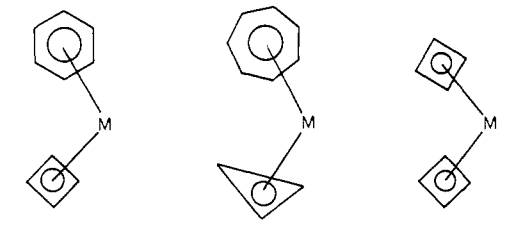
interesting features of this calculation is the fact that, in contrast to the isoelectronic system, $(\eta^5\text{-}C_5H_5)_2Si$, the bent-sandwich structure emerges as the ground-state geometry. The strong preference for the C_{2v} rather than D_{5h} (or D_{5d}) bis(pentahapto) structure in the case of the phosphenium ion is a result of the positive charge on the heteroatom and its consequent increased interaction with the cyclopentadienyl rings. In a D_{5h} structure, the HOMO is a degenerate pair of ring π orbitals of symmetry e_1''. Upon bending to a C_{2v} structure, the e_1'' MO becomes two single degenerate MO's, a_2 and b_2. Of these the a_2 ring-localized MO is precluded from interaction with P(3s) and P(3p) orbitals for symmetry reasons. The b_2 cyclopentadienyl ring MO can interact with a valence p_y orbital; however, as shown earlier, there is no perceptible interaction between these orbitals in the molecules $(\eta^5\text{-}C_5H_5)_2Si$ and $(\eta^5\text{-}C_5H_5)_2Sn$. The presence of a formal positive charge on the central atom in the case of $[(\eta^5\text{-}C_5H_5)_2P]^+$ increases greatly the interaction between the b_2 ring and $P(3p_y)$ MO's as evidenced by, e.g., the 1.3 eV gap between the $6a_2$ and $9b_2$ levels (Table IV).

Finally, and more speculatively, we note that bent-sandwich molecules with rings other than cyclopentadienyl might exist. Current efforts are focused on determining whether the 14 interstitial electron rule is applicable in cases such as:



M = Si, Ge, Sn M = Si, Ge, Sn M = S, Se, Te

Acknowledgment. The authors are grateful to the Robert A. Welch Foundation and the National Science Foundation (Grant CHE79-10155) for generous financial support. Gratitude is also expressed to Dr. J. F. Nixon of the University of Sussex for the He(II) data.

Registry No. $(Me_5C_5)_2Pb$, 80215-72-1; $(C_5H_5)_2Sn$, 1294-75-3; $(\eta^5\text{-}C_5H_5)_2Si$, 81770-35-6; $(\eta^1\text{-}C_5H_5)_2Si$, 81790-05-8; $(C_5H_5)_2Pb$, 1294-74-2; $(Me_2C_5)_2Sn$, 68757-81-3; $[(\eta^5\text{-}C_5H_5)_2P]^+$, 81770-36-7; $[(\eta^1\text{-}C_5H_5)_2P]^+$, 81790-06-9.

pH-Dependent Fluorescence Spectroscopy. 15.[1] Detection of an Unusual Excited-State Species of 3-Hydroxyxanthone†

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Contribution from the Institut für Organische Chemie der Karl Franzens-Universität, A-8010 Graz, Austria. *Received November 24, 1981*

Abstract: The solvent and acidity dependence of the absorption and fluorescence spectra of 3-hydroxyxanthone and 3-methoxyxanthone has been studied. 3-Hydroxyxanthone is shown to undergo adiabatic photodissociation in aqueous pH 7–2 solution. An unusual species has been detected in the pH 3 to H_0 −2 acidity range, which is characterized by its long-wave emission. This species is assumed to be a phototautomer or an exciplex, formed by proton transfer during the lifetime of the excited singlet state. No evidence for this species is apparent in the UV-absorption spectra. Particular broad-band emissions are found in protic organic solvents together with unexpected effects of acidification. The ground state and first excited singlet state pK_a's have been determined by either photometry or fluorimetry. The latter were also calculated by applying the Förster–Weller equation. The calculated values do not agree completely with the values obtained by fluorimetry, which may be the result of the noncorrespondence of the ground- and excited-state protolytic equilibria. A ground-state pK_a of 7.16 for 3-hydroxyxanthone together with a fluorescence quantum yield of 0.38 of its anion can make this compound a useful indicator for measuring physiological pH values.

3-Hydroxyxanthone is one of a number of related naturally occurring substances that were isolated from seeds of various plants.[2,3] It has been extracted from the plant *Kielmeyera excelsa*,[4] and other 3-hydroxy- and 3-methoxyxanthones are widely distributed in the plant kingdom.[5] We have focussed our interest on 3-hydroxyxanthone in continuation of our studies on the solvent and acidity dependence of the fluorescence spectra of natural products and because we expected it to be a useful indicator for the fluorimetric determination of physiological pH's.

Experimental Section

Compounds and Solvents. 3-Hydroxyxanthone and 3-methoxyxanthone were prepared according to the procedure given by Ullmann and Wagner.[6] They were triply recrystallized from ethanol. Stock solutions were prepared in methanol and were diluted with either triple-distilled water or buffer solution to contain finally not more than 10% methanol. For the measurements in sulfuric acid a stock solution was prepared in concentrated sulfuric acid, which was diluted with triple-distilled water to the desired acidity. H_0 values were taken from Hammett's book.[7] All solvents were of the best commercially available quality.

Spectra. The absorption spectra were run on a Uvikon 810 spectrophotometer (Kontron, Switzerland, wavelength accuracy ±0.5 nm, reproducibility ±0.1 nm) in buffered solutions at room temperature. The fluorescence spectra were recorded on an Aminco SPF 500 spectro-

† The IUPAC names for 3-hydroxyxanthone (Chemical Abstracts registry No. 3722-51-8) and 3-methoxyxanthone (Chemical Abstracts registry No. 3722-52-9) are 3-hydroxy-9*H*-xanthen-9-one and 3-methoxy-9*H*-xanthen-9-one, respectively. The trivial names will be used throughout this paper.

(1) Part 14. Reference 13.
(2) G. G. De Oliveira, A. A. L. Mesquita, O. R. Gottlieb, and M. T. Magahaes, *Ang. Acad. Bras. Cienc.*, **38**, 421 (1966); *Chem. Abstr.* **67**, 108528a (1967).
(3) O. R. Gottlieb, A. A. L. Mesquita, G. G. De Oliveira, and M. Texeira de Melo, *Phytochemistry*, **9**, 2537 (1970).
(4) D. de B. Correa, L. G. Fonseca e Silva, O. R. Gottlieb, and S. J. Goncalves, *Phytochemistry*, **9**, 447 (1970).
(5) F. M. Dean, "Naturally Occurring Oxygen Ring Compounds", Butterworths, London, 1963, Chapter 9.
(6) F. Ullmann and C. Wagner, *Liebigs Ann. Chem.*, **355**, 359 (1907).
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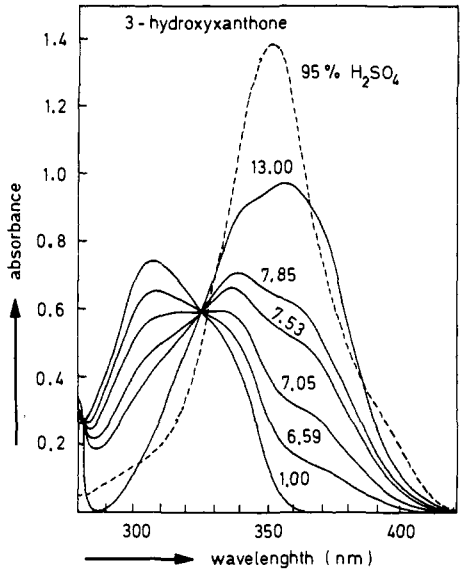

Figure 1. Acidity dependence of the absorption spectra of 3-hydroxyxanthone at room temperature (concentration 49.2 μM). Isosbestic wavelengths are at 325 nm for the system anion–neutral molecule and at 327 nm for the system neutral molecule–cation.

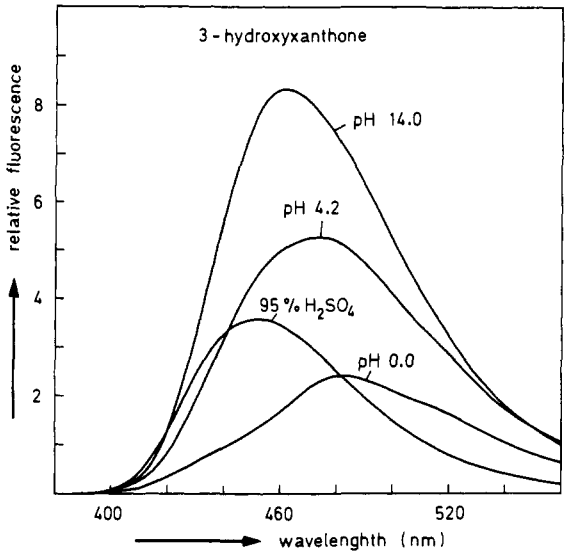

Figure 2. Fluorescence spectra of 3-hydroxyxanthone in pH 14.0, 4.2, and 0.0 and in concentrated sulfuric acid solution at room temperature (concentration 5.3 μM). Excitation at 326 nm.

fluorimeter (American Instrument Co., Md.) in rectangular quartz cells at 22 °C. The pH of the nondegassed solutions was adjusted externally by addition of either sulfuric acid or sodium hydroxide solution to avoid quenching by buffer ions. As suggested by Schulman,[8] fluorescence in each case was excited at an isobestic wavelength in the absorption spectra (327 nm for 3-hydroxyxanthone in the H_0 −10–0 range and 325 nm in the pH 0–14 range; 329 nm for 3-methoxyxanthone). This makes the fluorimetric titration correspond to the variation of relative fluorescence efficiency with pH.

Ground-state pK_a's were obtained by the spectrophotometric method in phosphate buffer at ionic strength $J < 0.05$. pK_a's below zero were determined in various diluted sulfuric acids. The excited-state pK_a's were taken from the inflexion points of the fluorescence titration curves.

Quantum yields were determined relative to quinine sulfate (ϕ_f 0.546 in 1 N sulfuric acid[9]) at optical densities lower than 0.2. For the integration the nanometer-linear readout of the instrument was converted to a wavenumber-linear spectrum by using a HP 9815 A desk computer and software provided by the American Instrument Co. All of the changes in the spectra with pH were found to be fully reversible.

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(9) J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, **75**, 991 (1971).

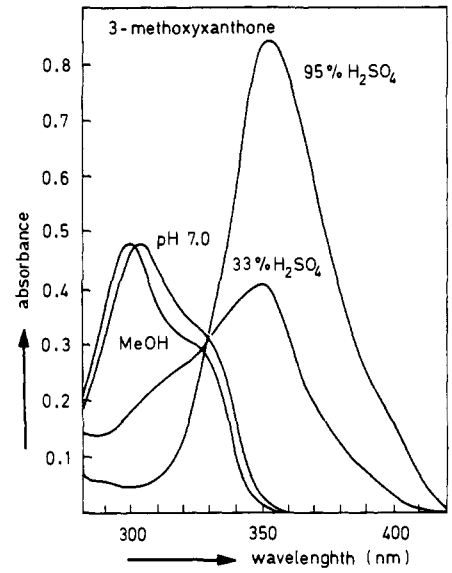


Figure 3. Absorption spectra of 3-methoxyanthone in methanol (pH 7.0, 33% and 95% sulfuric acid solution at room temperature, concentration 31.6 μM).

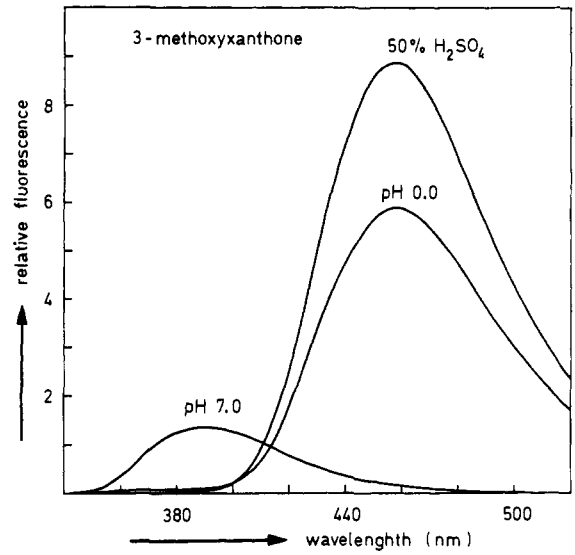
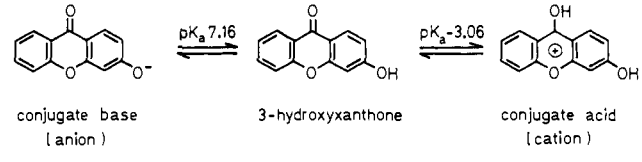


Figure 4. Fluorescence spectra of 3-methoxyxanthone in pH 7.0 and 0.0 and 50% sulfuric acid solution (concentration 3.2 μM). Excitation was accomplished at 329 nm, which is the isosbestic wavelength in absorption.

Scheme I. Structures of 3-Hydroxyxanthone Ground-State Species



Results and Discussion

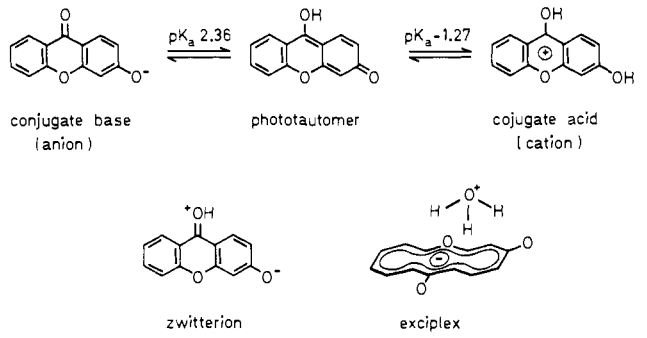
Three species of 3-hydroxyxanthone are evident from its pH-dependent absorption spectra (Figure 1). The respective equilibria are governed by two pK_a's, according to Scheme I. Both the cation and the anion fluorescence are evident in the fluorescence spectra (Figure 2). The anion fluorescence, maximizing at 461 nm, can be observed in the pH 14–2 range. The cation with its fluorescence maximum at 453 nm is evident in solutions of acidity bigger than H_0 −1. In the pH 3 to H_0 −1 range there is, however, a new species evident, having a fluorescence maximum at 481.5 nm. This band cannot be assigned to the fluorescent neutral molecule in view of the fluorescence maximum of 3-methoxyxanthone in neutral aqueous solution at 391 nm (Figures 3 and 4). Absorption,

Table I. Absorption, Excitation, and Fluorescence Maxima (nm) of 3-Hydroxyxanthone and 3-Methoxyxanthone at 22 °C (Concentration Range 15.0–50.1 μM)

solvent	λ_{max}^{abs}	log ε (cm^{-1} M^{-1})	λ_{max}^{exc}	λ_{max}^{flu}	fluorescent species
		3-Hydroxyxanthone			
95% sulfuric acid	350	4.45	352	453	cation
	272	3.81			
	250	4.55			
pH 0.0	~335 (sh)	4.00	335	515 (sh)	phototautomer
	307	4.18	317	481.5	
pH 7.0	~360 (sh)	3.78	365 (sh)	465.5	anion
	331	4.08	343		
	307 (sh)	4.08			
pH 10.0	354	4.28	355	461	anion
	335 (sh)	4.23			
pH 14.0	354	4.29	357	461	anion
	335 (sh)	4.23	340		
	278	3.83			
	259	3.75			
methanol/ sulfuric acid[a]	~325 (sh)	4.08	318	510	phototautomer
	304	4.18			
	261	4.00			
	234	4.61			
		3-Methoxyxanthone			
95% sulfuric acid	351	4.38	349	455	cation
pH 0.0	336 (sh)	4.01	337 (sh)	455	cation
	307	4.22	312		
pH 7.0	335 (sh)	4.05	337 (sh)	391	neutral molecule
	308	4.21	312		
pH 14.0	337 (sh)	*b*	337	391	neutral molecule
	305		312		

[a] 9 mL of methanol solution plus 1 mL of 0.1 N sulfuric acid. [b] Too insoluble for determination.

Scheme II. Structures of 3-Hydroxyxanthone Excited-State Species



excitation, and fluorescence data of 3-hydroxy- and 3-methoxyxanthone are compiled in more detail in Table I. The appearance of anion fluorescence in the pH 7–3 range can be explained by adiabatic photodissociation of 3-hydroxyxanthone during the lifetime of the excited singlet state. The unusual long-wave fluorescence in the pH 3 to H_0 −1 range requires further interpretation.

We assign this species to an excited-state tautomer. It may be formed by protonation of 3-hydroxyxanthone anion in acidic solution at the carbonyl oxygen rather than at the phenolate oxygen. The excited-state equilibria resulting from this assumption are given in Scheme II. The zwitterion shown there may contribute significantly to the phototautomer structure.

A similar behavior was shown to occur with 7-hydroxy-4-methylcoumarin (4-MU) and gave rise to unusual broad-band laser emission.[10]

(10) C. V. Shank, A. Dienes, A. M. Trozzolo, and J. A. Myer, *Appl. Phys. Lett.*, **16**, 405 (1970).

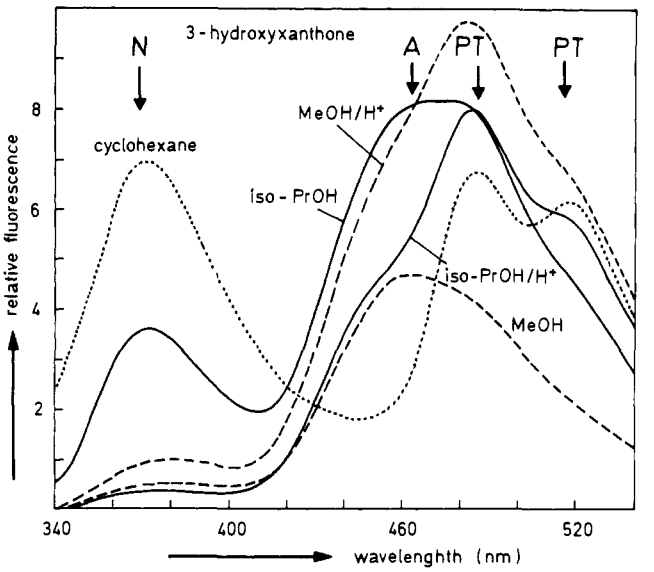


Figure 5. Fluorescence spectra of 3-hydroxyxanthone in methanol, 2-propanol, and cyclohexane (saturated with methanol). The addition of 0.1 N sulfuric acid (1 mL to 9 mL of the organic solvent) causes drastic changes in the emission spectra with practically no changes in the absorption spectra. A, N, and PT indicate the anion, neutral molecule, and phototautomer fluorescence bands.

However, exciplex formation between the anion and H_3O^+, as discussed in several other cases,[11-14] cannot be excluded. The formation of an exciplex as shown in Scheme II does not require a double proton transfer. It may be formed by a single proton transfer along a hydrogen bridge within the solvent cage. In other words, the phototautomer is formed by edge protonation of the anion and the exciplex by face protonation. The uncommon species thus formed will later on be referred to as the phototautomer.

On changing to organic solvents the fluorescence spectra of 3-hydroxyxanthone completely change their shape (Figure 5), but the absorption spectra are rather similar to the pH 6–2 spectra. In pure methanol fluorescence is observed from the excited neutral molecule (at 391 nm) and from the anion (at 461 nm). The latter again may be formed by photodissociation.

Acidification of the methanol solution (1 mL of 0.1 N sulfuric acid to 9 mL methanol solution) reduces the intensity of the anion emission in favor of a new, broad emission at 482 nm, together with a shoulder at 515 nm. This band again is assigned to the phototautomer.

In 2-propanol the fluorescence of neutral 3-hydroxyxanthone is much more distinct than in methanol, peaking at 371 nm. The fluorescence band with its maximum at 465 to 481 nm is still rather strong. Acidification as above gives rise to almost exclusive phototautomer emission at 483 nm.

The solubility of 3-hydroxyxanthone in cyclohexane is too poor to measure fluorescence spectra. It is not unlikely that xanthones (like several flavones[15]) are nonfluorescent in apolar solvents. Consequently the spectra were run in methanol-saturated cyclohexane. They show strong UV emission of the neutral molecule, together with two peaks at 484.5 and 514 nm. The first peak is assigned to the phototautomer, but interpretations for its formation in this solvent system are presently rather speculative.

It is noted that in the 2-propanol spectrum the phototautomer emission is also clearly evident as a shoulder at around 480 nm (Figure 5). So, with increasing dielectric strength of the solvent the relative intensities of the emissions of both the neutral molecule

(11) P. Zinsli, *J. Photochem.*, **3**, 55 (1974).
(12) G. S. Beddard, S. Carlin, and R. S. Davidson, *J. Chem. Soc., Perkin Trans. 2*, 262 (1977).
(13) O. S. Wolfbeis and R. Schipfer, *Photochem. Photobiol.*, **34**, 567 (1981).
(14) R. Schipfer, O. S. Wolfbeis, and A. Knierzinger, *J. Chem. Soc., Perkin Trans. 2*, 1443 (1981).
(15) V. Mallett and R. W. Frei, *J. Chromatogr.*, **54**, 251 (1971).

Table II. Ground State and First Excited Singlet State Dissociation Constants of 3-Hydroxyxanthone (3-HX) and 3-Methoxyxanthone (3-MX) at 22 °C[g]

species	ν_{max}^{abs} (cm^{-1})	ν_{max}^{flu} (cm^{-1})	0-0 transition (cm^{-1})	$pK_a(S_0)$[a]	$pK_a(S_1)$[b]	$pK_a(S_1)$[c]
3-HX conjugate acid	28 571	22 075	25 323	−3.06 ± 0.15[d]	2.07	−1.27 ± 0.1
3-HX	~29 851	~25 641	27 745	7.16 ± 0.04	1.28	2.36 ± 0.08
3-HX conjugate base	28 249	21 692	24 970			
3-MX conjugate acid	28 490	21 978	25 234	−3.16 ± 0.15[e]	2.09	1.16 ± 0.14
3-MX	~29 851	25 575	27 713			

[a] Including maximum deviation. [b] Calculated according to ref 16. [c] By fluorescence titration. [d] Reference 18 gives pK_a −3.0 (spectrophotometrically). [e] The methanol maximum was taken, as the neutral molecules fluorescence does not appear in aqueous solution. [f] Reference 18 gives pK_a −2.8 (spectrophotometrically). [g] The mean of the wavenumbers of absorption and fluorescence maxima were taken as 0-0 transitions.[17]

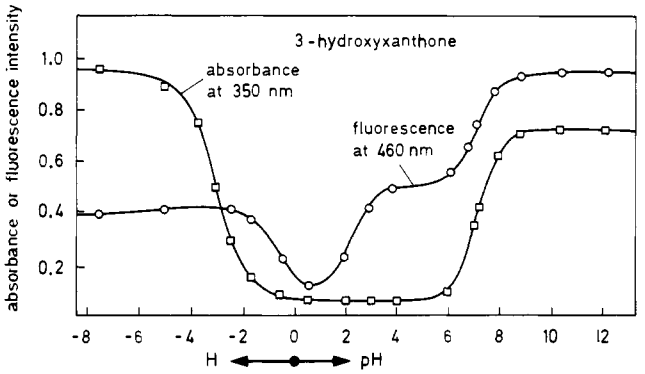


Figure 6. Plots of absorbance at 350 nm and of fluorescence intensity at 460 nm vs. acidity (pH or H_0). Excitation at 326 nm.

and the phototautomer decrease, but the one of the anion increases.

The appearance of two resolved bands in the cyclohexane–methanol spectra (at 484.5 and 514 nm) may be interpreted in terms of intimate and solvent-separated ion pairs, as was done by Beddard et al.[12] for 4-MU.

Ground and First Excited Singlet State pK_a Values. The ground-state pK_a's of 3-hydroxyxanthone and of the conjugate acids of 3-hydroxy- and 3-methoxyxanthone were determined photometrically to be 7.16, −3.06, and −3.16, respectively. From a plot of anion fluorescence intensity vs. pH two pK_a's can be obtained (Figure 6). The one (7.16) corresponds to the ground-state pK_a, the other to the excited-state pK_a.

From plots of cation fluorescence against Hammett acidity the excited-state pK_a's of the conjugate acids of 3-hydroxyxanthone (−1.17) and 3-methoxyxanthone (+1.16) were obtained, showing that these compounds are more basic at their carbonyl oxygen in the excited state than in the ground state. Plots of absorption and fluorescence intensity vs. pH or H_0 are shown in Figure 6. The results of the pK_a determinations are compiled in Table II.

The first excited singlet state pK_a's have also been calculated, applying the Förster–Weller equation.[16] The results correctly predict the direction of the pK_a changes, but the agreement with the experimental values is only fair (Table II). It should, however, be held in mind that the pK_a's resulting from the Förster–Weller calculations correspond to the excited-state equilibria shown in Scheme I, while the pK_a's obtained by fluorimetry correspond to the equilibria shown in Scheme II. The Förster–Weller calculations also correctly predict the phototautomerization, which was detected fluorimetrically in this work: In the first excited singlet state the phenolic hydroxy group is more acidic (pK_a 1.28) than the carbonyl group is basic (pK_a 2.07).

The fluorescence quantum yields of 3-hydroxyxanthone are strongly dependent upon the acidity of the solution, being 0.16 in 95% sulfuric acid and 0.38 in 0.1 N sodium hydroxide solution (excitation wavelength range 345–352 nm). The good fluorescence efficiency of 3-hydroxyxanthone together with its ground-state pK_a of 7.16 can make it a useful indicator for measuring physiological pH's fluorimetrically at excitation wavelengths around 350 nm.

Acknowledgment. This work was supported by a grant from the Fonds zur Förderung der wissenschaftlichen Forschung, Project No. 3807. E.F. thanks the Hermann F. Mark-Fonds for a stipend.

Registry No. 3-HX, 3722-51-8; 3-MX, 3722-52-9; 3-HX conjugate acid, 81898-32-0; 3-HX conjugate base, 81898-33-1; 3-MX conjugate acid, 81898-34-2.

(16) (a) Th. Förster, *Z. Elektrochem.*, **54**, 42 (1950); (b) A. Weller, *ibid.*, **56**, 662 (1952); (c) Review: Z. R. Grabowski and A. Grabowska, *Z. Phys. Chem. (Wiesbaden)*, **101**, 197 (1976).

(17) W. Bartok, P. Lucchesi, and N. Snider, *J. Am. Chem. Soc.*, **84**, 1842 (1962).

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