Ground-State Tautomerism and Excited-State Proton-Transfer Processes in 4,5-Dimethyl-2-(2′-hydroxyphenyl)imidazole in Solution: Fluorescence Spectroscopy and Quantum Mechanical Calculations

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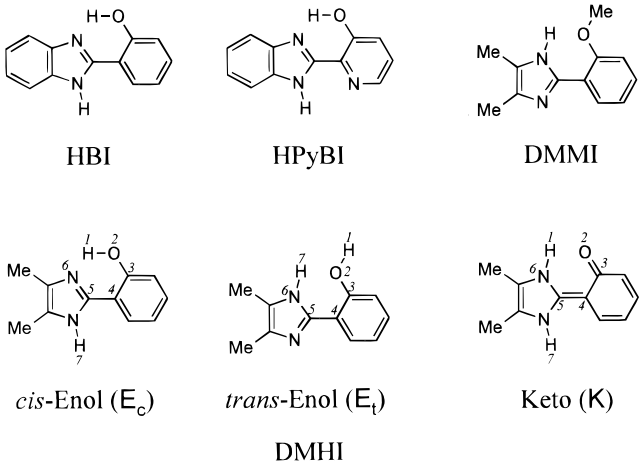
Ground-state tautomerism and excited-state proton transfer of 4,5-dimethyl-2-(2′-hydroxyphenyl)imidazole (DMHI) was investigated by means of UV−vis absorption spectroscopy, by steady-state and time-resolved fluorescence spectroscopy, and by quantum mechanical calculations. The behavior of DMHI in ethanol and water was studied in neutral, acidic, and basic conditions. Three ground-state species were detected in neutral solutions of DMHI: the *cis*-enol form with an intramolecular hydrogen bond, the *trans*-enol form that is hydrogen bonded to the solvent, and the keto tautomer. The relative proportions of these species depend strongly on the solvent: the keto tautomer was not detected in ethanol, whereas this tautomer is the predominant species in neutral water, where negligible amounts of the *cis*-enol form exist. These experimental findings could be reproduced by quantum mechanical calculations up to the B3LYP/6-311+G** level of theory combined with semiempirical calculations of solvation free energies, which allowed the energy diagram in the gas phase and in solution to be known. A combination of the supermolecular approach and the SMx methodology was necessary to account for the differential solvation of the different isomers in neutral aqueous solution. In the excited state, the *cis*-enol form always undergoes the fast excited-state intramolecular proton transfer to yield the keto tautomer. Upon photoexcitation of the *trans*-enol form and the keto tautomer, no excited-state reaction takes place. In acidic media, the photoexcited cation suffers deprotonation of the hydroxyl group, yielding the excited keto tautomer.

Introduction

In the past decades a wealth of research work has been conducted on proton transfer in excited electronic states and, in particular, in the field of excited-state intramolecular proton transfer (ESIPT).[1,2] Electronic molecular properties can change dramatically upon excitation to upper excited states. Very often this change in electronic molecular properties causes enhancement of the acid or basic character of the molecule. It is commonly accepted that aromatic nitrogens and hydroxyl groups become more basic and acidic, respectively, in the first-excited singlet state in comparison to the ground state.[3] Typical examples of this behavior are related molecules such as 4,5-dimethyl-2-(2′-hydroxyphenyl)imidazole (DMHI),[4] 2-(2′-hydroxyphenyl)benzimidazole (HBI),[4−8] 2-(3′-hydroxy-2′-pyridyl)benzimidazole (HPyBI),[9,10] 2-(2′-hydroxyphenyl)benzoxazole,[3,6,11,12] and 2-(2′-hydroxyphenyl)benzothiazole.[13,14] They behave in a similar manner in apolar solvents: the ground-state species is in the so-called *cis*-enol form (shown in Scheme 1), with an intramolecular hydrogen bond between the hydroxyl group and the N(3) of the azole. Upon photoexcitation to the first-excited singlet state, it undergoes an ultrafast proton transfer from the hydroxyl group to the nitrogen, as a consequence of the reverse acidities in the excited state. The proton-transfer reaction generates a tautomer usually called keto (see Scheme 1), which is the only fluorescent species in apolar solvents.

SCHEME 1: Molecular Structures of DMHI Tautomers and Conformers, DMMI, and Related Molecules Which Exhibit ESIPT



The behavior of the above-mentioned azole derivatives is very sensitive to the nature of the solvent and great differences have been observed in hydrogen-bonding solvents. In alcoholic solvents, most of these molecules show a conformational equilibrium between the *cis*- and *trans*-enol forms (Scheme 1). In the trans conformer, the strong O−H···N intramolecular hydrogen bond is replaced by intermolecular hydrogen bonds to the solvent and a weaker N−H···O intramolecular hydrogen bond. In the excited state, the *cis*-enol produces ESIPT and

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generates the keto tautomer. For the excited *trans*-enol, ESIPT is impossible and it deactivates emitting fluorescence.

High polarity and ability to form hydrogen bonds are special features of water that could cause different behavior compared to alcoholic solvents. A striking example was found in the study of HPyBI[10] in aqueous solutions. The *trans*-enol form of this species was found to be in negligible concentration in any solvent, but the keto tautomer was discovered to exist in the ground state in comparable amounts to the *cis*-enol form in neutral aqueous solutions. Later on, the ground-state keto tautomer was also detected in neutral aqueous solutions of HBI,[8] although in lesser amounts. No further cases have been reported in the literature on related molecules. In the excited state, the *cis*-enol always gives rise to ESIPT and produces the excited keto tautomer, also reached by direct excitation of the ground-state keto tautomer. In aqueous solution, the excited *trans*-enol form of HBI emits fluorescence and loses a proton, leaving the excited anion.[8]

With the aim of unraveling the molecular factors that favor the existence of the ground-state keto tautomer in aqueous solution, we have undertaken the investigation of the behavior of 4,5-dimethyl-2-(2′-hydroxyphenyl)imidazole. It is well-known that the basicity of the N(3) of the imidazole ring in aqueous solution is greater than that of the benzimidazole.[15] This factor could favor the keto structure (which is protonated in the azole ring) in relation to the enol forms. Moreover, DMHI presents the advantage of having a smaller molecular size than HBI and HPyBI, which facilitates high-level ab initio quantum mechanical calculations to study in depth the energy diagram in the gas phase and in solution. For these reasons we have undertaken the study of ground- and excited-state tautomerism and proton-transfer processes of DMHI in aqueous and ethanolic solution. The experimental study of this problem was done by means of UV−vis absorption and steady-state and time-resolved fluorescence spectroscopy. The model compound 4,5-dimethyl-2-(2′-methoxyphenyl)imidazole (DMMI, Scheme 1), in which intramolecular proton transfer is impossible, was also investigated. A quantum mechanical study was performed in the gas phase and in solution in order to know the relative energies of the different neutral forms of DMHI and how these energies can be affected by water solvation. "Solvation model" series (SMx) developed by Cramer and Truhlar[16−19] were employed as implemented in Spartan 4.1 programs to describe solute−solvent interactions in a semiquantitative way in combination with the supermolecular approach.[10,19−21]

This research work presents some novel results in the field of ground- and excited-state tautomerism of azoles in aqueous solutions. DMHI expands the range of different behaviors shown by related azoles in aqueous solution. A large proportion of DMHI is present as the keto tautomer in water, in equilibrium with the *trans*-enol form and a negligible proportion of the *cis*-enol form. This behavior could be reproduced by quantum mechanical calculations. SMx methodology, which accounts for polarization, dispersion, and solvent rearrangement effects, was not able to predict the differential solvation energy of the different enol and keto tautomers. Neither was the supermolecular approach able to predict the differential solvation energies of these species in the absence of a dielectric media that would allow the stabilization of molecules with highly separated charge densities as is the case of the keto form. In contrast, the combination of the SMx methodology and the supermolecular approach[19] seemed to resolve, almost quantitatively, the different solvation energies of keto and enol forms in neutral aqueous solutions. Such a result reveals the key role played by water in equilibria involving hydrogen-bonding species and molecules containing widely separated charges.

Experimental Section

DMHI was obtained by condensing salicylaldehyde with an equimolar amount of 2,3-butanedione in concentrated ammonium hydroxide.[22] The product was purified by repeated recrystallization from ethanol/water mixtures and characterized by elemental analysis and ^{1}H NMR spectroscopy in CCl_3D solution. DMMI was obtained by the same method using anisaldehyde instead of salicylaldehyde. Solutions were made up in spectroscopic grade ethanol (Scharlau) and double distilled water. All aqueous solutions contained 2% (v/v) of ethanol because DMHI and DMMI, which are poorly soluble in water, were initially dissolved in the alcohol. Acidity was varied with $HClO_4$, NaOH or NaH_2PO_4/Na_2HPO_4, NaAc/HAc, and NH_4ClO_4/NH_3 buffers (made up with Merck p.a. products). The total buffer concentration was always lower than 10^{-3} mol dm^{-3}. A slow ground-state reaction takes place in highly basified aqueous solutions of DMHI, detected by a progressive change in the UV−vis absorption spectrum. To avoid this problem, solutions used were always freshly prepared and we checked that the spectra remained unchanged during experiments. All experiments were carried out at 25 °C and none of the solutions were degassed.

Acidity constants were determined spectrophotometrically as described previously,[23] based on the pH dependence of UV−vis absorption spectra. pH was measured with a Radiometer PHM 82 pH meter equipped with a Radiometer Type B electrode. UV−vis absorption spectra were recorded in a Varian Cary 3E spectrophotometer. Fluorescence excitation and emission spectra were scanned in an 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 counting in an Edinburgh Instruments FL-900CD spectrofluorometer 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 in the Marquardt algorithm.

Ab initio and density functional theory (DFT) calculations were carried out with GAUSSIAN 94 programs.[24] The considerable size of the molecule restricted geometry optimization to the Hartree−Fock level of theory where the 6-31+G* basis set was employed. Harmonic frequency analysis was performed to characterize the stationary points as either minima or first-order saddle points and to obtain thermodynamic corrections. To refine the energies, single-point calculations on the B3LYP/6-311+G** level of theory were performed. Zero-point energy (ZPE) corrections for these calculations were carried out using the values obtained at the HF/6-31+G* level. ZPE were scaled.[25] For some structures we performed additional B3LYP/6-31+G* geometry optimizations and harmonic frequency analysis. Semiempirical calculations were carried out with MOPAC 93.[26] Solvation effects were modeled with the SMx methods as implemented in Spartan 4.1.[27]

Results of the Spectroscopic Measurements

1. UV−Vis Absorption Spectra of DMHI in Water and Ethanol. Figure 1a shows the UV−vis absorption spectra of DMHI in neutral, acid and basic ethanol, and Figure 1b shows the spectra at different pH values in aqueous solution. Although

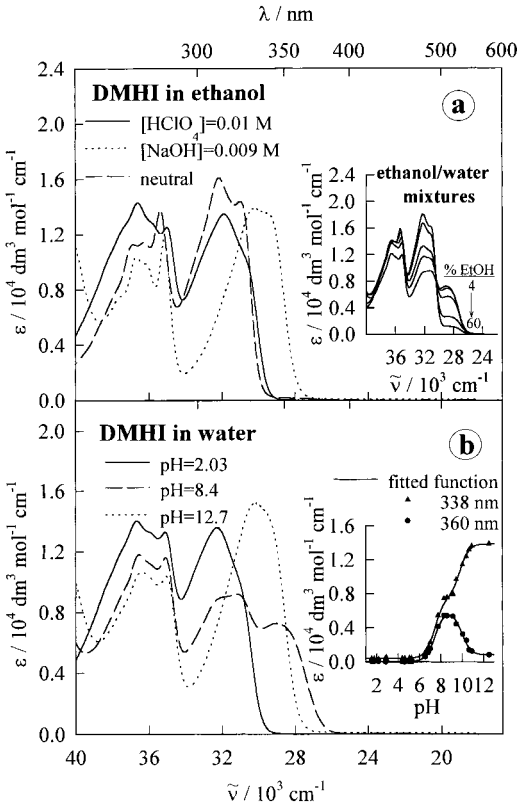


Figure 1. (a) UV−vis absorption spectra of DMHI in ethanolic solutions at various acidities. The insert shows the dependence of the absorption spectra of DMHI on solvent composition in neutral conditions. (b) UV−vis absorption spectra of DMHI in aqueous solution at various pH values. The insert shows the dependence of the DMHI molar absorption coefficient on the pH of the solution at 338 and 360 nm together with the fitted function.

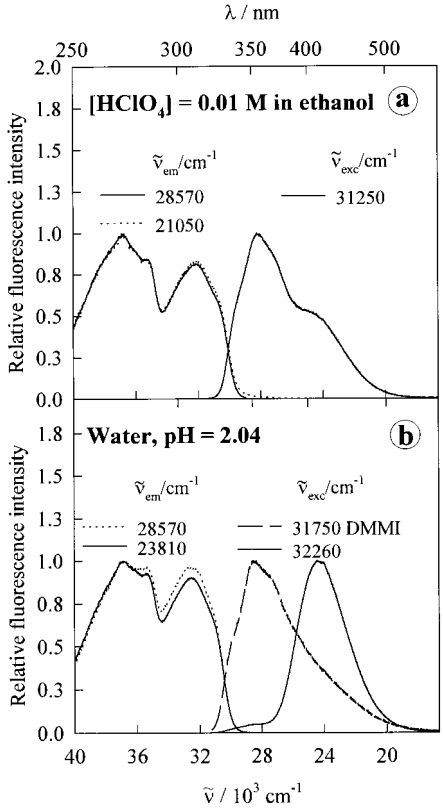


Figure 2. (a) Corrected fluorescence excitation and emission spectra of DMHI in acidified ethanolic solutions, [DMHI] = 1.1×10^{-5} mol dm^{-3}. (b) Corrected fluorescence excitation and emission spectra of DMHI in acidified aqueous solution, [DMHI] = 1.1×10^{-5} mol dm^{-3} and corrected fluorescence emission spectra of DMMI in the same conditions. The monitoring wavenumbers are shown.

the absorption spectra of DMHI in acid and basic ethanol coincide with those obtained in acid and basic water, respectively, this coincidence holds no longer in neutral solutions where a red-shifted absorption band was found in water but not in ethanol.

To get an insight into the influence of solvent on the intensity of the red-shifted absorption band peaking at 29 200 cm^{-1} in neutral aqueous solutions, we registered the UV−vis absorption spectrum of DMHI in different ethanol/water mixtures (see insert in Figure 1a). Increasing ethanol percentage resulted in a progressive extinction of the band peaking at 29 200 cm^{-1} and increase of the band at 32 000 cm^{-1}, showing an isosbestic point at 30 300 cm^{-1}.

The acidity constants of DMHI were obtained spectrophotometrically.[23] pK_a values obtained at several wavenumbers were 7.43 ± 0.03 and 9.86 ± 0.10 (see insert in Figure 1b). It has been checked that DMHI obeys the Lambert−Beer law at concentrations even higher than 3×10^{-4} mol dm^{-3}.

2. Fluorescence Spectra and Lifetimes in Acidic Media. The fluorescence excitation spectrum of DMHI in acidic ethanol and water was found to be unique and in very good agreement with the absorption spectrum. Nevertheless, the fluorescence emission was clearly dual (Figure 2).

In *acidified ethanol*, we observed a strong emission band peaking at 28 200 cm^{-1}, together with a shoulder with maximum at ∼25 000 cm^{-1} (Figure 2a). The strong emission band decayed monoexponentially with a fluorescence lifetime of 1.8 ns. At the red edge of the fluorescence emission spectrum, the decay profile was biexponential with lifetimes of 1.6 and 3.5 ns, the former showing negative amplitude.

In *acidified water*, the intensity ratio between the two emission bands is inverted with regard to ethanol solutions (Figure 2b). The fluorescence decay profile of the very weak emission band centered at 28 500 cm^{-1} coincided with the lamp pulse, which means that the fluorescence lifetime is shorter than the time resolution limit of our instrument, 0.1 ns, while the red-shifted band decayed with a lifetime of 3.0 ns.

DMMI in acidified aqueous solutions showed a single fluorescence emission band with normal Stokes shift, which decayed monoexponentially with a lifetime of 2.4 ns (Figure 2b).

3. Fluorescence Spectra and Lifetimes in Basic Media. The fluorescence spectra of DMHI were very similar in basic ethanol and water and both had normal Stokes shifts. The emission spectra showed maximum intensity at 26 000 cm^{-1} in ethanol and 26 600 cm^{-1} in water and decayed monoexponentially with a lifetime of 1.8 ns in both solvents. Excitation and emission spectra did not depend on the emission and excitation wavenumbers, respectively. In addition, corrected fluorescence excitation spectra showed a good agreement with the absorption spectra.

4. Fluorescence Spectra and Lifetimes in Neutral Media. DMHI showed dual fluorescence in *neutral ethanol* (Figure 3a). Two partially overlapped bands with maxima at 28 500 and 23 900 cm^{-1} were observed, with decay times of 2.3 and 3.5 ns, respectively. The fluorescence excitation spectra monitored at both emission bands were slightly different.

The fluorescence of DMHI in *neutral water* was also dual (Figure 3b), showing two overlapped bands peaking around ∼27 500 and 24 700 cm^{-1}, with lifetimes of 2.8 and 3.0 ns, respectively. The excitation spectra of the two emission bands

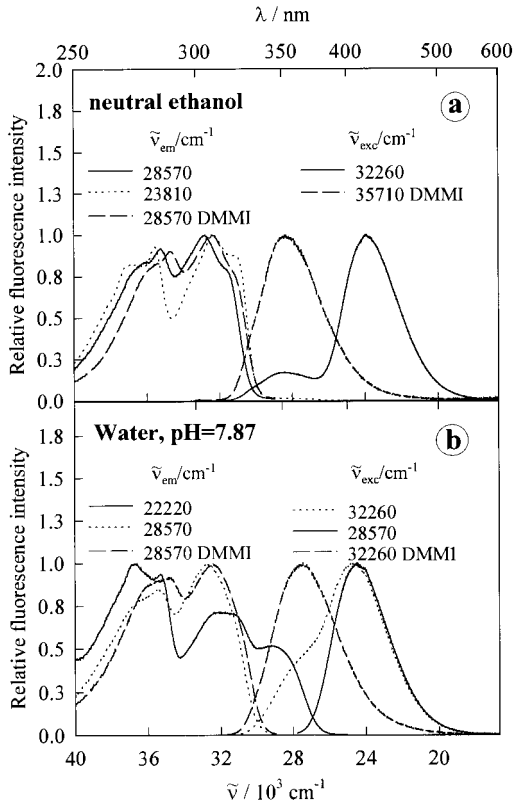


Figure 3. (a) Corrected fluorescence excitation and emission spectra of DMHI and DMMI in neutral ethanolic solutions, [DMHI] = 1.1×10^{-5} mol dm^{-3}. (b) Corrected fluorescence excitation and emission spectra of DMHI and DMMI in neutral buffered aqueous solution, [DMHI] = 1.1×10^{-5} mol dm^{-3}. The monitoring wavelengths are also shown.

were completely different. The excitation of the red-shifted emission band showed the characteristic peak of the absorption spectrum in aqueous solution at 29 200 cm^{-1}. Excitation at this wavenumber afforded just a single emission band, which decayed monoexponentially with a lifetime of 3.0 ns.

Figure 3a,b also shows the fluorescence emission spectra of DMMI in neutral ethanol and water. The emission spectra showed a single band which decayed monoexponentially with lifetimes of 2.2 ns in neutral ethanol and 2.4 ns in neutral water.

Discussion of the Spectroscopic Results

1. Analysis of the Absorption Spectra of DMHI. Acid−Base and Tautomeric Equilibria in the Ground State. Two acid−base equilibria with pK_a 7.43 and 9.86 were observed in aqueous solution of DMHI. These pK_a are in keeping with the values for the protonation of the N(3) of imidazole (6.993 at 25 °C and zero ionic strength)[15] and for the deprotonation of the hydroxyl group of the phenol (9.98 at 25 °C and zero ionic strength).[15] Therefore, the former acid−base equilibrium is thought to involve the cationic and neutral species, and the latter the neutral and anionic species (Scheme 2). It is noteworthy the difference in the first pK_a value for DMHI and HBI,[8] 7.43 and 5.48, respectively. Since DMHI shows a first pK_a value roughly equal to the unsubstituted imidazole (6.993), this effect is thought to be due to the electron-withdrawing effect caused by the set of aromatic carbons of the adjacent ring in the case of HBI.

The similarity between the absorption spectra of DMHI in water and ethanol, both in acidic and basic media, does not hold any longer in neutral solutions. A red-shifted band peaking at 29 200 cm^{-1} exists in water but not in ethanol or in other polar or nonpolar solvents.[4] The dependence of the absorption spectrum on water concentration (insert in Figure 1a), showing a clear isosbestic point, indicates the existence of a chemical equilibrium dependent on the solvent. A similar behavior was found for HPyBI,[10] and interpreted by the existence, only in water, of the keto tautomer in equilibrium with the enol form. If this explanation holds also for DMHI, we should assign the red-shifted band in the absorption spectrum of DMHI in aqueous solution to the neutral keto tautomer, which would be in equilibrium with the enol forms (Scheme 2). This equilibrium would be completely shifted to the enol forms in nonaqueous solutions, as the red-shifted absorption band disappears completely. Further evidence in favor of this hypothesis and identification of the enol conformation was obtained by the fluorescence measurements discussed in subsection 4.

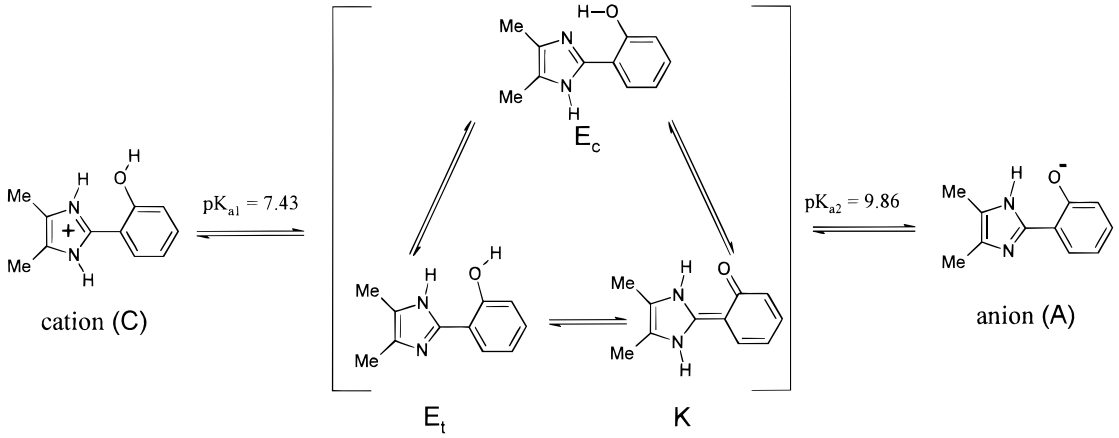
2. Analysis of Steady-State and Time-Resolved Fluorescence Data in Acidified Media. Although dual fluorescence was observed in acidified ethanol and water (Figure 2), just one precursor must be present in the ground state, given that the fluorescence excitation spectrum showed no dependence on the emission wavenumber and it coincided with the absorption spectrum in acidic media. The dual fluorescence must therefore be due to an excited-state process undergone by the excited cation, which is the only species present in the ground state in these conditions.

The fluorescence behavior of the O-methylated compound DMMI can throw light on the nature of the excited-state process operating in acid solutions of DMHI. The fluorescence emission of protonated DMMI showed just a single band with a normal Stokes shift (Figure 2b). It can therefore be concluded that O-methylation hinders the excited-state reaction operating for protonated DMHI. Furthermore, both band shape and spectral position of the higher-energy band of DMHI coincided with the single emission band of DMMI. According with these results, we suggest that the excited cation of DMHI transfers its hydroxyl proton to the solvent to afford the excited keto tautomer, which fluoresces with a great Stokes shift (Scheme 3).

This hypothesis is supported by the time-resolved fluorescence measurements. In ethanol, the fluorescence decay time measured at 28 600 cm^{-1} (1.8 ns), which should be assigned to the excited cation, matches the fluorescence rise time (1.6 ns) of the lower-energy band. This confirms that the species responsible for this band is formed from the cation in the excited-state. The fluorescence lifetime of the DMHI cation is also shorter than that of the DMMI cation (2.4 ns), according to the additional deactivation channel that deprotonation implies. A similar behavior should hold in aqueous solutions, although we could not clearly demonstrate it since the fluorescence lifetime of the cation is shorter than the resolution limit of our instrument and, as a result, it could not be shown that it matches the fluorescence rise time of the red-shifted band. The decay time of this band, assigned to the keto tautomer, is 3.5 ns in ethanol and 3.0 ns in water, similar to the values found for this species in neutral nonhydroxylic solvents.[4]

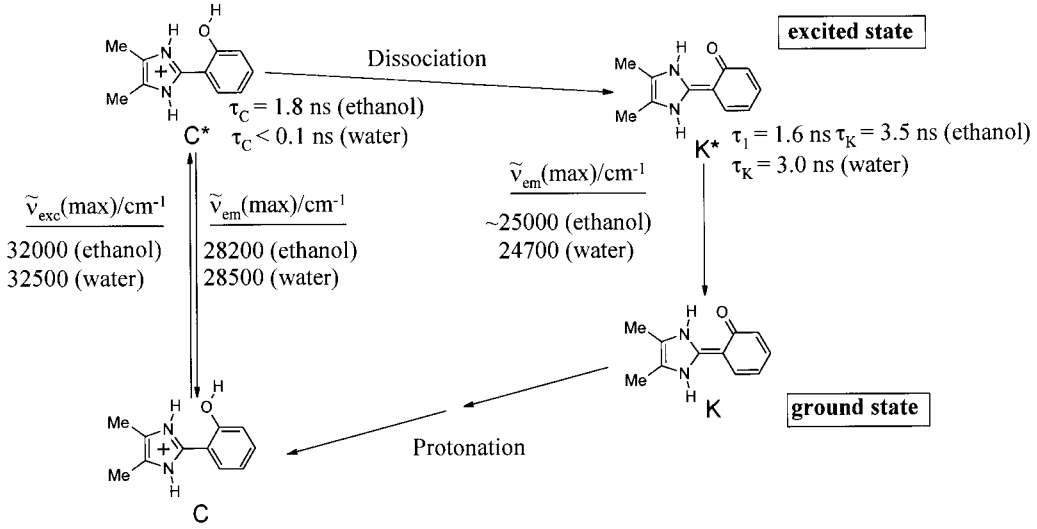
The lifetime measurements and the intensity ratio of the two emission bands in ethanol and water showed that the proton transfer to the solvent is far more efficient in water than in ethanol, as could be expected,[28] and was already previously found for the related molecules HBI[8] and HPyBI.[9] By comparing the behavior of DMHI, HBI, and HPyBI in acidic media, it is worth noting the much greater efficiency of the deprotonation for HBI[8] and HPyBI.[9] For these molecules, emission from the

SCHEME 2: Rotameric, Tautomeric, and Acid−Base Equilibria of Ground-State DMHI[a]



[a] According to the fluorescence measurements, E_c and E_t are the major forms in neutral ethanolic solutions, and E_t and K in neutral water.

SCHEME 3: Ground- and Excited-State Behavior of DMHI in Acidified Ethanolic and Aqueous Solutions



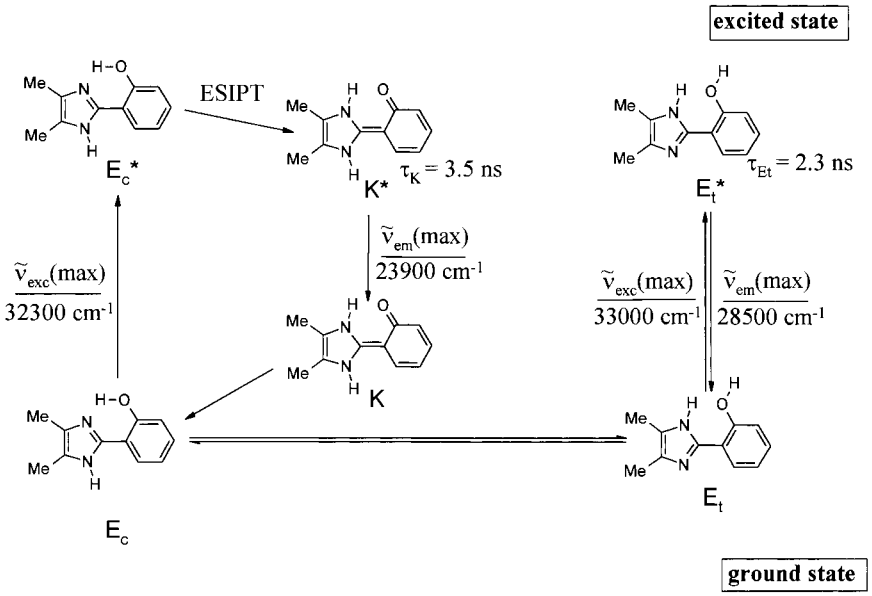
cation could only be clearly seen in nonprotic solvents like acetonitrile; in ethanol the emission is very weak and in water it could not be detected.

3. Analysis of Steady-State and Time-Resolved Fluorescence Data in Basic Media. At pH 11 (or NaOH concentrations nearby 0.001 M when working in ethanol solutions), around 99% of DMHI molecules are present in the ground state as anionic forms, which allows selective excitation of the DMHI anion. The fluorescence excitation spectra were in good agreement with the absorption spectra in both ethanol and water. The fluorescence emission spectrum showed a normal Stokes shift, no dependence on the excitation wavenumber, and a monoexponential decay. These results allow the assignment of the spectrum to the excited anion, which would have a lifetime of 1.8 ns.

4. Analysis of Steady-State and Time-Resolved Fluorescence Data in Neutral Media. In *neutral ethanol* the fluorescence emission spectrum consists of two bands (Figure 3a). The species responsible for these two bands must come from different species in the ground state, since the excitation spectra monitored at the two emission bands are different. The UV−vis absorption spectrum in neutral ethanol can be perfectly reproduced as a linear combination of both fluorescence excitation spectra. Despite the fact that both excitation spectra are rather similar, the species responsible for them must show some critical structural difference, as can be deduced from the great shift observed between their emission bands, probably due to an excited-state process.

The study of the O-methylated derivative again provided valuable information to identify the emitting species. As in acidified media, O-methylation prevents the excited-state process, as can be inferred from the absence of the red-shifted emission band in the fluorescence emission spectrum of DMMI in neutral ethanol (Figure 3a). This datum allows to locate the reaction site at the hydroxyl group of the phenyl moiety of the DMHI and suggests the excited-state reaction to be an intramolecular proton transfer from the hydroxyl group to the imidazole N(3). This hypothesis is supported by the similarity between the emission band peaking at 23 900 cm^{-1} in neutral ethanol and the red-shifted band registered in acidified solutions, and by the coincidence of its fluorescence decay times (3.5 ns), which allows this band to be assigned to the keto tautomer. The fluorescence excitation spectrum of the red-shifted band recorded in neutral ethanol is not very different from the excitation spectra of DMMI. Its precursor, therefore, has probably an enol-type structure. As in previous investigations,[8,10] we propose the *cis*-enol form (see Scheme 4), which would lead to the keto tautomer through a fast ESIPT. It must be pointed out that the excited keto tautomer comes from different precursors in the ground state in acidic and neutral ethanolic solutions, as can be deduced from the different excitation spectra of this emission band in both media (Figures 2a and 3a). In

SCHEME 4: Ground- and Excited-State Behavior of DMHI in Neutral Ethanolic Solution



acidic solution, the excited keto form builds up from the cation through loss of the hydroxyl proton to the solvent; in neutral solution, it comes from the *cis*-enol form through ESIPT.

It rests to assign the higher-energy band in the emission spectrum of DMHI in ethanol. This band is coincident in shape and position with the emission band of DMMI in neutral ethanol (Figure 3a). Furthermore, its fluorescence excitation spectrum and that of DMMI are very alike (Figure 3a). This set of results suggests that the species responsible for the higher-energy band has a similar electronic structure to the O-methylated compound and has therefore an enol-type structure, probably in a trans conformation, which hinders the excited-state intramolecular proton transfer, in line with previous investigations on related molecules.[6−8]

The fluorescence lifetimes clarify the dynamic aspects of the mechanism. The decay of the *trans*-enol species is monoexponential with a lifetime of 2.3 ns, which is comparable to the fluorescence lifetime of the O-methylated compound in neutral ethanol, 2.2 ns. The excited keto tautomer decays monoexponentially with a lifetime of 3.5 ns. No fluorescence rise time has been observed for the tautomer emission, which confirms that the tautomer is formed in the excited state through a very fast intramolecular process (ESIPT). As a result, the *cis*-enol form is confirmed as the only ground-state precursor of the keto tautomer in neutral ethanol. Our interpretation of the behavior of DMHI in neutral ethanolic solutions is summarized in Scheme 4.

The close proximity of the pK_a values of DMHI adds some complexity to the study of its behavior in *neutral aqueous solutions*, since it is not possible to isolate neutral forms from protonated or deprotonated species. Under such conditions it is necessary to carefully control the pH of the solution in order to avoid the presence of the anion in the ground state. Anion presence is not desired for several reasons: first, to reduce the number of emitting species and, second, to clear up if there is excited-state dissociation of the neutral species to yield the excited anion, as was previously found for HBI.[8] On the contrary, the presence of the cation in the ground state is preferred since its influence on the emission spectrum is easily known, as will be explained later. Therefore, a pH value of 7.87 was used for the analysis of the behavior of DMHI in aqueous solutions. In these conditions the concentration of the cation species is 16% and the anion concentration is just 1%.

DMHI exhibited dual fluorescence in aqueous solutions at pH 7.87 (Figure 3b). The relative contribution of each fluorescence band to the whole spectrum depends on the excitation wavenumber. The emission band with maximum at 24 700 cm^{-1} coincides with that registered in acidified water and it is slightly blue-shifted compared to that recorded in ethanol; it can therefore be assigned to the excited keto tautomer species. The decay of this band is monoexponential with a lifetime of 3.0 ns, which should be assigned to the decay of the excited keto form in neutral aqueous solution, as was equally found in acidic aqueous solution. The fluorescence excitation spectrum monitored at this emission band contains the contribution of the cation species. This contribution can be determined through knowledge of the pure excitation spectrum of the cation, obtained in acidic solutions under the same conditions. As the amount of cation in the ground state at pH 7.87 is known, its contribution to the excitation spectrum can be easily calculated and taken away from the whole spectrum. Figure 4 shows the fluorescence excitation spectrum obtained in this way for the neutral forms of DMHI that yield the excited keto tautomer, labeled as K. Comparison of this spectrum with that assigned to the *cis*-enol form in ethanol (Figure 3a) leads us to conclude that the *cis*-enol form must be present in very low concentration in neutral aqueous solution, since only a very weak shoulder is observed in the spectral region around 32 300 cm^{-1} where the *cis*-enol form reaches its absorption maximum. The peak at 29 200 cm^{-1} observed in the excitation spectrum of the red-shifted fluorescence band must be assigned to the keto tautomer since the enol forms and the cation do not absorb at this wavenumber and excitation there induces emission of the keto form with a mirror-image relationship with the absorption and normal Stokes shift (Figure 3b). This conclusion agrees with the previous assignment of the similar red-shifted band appearing in the absorption spectrum of DMHI in neutral aqueous solution. We have to conclude therefore that the ground-state keto tautomer is the major precursor of its excited form in neutral aqueous solution, with a negligible contribution from the *cis*-enol form. This result is in line with our expectation that the increase in basicity of the nitrogen should favor the presence of the keto

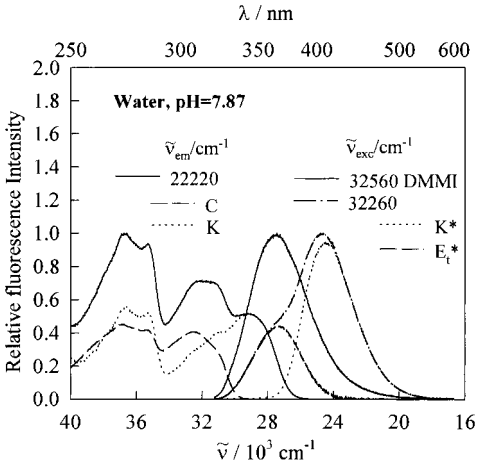
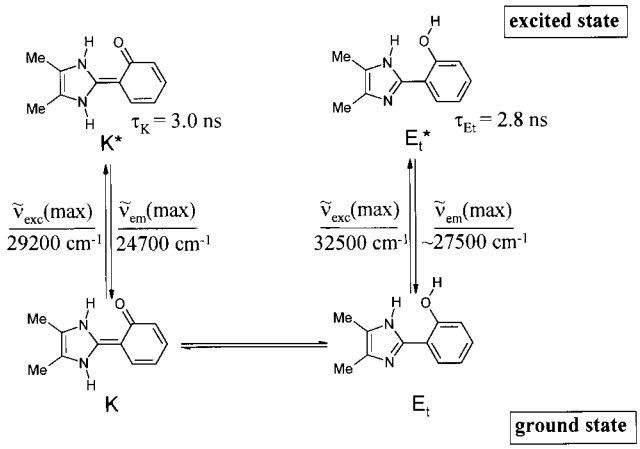


Figure 4. (Left) Decomposition of the excitation spectra of DMHI in neutral aqueous solutions as linear combination of the fluorescence excitation spectra of the cation (C) and keto (K) forms, [DMHI] = 1.1×10^{-5} mol dm^{-3}. (Right) Decomposition of the fluorescence emission spectra of DMHI in neutral aqueous solutions as linear combination of *trans*-enol (E_t*) and keto (K*) fluorescence emission spectra, [DMHI] = 1×10^{-5} mol dm^{-3}. The fluorescence emission spectra of DMMI in neutral aqueous solutions is also shown for comparison.

SCHEME 5: Ground- and Excited-State Behavior of DMHI in Neutral Aqueous Solution



form. For related molecules, only minor amounts of the keto form were detected (HBI),[8] or similar amounts to the *cis*-enol form (HPyBI).[10] This is the first case where the keto tautomer predominates clearly over the *cis*-enol form.

The emission band of DMHI in water peaking at ~27 500 cm^{-1} is quite similar to the emission band of DMMI, and the fluorescence excitation spectrum recorded at this emission band is almost equal to the excitation spectrum assigned to the *trans*-enol species registered in neutral ethanol (Figure 3). These bands are therefore attributed to the *trans*-enol species. The individual emission spectrum of the *trans*-enol form has been obtained normalizing at their red edge the dual fluorescence spectrum of DMHI obtained by excitation at 32 260 cm^{-1} with the spectrum of the keto form obtained by excitation at 28 570 cm^{-1}, followed by subtraction of the latter. The fluorescence emission spectrum for the *trans*-enol form determined in such a way (labeled E_t* in Figure 4) is in good agreement with the emission spectrum of DMMI. Our interpretation of the behavior of DMHI in neutral aqueous solutions is summarized in Scheme 5.

The fluorescence lifetime measurements are in keeping with the previous conclusions. On the one hand, the fluorescence decay of the *trans*-enol species, measured at 28 600 cm^{-1}, is monoexponential with a lifetime of 2.8 ns. This lifetime is even longer than in neutral ethanol, 2.3 ns, and that of the O-methylated compound, 2.4 ns. On the other hand, no contribution of the fluorescence lifetime of the anion has been observed until the pH of the solution was high enough to allow the presence of the anion in the ground state. Therefore, no evidence has been found of excited-state dissociation of the *trans*-enol form of DMHI, contrary to the behavior of the *trans*-enol form of HBI.[8] This difference is reflected in the fluorescence lifetimes of the *trans*-enol forms, much longer for DMHI (2.8 ns) than for HBI (0.4 ns), which reveals the additional deactivation channel of the excited HBI *trans*-enol. The weaker photoacidity of the *trans*-enol form of DMHI compared to that of HBI points to a higher excited-state pK_a* of the former species. An estimation of these pK_a* can be reached by using the Förster cycle.[29,30] As the spectra of the *trans*-enol and the anion are known, we can calculate a pK_a decrease upon excitation of 3 units for DMHI and 7 units for HBI.[8,30] From these results, which point in the expected direction, we cannot directly calculate the excited-state pK_a*, because the ground-state pK_a of the microscopic equilibrium between the *trans*-enol and the anion is not known. We only measured the macroscopic pK_a for dissociation of the enol and keto forms (9.86 for DMHI and 8.33 for HBI), although the values for the enol forms cannot be much different from these figures since coexistence of both neutral forms in equilibrium forces the microscopic acitidy constants to be of the same order of magnitude. We estimate therefore a pK_a* value of ~7 for DMHI and ~1 for HBI.

In view of the above considerations, the absorption spectra of DMHI at any pH should be the result of the individual contributions of the keto tautomer, the *trans*-enol form, the cation, and the anion. As the spectra of these species are known, the contribution of each species to the absorption spectra of DMHI can be easily calculated. The result of the spectral decomposition is shown in Figure 5 for two different pH values. Similar good-quality decompositions were obtained at other acidities. On increasing pH, the contribution of the cation decreases and that of the anion increases, as was to be expected. Moreover, the spectral contributions of the keto tautomer and the *trans*-enol form show a constant ratio, which supports the hypothesis about the nature of the absorbing species.

Results and Discussion of Quantum Mechanical Calculations. Ground-State Tautomeric and Conformational Equilibria in Gas Phase and Aqueous Solution

For the purpose of a thorough understanding of the behavior of DMHI, a detailed quantum mechanical investigation in the gas phase and in aqueous solution was carried out. Quantum mechanical calculations allow the relative energy of the neutral isomers to be known, and thus provide insight into the microscopic tautomeric and rotameric equilibria, which is our goal in the present investigation.

The *cis*-enol, *trans*-enol, and keto forms (Scheme 1) were characterized as energy minima of the potential energy surface through harmonic vibrational frequency analysis at the B3LYP/6-31+G* level of theory, no imaginary frequency being found. No other minima were found. All methods, except AM1, coincide in the prediction that the *cis*-enol form is the most stable one in the gas phase, followed by the *trans*-enol and the keto form (Table 1). Ab initio methods predict planar structures for the three isomers and correctly show the effect of hydrogen bonding and the formation of a pseudoaromatic ring in the *cis*-enol and keto forms. In the *cis*-enol form the O−H bond is

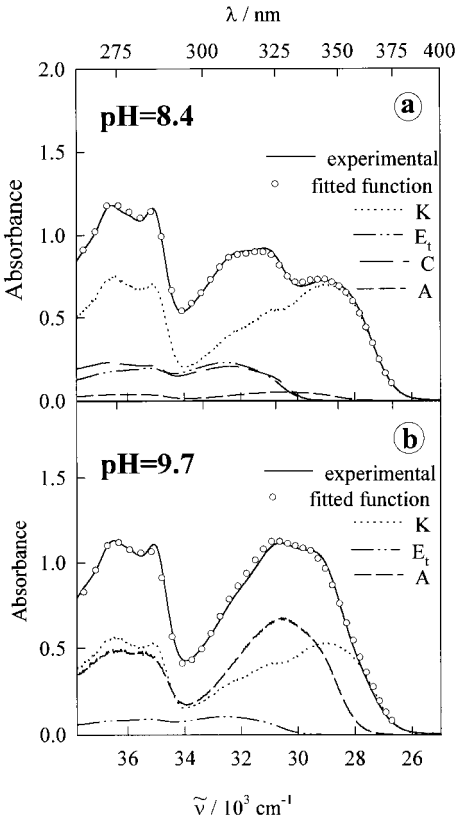


Figure 5. Decomposition of the UV−vis absorption spectra of DMHI in aqueous solution at several pH values as linear combination of the fluorescence excitation spectra of the keto (K), *trans*-enol (E_t), cation (C), and anion (A) forms [DMHI] = 1×10^{-4} mol dm^{-3}.

TABLE 1: Relative Energies (kcal mol^{-1}) of the Neutral Isomers of DMHI and the Transition State (ts) for the Ground-State Intramolecular Proton-Transfer Reaction in the Gas Phase[a]

method	*cis*-enol	keto	*trans*-enol	ts
AM1	0	+10.97	−0.36	+26.17
PM3	0	+13.25	+3.99	+27.37
RHF/3-21G	0	+8.27	+4.87	+7.38
RHF/6-31+G*	0	+10.98	+3.89	+13.80
B3LYP/6-311+G** (sp)	0	+10.33	+5.30	+5.90
B3LYP/6-31+G*	0	+8.34	+5.56	+6.92

[a] The molecular geometry for semiempirical calculations was forced to planarity. The B3LYP/6-311+G** (single point) calculations were performed on the B3LYP/6-31+G* geometry.

stretched in comparison to the phenol, and the interannular distance in the keto form is shortened in comparison to the enol forms (Table 2). The N−O distance in the *cis*-enol form is shorter than in the *trans*-enol form, suggesting a stronger H-bond[31] and explaining the relative stability of both rotamers in the gas phase. Semiempirical calculations resulted sometimes in structures deviating from planarity up to 45° between the two aromatic rings. Particularly odd results were obtained with AM1, which is due to the weakness of this method in describing N···H hydrogen bonds.[32] When semiempirical methods were used, the energies were calculated for planar structures, although they did not correspond to energy minima. Since the barrier for rotation around the interannular bond calculated with AM1 is quite small (∼1.6 kcal mol^{-1}), these errors are acceptable within the accuracy of the method. From these calculations we can conclude that the *cis*-enol form of DMHI should predominate in the gas phase at room temperature.

TABLE 2: Geometric Parameters and Dipole Moments Calculated at the B3LYP/6-31+G* Level of Theory for the Neutral Isomers of DMHI[a]

	cis-enol	*trans*-enol	keto
d_{H1-O2}	0.9967	0.9694	1.648
d_{C3-O2}	1.350	1.384	1.282
d_{O2-N6}	2.647	2.712	2.526
d_{H1-N6}	1.756		1.056
d_{C4-C5}	1.459	1.465	1.427
d_{O2-H7}		2.067	
$\theta_{O2-H1-N6}$	146.76		137.06
$\theta_{H1-O2-C3}$	108.76	109.54	106.64
$\theta_{H7-O2-C3}$		108.81	
μ/D	1.059	0.39	2.468

[a] Distances (d) are in Å and angles (θ) in degrees. See Scheme 1 for numbering.

TABLE 3: Relative Energies (kcal mol^{-1}) of the Neutral Isomers of DMHI in Aqueous Solution Calculated by the SMx Methods, by the AM1 Supermolecular Approach, and by the AM1-SM2 Method Using the Supermolecule as Solute[a]

method	*cis*-enol	keto	*trans*-enol
PM3-SM3	0	−0.19	+5.94
AM1-SM2	0	+2.67	+6.13
AM1 + 2 water	0	+5.25	+1.17
AM1 + 3 water	0	+1.01	−1.92
AM1 + 4 water	0	+0.65	−2.32
AM1-SM2 + 2 water	0	+1.27	−0.67
AM1-SM2 + 3 water	0	−0.33	−0.65
AM1-SM2 + 4 water	0	−0.13	−0.28

[a] The molecular geometry was forced to planarity. Energies were calculated as the sum of the B3LYP/6-31+G* (ZPE corrected) and the solvation free energy predicted by the method.

A way to predict the relative stability of the different conformers in aqueous solution is to combine high-level ab initio gas-phase calculations with free energy of solvation obtained by an appropriate model. We used the B3LYP/6-31+G* energies corrected with thermodynamic contributions from the same level to provide the gas-phase free energies. Solvation free energies were estimated as the energy difference between AM1 or PM3 planar optimized geometries in the gas phase and in aqueous solution. The SMx solvation methods of Cramer and Truhlar[16−19] as implemented in Spartan 4.1 were used. These solvation models not only account for the polarization effect of the solvent but also for structural changes in the solvent and specific solute−solvent interactions. The results of the relative energies of the neutral forms of DMHI are summarized in Table 3.

Because of the higher polarity of the keto tautomer (Table 2), the SMx methods predict that this form is much more stabilized than the *cis*-enol form by the dielectric medium (Table 3).[33−35] The relative energy of the *trans*-enol form is higher compared with the results obtained in the gas phase, a fact that does not correspond to our interpretation of the experimental findings. According to the solvation energy partition proposed by Cramer and Truhlar in the SMx methods, the part of solvation energy due to the solute−solvent specific interactions was parametrized assuming that it is proportional to the solvent-accessible surface area of the solute. For the estimation of these interactions, a united atom approach was assumed for hydrogen atoms attached to heavy atoms (i.e., hydrogen atoms are assumed to have zero surface tension, zero surface area, and zero volume, and each AH_n group, where A is a heavy atom, is treated as a single group with properties that depend on n). As a result, the solvent-accessible surface area should be roughly

the same for the *cis*- and *trans*-enol rotamers, and it is expected that the SMx methods were not able to account for their different specific solute−solvent interactions.[16] Thus, only polarization effects would differentiate both rotamers in solution within the SMx models, and the increase in their energy difference in aqueous solution predicted by these methods is probably due to the higher dipole moment of the cis rotamer (Table 2).

To improve our solvation model, we used the supermolecular approach[10,20,21] for the estimation of the relative stability of the neutral forms in aqueous solution; that is, we placed explicitly solvent molecules in the area of interest. For that purpose, we located successively two, three, and four water molecules around the intramolecular hydrogen bond and the lone pair of the imidazole nitrogen and carried out semiempirical calculations. In this way, good results were obtained with AM1 for the relative stability of the neutral forms compared to our best DFT calculations in the gas phase. As in the case of the optimization in the gas phase, the molecular geometry was forced to planarity. As summarized in Table 3, assuming that the basis set superposition error (BSSE) cancels,[21] the results of the AM1 calculations account for the differential solvation effect of the *trans*-enol form with respect to the *cis*-enol form. The solvation energy increased on increasing the number of interacting water molecules. A test showed that a fifth water molecule mainly interacts with a water molecule of the first-solvation shell and no improvement is achieved in the solvation energy.

PM3 did not show convergence in the solvation energy upon increasing the number of water molecules in the first-solvation shell, in contrast to the results obtained with AM1. The same behavior was observed for the PM3-SM3 method employing the supermolecule as a solute. In addition, the PM3-optimized supermolecules showed anomalous geometries, placing water molecules inside the intramolecular hydrogen-bond areas, which is thought to be due to the weakness of the model in treating hydrogen bonds. Moreover, PM3 tends to make aromatic N atoms far too electropositive, which is due to the unrealistic partial charges on N predicted by this method.[19] Therefore, the PM3 results were not taken into consideration.

While the AM1 supermolecular approach accounts better than SMx methods for explicit hydrogen bonding to the solvent, it cannot describe properly the stabilization of highly dipolar substrates like the keto tautomer since it does not account for the polarization effect of the solvent.[20,33−36] Progressive incorporation of water molecules to the first-solvation shell (Table 3) yields the stabilization of the *trans*-enol form with respect to the *cis*-enol form. Nevertheless, this stabilization is thought to be overestimated when theoretical results are compared to experimental findings. According to the energy diagram predicted by the AM1 supermolecular approach (AM1 + 4 water, Table 3), the keto tautomer and the *cis*-enol species would hardly be observable in neutral aqueous solutions at room temperature, contrary to the experimental findings. To refine our results, we performed additional AM1-SM2 calculations with the supermolecule as a solute. This methodology was previously suggested by Cramer and Truhlar[19] and has shown a very good performance since it combines both the effect of specific solute−solvent interactions and dispersion−cavitation−polarization effects. As seen in Table 3 (AM1-SM2 + 4 water), we can now predict correctly the relative stability of all forms in accordance with the experimental results. Due to restricted computational resources, we could not prove if a greater amount of water molecules would change the results significantly, but a statement on the relative stability of all forms present in neutral water can be made within the accuracy of the model. In the gas phase, AM1 describes incorrectly the *trans*-enol form as the most stable one, a trend which remains in the solvated supermolecular calculations. The solvation model 2, SM2, tends to correct the errors of AM1 by means of its parametrization[17] and so the energy of the *trans*-enol form with respect to the *cis*-enol form is increased. We have no value for the amount of correction, so we cannot exclude that the excellent results obtained with AM1-SM2 are to some extent coincidental. In spite of this we can consider our results as encouraging. Again assuming that the BSSE cancels, the stabilization of the keto tautomer and the *trans*-enol form with respect to the *cis*-enol form in aqueous solution is described correctly, although the calculated energy differences predict a population distribution which would not match quantitatively the experimental findings: the *cis*-enol form is present in negligible concentration in neutral aqueous solution at room temperature, whereas the calculations suggest a substantial population of all three isomers. It should be emphasized once more that a satisfactory description of these equilibria in solution requires the specific interactions between solute and solvent as well as dielectric stabilization of polar species.

Conclusions

In neutral ethanolic solutions, the *cis*- and *trans*-enol forms of DMHI were found in equilibrium in the ground state. However, in neutral aqueous solutions, the *trans*-enol and keto forms were the only species detected. This is a remarkable result, since no similar behavior has been described for related molecules. This points to the fact that an increment in the basicity of the azole ring lowers the energy of the keto form in neutral aqueous solutions. Quantum mechanical calculations in the gas phase and in aqueous solution showed that this change in the relative energies of the three neutral isomers of DMHI is due to their specific interactions with the solvent together with the differential stabilization of these dipolar species in highly polar media.

In the first-excited singlet state, the *cis*-enol form undergoes ESIPT to yield the excited keto tautomer, which deactivates emitting fluorescence. In neutral aqueous solutions, the excited keto tautomer is formed by direct excitation. Contrary to the behavior found for HBI in aqueous solution, the *trans*-enol species does not dissociate at the hydroxyl group due to its weak acidity in the excited state.

Upon excitation to the first-excited singlet state, the cation of DMHI dissociates at the hydroxyl group to afford the excited keto tautomer. This excited-state process has been found in acidic ethanol and water, although its quantum yield is much higher in water than in ethanol.

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