Phototautomeric Equilibrium in the Lowest Excited Singlet State of 3-Hydroxyacridone

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The pH dependencies of the absorption and fluorescence emission of 3-hydroxyacridone (3-HA) were studied. Three species (the cation, the neutral molecule, and the anion) were identified in absorption. The same species, albeit at different pH ranges, were identified in fluorescence. Their decay times are highly different. In addition, a new species, having an unusually long wavelength emission that has no equivalent in the ground state was identified in the pH 4 to H_0 −4 range. This species is assigned to an excited-state tautomer formed predominately by adiabatic double-proton transfer during the lifetime of the excited state. Two mechanisms are operative: At pH 4 to H_0 0, the neutral (uncharged) species is photoprotonated at the carbonyl group and quickly undergoes photodissociation at the hydroxy group. In more acidic solutions (H_0 −3 to H_0 −2), the cation is exclusively excited, followed by photodissociation from the 3-hydroxy group to form the phototautomer. In the region H_0 −2 to H_0 0, both mechanisms are operative, and therefore, the continuity of the fluorimetric titration curve of the cation suggests that the photoexcited cation and the phototautomer are in equilibrium, a photochemical and spectroscopic rarity.

1. Introduction

Owing to their strong fluorescences, acridone and N-alkylacridones have been investigated by numerous photophysical methods. The effects of pH,[1,2] temperature,[3] and solvents[1,3,4] have been studied in detail. Substituted acridones, by contrast, have received very little attention, although there are qualitative reports on the intense green fluorescence of some naturally occurring 3-hydroxy- and 3-methoxyacridones.[5] Conceivably, hydroxy-substituted acridones may undergo excited-state reactions leading to unusual tautomeric species, as shown previously for certain coumarins,[6] xanthones,[7] and salicylic acids as well as cinnamic acids,[8,9] nitrogen heterocyclic compounds,[10−13] and natural products such as the harmala alkaloids.[14,15] Phototautomerism is the result of excited-state proton transfer from the proton of a hydroxy group to the oxygen of a carbonyl group or to a nitrogen atom.[12,16] Phototautomerism forms the basis not only of widely tunable lasers but also of advanced fluorescent bioprobes. We report here on the fluorescence of 3-hydroxyacridone (3-HA) and 3-methoxyacridone (3-MA) in acidic and near-neutral aqueous solutions. This study revealed the formation (via an unusual path) of a highly unusual excited-state (phototautomeric) species with no equivalent in its ground state.

2. Experimental Section

Compounds and Solvents. 3-Hydroxyacridone (3-HA) and 3-methoxyacridone (3-MA) were prepared according to published procedures.[17,18] They were recrystallized from methanol and further purified by preparative TLC on silica gel to remove fluorescent byproducts, until one spot was formed on TLC. Their identity was proven by melting points, IR, and NMR spectra. Stock solutions were prepared in methanol and were diluted with sulfuric acid, sodium hydroxide, phosphate, or acetate buffer to finally contain not more than 10% methanol. All of the solvents were checked to exhibit negligible background fluorescence.

Spectra and Decay Time Measurements. The absorption spectra were recorded on a Hitachi (U-3000) UV−vis spectrophotometer, and fluorescence excitation and emission spectra were measured on an Aminco−Bowman Series 2 luminescence spectrometer (SLM−Aminco, Rochester, NY 14625) in rectangular ($1 \times 1 \times 3$ cm^3) quartz cells at 22 °C. The pH of the nondegassed solutions was adjusted by addition of either sulfuric acid or sodium hydroxide solution or by the titration of a weakly buffered aqueous solution with either acid or base. Fluorescence decay time measurements were carried out on an ISS K2 multifrequency phase-modulation fluorometer using a 150 W continuous xenon lamp (PS 300−1, ILC technology) as the excitation light source, two signal generators (2022D from Maroni Instruments) and a filter (Lee Filters, special medium blue, Nr 363). The lifetimes were referenced against a dilute solution of glycogen.

Dissociation Constants. Ground-state pK_a values in the near-neutral pH range were obtained by the spectrophotometric method in phosphate buffers at ionic strength below 0.05. The pK_a of the cation was obtained from plots of absorbance at 345 and 409 nm versus the Hammett acidity (H_0) of the sulfuric acid solution. The properties of the excited states of 3-HA and 3-MA were obtained from fluorescence titration curves. The

† The IUPAC names are 3-hydroxy-9(10H)-acridone for 3-hydroxyacridone (Chem. Abstr. Reg. No. 20168-55-2) and 3-methoxy-9(10H)-acridone for 3-methoxyacridone (Chem. Abstr. Reg. No. 61736-68-3). The trivial names and respective acronyms (3-HA and 3-MA) will be used throughout this paper. The registry numbers are provided by the author.

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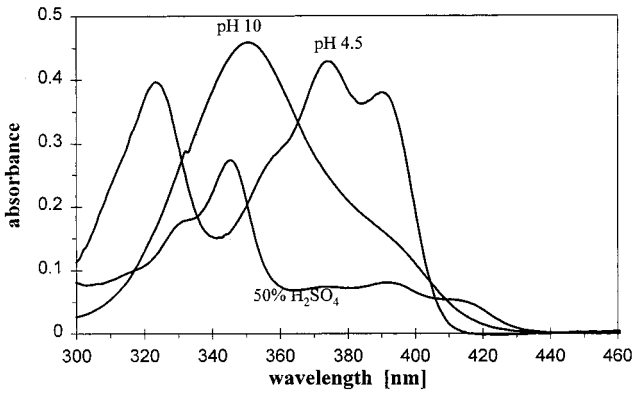


Figure 1. Absorption spectra of 3-hydroxyacridone in aqueous solutions of pH 4.5 (72.2 μM), pH 10 (36.1 μM), and 50% sulfuric acid (18.0 μM) at room temperature.

excitation was performed at 395 nm, which is an isosbestic wavelength between the cation and the neutral molecule in the absorption of 3-HA. This makes the fluorimetric titration correspond to the variation of relative fluorescence efficiency with pH. All of the changes in the spectra with pH were found to be fully reversible.

3. Results

Absorption Spectra. Figure 1 shows the absorption spectra of 3-HA in water solution at three representative acidities. The spectral maxima and molar absorptivities are given in more detail in Table 1. Although the absorption spectra of the uncharged and protonated molecules show some fine structure (also found in emission), the anion band is rather broad. The absorption spectra of 3-HA and 3-MA in methanol agree with published data.[19] The absorption spectra of 3-MA are similar to those of 3-HA in acidic and neutral solutions (Table 2). There is, of course, no phenolate absorption to be observed in alkaline solutions of 3-MA.

Fluorescence Spectra. Figure 2 shows the fluorescence spectra of 3-HA at selected pH values. From H_0 −4 to pH 12, four bands are evident, designated as N, C, A, and PT. Among these, the N and C bands are by far the most intense. The spectrum in the pH 4 solution is similar to that found in methanol, and it shows vibrational resolution, which is the mirror image of the corresponding long-wavelength absorption band. The uncorrected excitation spectra at any analytical emission wavelength are in acceptable agreement with the respective absorption spectra at the same pH.

Although the directions in the shifts in the absorption maxima with pH are the same as in the fluorescence for species A, N, and C, there is an unusual additional green component (PT) evident in the fluorescence spectra of unbuffered pH 0−2 solutions that has no equivalent in absorption. The excitation spectrum of this band agrees with the absorption spectrum at this pH value, such that it cannot be considered as an impurity. By comparison to the strong fluorescences of the neutral molecule and the cation, its fluorescence is rather weak. However, in buffered solution, such as in 1 M acetic acid sodium acetate of pH 4.5, the green emission at 520 nm can be easily detected along with the strong violet emission of the excited neutral molecule (Figure 3). No such band can be observed in the pH-dependent emission spectra of 3-MA. Rather, its fluorescence maxima in the pH 9 to 2 range are quite similar to those of the short-wave component in the spectra of 3-HA in this acidity range.

Also, no such effects are observed in pure organic solvents. Aside from small solvatochromic shifts, the absorption and emission spectra of both 3-HA and 3-MA are identical with the spectra of aqueous solutions of pH 3−6. However, for cases in which 1 N sulfuric acid (1 drop to 3 mL) is added to a methanol solution of 3-HA, the tautomer band at 520 nm can be identified along with the fluorescence from 3-HA (Figure 3).

Decay Time Studies. Highly different decay times are found for the isolated cationic (16.6 ns), neutral (13.6 ns), and anionic species (1.3 ns), respectively. All of the species have monoexponential decay. The data are also summarized in Table 1. The measurement of 16.6 ns for the cation pertains to the whole band. The shoulder at 438 nm is a vibronic feature of the cation emission.

pK_a Determinations. Depending upon the excitation wavelengths, the fluorescence titration curves can provide either ground-state pK_a values or a composite of parameters related to the chemistry and photophysics of the lowest excited singlet state. The pK_a obtained by fluorescence titration at an excitation wavelength of 420 nm (which excites the cation exclusively) is in agreement with the ground-state pK_a obtained by photometry.

However, for cases in which the excitation is performed at 395 nm, which is the isosbestic point of absorption in the pH 4 to H_0 −4 range (fluorescence intensity is monitored at 411 nm), the fluorescence titration curves shown in Figure 4 are obtained.

4. Discussion

The absorption and fluorescence spectra of the corresponding species derived from 3-HA and 3-MA are similar, whereas their absorptiometric titration behavior differs. Unlike in the case of 3-HA, there is no dissociable hydroxy group in 3-MA. The pK_a of neutral 3-HA, evaluated spectrophotometrically, was found to be 7.61 ± 0.04. From previous work on unsubstituted acridones,[2,16] it is known that acridones do not undergo either ground-state reactions or excited-state reactions at the NH group at pH's lower than 12.

The ground-state pK_a values corresponding to interconversion of the cation and neutral molecule (due to protonation at the carbonyl group) were evaluated spectrophotometrically and found to be −0.11 ± 0.03 for 3-HA and −0.25 ± 0.03 for 3-MA. The slightly more positive pK_a of 3-HA is not really significant but is likely to be due to the partial release of positive charge in the cation derived from hydrogen bonding of the 3-hydroxy proton with the solvent.

The fluorimetric titration behaviors of the two compounds are quite different. 3-HA fluoresces in its neutral and anionic forms, but the fluorimetric titration of the interconversion between these two species exactly duplicates the absorptiometric titration in the pH region from 5 to 10, indicating that there is no photodissociation of the 3-hydroxy group. This suggests the following expressions,[20]

$$\frac{\varphi_N}{\varphi_N^0} = \frac{1}{1 + k_{NA}\tau_N^0} \tag{1}$$

and

$$\frac{\varphi_A}{\varphi_A^0} = \frac{k_{NA}\tau_N^0}{1 + k_{NA}\tau_N^0} \tag{2}$$

which relate φ_N/φ_N^0 and φ_A/φ_A^0 (the respective relative quantum yields of fluorescence of the neutral molecule and anion

TABLE 1. Absorption, Excitation, and Fluorescence Maxima (nm) and Fluorescence Decay Times of 3-HA at 22 °C

solvent	λ_{max}^{abs}	ϵ [$M^{-1}cm^{-1}$]	λ_{max}^{exc}	λ_{max}^{flu} (assignment)	decay time [ns] (Chi-Square)
methanol	385	5150	386	456 (sh) (neutral species)	8.7
	368	5250	368	426	(0.7)
	319	4250	326	406	
chloroform	363		382	450 (sh) (neutral species)	
	347		365 (sh)	420	
			315	400	
50% H_2SO_4	416 (sh)	2450	416	460 (cation)	16.6
	390	4000	396	438 (sh)	(3.5)
	376	3500	351		
	346	13 200			
1N H_2SO_4	390	4900	393	520 (phototautomer)	1.8
	373	5000	370	460 (sh) (cation)	(3.4)
	345	7100	325	431, 410	
pH 4.5	389	3450	392	460 (sh) (neutral species)	
	374	4000	370	431, 410	13.6
	356	1900	325		(0.4)
pH 10	400 (sh)	3600	393	470 (anion)	
	352	12 800	363		
pH 12	400 (sh)	3600	393	478 (dianion)	
	352,	12 800	363		
1 M NaOH	400 (sh)	3600	393	478 (dianion)	1.3
	343	8600	357		(6.6)

in the near-neutral pH region) to k_{NA} (the rate constant for the photodissociation of the neutral molecule) and to τ_N^0 (the excited-state lifetime of the neutral molecule in the absence of proton transfer). The value of $k_{NA}\tau_N^0$ must be smaller than ~0.02, which represents the limit of the ability of the instrumentation to detect the dissociation of the neutral molecule. Because $\tau_N^0 = 13.6$ ns, $k_{NA} = 1.5 \times 10^6$ s^{-1}. A Förster cycle-based[21] estimation of $pK_A^* = 3.6$, coupled with an estimate of the rate constant for reprotonation of the excited anion k_{AN} as 1×10^{10} L $mol^{-1}s^{-1}$ (assuming a diffusion-controlled reprotonation), gives a crude estimate of k_{NA} at 2.5×10^6 s^{-1}.

The most remarkable differences between the fluorimetric titration behaviors of the hydroxy and methoxy compounds are seen in the pH 4 to H_0 −2 region. Between pH 4 and H_0 0 the fluorimetric titration of 3-MA is quite unremarkable. With decreasing pH, the blue fluorescence of the neutral molecule falls with the concomitant rise in the blue-green emission of the cation. The decrease in one is reciprocal to the rise in the

TABLE 2: Absorption, Excitation, and Fluorescence Maxima (nm) of 3-MA at 22 °C

solvent	λ_{max}^{abs}	ϵ [$M^{-1}cm^{-1}$]	λ_{max}^{exc}	λ_{max}^{flu} (assignment)
methanol[a]	382	5900	385	470 (sh) (neutral species)
	364	2600		438
	347 (sh)	2950		418
	316	4000		
	300 (sh)			
	273			
1 N H_2SO_4	385	5600	390	472 (cation)
	369	6050		445 (sh)
	346	3900		
	323	6300		
pH 7	385	5750	390	465 (sh) (neutral species)
	370	6050		433
	350 (sh)	2900		413
	322			
pH 12	387	5750	390	505 (sh) (anion)
	370	6050		475 (anion and neutral species)
	350 (sh)	2900		448 (neutral molecule)
	323			420[b]

[a] Brockmann et al. give 382, 365, 316, 265, and 251 nm for the maxima in methanol.[19] [b] This band disappears in pH 13 solution that exhibits fluorescence maxima at 451, 476, and 507 nm. In absorption, a new band begins to arise at 415 nm. The anion absorption in 20% NaOH has maxima at 410, 385, and 363 nm.

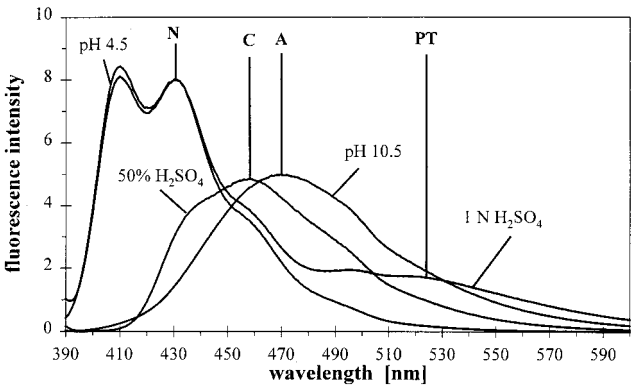


Figure 2. Fluorescence emission spectra of 3-hydroxyacridone in aqueous solutions of pH 4.5 and 10.5, in 1 N, and in 50% sulfuric acid at room temperature. Excitation wavelengths are 395 nm for pH 4.5, 1 N, and 50% sulfuric acid solution and 352 nm for pH 10.5. To make the intensities comparable, the instrument parameters were changed.

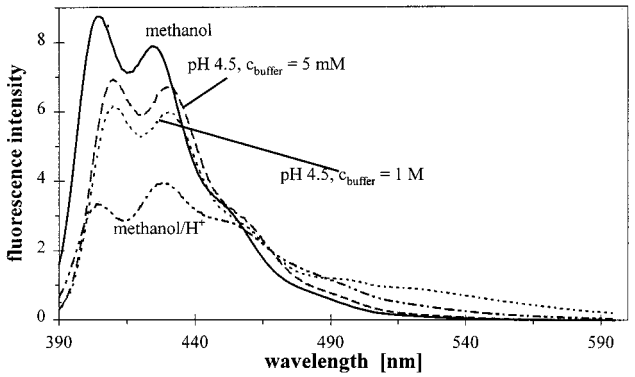


Figure 3. Fluorescence emission spectra of 3-hydroxyacridone in buffered solutions of pH 4.5 (buffer concentration $c_{buffer} = 5$ mM and 1 M, respectively), in methanol and in methanol plus a drop of 1 N sulfuric acid.

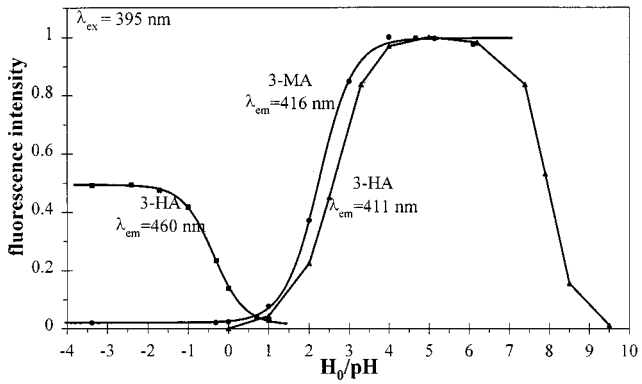


Figure 4. Plots of fluorescence intensity of 10^{-5} M solutions of, respectively, 3-HA and 3-MA versus pH or Hammett acidity H_0 when excited at λ_{exc} 395 nm: (■) fluorescence of 3-HA at 460 nm; (▲) fluorescence of 3-HA at 411 nm; (●) fluorescence of 3-MA at 416 nm.

other, which occurs at pH > pK_a, indicating the protonation of the neutral molecule in the lowest excited singlet state (with rate constant k_{NC}) possibly followed by redissociation of the conjugate acid (with rate constant k_{CN}). The variations of the relative quantum yields of fluorescence of the neutral molecule (φ_N/φ_N^0) and of its conjugate acid (φ_C/φ_C^0) with the hydrogen ion concentration [H^+] are given by eqs 3 and 4

$$\frac{\varphi_N}{\varphi_N^0} = \frac{1 + k_{CN}\tau_C^0}{1 + k_{CN}\tau_C^0 + k_{NC}\tau_N^0[H^+]} \quad (3)$$

$$\frac{\varphi_C}{\varphi_C^0} = \frac{k_{NC}\tau_N^0[H^+]}{1 + k_{CN}\tau_C^0 + k_{NC}\tau_N^0[H^+]} \quad (4)$$

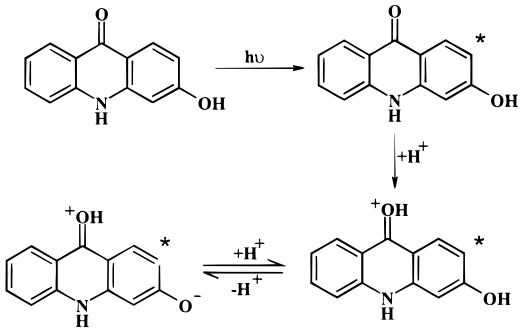
where τ_C^0 and τ_N^0 are the respective lifetimes of the cation and the neutral molecule in the lowest excited singlet state, which yields

$$\frac{\varphi_C/\varphi_C^0}{\varphi_N/\varphi_N^0} = \frac{k_{NC}\tau_N^0[H^+]}{1 + k_{CN}\tau_C^0} = 177 \pm 3 \quad (5)$$

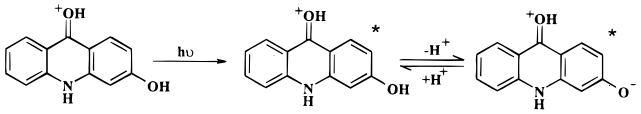
This is approximately the reciprocal of [H^+] at the inflection point in the fluorimetric pH titration of 3-MA.

For 3-HA, the fluorimetric titration curve in the acidic pH region is very different from that of 3-MA. Between pH 4 and H_0 0, the blue fluorescence of the neutral molecule decreases with decreasing pH, as does the cation derived from the methoxy compound. This decrease is centered at [H^+] = $2.5 \pm 0.4 \times 10^{-3}$ M. However, in this pH region, there is no concomitant appearance of the blue-green fluorescence of the cation. Rather, the blue fluorescence of the neutral molecule is supplanted by a weak-green emission between H_0 and pH 1 and then by the intense blue-green emission from the cation, which first appears at about pH 1 and increases to a maximum at about H_0 −3, after which, it remains constant in intensity. The weak-green fluorescence between H_0 and pH 1 is eclipsed by the long-wavelength tailings of the much more intense emissions of the cation and neutral molecule at lower H_0 and at higher pH, respectively. The green emission can also be observed at about pH 4.5 in concentrated (∼2 M) acetic acid−sodium acetate buffers, in which acetic acid is the proton donor and the acetate anion and/or water may function as the proton acceptor.

SCHEME 1



SCHEME 2



These observations lead to the following conclusions: (a) The photoprotonation of the neutral molecule at pH ≤ 4 is followed by the immediate photodissociation of the excited cation from the 3-hydroxy group, to form a phototautomer that is not stable in the electronic ground state and, therefore, not observable absorption (see Scheme 1). (b) For the most acidic solutions (H_0 −3 to H_0 −2), in which the cation is exclusively excited, the deviation of (φ_C/φ_C^0) from unity is due to the direct excitation of the cation followed by photodissociation from the 3-hydroxy group to form the phototautomeric zwitterion (or the uncharged vinylog thereof), according to Scheme 2. In the region from H_0 -2 to H_0 0, both mechanisms are operative, and therefore, the continuity of the fluorimetric titration curve of the cation suggests that the photoexcited cation and the phototautomer are in equilibrium. This is a photochemical and spectroscopic rarity that contrasts the situation of related hydroxycarbonyls[6−8] and, of course, of hydroxy-substituted heterocyclic compounds containing nitrogen atoms of high excited-state basicity.[13,22]

Although it has been impossible to unequivocally confirm that the green emission is arising from a phototautomer, the fact that it lies at wavelengths longer than either the cation or anion of 3-HA supports this conclusion. Both protonation and dissociation of 3-HA lead to red shifts in both the absorption spectra and the fluorescence spectra. It would be reasonable to expect the simultaneous protonation of the vinylogous amidic oxygen atom and dissociation of the phenolic 3-hydroxy group of the neutral molecule to give rise to a species, absorbing and emitting at wavelengths longer than those in either the cation or the anion. The appearance of the same green emission in concentrated acetic acid−sodium acetate buffers at pH 4.5 is likely because of the direct photoprotonation by acetic acid, followed by photodissociation of the 3-hydroxy group, which is possibly assisted by the acetate ion. A concerted mechanism involving two molecules of 3-HA is very unlikely to occur in view of the low concentrations applied in this work.

If equilibrium is attained between the cation and the phototautomer of 3-HA in the lowest excited singlet state, the ratio of their respective relative quantum yields of fluorescence (φ_C/φ_C^0 and $\varphi_{PT}/\varphi_{PT}^0$) is given by

$$\frac{\varphi_C/\varphi_C^0}{\varphi_{PT}/\varphi_{PT}^0} = \frac{k_{PTC}\,\tau_{PT}^0 h_0}{k_{CPT}\,\tau_C^0} \quad (6)$$

where k_{CPT} and k_{PTC} are the respective rate constants for dissociation of the excited cation to form the phototautomer and reprotonation of the phototautomer, τ^0_{PT} is the lifetime of the excited phototautomer in the absence of excited-state proton transfer and h_0 is the Hammett acidity of the solution (as a natural number). For this reaction, $k_{CPT}/k_{PTC} = K^*_a$, the excited-state dissociation constant, K^*_a can be calculated as

$$K^*_a = h_0 \frac{\varphi_{PT}/\varphi^0_{PT}}{\varphi_C/\varphi^0_C} \frac{\tau^0_{PT}}{\tau^0_C} \tag{7}$$

or in a logarithmic form

$$pK^*_a = H_0 - \log \frac{\varphi_{PT}/\varphi^0_{PT}}{\varphi_C/\varphi^0_C} - \log \frac{\tau^0_{PT}}{\tau^0_C} \tag{8}$$

Using the data from the fluorimetric titration curve of 3-hydroxyacridonium cation and the measured decay times of 16.6 ns for the cation and 1.8 ns for the phototautomer (the latter figure was measured at H_0 −0.02 and may be somewhat high because there may be some residual emission from the cation at that acidity), a pK^*_a of -0.11 ± 0.03 was obtained for the equilibrium between the excited cation and the phototautomer. By coincidence, this value is the same as the ground-state pK_a governing the protonation of 3-HA.

We believe that these mechanisms of excited-state proton transfer may be of use in the design of pH tunable dye lasers. The ground-state pK_a of 3-HA (7.61) suggests its use as a fluorescent probe for physiological pH's.

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