Photochromic Properties of 3-Methyl-Substituted Flavylium Salts

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The photochromic properties of some 3-methyl-substituted synthetic flavylium compounds have been investigated. The main structural feature in these compounds is the existence of a steric effect, caused by the methyl substituent, that forces the phenyl ring to move out of coplanarity with the benzopyrylium moiety. The X-ray structure of 3-methylflavylium tetrafluoroborate shows a torsion angle of 40.4° between the benzopyrylium and the benzene ring. In 3-methyl-substituted synthetic flavylium compounds, the steric effect impedes the formation of *trans*-chalcone. In the case of the 4′-hydroxy-3-methylflavylium ion, for example, the *trans*-chalcone form could only be obtained (up to a maximum yield of 60%) by protonation of the *trans*-chalcone anion, obtained in turn by irradiation of anionic *cis*-chalcone in basic solution. The multistate/multifunctional properties of the 4′-hydroxy-3-methylflavylium compound are also discussed in detail. For the 4′,7-dihydroxy-3-methylflavylium ion, a detailed investigation of the kinetic and thermodynamic properties of the various forms was performed by means of pH jumps and photochemical experiments.

Introduction

Anthocyanins,[1,2] one of the most important sources of colour in flowers and fruits, undergo structural transformations accompanied by colour changes upon variation in pH and stimulation with light.[3,4] Synthetic flavylium salts, which possess the basic structure of natural anthocyanins, undergo similar structural transformations and colour changes with changes in pH[7,8] or upon light excitation.[9−12]

The scheme of Figure 1 can be used to explain in simplified terms the thermal and photochemical behaviour of flavylium-type (either natural or synthetic) compounds.[1−12] Depending on the nature of the substituents, however, other forms can also be involved (see below). In strongly acid solution the flavylium cation AH^+ is the most stable species. In moderately acidic or neutral solution, the thermodynamically stable species is usually the neutral *trans*-2-hydroxychalcone form **Ct**, which is obtained from AH^+ through two intermediate compounds, **B2** and **Cc**. Compound **B2** is a hemiacetal species, obtained by hydration of the flavylium cation at the 2-position, while **Cc** is a *cis*-2-hydroxychalcone, formed from the hemiacetal **B2** through a tautomeric process. The interesting feature of these systems is that the **AH**$^+$ and **B2** forms can be reversibly interconverted by variation of the pH,[7,8] whereas **Cc** and **Ct** can be interconverted by photoexcitation.[9−12] Since the **B2** and **Cc** forms are in tautomeric equilibrium, it follows that pH and light stimuli can be used to cause interconversion of the four fundamental forms. Systems of this kind, capable of existing in several forms that can be interconverted by different external stimuli, are referred to as *multistate/multifunctional* systems.[15] An interesting aspect of such systems is that they exhibit properties suitable for playing the role of optical memories with multiple storage and nondestructive readout capacities (*write*-*lock*-*read*-*unlock*-*erase* cycles, Figure 2, a). In these systems, light is used to convert a species, **X**, into another species, **Y** (*write*); a second stimulus (such as a proton, as in Figure 2, a) is then employed to transform **Y** (which would otherwise be reconverted back into **X** either thermally or by a direct photon-reading process) into **Z**, another stable state of the system (*lock*), that can be optically detected without being destroyed (*read*). Through this process, the change caused by the writing photon is safeguarded. When the written information is to be erased, **Z** is reconverted back into **Y** (*unlock*; by addition of a base, for example, as in Figure 2, a), and **Y** is then reconverted back into **X** (*erase*). Figure 2, b shows how flavylium compounds behave according to such a *write*-*lock*-*read*-*unlock*-*erase* scheme. In order to avoid spontaneous erasure of the written information, **Y** must persist long enough to wait for the second stimulus and the occurrence of the locking reaction. Some flavylium compounds exhibit an energy barrier that slows down the **Cc** → **Ct** back iso-

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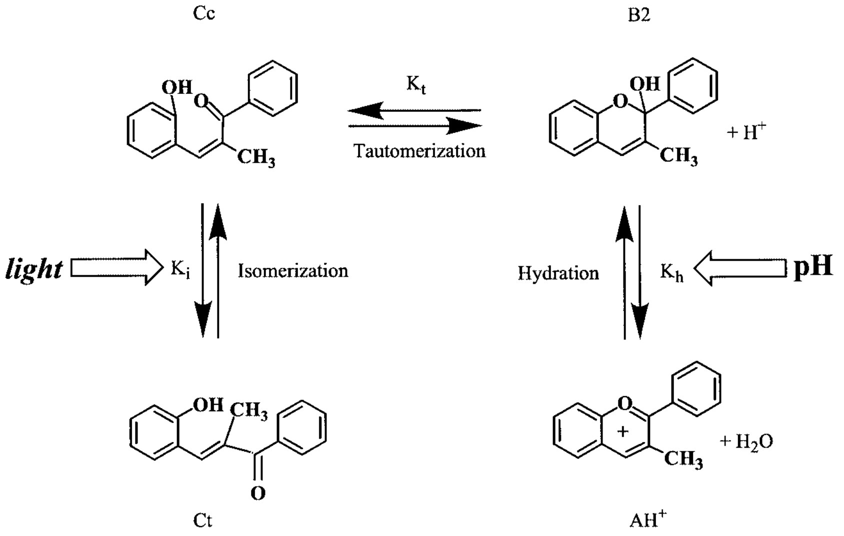

Figure 1. Structural transformations of the flavylium-type compounds; only the most important forms are shown

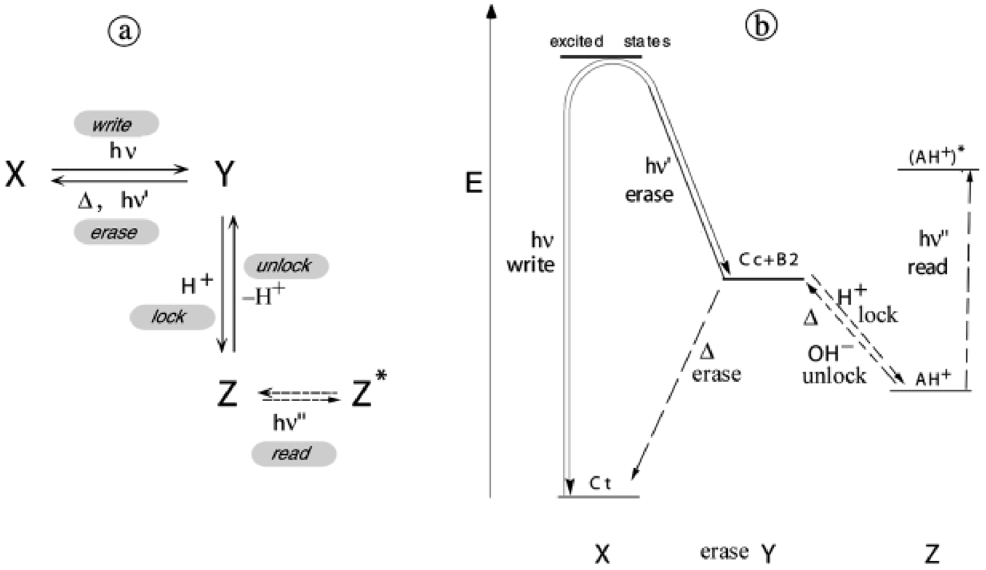

Figure 2. (a) Schematic representation of a *write-lock-read-unlock-erase* dual-mode system; (b) schematic energy level diagram for the species involved in a *write-lock-read-unlock-erase* cycle; for more details, see text

merization reaction and so allows the locking reaction to take place. It should also be noted that a high-energy barrier in the isomerization reaction improves the stability of the nonwritten and written information, allowing the system to remain in the **X** or **Z** states, respectively, for longer times.

Systematic studies[12−16] performed by the use of pH jump methods, continuous irradiation, and flash photolysis techniques have clearly shown that substituents in the 4′- or 7-positions have a strong influence in determining the thermal and photochemical reactivity of synthetic flavylium compounds. Specifically, it has been found that the energy barrier to isomerization (either **Cc** → **Ct** or **Ct** → **Cc**) can be increased by the presence of a hydroxy substituent in the 4′-position of the flavylium backbone and lessened by a hydroxy substituent in the 7-position.

We have already reported that the 4′-methoxyflavylium ion may be used as a basis for a *write-lock-read-unlock-erase* photochromic cycle[14] and that suitably designed 4′-substituted flavylium ions may i) behave as logic gates,[15,17] ii) exhibit intricate patterns of chemical processes,[15] and iii) even be used as components of a rudimentary neural network.[17]

In order to extend our knowledge on the reversible transformation processes that take place in flavylium compounds and to improve the stability of the written information when such compounds are used for optical information storage, we have investigated three compounds of the 3-methylflavylium family: namely 3-methylflavylium, 4′-hydroxy-3-methylflavylium, and 4′,7-dihydroxy-3-methylflavylium (Figure 3). We were prompted to study these compounds by the observation by Devine and McClelland[18] that the *cis*/*trans* isomerization rate for the 3-methylflavylium derivative was thirty times slower than that of the unsubstituted compound. In this work, we report the results of the investigation of the thermal and photochemical properties of these three 3-methyl-substituted flavylium compounds in acidic, neutral and basic solutions.

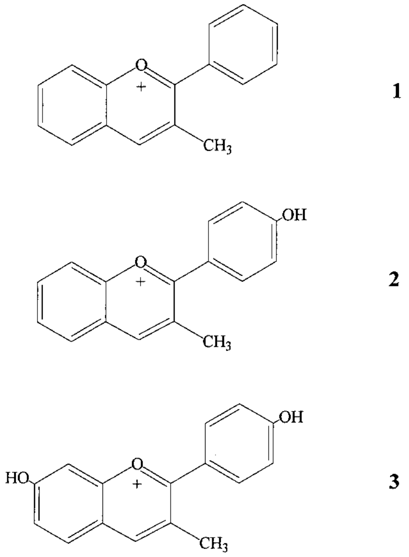

Figure 3. Structural formulae of the 3-methylflavylium compounds studied in this work

Kinetic Scheme

Our results, together with those previously reported by Devine and McClelland,[18] have been elaborated by means of the kinetic scheme[11] commonly used to determine the kinetic and thermodynamic parameters that control the intricate network of the reactions of flavylium compounds:

$$\mathbf{AH^+} + H_2O \overset{K_h}{\rightleftharpoons} \mathbf{B2} + H_3O^+ \qquad (1)$$

$$\mathbf{B2} \overset{K_t}{\rightleftharpoons} \mathbf{Cc} \qquad (2)$$

$$\mathbf{Cc} \overset{K_i}{\rightleftharpoons} \mathbf{Ct} \qquad (3)$$

This set of equations can be simplified to give a single acid/base equilibrium, Equation (4), in which the acidic flavylium cation $\mathbf{AH^+}$ is in equilibrium with its conjugate base **CB**, constituted by the sum of the species **B2**, **Cc** and **Ct**.

$$\mathbf{AH^+} + H_2O \overset{K'_a}{\rightleftharpoons} \mathbf{CB} + H_3O^+ \qquad (4)$$

$$K'_a = K_h + K_hK_t + K_hK_tK_i \qquad (5)$$

As shown previously[11] the molar fraction distribution of the acidic form $\mathbf{AH^+}$ can be obtained from Equation (6). If the pH is sufficiently high that all the compounds may be considered to be in the **CB** form, the molar fractions of each component of **CB** can easily be calculated by Equation 7, where C_0 is the total concentration and K'_a is defined in Equation (5).

$$\frac{[AH^+]}{C_o} = \alpha = \frac{[H^+]}{[H^+] + K'_a} \qquad (6)$$

$$\frac{[B2]}{C_o} = \frac{K_h}{K'_a}; \quad \frac{[Cc]}{C_o} = \frac{K_hK_t}{K'_a}; \quad \frac{[Ct]}{C_o} = \frac{K_hK_tK_i}{K'_a} \qquad (7)$$

In basic media, however, two more species, namely the anionic forms of *cis*- and *trans*-chalcone, $\mathbf{Cc^-}$ and $\mathbf{Ct^-}$, have to be taken into consideration (Figure 4).

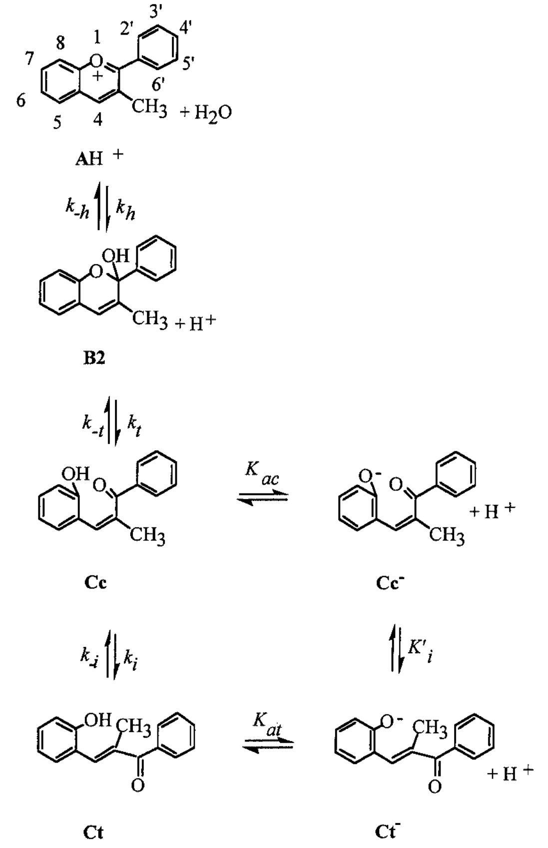

Figure 4. A more complete scheme showing the structural transformations taking place in the 3-methylflavylium compound

$$\mathbf{Cc} + OH^- \overset{K_{ac}}{\rightleftharpoons} \mathbf{Cc^-} + H_2O \qquad (8)$$

$$\mathbf{Ct} + OH^- \overset{K_{at}}{\rightleftharpoons} \mathbf{Ct^-} + H_2O \qquad (9)$$

When OH substituents are present, a more complex kinetic scheme has to be considered, as discussed below.

Results and Discussion

3-Methylflavylium Ion

The thermal reactions of the 3-methylflavylium ion had previously been investigated by Devine and McClelland.[18] They found that very acidic solutions were required to maintain the 3-methylflavylium compound in the coloured cationic **AH**$^+$ form. This behaviour was explained[18] on the basis of a steric interaction between the 3-methyl substituent and the *ortho*-(2′-)hydrogen atom of the phenyl ring, which forced the phenyl ring out of coplanarity with the benzopyrylium moiety, thereby decreasing the delocalization of the positive charge and the stability of the cation. Moreover, they also found by use of stopped flow analysis that at pH = 7, at which all the 3-methylflavylium cation (**AH**$^+$) had disappeared ($\beta = 1$), the equilibrated mixture only contained **B2** and **Cc**, with **B2** being by far the predominant species (ca. 98%).

We have re-examined several aspects of the thermal behaviour of this system and we have obtained results that substantially agree with those reported by these authors. In strongly acid solution (e.g., pH = 1), the 3-methylflavylium ion (**AH**$^+$) was the thermodynamically stable form of the system. This species is coloured (yellow) and its absorption spectrum shows an intense band with λ_{max} = 385 nm (ε = 28000 $\text{M}^{-1}\cdot\text{cm}^{-1}$). When aqueous solutions of **AH**$^+$ were subjected to pH jumps from pH = 1 to higher pH values the absorption spectra showed a continuous decrease of the 385-nm band up to pH = 3 (Figure 5). From the normalised changes in absorbance at 385 nm vs. pH (Figure 5, inset) we obtained the molar fraction of **AH**$^+$ vs. pH. From these data, a value of ca. $6\cdot10^{-2}$ for K'_a was obtained by use of Equation (5). Moreover, practically no **Ct** had formed after 15 d at room temperature or 6 d at 60 °C, since the absorption spectra obtained a few seconds after the pH jump (Figure 5) remained constant over these periods.

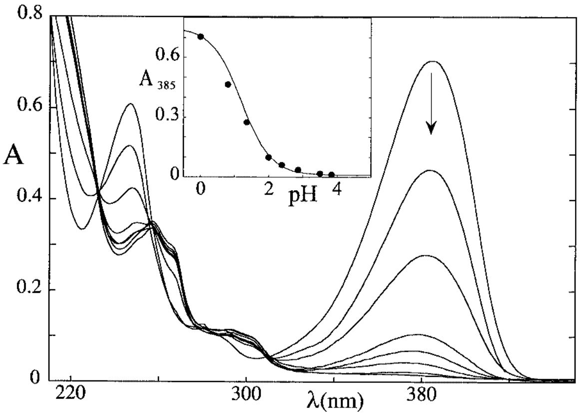

Figure 5. Absorption spectra of thermally equilibrated aqueous solutions of the 3-methylflavylium compound as a function of pH; the inset shows the molar fraction of **AH**$^+$ as a function of pH

In conclusion, the disappearance of **AH**$^+$ with an increase in pH had resulted almost exclusively in the hemiacetal **B2** (98%), on both short (seconds) and long (weeks) timescales. This suggested the conclusion that $K'_a \approx K_h \approx 6\cdot10^{-2}$. Such a K'_a value would be considerably lower than that found for the unsubstituted flavylium compound, which would mean that, as pointed out by Devine and McClelland,[18] more highly acidic solutions would be required to maintain the 3-methylflavylium compound in the cationic form. The X-ray structure (see supplementary material deposited with the CCDC for the details of the crystal structure) performed on crystals of 3-methylflavylium tetrafluoroborate obtained in this work confirmed McClelland's interpretation.[18] In fact, the torsion angle between the benzopyrylium moiety and the phenyl ring in this compound was 40.4° (Figure 6). Such a value is considerably higher than those found for 4′,6,7-trihydroxyflavylium (3-H substituted)[24] and 3,3′,4′,5,7-pentahydroxyflavylium, (3-OH substituted)[25] salts, which showed phenyl rings out of the benzopyrylium planes by 5.9[24] and 10.1°,[25] respectively.

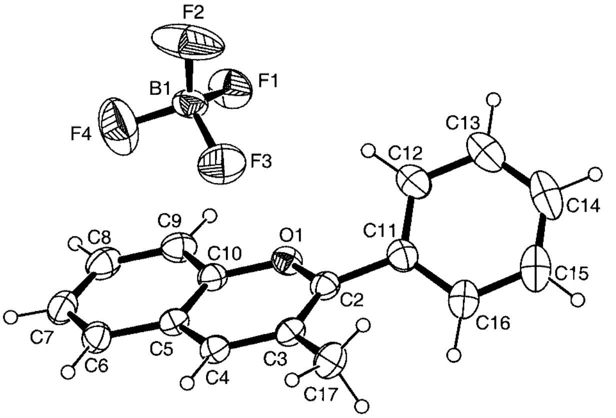

Figure 6. X-ray structure of a single crystal of the 3-methylflavylium compound

In addition to verifying McClelland's work in acidic and neutral aqueous solutions, we studied the thermal properties of 3-methylflavylium cation in basic media. The absorption spectra obtained immediately after a pH jump from 1 to basic pH values showed the formation of a new species, the characteristic band of which, at ca. 390 nm (ε = 4000 $\text{M}^{-1}\cdot\text{cm}^{-1}$), grew over the pH range of 9.0−12.5. This species was thermodynamically not stable, slowly converting into a second species that exhibited an absorption maximum at 395. The first-order rate constant for this conversion was temperature-dependent; at pH = 12.5 the values of k_{obs} at different temperatures (0.40, 0.87, and 1.46 h^{-1} at 50, 60, and 65 °C, respectively) yielded an activation energy of 77 $\text{kJ}\cdot\text{mol}^{-1}$. A reverse pH jump back to pH = 1 performed on an aqueous solution previously equilibrated for 15 d at room temperature and pH = 12.5 resulted in the appearance of the flavylium cation band (385 nm) in two distinct steps. In the first of these, immediately after the pH jump, ca. 13% of the expected total amount of **AH**$^+$ was formed, while the remaining 87% of **AH**$^+$ was formed in the subsequent, slower, step (several minutes at 65 °C). These results can be accounted for by the assumption that the first species obtained at pH > 9.0 was the *cis*-chalcone anion (**Cc**$^-$), which slowly reacted to give the *trans*-chalcone anion (**Ct**$^-$), until an equilibrium was attained. In the subsequent reverse pH jump (to pH = 1), the unchanged **Cc**$^-$ immediately gave the flavylium cation **AH**$^+$, while more time was required for the formation of **AH**$^+$ from **Ct**$^-$ (several minutes at 65 °C). The composition of the equilibrated mixture (**Cc**$^-$ 13%, **Ct**$^-$ 87%), and, in turn, the equilibrium constant for the anion isomerization [$K^-{}_i = 6.7$, from Equation (5)] at pH = 12.5 can thus be evaluated from the concentrations of flavylium cation obtained in the two steps.

$$\mathbf{Cc}^- \rightleftarrows \mathbf{Ct}^- \quad K^-{}_i \tag{10}$$

The second, slower step, the conversion of the *trans*-chalcone (**Ct**) into the flavylium cation (**AH**$^+$) via the *cis*-chalcone (**Cc**), was also found to be temperature-dependent; its rate constants at different temperatures (0.075, 0.225, and 0.5 h^{-1} at 50, 60, and 65 °C, respectively) allowed an energy barrier of 110 kJ·mol^{-1} to be determined.

These results show that while the flavylium cation can be obtained thermally from *trans*-chalcone (via *cis*-chalcone), no measurable amount of *trans*-chalcone was detected after a pH jump from 1 to 3 < pH < 9 at the same temperature and in an identical period of time. This apparent contradiction can be explained by the fact that the equilibrium constant of Equation (2) favours the hemiacetal at the expense of *cis*-chalcone, thus limiting the rate of the *cis* → *trans* isomerization reaction, which is proportional to the concentration of *cis*-chalcone at the equilibrium. Therefore, the most efficient way to obtain *trans*-chalcone is from reacidification of the anionic *trans*-chalcone formed by thermal isomerization of the *cis*-chalcone anion at pH = 12.

Photochemical Experiments

Continuous Irradiation

Aqueous solutions of the 3-methylflavylium compound, previously equilibrated in the dark at pH = 12.5 (87% **Ct**$^-$ and 13% **Cc**$^-$), were irradiated at 313 nm until a steady state was reached. The absorbance changes were consistent with a partial conversion of **Ct**$^-$ to **Cc**$^-$, with an initial quantum yield of 0.043. Reacidification of the irradiated solution to pH = 0 resulted in instantaneous formation of flavylium cation (42% of the initial concentration), showing that, at the steady state, there were 42% and 58% of **Cc**$^-$ and **Ct**$^-$, respectively. Subsequent irradiation (again at 313 nm) resulted only in a further, partial recovery of **AH**$^+$ (up to 60%), showing that a degradation reaction (ca. 40%) was taking place.

In contrast, irradiation of an aqueous solution of the 3-methylflavylium compound previously equilibrated at pH = 12.5 and reacidified to pH = 0 (ca. 87% **Ct** and 13% flavylium cation) showed the formation of the flavylium cation, with an initial quantum yield of 0.5 (Figure 7). Only 94% of the **AH**$^+$ could be recovered, showing that some degradation was occurring even in this case. When reacidification of the solution, previously equilibrated at pH = 12.5, was stopped at pH = 6.2 (instead of at pH = 0), the photochemical reaction almost completely converted the *trans*-chalcone into the hemiacetal species, with an initial quantum yield of 0.18. A subsequent pH jump from pH = 6.2 to 0 showed that even in this case some degradation (ca. 5%) occurred.

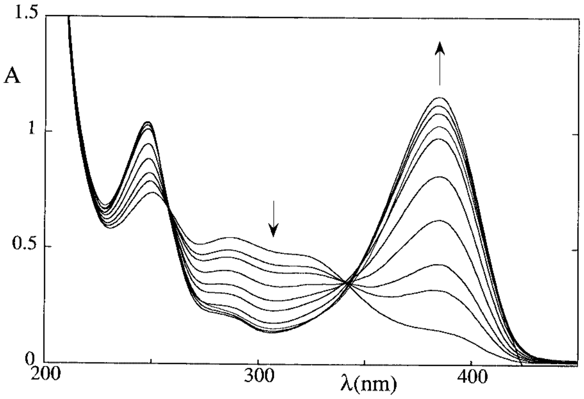

Figure 7. Spectral changes upon continuous irradiation (365 nm) of aqueous solutions of the 3-methylflavylium compound previously equilibrated at pH = 12.5 and then reacidified to pH = 0

Most probably, the degradation observed in the experiments described was due to a photochemical side reaction, as can be argued by the fact that even irradiation of a freshly prepared solution of flavylium cation at 313 nm in HCl (1 M) results in a slight, permanent photo-bleaching of the **AH**$^+$ absorption band (at 380 nm).

4′-Hydroxy-3-methylflavylium Compound

Equilibria in the Dark

In acidic and neutral aqueous solutions, the 4′-hydroxy-3-methylflavylium ion underwent the structural transformations shown in Figure 8. Besides the four species found for the 3-methylflavylium compound **AH**$^+$, **B2**, **Cc**, and **Ct** in the case of the 4′-hydroxy-3-methylflavylium, another species − the quinoidal base (**A**) obtained by simple deprotonation of the hydroxy group in the 4′-position − has to be considered (Figure 8 and Equation (11)].

$$\mathrm{AH^+ + H_2O \overset{K_a}{\rightleftharpoons} \mathbf{A} + H_3O^+} \tag{11}$$

The presence of an equilibrium according to Equation (11) modifies the previously discussed kinetic scheme. One more equilibrium constant has to be considered, and Equation (5) converts into Equation (12).

$$K'_a = K_a + K_h + K_hK_t + K_hK_tK_i \tag{12}$$

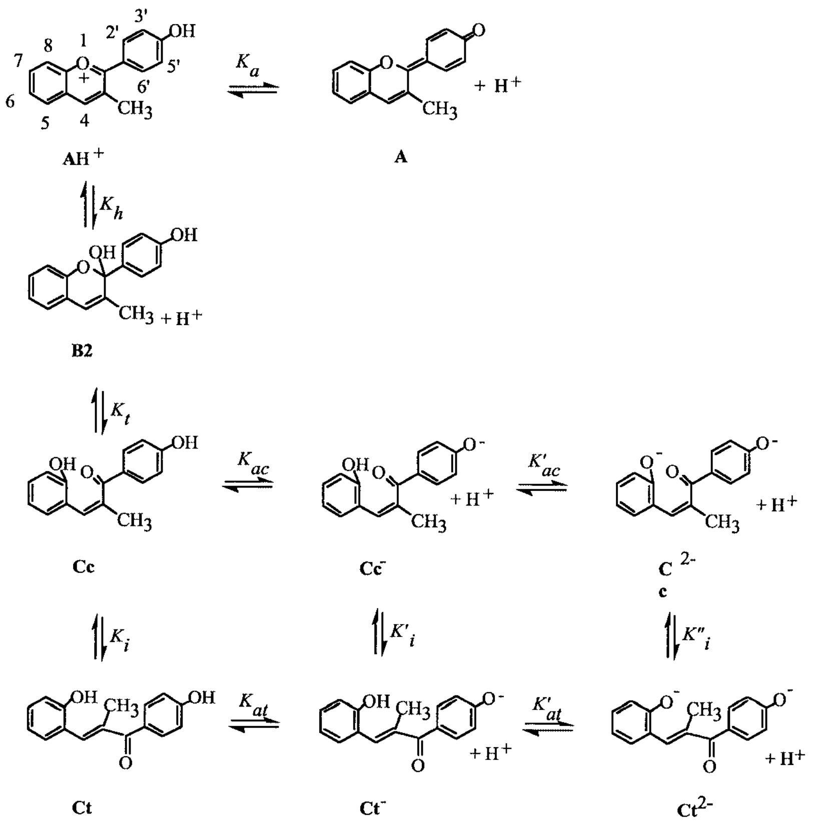

Figure 8. Structural transformations taking place in the 4′-hydroxy-3-methylflavylium compound

Moreover,

$$[\mathbf{CB}] = [\mathbf{A}] + [\mathbf{B2}] + [\mathbf{Cc}] + [\mathbf{Ct}] \tag{13}$$

and

$$\frac{[A]}{C_o} = \frac{K_a}{K'_a} \tag{14}$$

The absorption spectra of some species involved are shown in Figure 9.

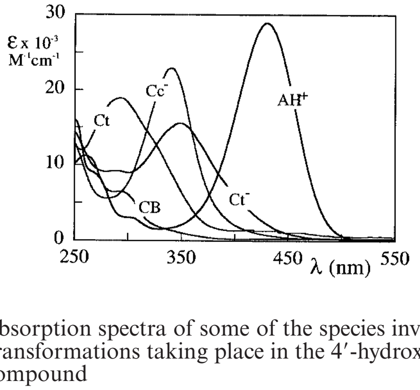

Figure 9. Absorption spectra of some of the species involved in the structural transformations taking place in the 4′-hydroxy-3-methylflavylium compound

In order to evaluate the K'_a value, UV/Vis absorption spectra of dark-equilibrated solutions of the 4′-hydroxy-3-methylflavylium compound were recorded as a function of pH (= 1−7) (Figure 10). Since the deprotonation reaction [Equation (11)] was faster than the hydration reaction giving **B2** [Equation (1)], pH jumps from pH = 1 to higher pH values caused the initial, fast formation of **A**. Species **A** then disappeared as rapidly as it was formed, giving rise to other forms. The lifetime of **A** was so short that we were unable to obtain its absorption spectrum, observing only a transient change of the colour of the solutions exposed to the pH jump.

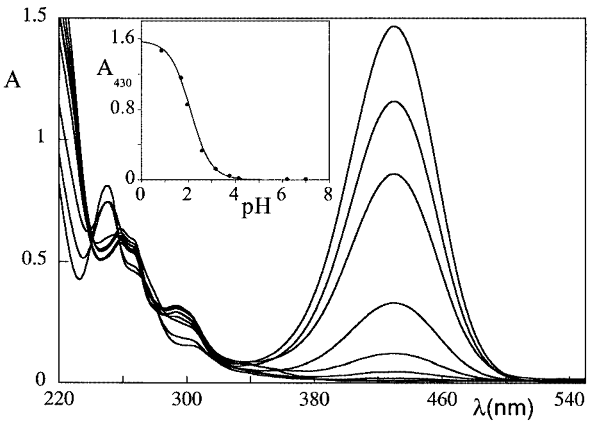

Figure 10. Absorption spectra of thermally equilibrated aqueous solutions of the 4′-hydroxy-3-methylflavylium compound as a function of pH in the range 0−7; the inset shows the molar fraction of AH^+ as a function of pH

The absorption spectra of the solutions (taken after the decay of **A**) showed that the intensity of the characteristic absorption band of $\mathbf{AH}^+$ at ca. 430 nm decreased with increasing pH, as expected on the basis of Equation (4). The behaviour of the absorption at 430 nm as a function of pH, shown in the inset of Figure 10, gave a value of ca. $8\cdot10^{-3}$ for K'_a. Moreover, the absorption spectra of **CB** obtained at various pH values strongly suggested that the equilibrated solutions contained neither **A** nor **Ct**. These results were confirmed by ^{1}H NMR measurements on aqueous (D_2O) solutions of the 4′-hydroxy-3-methylflavylium compound in the pH range 1−4: a gradual decrease in the intensity of the peaks corresponding to the flavylium cation was observed with increasing pH, with a parallel increase in a single set of peaks. An equilibrated solution at pH = 1.0, for example, showed the peak corresponding to proton 4 of the species $\mathbf{AH}^+$ at $\delta = 9.10$ ppm. At pH = 2.18[26] this proton had maintained the chemical shift, but its relative area had diminished to 66%. The invariance of the chemical shift confirmed the absence of quinoidal base. In the same spectrum (pH = 2.18) the peak corresponding to proton 4 for **CB** appeared at $\delta = 6.50$ ppm, with a relative area of 34%. This result indicated that **CB** consisted either of only one species or of a mixture of species in fast equilibrium on the NMR timescale. Moreover, the small chemical shift of proton 4 relative to the other protons indicated that this species was not **Ct**. In cases of other hydroxyflavylium compounds,[15] **Cc** and **B2** were found to be in fast equilibrium, but in this case **CB** may have consisted mainly of species **B2**, by analogy with the 3-methylflavylium compound.

As found for 3-methylflavylium, the absorption spectra of all the solutions of the 4′-hydroxy-3-methylflavylium shown in Figure 10 remained almost constant for a month at room temperature. At higher temperatures (40−65 °C), however, the absorption spectra showed that the *trans*-chalcone species could slowly be formed at neutral pH. The observed rate constants at various temperatures (Table 1) gave an activation energy of 68 kJ·mol^{-1} for the **Ct**-forming process. Nevertheless, some degradation also occurred with increasing temperature.

Table 1. Observed rate constants for the *cis*/*trans* (and *trans*/*cis*) isomerization reactions of 4′-hydroxy-3-methylflavylium ion against temperature

T [°C]	$k_{obs}(\mathbf{Cc} \rightarrow \mathbf{Ct})$ [min^{-1}]	$k_{obs}(\mathbf{Ct} \rightarrow \mathbf{Cc})$ [min^{-1}]
40	$2.2\cdot10^{-4}$	$4.1\cdot10^{-4}$
55	$8.5\cdot10^{-4}$	$15.0\cdot10^{-4}$
60	$11.1\cdot10^{-4}$	$22.1\cdot10^{-4}$
65	$16.0\cdot10^{-4}$	$25.0\cdot10^{-4}$

As shown later, it was possible to use a photochemical reaction, followed by an HPLC separation procedure, to obtain pure samples of *trans*-chalcone (**Ct**), thus permitting its thermal and photochemical behaviour to be studied. Its spectrum is shown in Figure 9. Solutions of the *trans*-chalcone form of the 4′-hydroxy-3-methylflavylium were almost stable when kept in the dark; a 5% decrease in the absorption band at 290 nm was observed after 10 d at pH = 1. The rate of **Ct** disappearance increased with increasing temperature and the spectral changes at pH = 1 were compatible with the conversion of **Ct** into $\mathbf{AH}^+$. The change of the observed rate constant with temperature (Table 1) gave an activation energy of 65 kJ·mol^{-1} for the $\mathbf{AH}^+$-forming process.

The thermal properties of the 4′-hydroxy-3-methylflavylium compound were also studied in basic media upon pH jumps from pH = 1 to various pH values in the range 6−13.

In the pH range 6−12, the spectral variations (Figure 11) showed the formation of a species with an absorption band at 350 nm. At pH values higher than 12, further absorbance changes (not shown in Figure 11) were observed. The spectral changes observed immediately upon the pH jump remained stable upon standing in the dark at room temperature for a long time (at least 5 d). This behaviour suggested that two new, stable species, which could be identified with the mono- and dianionic forms of *cis*-chalcone ($\mathbf{Cc}^-$ and $\mathbf{Cc}^{2-}$), were formed. In order to check the correctness of this assignment, a solution of $\mathbf{AH}^+$ at pH = 1 was submitted to a pH jump to 11. After this solution had been left to stand in the dark at this pH value for 1 d, a reverse pH jump back to pH = 1 was carried out. The flavylium cation was totally recovered, indicating, besides complete reversibility of the system, the exclusive presence of anionic *cis*-chalcone even after standing for 1 d at pH = 11. The pH dependence of the intensity of the band at 350 nm in the absorption spectrum of $\mathbf{Cc}^-$ (Figures 9 and 11) allowed us to determine a value of $4\cdot10^{-9}$ for the dissociation constant of **Cc**, K_{ac} (inset of Figure 11). Since the absorption spectrum of $\mathbf{Cc}^{2-}$ was not clearly isolated, only an estimation ($< 10^{-13}$) for the dissociation constant of $\mathbf{Cc}^-$ was possible.

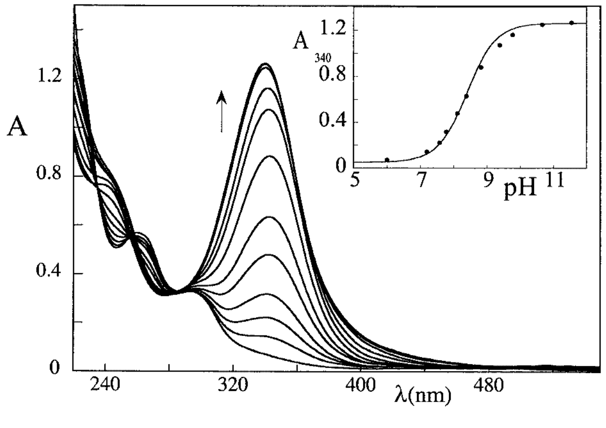

Figure 11. Absorption spectra of aqueous solutions of the 4′-hydroxy-3-methylflavylium compound as a function of pH in the range 6−12; the inset shows the molar fraction of $\mathbf{Cc}^-$ as a function of pH

High-temperature experiments (60 °C) were performed in an attempt to produce $\mathbf{Ct}^-$ from $\mathbf{Cc}^-$ through a thermal reaction. For example, a solution of 4′-hydroxy-3-methylflavylium kept in the dark at pH = 11 and 60 °C for 3 d showed a decrease in the 350-nm band of **Cc**$^-$. The solution was then reacidified to pH = 1, resulting in a 56% recovery of the initial flavylium cation concentration. Irradiation of this solution at 365 nm (see later) produced a further recovery of the initial flavylium cation concentration, reaching a total value of 80%. About 20% of the flavylium cation was missing after this experiment, showing that solutions of 4′-hydroxy-3-methylflavylium were not stable at high temperatures at basic pH values. This result prevented the determination of the activation energy of the **Ct**$^-$-forming process. Nevertheless, an activation energy exists, precluding the formation of **Ct**$^-$ at room temperature. While kinetic barriers for *cis*/*trans* isomerization of chalcones are fairly common in the acidic region, in our case such a barrier was also present at basic pH values.

Photochemical Experiments

Aqueous solutions of 4′-hydroxy-3-methylflavylium, previously equilibrated in the dark at pH = 11.0, were irradiated at 365 nm. The spectral changes found showed that **Cc**$^-$ disappeared by a clean process until a photostationary state was reached. ^{1}H NMR investigations of the irradiated solutions showed the appearance of a new set of peaks, consistent with the formation of the anionic *trans*-chalcone species. This result was confirmed by the following experiment. The solution irradiated at pH = 11 was reacidified to pH = 1, with immediate formation of 40% of the initial flavylium cation. The missing 60% was the result of the photoconversion of **Cc**$^-$ into a new species, indicated by **X**. The mixture at pH = 1 (60% new species generated from **X** and 40% **AH**$^+$) was again irradiated at 365 nm, with complete recovery of the initial concentration of the flavylium cation. This result showed that the species indicated by **X** was **Ct**$^-$, formed by irradiation of **Cc**$^-$ at pH = 11. Upon protonation, **Ct** was then obtained. HPLC separation procedures performed on the mixture at pH = 1 (60% **Ct** and 40% **AH**$^+$) allowed us to obtain pure samples of **Ct** and to study its photochemical behaviour.

Similar results were obtained after reacidification (to pH = 1.0) of solutions irradiated at pH = 6 (**Cc** irradiation) and 13.3 (**Cc**$^{2-}$ irradiation), but the extent of the *cis*/*trans* conversion was smaller (14 and 39%, respectively).

The quantum yield of the photochemical conversion of **Cc**$^-$ into **Ct**$^-$ at pH = 11.0 by 365-nm light was 0.020. Irradiation of a solution of **Ct**$^-$ (obtained by deprotonation of **Ct** at pH = 11.0) caused the formation of **Cc**$^-$ with a quantum yield of 0.013.

Irradiation of **Ct** at 365 nm at pH = 1.0 caused the formation of **AH**$^+$ with a quantum yield of 0.15 (Figure 12).

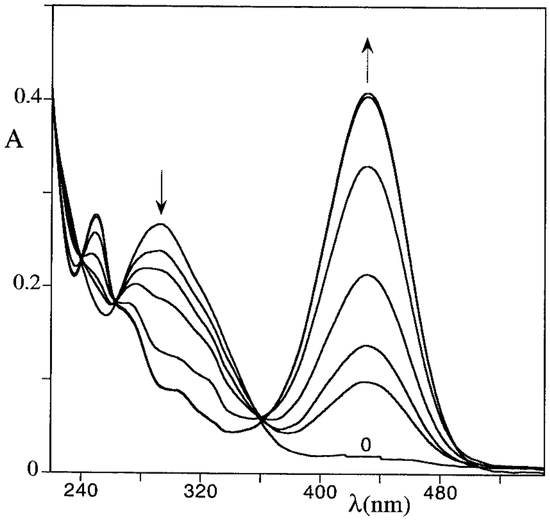

Figure 12. Spectral changes upon continuous irradiation (365 nm) of pure samples of the 4′-hydroxy-3-methylflavylium **Ct** form at pH = 1 (for details see text)

In conclusion, the 4′-hydroxy-3-methylflavylium compound exhibited a quite intricate network of chemical processes in response to pH and light stimulation, as has also been observed for other compounds of the flavylium family. This compound, however, presented two quite peculiar features: (i) the *trans*-chalcone form was only accessible through reacidification of the *trans*-chalcone anion, obtained by continuous irradiation of the *cis*-chalcone anion, and (ii) spectral overlap (Figure 9) prevented complete conversion of **Cc**$^-$ into **Ct**$^-$, a stationary state being reached. These features can be fully appreciated by reference to Figure 13, which illustrates a cyclic process, related to information processing, based on this system. Starting from **Cc**$^-$ at pH = 11, light excitation (“writing” process) causes conversion into **Ct**$^-$ (up to a maximum of 60%, corresponding to the photostationary state for λ_{exc} = 365 nm). The **Ct**$^-$ form is completely stable in the dark, but cannot be “read” without being “erased”. This drawback, however, can be avoided by a pH jump from 11 to 1 (“locking”), which causes complete conversion of **Ct**$^-$ to **Ct**. It must be taken into account that bringing the system to pH = 1 also causes the conversion of the residual **Cc**$^-$ form (which cannot be less than 40% of the original concentration, see above) into the coloured **AH**$^+$ species, the amount of which can readily be measured. A light input (365 nm) can then be used to convert **Ct** into the **AH**$^+$ form (“develop”). By “reading” the amount of **AH**$^+$ again, it is possible to derive the amount of **AH**$^+$ originating from **Cc**$^-$ via **Ct**$^-$ and **Ct** by difference. “Erasure” of the written information and complete recovery of the initial amount of **Cc**$^-$ can then be achieved by changing the pH to 11.

4′,7-Dihydroxy-3-methylflavylium

Equilibria in the Dark

In acidic and neutral aqueous solutions the 4′,7-dihydroxy-3-methylflavylium compound underwent the structural transformations giving rise to the five species seen for the 4′-hydroxy-3-methylflavylium compound (**AH**$^+$, **A**, **B2**, **Cc**, and **Ct**). The flavylium cation (**AH**$^+$) was the predominant species in very acidic media (pH = 1). As expected, the characteristic absorption band of the flavylium cation (**AH**$^+$) at ca. 450 nm decreased with increasing pH. Fig-

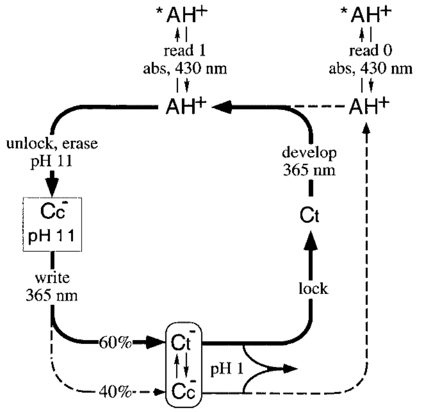

Figure 13. Example of *write-lock-read-unlock-erase* cycles based on the 4′-hydroxy-3-methylflavylium compound

ure 14 shows the absorption spectra vs. pH of thermally equilibrated solutions of the 4′,7-dihydroxy-3-methylflavylium cation (30 min after the pH jump; see below). The behaviour of the absorption at 430 nm as a function of pH, shown in the inset of Figure 14, gives a value of ca. $2.5 \cdot 10^{-4}$ for K'_a. The equilibrated solution in the pH range 5−7 (i.e., when the flavylium cation was completely converted into **CB**) contained approximately 20% **A**.

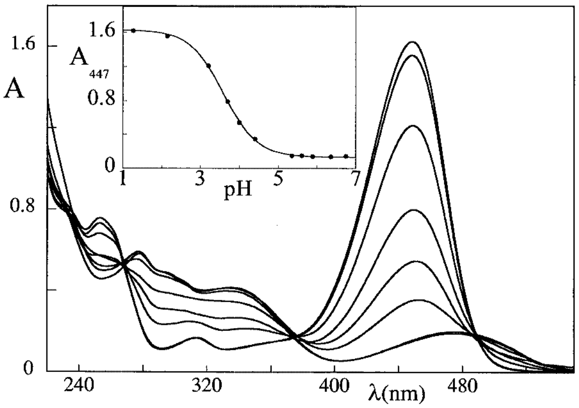

Figure 14. Absorption spectra of aqueous solutions of the 4′,7-dihydroxy-3-methylflavylium compound as a function of pH in the range 1−7; the inset shows the molar fraction of $\mathbf{AH}^+$ as a function of pH

Since the proton transfer was once again faster than the hydration reaction, pH jumps from 1 to higher pH values gave rise to the initial formation of a quinoidal base, which then disappeared to reach the final thermal equilibrium, with ca. 20% of base **A**, in ca. 30 min. A detailed investigation of the disappearance process of **A**, performed by means of several pH jumps from 1 to pH values in the range 1−7, showed that the quinoidal base disappeared through two different kinetic processes. The first one, which was pH-dependent, corresponded to the hydration reaction, and gave an observed rate constant of $3 \cdot 10^{-2}$ s^{-1} at pH = 5. Extrapolation of the absorbance of **A** to $t = 0$, yielded a value of 0.20 for the ratio between the final and initial concentrations of **A**. This ratio is the molar fraction of **A** (at $\beta = 1$) that, when used in Equation (14), yields a value of $5 \cdot 10^{-5}$ for K_a. The second process, found to be pH-independent, could be identified as the *cis* → *trans* isomerization reaction and showed an observed first-order rate constant with a value of $3.2 \cdot 10^{-3}$ s^{-1}.

Attempts were also made to study the behaviour of 4′,7-dihydroxy-3-methylflavylium in basic media, in which behaviour different to that of 4′-hydroxy-3-methylflavylium was expected, because of the presence of one more hydroxy group. With pH jumps from pH = 1 to various pH values in the range 7−11, the absorption spectra showed the growth of an absorption band at 525 nm with increasing pH. The 525-nm absorption band then disappeared, with parallel appearance of new absorption bands at lower wavelengths (Figure 15). At pH = 11, the disappearance of the absorption band at 525 nm (inset of Figure 15), followed first-order kinetics, with an observed rate constant of $4.2 \cdot 10^{-3}$ s^{-1}. The products formed were not stable; irreversible side reactions took place, resulting in the degradation of 75% of the initial 4′,7-dihydroxy-3-methylflavylium compound over 3 d.

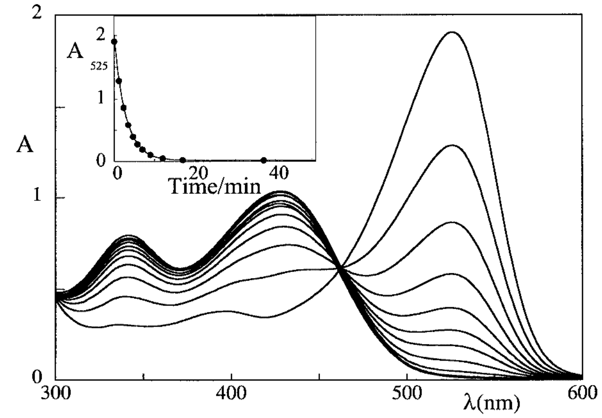

Figure 15. Spectral variations observed after pH jumps from 1.0 to 11.0 for aqueous solutions of the 4′,7-dihydroxy-3-methylflavylium compound as a function of time; the inset shows that the change in absorbance at 525 nm follows first order kinetics

For shorter time periods, however, the system was almost reversible. Reverse pH jumps from basic media to pH = 1 carried out on solutions that had previously been equilibrated in the dark for 30 min caused a complete recovery of the initial flavylium cation concentration in 1 h.

Photochemical Experiments

Continuous irradiation (at 365 nm) of a thermally equilibrated solution at pH = 5 showed an increase in the intensity of the band (370 nm) of the quinoidal base up to 50% of its value. The thermodynamic equilibrium was then reached upon standing for 30 min in the dark. At pH = 11, irradiation at 365 nm of a previously equilibrated solution did not cause any reaction.

White-light pulsed irradiation of a solution equilibrated at pH = 4.64 showed a decrease in absorbance at 340 nm (absorption band of **Ct**) over a very short period, not detectable by our apparatus. At 475 nm (absorption band of **A**) an increase in absorbance was observed over 60 s, with first-order kinetics and an observed rate of 0.055 s^{-1}. During this time no recovery of **Ct** absorption was detected. Over a longer timescale (15 min), however, the *trans*-chalcone absorbance at 340 nm was recovered in a first-order process with a rate constant of 0.0033 s^{-1}. This value was in excellent agreement with that (0.0032 s^{-1}) determined for the slow decay component of the quinoidal base in pH jump experiments.

This behaviour showed that no **Ct** was formed immediately after the light pulse, contrary to what had been observed for the similar 4′,7-dihydroxyflavylium compound. This result is further clear evidence of the presence of an energy barrier in the isomerization process, as already suggested by the results relating to the other two methylflavylium compounds.

Conclusions

The presence of a methyl group in the 3-position causes a decrease of the pK'_a value of the flavylium ion because of steric effects. In addition, the presence of such a substituent prevents the thermal and photochemical formation of *trans*-chalcone in acidic and neutral media, because the **CB** species [Equation (13)] mainly consists of the hemiacetal and contains only 2% of *cis*-chalcone. In basic solutions, however, the anionic form of the *trans*-chalcone can be obtained as a major product (90%), resulting in the *trans*-chalcone on reacidification.

The introduction of a 4′-hydroxy substituent in 3-methylflavylium increases the difficulty of the *cis*/*trans* isomerization, as found for previously studied flavylium compounds.[13−15] In this case, *trans*-chalcone could not be obtained at acidic or neutral pH values, either thermally (at room temperature) or photochemically. Moreover, in basic solution the *trans*-chalcone anion was only accessible photochemically, but only in a molar fraction of 0.6, due to the achievement of a photostationary state.

In spite of the reduced efficiency of these systems with regard to light stimulation, some interesting features concerning *write*-*lock*-*read*-*unlock*-*erase* cycles have been found.

The introduction of a 7-hydroxy substituent into the 4′-hydroxy-3-methylflavylium compound makes the *cis*/*trans* isomerization reaction easier, as already found for other 7-hydroxy-substituted analogues.[12a,13] The isomerization process, however, remains slower than the hydration reaction, contrary to what happens in the case of the 4′,7-dihydroxyflavylium ion.[12a] This again shows that methyl substituents in the 3-position hinder the *cis*/*trans* isomerization reaction.

Experimental Section

The tetrafluoroborate salt of 3-methylflavylium and the chloride salts of 4′-hydroxy-3-methylflavylium and 4′,7-dihydroxy-3-methylflavylium were prepared by published procedures.[19] All other chemicals used were of analytical grade. The experiments were carried out in water at 25 °C. The pH of the solution was adjusted by addition of HCl ($pH < 2$) and of small amounts of NaOH or buffer for higher pH values. All the pH jump experiments were carried out with buffer solutions to assure that the pH was maintained in the successive reactions. Measurements of pH were performed with a Metrohm 713 pH meter. UV/Vis absorption spectra were recorded with a Perkin−Elmer lambda 6 spectrophotometer. Light excitation was carried out with a medium-pressure mercury arc lamp, and the excitation bands were isolated with interference filters (Oriel). The incident light intensity was measured by ferrioxalate actinometry.[20] Flash photolysis experiments were performed as previously described.[12a] The ^{1}H NMR experiments[21] were carried out with a Bruker ARX 400 spectrometer operating at 400.13 MHz. The HPLC equipment consisted of an L-6200A Merck-Hitachi intelligent pump, with a Rheodyne 7125 injection valve, fixed volume of 20 μL. The effluent was monitored by means of a Merck-Hitachi L-4500 diode array UV/Vis detector. Quantitative measurements were made with a Merck-Hitachi D-6000 interface connected to a computing integrator. The temperature was kept constant with an L-5025 Merck-Hitachi column thermostat. The dark red crystals of the 3-methylflavylium tetrafluoroborate salt ($C_{16}H_{13}OBF_4$) used for the structure determination were obtained from acetic acid/acetic anhydride/tetrafluoroboric acid/water. The chosen crystal (crystal size 0.48 × 0.44 × 0.20 mm) was mounted on a glass fibre by using an epoxy resin. Data were collected with an Enraf Nonius TurboCAD4. Cell parameters were refined from the setting angles of 25 reflections (θ range 19.04−42.66°). Reflections were measured from nonprofiled $\omega/2\theta$ scans. Of the 3131 reflections measured, all of which were corrected for Lorentz and polarisation effects and for absorption (maximum and minimum transmission coefficients of 0.8078 and 0.7193), 2909 [$R(\text{int}) = 0.0379$] independent reflections exceeded the significance level $F/\sigma(F) > 2.0$. The structure was solved by direct methods and refined by full-matrix, least-squares methods on F^2. Refinement converged at a final $R1 = 0.0603$, $wR2 = 0.1725$ [$I > 2\sigma(I)$]. Minimum and maximum final electron densities −0.380 and 0.541e·Å^{-3}. The computing structure solutions were taken from SIR97,[22a] the computing structure refinement from SHELXL-97,[22b] the computing molecular graphics from ORTEP-3[23a] for Windows, and the computing publication material from WinGX publication routines.[23b] CCDC-188959 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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