the wavenumber decreased.

The excitation of compounds **1** and **2** at band II of the absorption spectrum (∼28000 cm^{-1}), absent for compound **3**, led to an emission band around 24000 cm^{-1} (Figure 3, parts a and b). For compound **2**, an additional band located around 20800 cm^{-1} was also observed (Figure 3b). The excitation spectra recorded for this emission band showed contributions

TABLE 1: Relative Energies[a] and Dipole Moments (in Parentheses) for Various Conformers[b] and Tautomers of 1 in the Ground State, As Obtained at Various Levels of Quantum Mechanical ab Initio Calculations

form	RHF/3-21G	RHF/6-31G	RHF/6-31G*	RHF/6-31+G*
N	0.000	0.000	0.000	0.000 (2.06 D)
$\mathbf{N}_{\text{OH rotamer}}$	5.297	5.860	4.583	4.443 (3.93D)
T	2.296	2.066	3.026	2.719 (7.45D)

[a] Energies in kcal mol^{-1} at 0 K. [b] **N** represents the conformer of N with the hydroxy group pointing toward the pyridine nitrogen (represented in Chart 1), and $\mathbf{N}_{\text{OH rotamer}}$ represents the conformer with the OH pointing away from the nitrogen.

TABLE 2: Fluorescence Quantum Yields ϕ[a] of 1 and 2 in Various Solvents at 298 K

	compound **1**		compound **2**	
solvent	ϕ_I	ϕ_{II}	ϕ_I	ϕ_{II}
acetonitrile	0.21	0.013	0.22	0.005
ethanol	0.20	0.10	0.17	0.025
water[b]	0.074	0.065	0.058	0.057

[a] Under excitation in band I (ϕ_I) and band II (ϕ_{II}). [b] pH = 6.78 for **1** and pH = 6.80 for **2**.

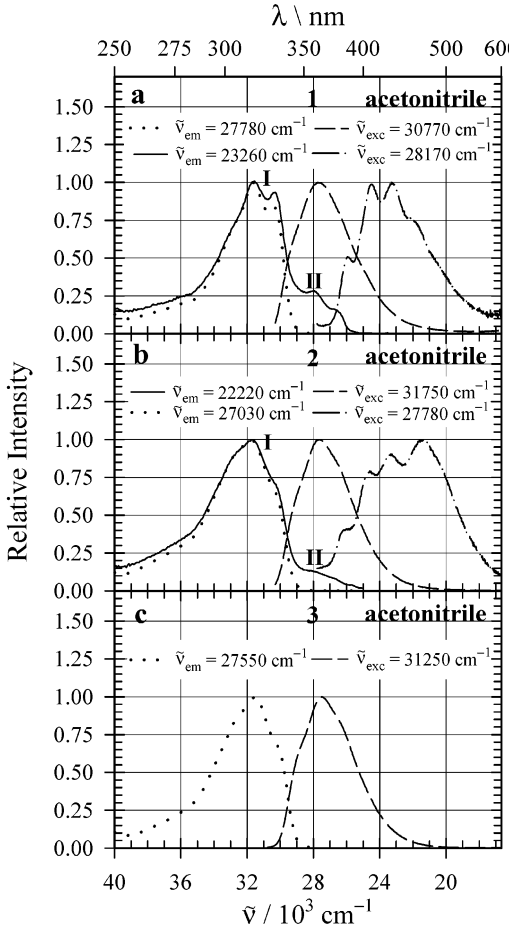


Figure 3. Normalized fluorescence excitation and emission spectra of **1** (a), **2** (b), and **3** (c) in acetonitrile. [**1**] = 7×10^{-6} mol dm^{-3}, [**2**] = 3×10^{-6} mol dm^{-3}, [**3**] = 3×10^{-6} mol dm^{-3}.

of bands I and II. The ratio between them depended on the monitoring emission wavenumber. Furthermore, compound **2** showed a lower contribution of band II compared to **1** for the same emission wavenumber.

Under excitation at band II, the fluorescence decay of **1** and **2** became triexponential. The values of the lifetimes were very

TABLE 3: Fluorescence Decay Times τ and Associated Percentages (in Parentheses) of 1 in Various Solvents[a] at 298 K

solvent	$\tilde{\nu}_{exc}/cm^{-1}$	$\tilde{\nu}_{em}/cm^{-1}$	τ_1/ns	τ_2/ns	τ_3/ns	χ^2
acetonitrile	31 700	27 000	1.67 ± 0.01			1.10
	31 700	24 100	1.60 ± 0.02 (92%)	3.2 ± 0.3 (8%)		1.11
	31 700	23 200	1.66 ± 0.01 (71%)	4.6 ± 0.4 (11%)	0.03 ± 0.01 (18%)	1.07
	29 400	23 800	1.63 ± 0.02 (68%)	4.1 ± 0.2 (10%)	0.07 ± 0.01 (22%)	1.03
	28 600	23 200	1.67[b](6%)	3.8 ± 0.5 (14%)	0.16 ± 0.01 (80%)	0.95
ethanol	31 700	27 700	1.04 ± 0.03			1.05
	31 700	24 100	1.28 ± 0.03 (47%)	3.4 ± 0.1 (18%)	0.12 ± 0.02 (35%)	1.04
	31 700	22 200	2.12 ± 0.02 (65%)	6.6 ± 0.1 (15%)	0.12 ± 0.02 (20%)	1.05
	31 700	21 300	2.07 ± 0.01 (62%)	6.90 ± 0.05 (28%)	0.25[b](10%)	1.07
	27 000	24 100	1.04[b](−χ)	2.50 ± 0.03 (24%)	0.25 ± 0.01 (76%)	1.06
water	31 700	27 000	1.36 ± 0.04 (68%)	2.7 ± 0.1 (15%)	0.30 ± 0.05 (17%)	0.98
pH 6.23	31 700	22 200	1.5 ± 0.2 (9%)	5.3 ± 0.2 (17%)	0.27 ± 0.01 (74%)	1.00
	29 900	23 800		3.6 ± 0.1 (13%)	0.340 ± 0.006 (87%)	1.09
	29 900	22 200		4.0 ± 0.1 (13%)	0.310 ± 0.005 (87%)	1.07

[a] [**1**] = (1−3) × 10^{-5} mol dm^{-3}. [b] Fixed. [c] The associated amplitude is very small and negative and the percentages of the other two lifetimes have been calculated assuming a biexponential decay.

TABLE 4: Fluorescence Decay Times τ and Associated Percentages (in Parentheses) of 2 in Various Solvents[a] at 298 K

solvent	$\tilde{\nu}_{exc}/cm^{-1}$	$\tilde{\nu}_{em}/cm^{-1}$	τ_1/ns	τ_2/ns	τ_3/ns	χ^2
acetonitrile	31 700	27 000	1.65 ± 0.01			1.05
	31 700	24 100	1.71 ± 0.03			1.07
	29 400	24 100	1.62 ± 0.03 (63%)	4.52 ± 0.07 (20%)	0.08 ± 0.01 (17%)	1.09
	29 400	22 200	1.75 ± 0.02 (65%)	4.7 ± 0.2 (11%)	0.06 ± 0.01 (24%)	1.19
ethanol	31 700	27 000	1.55 ± 0.03			1.08
	31 700	24 100	1.57 ± 0.01 (76%)	4.3 ± 0.1 (9%)	0.03 ± 0.01 (15%)	1.04
	31 700	22 200	1.70 ± 0.02 (59%)	4.9 ± 0.1 (21%)	0.03 ± 0.01 (20%)	1.06
	29 400	24 100	1.61 ± 0.02 (64%)	4.4 ± 0.1 (20%)	0.08 ± 0.04 (16%)	1.10
	29 400	22 200	1.80 ± 0.03 (39%)	4.7 ± 0.1 (37%)	0.06 ± 0.01 (24%)	1.08
water	31 700	27 000	1.8 ± 0.1 (56%)	3.6 ± 0.2 (9%)	0.07 ± 0.01 (35%)	1.10
pH 6.11	31 700	24 100	1.4 ± 0.1 (13%)	3.6 ± 0.1 (12%)	0.10 ± 0.01 (75%)	1.18
	31 700	22 200	1.4 ± 0.1 (9%)	4.4 ± 0.1 (15%)	0.09 ± 0.01 (76%)	1.07
	29 400	24 100	1.0 ± 0.2 (7%)	3.4 ± 0.2 (12%)	0.15 ± 0.01 (81%)	0.99
	29 400	22 200	0.9 ± 0.1 (10%)	3.9 ± 0.1 (14%)	0.11 ± 0.01 (76%)	1.13

[a] [**2**] = (1−4) × 10^{-5} mol dm^{-3}.

similar in both compounds, with the lifetime of 1.6−1.7 ns matching that of the fluorescence decay under excitation at band I. Although the lifetime values were very similar in both compounds, the contributions of the three components showed different patterns. Thus, the main contribution for compound **1** corresponded to a lifetime of 0.16 ns (80%) while for compound **2** it was a lifetime of 1.6 ns (65%). The third component showed a lifetime of 3.8−4.7 ns and a contribution less than 20% for both compounds.

Ethanol. The fluorescence behavior of compounds **1** and **2** in ethanol (Figure 4, Tables 3 and 4) was qualitatively similar to the previously reported in acetonitrile, with the exception of the following: (i) under excitation at band I compound **1** showed a shoulder with maximum at ∼20000 cm^{-1}, not observed for compound **2** in this solvent or for neither of them in acetonitrile; (ii) in both compounds the excitation spectra obtained at emission wavenumbers lower than 27000 cm^{-1} showed a bigger contribution of band II in comparison to acetonitrile; (iii) the fluorescence quantum yield under excitation at band II was higher than in acetonitrile (Table 2); (iv) the fluorescence decay of compound **1** at 27000 cm^{-1} when exciting at band I was faster (1.04 ns) than in acetonitrile (1.67 ns), while for **2** remained very similar (1.55 ns); (v) for compound **1**, and only under excitation at band I, a very long lifetime (6.6−6.9 ns) was detected at low emission wavenumbers.

Neutral Aqueous Solution. The excitation and emission spectra of **1** and **2** in neutral aqueous solution are shown in Figure 5, parts a and b, respectively. The emission spectra showed for both compounds a much weaker dependence with the excitation wavenumber than that observed in acetonitrile and ethanol, the spectra obtained differing only slightly in the high-energy region. However, the excitation spectra showed a stronger dependence with the emission wavenumber. A fluorescence excitation band with maximum at 29800 cm^{-1} for **1** and at 31250 cm^{-1} for **2** was obtained with the emission monochromator positioned at 22730 and 24100 cm^{-1}, respectively. When the excitation was collected at the blue edge of the emission spectra ($\tilde{\nu}_{em}$ = 27000−28000 cm^{-1}), an excitation band with maximum at 31700 cm^{-1} for **1** and 33100 cm^{-1} for **2** was observed. The fluorescence quantum yields are shown in Table 2.

The fluorescence decay of **3** in aqueous solution at pH 6.72 was monoexponential with a lifetime of 2.32 ns. A triexponential decay was obtained for **1** when exciting at 31700 cm^{-1} (Table 3). A decay time of 1.36 ns was obtained, its percentage decreasing as the emission wavenumber decreased, together with a decay time of around 0.30 ns, showing its maximum contribution to the decay at low emission wavenumbers. The third component, 2.7−5.3 ns, contributed less than the other two to the global decay. At $\tilde{\nu}_{exc}$ = 29900 cm^{-1} the fluorescence decay was biexponential at both 23800 cm^{-1} and 22200 cm^{-1} emission wavenumbers. The main component showed a decay time of ∼0.30 ns (> 87%) and the second decay time had a value of ∼4 ns.

For **2** (Table 4), the fluorescence decay at pH = 6.11 was triexponential independently of the excitation wavenumber. A decay time of 1.8 ns with a 56% contribution was obtained at $\tilde{\nu}_{em}$ = 27000 cm^{-1} under excitation at 31700 cm^{-1}. A short lifetime of ∼0.1 ns was also observed with its contribution increasing on going from high to low wavenumbers (35−81%). The third component had a lifetime of ∼4 ns and showed a very small contribution (9−15%) at all emission wavenumbers.

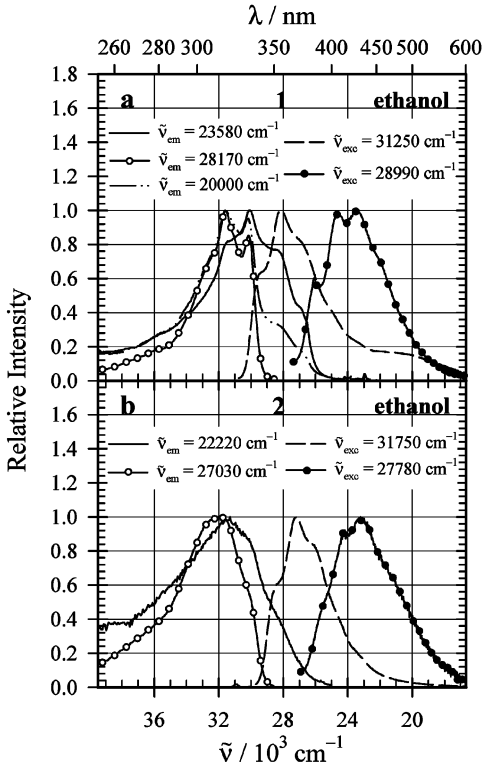


Figure 4. Normalized fluorescence excitation and emission spectra of **1** (a) and **2** (b) in ethanol. [**1**] = 4 × 10^{-6} mol dm^{-3}, [**2**] = 3 × 10^{-6} mol dm^{-3}.

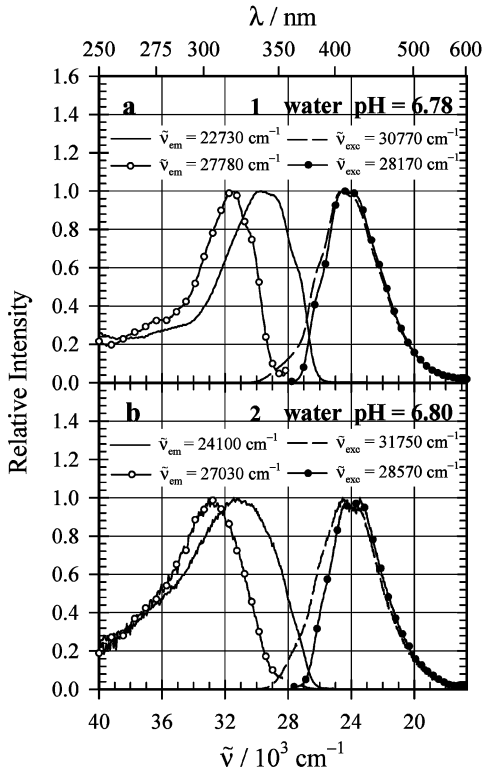


Figure 5. Normalized fluorescence excitation and emission spectra of **1** (a) and **2** (b) in neutral aqueous solution. [**1**] = 7 × 10^{-6} mol dm^{-3}. [**2**] = 3 × 10^{-6} mol dm^{-3}.

Discussion

1. Interpretation of the Absorption Spectra: Tautomeric Equilibrium in the Ground-State Modulated by the Solvent. The absorption spectra of **1** and **2** in acetonitrile and ethanol (Figure 1) were broader than those recorded for **3**, suggesting more than one species in the ground state. Furthermore, the increase in the absorption at ∼30000 cm^{-1} (band I) and the isosbestic point detected on lowering the temperature from 290 to 160 K (Figure 2) indicate that in ethanol there is an equilibrium between two species in the ground state. Also, they must be neutral as they do not match any of the spectra reported previously for the monoanions[50] in the same solvents or for the monocations[47] in acetonitrile.

To identify these species, we must consider all the possible neutral structures for these compounds. Three different candidate structures for **1** can be proposed (Chart 1): the lactim or normal form **N**, the lactam tautomer **T** resulting from a proton translocation between the hydroxyl group and the pyridine nitrogen, and a second tautomer **X** originated by proton transfer from the benzimidazole NH to the pyridine nitrogen. Tautomer **X** can be ruled out because compound **2**, methylated at the benzimidazole NH, cannot give **X** but showed absorption spectra similar to those for **1**. This points to **N** and **T** being responsible for the absorption bands I and II obtained for **1** and **2** in acetonitrile and ethanol. The tautomeric lactim−lactam equilibrium is analogous to that reported for 2-hydroxypyridine and derivatives.[13,39−46] Various levels of RHF ab initio ground-state calculations were performed in gas phase for **T** and two conformers of the lactim form of **1**: **N** (shown in Chart 1), and $\mathbf{N}_{\text{OH rotamer}}$, with 180° rotation of the OH group. Table 1 shows the results of these calculations. It is clearly seen that, independently of the calculation level, **N** is the more stable structure in the gas phase followed by **T**, this species being between 2.00 and 3.00 kcal mol^{-1} less stable. From these figures, a percentage of ∼2% of **T** in the ground sate is calculated in equilibrium at room temperature.

Nevertheless, the stability of **N** and **T** is probably different in solution. It is shown in Table 1 that the dipole moment of **T** (7.45 D) is higher than that of **N** (2.06 D). According to this, **T** is expected to be more stabilized than **N** by polar solvents. It is important to point out that the ^{1}H NMR spectra of **1** and **2** in DMSO-d_6 (see the Appendix) showed broad signals for the protons in ortho and para positions with respect to the OH group, in agreement with the existence of equilibrium between **N** and **T**.

We still must assign absorption bands I and II to **N** and **T**. This can be done taking into account that compound **3**, methylated both at the hydroxyl group and at the benzimidazole NH, can only exist as the lactim form. Thus, on the basis of similarities in position and shape between the absorption spectrum of **3** and band I of compounds **1** and **2**, it is reasonable to propose that band I is due to **N,** whereas band II corresponds to the absorption of **T**.

From the temperature dependence of the absorption spectra of **1** in ethanol (Figure 2) the values of $\Delta H°$ and $\Delta S°$ for the equilibrium $\mathbf{N} \leftrightarrows \mathbf{T}$ can be estimated if we assume that, in the range 160−290 K, $\Delta H°$ and $\Delta S°$ do not depend on temperature. Assuming an ideal behavior of the very diluted solutions, the temperature dependence of the absorbance at any wavenumber can be calculated using eqs 1 and 2.

$$A^{\tilde{\nu}} = \frac{A_N^{\tilde{\nu},0} + A_T^{\tilde{\nu},0}K}{1+K} \quad (1)$$

$$K = \frac{[T]}{[N]} = \exp\left[-\frac{\Delta H° - T\Delta S°}{RT}\right] \quad (2)$$

In these equations, $A_N^{\tilde{\nu},0}$ and $A_T^{\tilde{\nu},0}$ are the absorbance values, at any wavenumber, of **N** and **T** when the equilibrium is

completely shifted to **N** or **T** respectively, and K is the equilibrium constant defined by eq 2. Equations 1 and 2 were fitted to the experimental absorption data at various wavenumbers. A global fit within the range 41670−28250 cm^{-1} was carried out, the results at 28250 and at 31450 cm^{-1} being shown in the inset of Figure 2. The optimization procedure led to $\Delta S° = 44 \pm 4$ J mol^{-1} K^{-1} and $\Delta H° = (11.2 \pm 0.8)$ kJ mol^{-1}. With these results, the equilibrium constant K is calculated to be 0.04 ± 0.03 at 160 K and 2.2 ± 1.2 at 298 K. It is clearly seen that whereas **N** is the more stable form at low temperatures, **T** becomes the more stable tautomer as the temperature is increased, the entropic term favoring the stabilization of **T**. The value of $\Delta H°$ can also be estimated from the electronic energies of **N** and **T** obtained from ab initio calculations (Table 1). To calculate the enthalpy of **N** and **T**, the contributions of the translational, vibrational, and rotational enthalpy must be taken into account. As the molecular structures of **N** and **T** are very similar, we can assume that those terms cancel each other in the calculation of $H_T° - H_N°$, $\Delta H°$ being estimated as the difference between the electronic energy of **N** and **T**. In this way, a value for $\Delta H°$ between 9 and 13 kJ mol^{-1} is calculated. This figure is in good agreement with that obtained experimentally, although the theoretical value corresponds to the gas-phase equilibrium.

With regard to the behavior of **1** and **2** in neutral aqueous solution, it is not clear from the absorption spectra if an equilibrium between **N** and **T** exists, as in acetonitrile and ethanol, or if there is only one absorbing species. The study of the fluorescence excitation and emission spectra of **1** and **2** in aqueous solution, discussed in the next section, will allow us to clarify this point. Furthermore, the analysis of the fluorescence spectra of these compounds in acetonitrile and ethanol supports the interpretation of the absorption spectra in these solvents.

2. Interpretation of the Fluorescence Spectra in Acetonitrile: No Significant Excited-State Proton Transfer. The fluorescence spectra of **1**, **2**, and **3** obtained in acetonitrile are shown in Figure 3. The excitation of compounds **1** and **2** at the maximum of band I led to a single fluorescence band that overlapped the excitation spectrum and decayed monoexponentially at the maximum with lifetimes of 1.67 and 1.65 ns (Tables 3 and 4), respectively. From the similarities in the fluorescence position and lifetime obtained for **1** and **2** with those recorded for **3** (1.73 ns), and taking into account that the latter can only exist as the lactim form, we can conclude that under these conditions the fluorescence observed for **1** and **2** is mainly due to the excited lactim form **N***. Furthermore, from the resemblance in the lifetime values of the three compounds, we can rule out the possibility of **N*** undergoing any other deactivation process apart from the fluorescence decay.

For compound **1**, excitation of **T** at the absorption band II led to a structured emission spectrum (Figure 3a) that overlapped the absorption band. This indicates that the fluorescence emission is mainly due to **T***. The excitation spectrum of this fluorescence band showed contributions of absorption bands I and II, with the ratio between them strongly dependent on the emission wavenumber. The fluorescence quantum yield (Table 2) obtained under excitation in band II, attributed to **T**, is around 20 times lower than that measured under excitation in band I, due to **N** + **T**, implying that $\phi_N \gg \phi_T$. This difference in fluorescence quantum yield values allowed some fluorescence from **N*** being detected at the emission maximum of **T***. It has not been possible to obtain the excitation spectrum of **T*** alone because the **N*** emission overlapped almost entirely the emission band of **T***. Only at wavenumbers around 17000 cm^{-1} this may be possible, however the sensitivity of our photomultiplier drops substantially in that region. At $\tilde{\nu}_{em} < 24000$ cm^{-1}, a triexponential fluorescence decay was obtained for compound **1** independently of the excitation wavenumber. One of the lifetimes observed, 1.6 ns, corresponds to **N***, its contribution being again due to the detection of **N*** emission within this wavelength region. A component of ∼0.1 ns showing its main contribution (80%) at $\tilde{\nu}_{em} = 23\,200$ cm^{-1} under excitation in band II was also obtained and must correspond to **T***. Finally, a third component of ∼4 ns with a minor contribution to the global decay (<14% at all emission wavenumbers) was also detected. The low participation of this component makes it impossible for us to prove its identity, but we believe that this lifetime corresponds to a trace impurity present in the sample, probably similar to that detected in the spectrum of compound **2**, as a similar component lifetime was also found for compound **2** (see next paragraph).

The behavior of **2** under excitation in band II was very similar to that of **1** except for the fact that the fluorescence spectrum showed, besides the band due to **T***, a second band at around 21000 cm^{-1} which was absent for **1**. As we suspected that this band could be due to an impurity, we synthesized a new sample of **2** and measured again the fluorescence (spectrum shown in Figure 3b). We found that the intensity of the band at ∼21000 cm^{-1} diminished, whereas the rest of the spectrum did not change, which strongly supports our hypothesis that this band is due to an impurity. The very low fluorescence quantum yield of **T*** would allow the detection of residual emission from a trace impurity of high fluorescence quantum yield. The observation of the fluorescence emission band of the impurity only for compound **2** can be due to the fact that the fluorescence quantum yield under excitation in band II (which at least for **2** involves, besides **T***, the species fluorescing at about 21000 cm^{-1}) is lower for **2** (0.005) than for **1** (0.013), this meaning that ϕ_T is much smaller for **2** than for **1**. The fluorescence decay of **2** at low emission wavenumbers was, as for **1**, triexponential, showing the contributions of **N*** (component of 1.6 ns), and **T*** (component of lifetime < 0.1 ns). The third component of ∼4.6 ns must be assigned to the fluorescence of the trace impurity, showing a similar lifetime as that detected for compound **1**.

Although the pure excitation spectrum of **T** was not known, the absorption spectrum of both **1** and **2** could be satisfactorily decomposed as a sum of the contributions of **N** (corrected excitation spectrum obtained at $\tilde{\nu}_{em} = 27780$ cm^{-1}) and that of a mixture of **N** and **T** (corrected excitation spectrum obtained at $\tilde{\nu}_{em} = 23260$ cm^{-1}, showing the maximum contribution of the lactam tautomer). Figure 6a shows the results for **1**. The good agreement between the experimental and calculated absorption spectra of **1** (and also of **2**, results not shown) supports the initial assumption that only two species are present in the ground state. Furthermore, the excitation spectrum attributed to **N** + **T** showed a higher contribution of **N** than that observed in the absorption spectrum due to the fact that $\phi_N \gg \phi_T$, this resulting in a negative contribution of the pure excitation spectrum of **N** to calculate the absorption spectrum.

3. Interpretation of the Fluorescence Spectra in Ethanol: Excited-State Proton Transfer from the Benzimidazole to the Pyridine Ring. The behavior of **2** in ethanol (Figure 4b, Table 4) resembles that observed in acetonitrile. The excitation of **2** at band I led mainly to the emission of the lactim form **N*** with maximum at 27000 cm^{-1} and a lifetime of 1.55 ns, very similar to that obtained for **3** in ethanol (1.67 ns). Under excitation at band II the emission band of the lactam tautomer

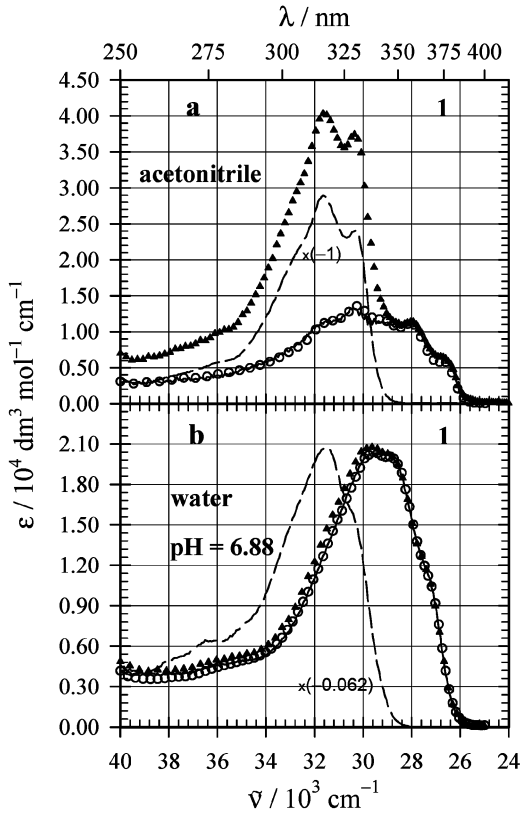


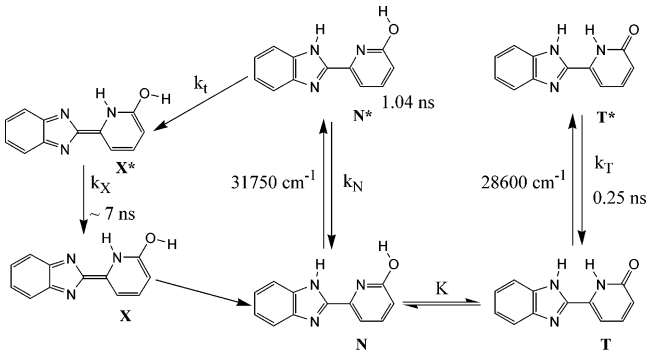
Figure 6. (a) Experimental (○) absorption spectrum of **1** in acetonitrile together with the calculated (—) spectrum obtained by fitting a linear combination of the fluorescence excitation spectrum at $\tilde{\nu}_{em}$= 27780 cm^{-1} (− −, negative contribution) and that corresponding at $\tilde{\nu}_{em}$= 23260 cm^{-1} (▲▲▲). (b) Experimental (○) absorption spectrum of **1** in aqueous solution of pH 6.88 together with the calculated (—) spectrum obtained by fitting a linear combination of the fluorescence excitation spectrum at $\tilde{\nu}_{em}$= 27780 cm^{-1} (− −, small negative contribution; the factor represents the number by which the spectrum should be multiplied to obtain the real contribution) and that corresponding at $\tilde{\nu}_{em}$= 22730 cm^{-1} (▲▲▲). [**1**] = 7 × 10^{-6} mol dm^{-3} for fluorescence.

T* was observed, the fluorescence quantum yield obtained being about 4 times higher than in acetonitrile, but even so much lower than that of the lactim form (Table 2). At $\tilde{\nu}_{em} \leq$ 24000 cm^{-1} the fluorescence decay of **2** was triexponential (Table 4). The first exponential showed the characteristic decay time of **N*** and a decreasing contribution as the emission wavenumber decreased, this species being detected at these emission wavenumbers due to its high fluorescence quantum yield. A decay time τ < 0.1 ns showing its main contribution to the decay at ∼22000 cm^{-1} was also detected, and is assigned to **T***. The third decay time of 4−5 ns might be assigned again to an impurity. Because of the higher quantum yield of **T*** in ethanol than in acetonitrile, the emission band of the impurity cannot be detected in the spectrum.

The behavior of **1** under excitation at band I (Figure 4a) is different from that of the *N*-methyl derivative **2** shown above. Thus, besides the characteristic fluorescence band due mainly to the lactim form **N***, a shoulder undetectable when exciting at band II and with maximum at ∼20000 cm^{-1} was observed. At 27700 cm^{-1} the fluorescence decay due only to **N*** is monoexponential with a lifetime of 1.04 ns, about 1.6 times lower than the lifetime obtained for the same tautomer in compounds **2** and **3**. This suggests an additional deactivation channel as compared to the lactim form of **2** and **3**.

Under excitation at band II (ascribed to **T**) and at 24100 cm^{-1} emission wavenumber, the fluorescence decay of **1** was triexponential, the major component showing a lifetime of 0.25 ns, originating from **T***. The decay was also triexponential within the region 24100−21300 cm^{-1} under excitation at band I. One component had a decay time of 0.1−0.2 ns, matching the decay time of the lactam tautomer **T***. The other two decay times depended on the monitoring emission wavenumber. Thus, a component with a decay value of 1−2 ns was observed, whereas the other decay time had a value ranging from ∼3 to ∼7 ns. The decay time of 1−2 ns should be ascribed to **N***, the dependence of this value with the emission wavenumber being probably due to the existence of some correlation in the fit due to the detection, as in acetonitrile, of the lifetime of ∼4 ns assigned to the trace impurity. The last component showed a long lifetime (>6.6 ns) only in the emission region of the shoulder located at 20000 cm^{-1} and under excitation at band I, where **N** absorbs. All these suggest that this long lifetime corresponds to the species responsible for the emission at 20000 cm^{-1}, which must be formed upon excitation of the lactim tautomer **N**. Further evidence can be obtained by analyzing the excitation spectra. It is observed in Figure 4a that at $\tilde{\nu}_{em}$ = 23580 cm^{-1} (maximum of **T*** fluorescence band), the excitation spectrum showed a higher intensity of band II and a lower one of band I as compared to those observed in the excitation spectrum obtained at $\tilde{\nu}_{em}$ = 20000 cm^{-1}. This confirms that the precursor of the species fluorescing at around 20000 cm^{-1} is the lactim form **N**.

SCHEME 1: Excitation and Deactivation of 1 in Ethanol



Moreover, the lifetime of the excited lactim form **N*** of **1**, 1.04 ns, is clearly shorter than that of this species for compounds **2** (1.55 ns) and **3** (1.67 ns), suggesting that the lactim form of **1** experiences an additional deactivation channel as compared to the other two compounds. This process must correspond to the formation, from **N***, of a new fluorescent species **X***, responsible for the emission at ∼20000 cm^{-1} with long lifetime. This new species must be neutral, since its fluorescence does not match any of the monoanions[50] or monocations[47] of **1**. We can get information about its nature if we take into account that the lactim tautomer of **2**, where the benzimidazole NH group has been replaced by NMe, does not undergo any process in the excited state. This points to the benzimidazole NH group as the active site and suggests that the process taking place for **1** is a solvent-assisted excited-state proton transfer from the benzimidazole NH to the pyridine nitrogen (see Scheme 1). This process is favored by the increase of basicity of the pyridine nitrogen in S_1 state, as it has been reported for 2-(2′-pyridyl)-benzimidazole,[36−38] 2-(3′-hydroxy-2′-pyridyl)benzimidazole,[48] and 1-methyl-2-(3′-hydroxy-2′-pyridyl)benzimidazole.[49] A similar excited-state process was demonstrated for the monoanion of compound **1**[50] and 2-(2′-pyridyl)benzimidazole in neutral[36,37] and protonated form.[38] It is worth stressing that process **N*** → **X*** probably does not take place intramolecularly, but needs

the assistance of one or more molecules of the hydroxylic solvent, which would act as bridge between the acid and the basic site. This is suggested by the fact that the process does not takes place in acetonitrile, and was demonstrated for the similar processes taking place for 2-(2′-pyridyl)benzimidazole.[36−38]

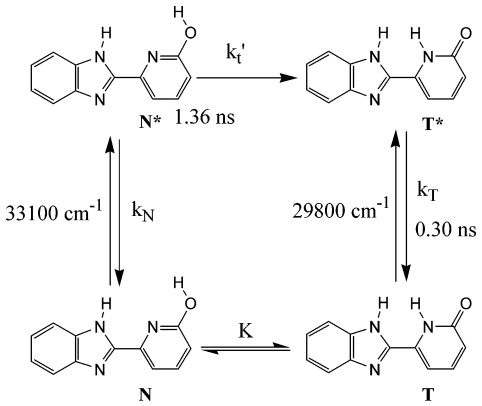
The rate constant, k_t, of process **N*** → **X*** can be estimated as follows. As the fluorescence lifetime of **N*** in acetonitrile, solvent where no process **N*** → **X*** occurs, is about the same for **1**, **2** and **3** (1.67, 1.65, and 1.73 ns, respectively) we can consider the fluorescence lifetime of the lactim form of **1** in ethanol in the absence of phototautomerization to be that of compound **3** in the same solvent ($\tau_N^0 = k_N^{-1} = 1.67$ ns). Taking $\tau_N = (k_N + k_t)^{-1}$ to be 1.04 ns for **1**, k_t is calculated to be 3.6×10^8 s^{-1}. Similar values were obtained for the similar phototautomerization process observed for the monoanion **NA*** of **1** in ethanol (2.0×10^9 s^{-1}) and water (1.52×10^8 s^{-1}).[50]

Finally, as not only the basicity of the pyridine nitrogen, but also the acidity of the OH group increase in the excited state, we cannot rule out that a fraction of the excited **N*** species experience in this protic solvent a proton transfer from the hydroxyl group to the pyridine nitrogen to give **T***. We will show in the next section that this process can be inferred for **1** and **2** in neutral aqueous solution. From the data available, process **N*** → **T*** cannot be verified although the fact that the decay time of the lactim form of **2** (which cannot yield **X*** but may, as **1**, experience the process **N*** → **T***) is almost coincident with that of compound **3** (which cannot suffer that process), suggests that the phototautomerization **N*** → **T*** does not take place to a great extent in this solvent.

4. Interpretation of the Fluorescence Spectra in Aqueous Solution: Excited-State Proton Transfer within the Pyridine Moiety. In contrast to what happens in acetonitrile and ethanol, the fluorescence emission spectra of **1** and **2** in neutral aqueous solution (Figure 5) showed only a minor dependence on the excitation wavenumber. Under excitation at the maximum of the absorption spectrum (∼29500 cm^{-1}), we detected a single emission band with maximum at ∼24000 cm^{-1} that decayed with a component (>80%, Tables 3 and 4) with lifetimes of 0.3 (**1**) and 0.1 ns (**2**). Both lifetimes and emission maxima are very similar to those obtained for the lactam form **T*** in acetonitrile and ethanol. The excitation spectrum performed at the maximum of **T*** overlapped the emission band and was very similar to the absorption spectrum (see Figure 6b). All these suggest the lactam tautomer as the main species in both ground and excited states. This is consistent with the results of the quantum-mechanical calculations, showing that the dipole moment is higher for **T** than for **N**, and therefore **T** becomes more stabilized in polar solvents. Similar results were obtained for other hydroxypyridines.[13,39−45] Although the lactam tautomer is the main species in ground and excited states, a weak emission from **N*** was detected at 27000 cm^{-1}, decaying with lifetimes of 1.36 (**1**) and 1.8 ns (**2**). The excitation spectra carried out at 27000 cm^{-1} matched that of **3**, which can only exist as the latim form.

The possibility that process **N*** → **T*** takes place in this protic solvent remains to be considered. More insight about this topic can be gained from the comparison of the fluorescence lifetime of **N*** for compounds **1** and **2** with that of **3**, for which that process is not possible, and from the comparison of the excitation and absorption spectra for **1** and **2**. The fluorescence lifetime of **N*** is 2.32 ns for **3**, but only 1.36 ns for **1**, this difference pointing to the existence of an extra deactivation channel for **1**. As for acetonitrile and ethanol, we tried to decompose the absorption spectrum of **1** as the sum of the contributions of the excitation spectra obtained at 27780 and 22730 cm^{-1}. The results are shown in Figure 6b. It is seen that the absorption spectrum almost coincides with the excitation spectrum obtained at 22730 cm^{-1}, except at high wavenumbers where **N** absorbs, the excitation spectrum showing a somewhat higher intensity than the absorption spectrum in that region. This means that part of the fluorescence from **T*** originates from **N**. There are two possibilities to explain this: (i) Under excitation at wavenumbers where **N** absorbs, a small part of the fluorescence detected at 22000 cm^{-1} is due to **N***, and (ii) all the fluorescence detected corresponds to **T*** but a fraction of this species comes from the excitation of **N** through a phototautomerization process **N*** → **T***. The amount of **N*** emission detected at 22000 cm^{-1}, which corresponds to the tail of its fluorescence spectrum, must be undetectable because even when **N** is excited at its absorption maximum the amount of emission detected is very weak. For this reason, we can rule out point i. Furthermore, point i will not explain the short fluorescence lifetime of **N*** as compared to **3**, whereas point ii seems more plausible as it explains the decrease in the lifetime of **N*** as compared to **3**, in which process **N*** → **T*** cannot take place.

SCHEME 2: Excitation and Deactivation of 1 in Neutral Aqueous Solution



We can conclude that in neutral aqueous solution a water-assisted phototautomerization of **N*** to yield **T*** takes place for **1** as shown in Scheme 2. A similar excited-state proton transfer from the hydroxyl group to the pyridine nitrogen has been detected by us for the monocations of **1** and **2** in acetonitrile in the presence of small amounts of alcohols[47] and water,[56] the process being more efficient for **1** than for **2**. As regards compound **2**, the excitation spectrum obtained at $\tilde{\nu}_{em} = 22200$ cm^{-1} is blue shifted with respect to the absorption spectrum, and the fluorescence lifetime of **N*** (1.8 ns) is also lower than that of **3**. From both findings we propose that process **N*** → **T*** also takes place for **2**.

With respect to the mechanism of the phototautomerization, there are three different processes that could yield **T*** from **N***: (1) deprotonation of **N*** at the hydroxyl group to give normal anion **NA*** which then protonates at the pyridine nitrogen to yield **T***; (2) protonation of **N*** at the pyridine nitrogen leading to a monocation which then deprotonates at the hydroxyl group giving **T***; (3) a water-assisted multiprotonic transfer from the hydroxyl group to the pyridine nitrogen.

Process 1 can be ruled out on the following grounds. At pH = 6, the H^+ concentration is too low for the protonation of **NA*** by H^+ to compete with the deactivation of **NA***. Water would have to act as acid to protonate **NA*** yielding **T***. This process could also take place at higher pH, but we have demonstrated recently[50] that direct excitation of the normal anion **NA** in aqueous solution does not lead to **T*** but to a tautomeric

lactam anion **TA*** as result of a proton transfer from the benzimidazole NH to the pyridine nitrogen, both fluorescence from **NA*** and **TA*** being observed. This fact rules out the existence of process 1.

The small amount of **N** present in the ground state makes it impossible to decide between mechanisms 2 and 3 for this phototautomerization, but process 2 seems to be less probable as we have never detected that intermediate monocation in acidified solvents. Nevertheless, if we assume that the same mechanism holds for **1** and **2**, we can estimate the rate constant k_t' for this process as follows. As the fluorescence lifetime of **N*** in acetonitrile, a solvent where no process **N*** → **T*** occurs, is about the same for **1**, **2** and **3** (1.67, 1.65, and 1.73 ns respectively), we can consider the fluorescence lifetime of **N*** in the absence of phototautomerization for **1** and **2** in aqueous solution to be that of compound **3** in the same solvent ($\tau_N^0 = k_N^{-1} = 2.32$ ns). Taking $\tau_N = (k_N + k_t')^{-1}$ to be 1.36 ns for **1** and 1.80 ns for **2**, k_t' is calculated to be 3.0×10^8 s^{-1} for **1** and 1.2×10^8 s^{-1} for **2**, indicating that the process **N*** → **T*** is more efficient for **1** than for **2**. This is in agreement with our previous finding that the analogous lactim−lactam phototautomerization taking place for the monocations of these compounds in acetonitrile, assisted by alcohols, is more efficient for **1** than for **2**,[47] and must be due to the presence of a methyl group at the benzimidazole nitrogen N(1) in compound **2**.

To sum up, the behavior of **N*** in compound **1** found in our studies is strongly dependent on the solvent. **N*** does not tautomerize in acetonitrile but undergoes a proton transfer from the benzimidazole NH to the pyridine nitrogen in ethanol (**N*** → **X***) and a proton transfer from the hydroxyl group to the pyridine nitrogen in water (**N*** → **T***). The reason for this behavior lies in the protic properties of the solvent and the solvation dynamics, which decisively influence the rates of the various competing processes. In acetonitrile, the phototautomerization of **1** must be very slow, and was in fact not detected, because the process does not occur intramolecularly, and the aprotic solvent does not facilitate the proton transfer through bridges of solvent molecules. In the protic solvents ethanol and water, formation of a hydrogen-bonded bridge between the acid (NH or OH) and the basic (pyridine N) sites through solvent molecules is allowed, the rate of the competing phototautomerization processes being dependent on the nature of the solvation complexes and its dynamics. We have demonstrated for the anion of **1** (deprotonated at the OH group) that the proton transfer from the benzimidazole NH to the pyridine N is much faster in ethanol than in water,[50] the process involving in ethanol as first step a solvent rearrangement to form an adequate solvate which yields the tautomer in a very fast process. The rate of the similar tautomerization process **N*** → **X*** is also probably much faster in ethanol than in water, thus allowing the observation of process **N*** → **X*** in ethanol and process **N*** → **T*** in water.

Conclusions

In this article, we have studied the ground- and excited-state behavior of **1** and **2** in acetonitrile, ethanol, and neutral aqueous solutions. We have shown that in these solvents there is an equilibrium for both compounds in the ground state between the lactim or normal form **N** and its lactam tautomer **T**, the equilibrium shifting toward the tautomer **T** as the polarity of the solvent increases.

Under excitation of **T**, fluorescence from **T*** is observed for **1** and **2** in all the solvents. The behavior of **N*** is however solvent dependent. In acetonitrile, under excitation of **N** only fluorescence from **N*** is observed for both compounds. In ethanol, a fraction of the excited normal form of **1** experiences a proton transfer from the benzimidazole NH to the pyridine nitrogen to give **X*** as a result of the increase of basicity of the pyridine nitrogen in the excited state. This process cannot take place for **2**. In neutral aqueous solution a different phototautomerization is observed. Both for **1** and **2**, part of the excited **N*** molecules undergo a proton transfer from the hydroxyl group to the pyridine nitrogen yielding the lactam tautomer **T***. Both phototautomerization processes are assisted by the solvent.

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Appendix

Compound 3. ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 7.95 (dd, 1H, $J = 7.5$ Hz, 1.5 Hz), 7−90 (t, 1H, $J = 7.5$ Hz), 7.68 (ddd, 2H), 7.29 (dd, 2H, $J = 7.6$ Hz, 1.2 Hz), 6.95 (dd, 1H, $J = 7.5$ Hz, 1.5 Hz), 4.27 (s, 3H, N−CH_3), 3.99 (s, 3H, O−CH_3).

Compound 2. ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 12.9 (s, N−H), 11.1 (s, O−H), 7.74 (t, 1H, $J = 8.4$ Hz), 7.68 (ddd, 2H, $J = 7.6$ Hz, 1.4 Hz, 0.7 Hz), 7.46 (broad d, 1H, $J = 8.4$ Hz), 7.28 (dd, 2H, $J = 7.6$ Hz, 1.4 Hz), 6.66 (d, 1H, $J = 8.4$ Hz), 4.15 (s, 3H, N−CH_3).

Compound 1. ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 12.9 (s,N−H), 11.1 (s, O−H), 7.70 (t, 1H, J∼7.5 Hz), 7.64 (broad band, 2H), 7.45 (broad band, 1H), 7.22 (broad m, 2H), 6.65 (broad d, 1H, $J \sim 7.5$ Hz).

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