

Luminescent Heterocycles. IX

pH Dependent Fluorescence Spectra of Chromone, 2-Methylchromone and 7-Hydroxy-2-Methylchromone [1]

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Chromones fluorescence, ground and excited state pK_a , tautomerism

The fluorescence spectra of the title compounds have been measured in the pH 9 to $H_0 - 9$ acidity range at 25 °C. Chromone and its 2-methyl derivative fluoresce intensely from their protonated form, whereas the uncharged molecules are nonfluorescent. Förster cycle calculations show chromone to be more basic in its first excited singlet state than in its ground state, but protonation proceeds incompletely during the lifetime of the excited molecule.

7-Hydroxy-2-methylchromone (7-HMC) fluoresces in water, depending upon the solvent acidity, from its anion and a zwitter-ion (or phototautomer or exciplex), but not from the neutral molecule. pK_a values are given for both the ground and excited state and drastic differences are found: The 7-HMC anion in its S_1 -state is first protonated at the carbonyl oxygen rather than at the phenolate oxygen.

Attention is drawn to the extrem solvent and acidity sensitivity of the fluorescence maxima and intensities of 7-hydroxy-chromones with respect to possible analytical determinations of naturally occurring derivatives using luminescence spectroscopy.

Introduction

Solvent and pH dependent fluorescence spectroscopy has proved to be a valuable tool for studying primary photochemical processes, in particular photodissociations and photoprotonations [2]. Since the pioneering work of Förster and Weller it is well established that excited state dissociation constants may differ drastically from the corresponding ground state values [3].

An intriguing phenomenon is phototautomerism: Following charge transfer excitation, a molecule is protonated at one site and deprotonated at another site to form a tautomeric molecule. This often results in an anomalous large Stokes shift of the fluorescence band.

One may distinguish between intramolecular phototautomerism (e.g. of ortho-hydroxy arylketones or salicylic acid) and intermolecular phototautomerisms, which most often occur in heterocyclic systems having clearly separated acidic and basic centers. Due to the variety of simultaneous prototropic processes, several fluorescence bands are observed ("multiple fluorescence"),

which cover a broad region in the uv and visible spectrum, thus providing a possibility for broad band laser tuning.

Our interest in photoinitiated tautomerisations has now led us to study the fluorescence behaviour of several chromones (4H-benzopyrones). We have expected especially 7-hydroxychromones to undergo photodissociations as well as tautomerisations in their first excited singlet state. The use of pyrylium salts as Q-switches has been described [4]. The fluorescence spectra of chromones have been reported qualitatively in concentrated sulfuric acid [5], in organic solvents [6, 7] (and also their metal chelates [7]), and more detailed in nonaqueous solutions at 77 K [8, 9]. No investigation on the pH and Hammett acidity dependent fluorescence of chromone has been published so far, contrary to the many papers dealing with the isomeric coumarines (2H-benzopyrones) [10–13] and flavones (2-phenylchromones) [9, 14–18] including their analytical applications [7, 16, 17, 19–22].

This paper presents results obtained on chromone, its 2- and 3-methyl derivatives and on 7-hydroxy-2-methylchromone.

Experimental Section

The compounds were prepared according to known methods [23–26]. Fluorescence spectra were recorded on a Perkin-Elmer-Hitachi MPF 44

* Dedicated to Prof. Dr. O. E. Polansky on the occasion of his sixtieth birthday.

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at 25 °C in nondeaerated solutions. Analytical grade sulfuric acid and commercial buffer solutions were used throughout, except those containing chloride ions, which were found to quench fluorescence to some extent.

Results

1. Chromone, 2-methylchromone and 3-methylchromone

Chromone is nonfluorescent in neutral aqueous solution, as it is in other nonacidic solvents. On acidification chromone begins to fluoresce intensely with a maximum at 419 nm. This band undoubtedly arises from the pyrylium cation 1 P. In sulfuric acid the fluorescence intensity grows with increasing Hammett acidity H_0 to a maximum at $H_0 > 4$ (Figure 1).

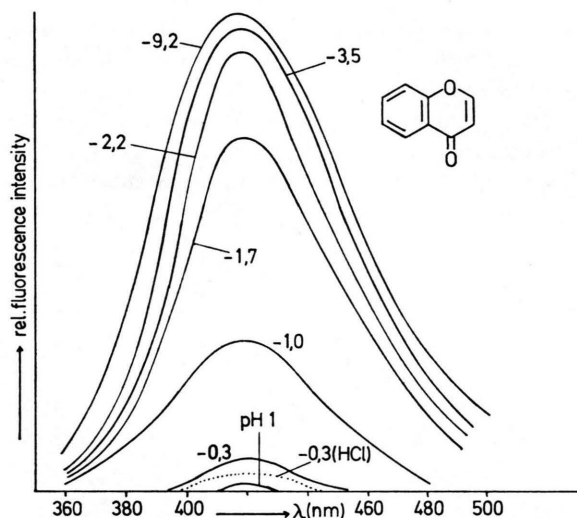
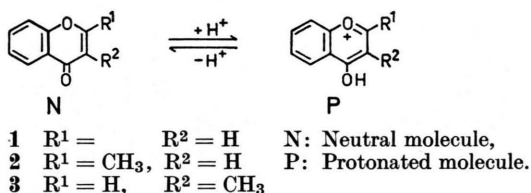
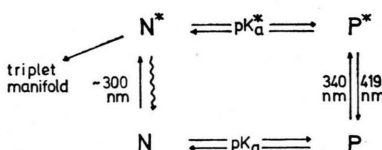


Fig. 1. Fluorescence spectra of chromone in sulfuric acid of different Hammett acidity H_0 . The H_0 values have been taken from L. P. Hammett, *Physikal. Organ. Chemie* (Verlag Chemie, Weinheim 1970), Table 9.2, p. 270. Concentration 1.6×10^{-4} Mol. dm⁻³, excitation wavelength 318 nm from $H_0 = 0$ to -2.5 , and 335 nm from $H_0 = -2.6$ to -9 . The uv absorption spectra are given in Refs. [8], [10]. The dotted line refers to a hydrochloric acid solution of chromone having $H_0 = -0.3$ and clearly shows the reduced fluorescence intensity due to chloride ion quenching, as compared to the sulfuric acid solution.

The ground state pK_a of chromone is reported to be -2.0 [27]. There is evidence from Förster cycle calculations, that pK_a^* (the pK_a for the first excited singlet state) is bigger than $+0.5$. In other words, chromone is more basic in its excited state than in its ground state.

The thermodynamic equilibria between N and P as well as N^* and P^* are governed by the respective pK_a values (Scheme 2).



Scheme 1. Possible reaction sequences for chromone in its ground and excited states. An asterisk indicates the excited state.

Fluorescence at $H_0 = -1$ would be expected to occur from the excited ion P^* with maximum intensity due to complete protonation following photoexcitation ($N \rightarrow N^* \rightarrow P^* \rightarrow \text{fluorescence} \rightarrow P \rightarrow N$). But only 60% of the maximum intensity, which is found in strong sulfuric acid, is observed, and incomplete protonation during the lifetime of the S_1 -state ($< 10^{-8}$ sec) has to be assumed. The equilibrium $N^* \rightleftharpoons P^*$ is not fully established during this time.

Two reasons may account for this: a) Solvent-solvate proton exchange leads to quenching of the excited state and prevents fluorescence. This quenching effect is more pronounced in intermolecular rather than in intramolecular prototropisms. Moreover, the fluorescence intensity is also reduced by some anions and is, e.g. in 1 N HCl, remarkably lower than in 1 N sulfuric acid.

b) Gallivan [8] has shown chromone to have at least two phosphorescent triplet states energetically close to the S_1 -state, having lifetimes of some 10^{-2} sec. As a result, singlet excited chromone can undergo efficient intersystem crossing (isc) to its triplet state and by that escapes protonation.

Contrarily, when ground state protonated chromone (P) is excited to form P^* , the fluorescence is extremely intense, as no more prototropic processes are involved, and isc is practically suppressed.

The intensity of the pyrylium salt fluorescence band at 419 nm is excitation wavelength dependent too. The most effective λ_{exc} shifts from 318 nm in less acidic solution to 335 nm in sulfuric acid of

$H_0 < -2.5$ due to excitation of ground state protonated chromone.

Results similar to those of chromone have been obtained for its 2-methyl and 3-methyl derivatives (2N and 3N). Both begin to fluoresce strongly in acidic solution, but again the maximum fluorescence intensity is observed only in relatively strong acid (2-methylchromone: $H_0 < -1$, $pK_a = -1.16$ [27]; 3-methylchromone: $H_0 < -2$). Their fluorescence maxima and relative fluorescence intensities are compiled in Table 1. The strong fluorescence of chromones in acidic solution suggests their use as laser dyes in the near visible region, alternatively to coumarines.

7-Hydroxy-2-methylchromone (7-HMC)

Introducing a 7-hydroxy group into 2-methylchromone gives rise to particularly effective charge transfer excitation and consequently to changes in electron densities. Therefore this compound was of special interest to us. Its isomer, 4-methylumbelliferone (4-MU) exhibits phototautomerism [10, 28, 29] (or, according to Zinsli, exciplex formation with H_3O^+). As a result, broad band laser emission from acidified 4-MU solutions has been reported [31, 32]. Similar observations have been made with 4,7-dihydroxycoumarin [11] and 7-hydroxylepidone [33], but not with 4-hydroxycoumarin [11, 34].

7-HMC shows fairly strong fluorescence in both polar and unpolar aprotic solvents with a Stokes shift of approximately 75 nm. So, for instance, in

Table 1. Fluorescence data on some chromones in 60% sulfuric acid ($H_0 = -4.46$).

Compound	$\lambda_{\max}(\text{flu})$	$\lambda_{\max}(\text{exc})$	$c \cdot 10^{-4}$ (mol dm ⁻³)	Rel. intensity ^a
Chromone	419	336	4.5	0.72
2-Methylchromone	409	334	2.6	0.75
3-Methylchromone	421	346	2.91	0.69
7-Hydroxy-2-methylchromone	426	359	2.9	0.86

^a calculated from the values of band height, concentration, molar absorptivity at λ_{exc} , and slit width.

benzene 7-HMC has a long wave absorption band at 302 nm and a fluorescence maximum at 379 nm.

Drastic changes in the fluorescence spectra are observed on altering to protic solvents like alcohols or water. The emission spectra of aqueous solutions of 7-HMC are extremely sensitive towards acidity changes and cover a broad region of the short wave visible range (Figure 2).

Additionally an enormous increase in fluorescence intensity of 7-HMC is observed in water, as compared to unprotic solvents. This, together with broad band emission in acidified solution and a lack of concurrent isc processes makes 7-HMC an interesting laser dye.

The electronic absorption spectra (Fig. 2) indicate three ground state species to be present in the pH 10 to $H_0 - 9$ region, depending on the actual acidity.

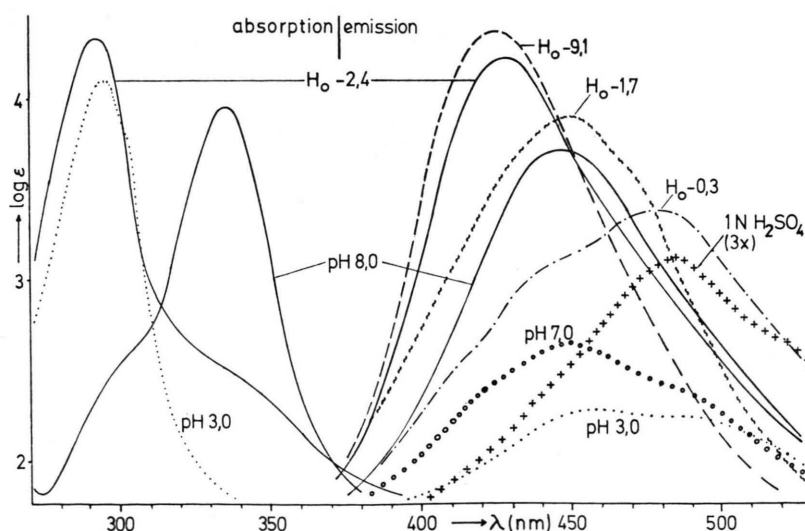


Fig. 2. Changes in the absorption and fluorescence spectra of 7-hydroxy-2-methylchromone in water of different acidity. Conc. $1.5\text{--}4.5 \times 10^{-4}$ mol dm⁻³. For the uv spectra see also Refs. [36], [37].

The anion *A*, absorbing with a maximum at 336 nm, is present in solutions of pH > 7. In neutral or slightly acidic solution 7-HMC exists as an uncharged molecule. The ground state pK_a for the phenolic hydroxyl group was calculated from the uv spectra to be 7.4 ± 0.2 .

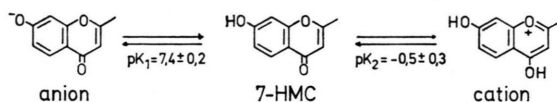
Neutral 7-HMC absorbs strongly ($\log \varepsilon = 4.06$) at 295 nm with a shoulder at 304 nm. In more acidic solution the positively charged molecule is formed, recognizable by a new long wave absorption band at 335 nm. In solutions of $H_0 - 3$ the molecule is fully protonated. The corresponding ground state pK_a was calculated to be -0.4 ± 0.3 .

The changes in the pH and H_0 dependent fluorescence spectra of 7-HMC do not parallel those of the absorption spectra (Figure 2). The strong anion fluorescence at 445 nm is still present, albeit at reduced intensity on acidification to pH 6, even when the neutral molecule is excited at $\lambda_{\text{exc}} = 308$ nm. The spectra of slightly acidic 7-HMC solutions reveal, however, a new red shifted band at 480–490 nm. At pH 3 this band becomes as strong as the anion band and at pH 1 up to 30% sulfuric acid it is the predominant one. Simultaneously the anion band at 450–460 nm decreases steadily. The new band at 480–490 nm is accompanied by a shoulder at 530 nm of unknown origin. The latter is somewhat more expressed in acidified methanol. 4-Methylumbelliferone shows a similar shoulder at 520 to 530 nm in acidified methanol [32]. No emission band originating from the excited neutral molecule can be observed in the 380 nm region.

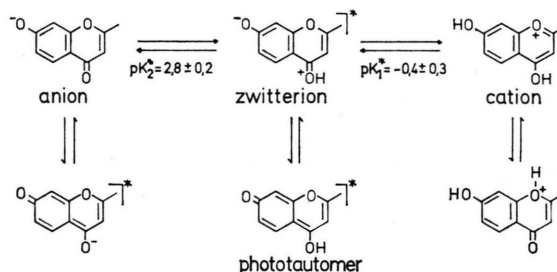
In strong sulfuric acid ($H_0 < -1.5$) the pyrylium cation is the predominant fluorescent species with an emission maximum at 426 nm. (Because of a relatively strong shoulder at 470 nm the cation fluorescence band in 30% H_2SO_4 lies at 439 nm, but is shifted successively towards 426 nm in more concentrated acid.

To interpret these phenomena one has to assume prototropic reactions of the molecule in its excited state prior to fluorescence. Furthermore, no emission from the neutral molecule itself is observed in aqueous solution at all. That indicates the first excited singlet state of 7-HMC to be thermodynamically unstable in water. When 7-HMC is excited, it will undergo reactions involving one or more prototropic steps to form an excited new molecule prior to energy loss. That molecule must

account for the fluorescence band at 487 nm. It is reasonable to assume a "phototautomeric" or "exciplex" [30] species whose fluorescence appears at longer wavelengths than that of the anion.



Scheme 2. Ground state equilibria of 7-hydroxy-2-methylchromone.



Scheme 3. First excited singlet state equilibria of 7-hydroxy-2-methylchromone (7-HMC). An asterisk indicates the molecule to be existent in the excited state only.

Again two dissociation constants describe the protolytic equilibria of S_1 -7-HMC, but they differ dramatically from the ground state constants (Scheme 2 and 3).

On acidimetric titration of anionic 7-HMC*, the carbonyl oxygen is rather more protonated than the phenolate oxygen with its excited state pK_1^* of -0.4 ± 0.3 . In other words, the difference between pK_1 and pK_1^* is as much as 8.7 ± 0.5 logarithmic units. All the pK_a 's under consideration are summarized in Table 2.

We have noted that such huge pK_a differences have to be expected when excitation is followed by efficient charge transfer from a donor function (e.g. hydroxy or amino groups) to another single π -bound heteroatom (e.g. C=O or C=N groups). As a result the basicities of the carbonyl and the hydroxyfunctions may become reversed in the

Table 2. pK_a 's of 7-hydroxy-2-methylchromone for the ground state (pK) and for the S_1 -state (pK^*), determined from the acidity dependent absorption and fluorescence spectra at 25 °C.

Functional group	pK_a	pK_a^*
—OH	7.4 ± 0.2 (pK_1)	-0.4 ± 0.3 (pK_1^*)
—CO—	-0.5 ± 0.3 (pK_2)	2.8 ± 0.2 (pK_2^*)

S₁-state. This is apparently the case with 7-HMC and 4-methylumbelliferone.

When, on the other hand, charge is not transferred to a single heteroatom, but delocalized over several ones, no drastic *pK_a* differences will be observed. It is known from umbelliferone and its derivatives, that an electron withdrawing substituent in 3-position reduces the excited state basicity of the carbonyl oxygen and no phototautomerism will occur then [12].

In a similar way 7-hydroxychromones with an electron attracting substituent in the pyrone ring have only limited ability to form phototautomers, and rather fluoresce from the unchanged excited molecule at relatively short wavelengths [35].

It is interesting to note that 7-HMC fluoresces in methanol from its anion too (Fig. 3) though this solvent to be a comparatively weak dielectric. This is in contrast to the fluorescence mechanisms of 4-MU, which in methanol emits mainly from the excited neutral molecule. A minor reaction path leads to tautomerization and consequently to long wave emission. 7-HMC does not emit from its "normal" S₁-state at all.

In methanol a band at 480 to 490 nm shows the presence of a second species additionally. This band becomes very strong in acidified methanol, whereas the 455 nm anion band disappears. It is rather broad (485–500 nm) and possibly consists of two bands.

Presumably the phototautomer is responsible for that emission. Excited state ion pair (ESIP)

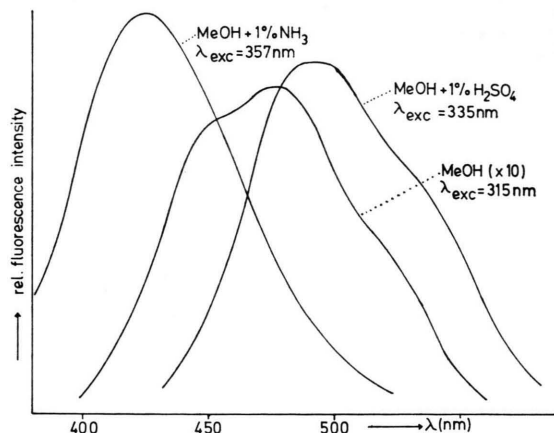


Fig. 3. Fluorescence spectra of 7-hydroxy-2-methylchromone (7-HMC) in methanol, methanol-NH₃ and methanol-HCl. Conc. = $2.5\text{--}6.0 \times 10^{-4}$ mol dm⁻³.

formation between methanol-H⁺ and anionic 7-HMC cannot be excluded and many account for the broadness of the luminescence band.

Finally, with respect to a possible fluorimetric detection of naturally occurring 7-hydroxychromones, once more attention is drawn to the fact that most of the fluorescence maxima are extremely acidity dependent. Careful pH adjustment is necessary to obtain reproducible results. Additionally the fluorescence intensities can vary strongly even at identical pH, since several anions (like chloride), which may be present in commercial buffer solutions, can quench the fluorescence.

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