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2′-Hydroxyflavylium: introducing flavanones into the flavylium network of chemical reactions

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Abstract

Chalcones possessing a hydroxyl group in position 2 cyclize to form flavylium salts in acidic media, this reaction being reversible under neutral–basic conditions. On the other hand, chalcones possessing a hydroxyl group in position 2′ cyclize to form flavanones in basic media. By synthesizing 2′-hydroxyflavylium tetrafluoroborate, it was possible to obtain *trans*-2,2′-dihydroxychalcone that in solution can evolve to 2′-hydroxyflavanone or back to 2′-hydroxyflavylium depending on the pH. The several equilibria established in aqueous solution were fully characterized. The importance of including flavanones into the flavylium network of chemical reactions is briefly exploited.

1. Introduction

Chemical systems possessing two or more chemical species that can be interconverted by external stimuli such as light, pH variations, redox potential (multistate systems) are a matter of great interest due to the possibility of conceiving switches,[1] optical memories[2] or even models that mimick the activity of a neuron.[3] In particular, the possibility of connecting two or more switches in the same system is a step further to envisage logic gates at the molecular level.

The network of reversible chemical reactions originated by flavylium ions in aqueous solutions is a paradigmatic example of such kind of systems, Scheme 1. This general network is also followed by anthocyanins, the molecules responsible for the majority of red and blue colours of flowers and fruits.

As shown in Scheme 1, the flavylium cation, $\mathbf{AH}^+$, is the dominant species at low pH values. The quinoidal base, **A**, results from deprotonation of $\mathbf{AH}^+$; the hemiketal species, **B**, is obtained by hydration in the 2 position of the flavylium cation; *cis*-2-hydroxychalcone, **Cc**, is an open form tautomer of the hemiketal **B**, and *trans*-2-hydroxychalcone, **Ct**, results from the cis–trans isomerization of **Cc**. In basic media, unprotonated hemiketals and chalcones can be formed.

It is known that flavanones make part of the flavanoid biosynthesis pathways, being formed upon intramolecular cyclization of chalcones through an enzymatic reaction involving chalcone isomerase.[4] On the other hand, they are biosynthetic precursors of anthocyanins. Flavanones can also be synthesized by non-enzymatic mechanisms from the adequate chalcones.[5] In general, these syntheses start from chalcones lacking hydroxyl groups at position 2, thus preventing the intramolecular formation of hemiketals and by consequence of flavylium cations.[6] On contrary, by using chalcones possessing hydroxyl groups in both positions 2 and 2′, they can either cyclize to flavylium salts in acidic media or to flavanones in moderately basic media. Herein, the extension of the flavylium network of chemical reactions to the formation of flavanone species is reported.

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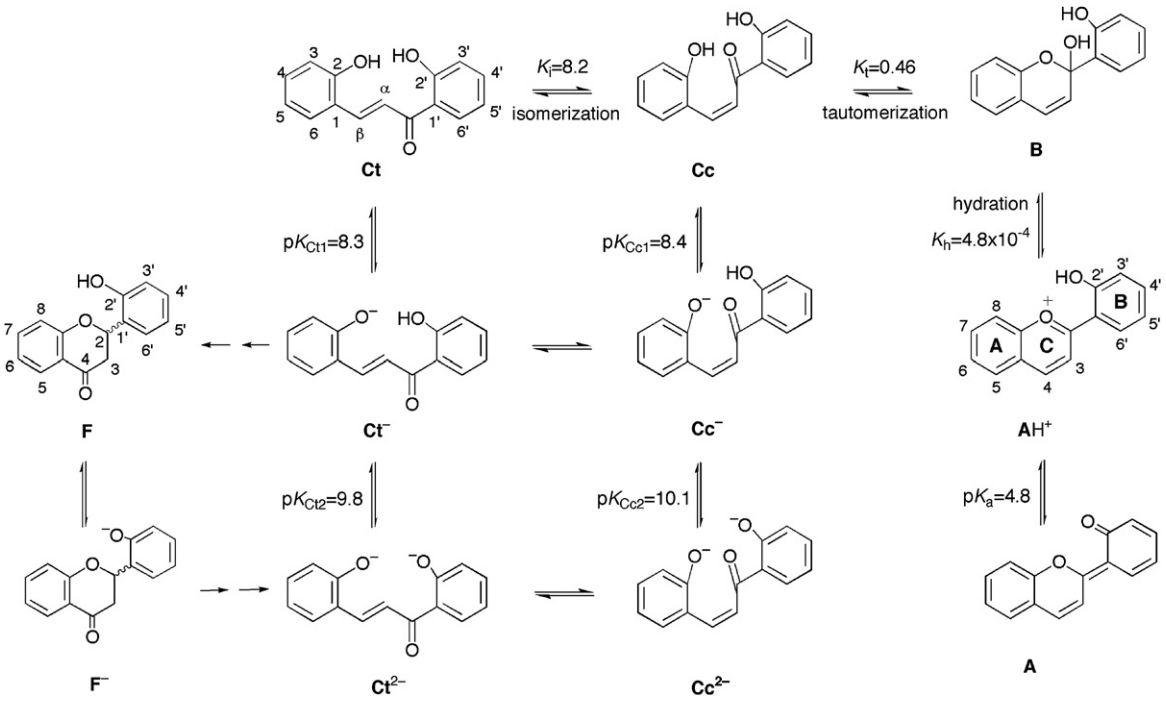


Scheme 1. Species originated by 2′-hydroxyflavylium tetrafluoroborate in aqueous solution.

2. Results and discussion

The synthesis of 2′-hydroxyflavylium tetrafluoroborate was accomplished by condensation of salicylaldehyde with 2′-hydroxyacetophenone, following known methods.[7] Full assignment of the ^{1}H NMR spectrum was made on the basis of the COSY spectrum, on the known fact that 4-H usually appears as the lowest field signal in the NMR spectra of flavylium salts,[8] and that either 3′-H or 5′-H is expected to appear at the highest field. These assignments were confirmed through a NOESY spectrum where a cross peak between 3-H and 6′-H was observed. HMQC and HMBC experiments allowed full assignment of the ^{13}C NMR spectrum (see Supplementary data for a list of HMBC connectivities).

trans-2,2′-Dihydroxychalcone was obtained from the flavylium salt by dissolution in 0.1 M NaOH, waiting for full conversion to $\mathbf{Ct}^{2-}$ and neutralization. The assignments of the NMR signals were based on COSY, HMQC and HMBC spectra. In particular, the connectivities of 6′-H with 2′-C and with the carbonyl carbon in the HMBC spectrum unequivocally identify 6′-H, and thereafter it is straightforward to assign the other ring B protons using the COSY spectrum. Similarly, the connectivity between 6-H and β-C in the HMBC spectrum leads to full assignment of ring A protons. An interesting feature in the ^{1}H NMR spectrum of this chalcone is the chemical shifts of the two hydroxyl protons, appearing at δ 12.9 and 5.9 ppm. The low field of the former and the large difference between both signals indicate that the hydroxyl group in position 2′ is H-bonded to the carbonyl group. This suggests that the hydroxyl group in position 2 is more acidic, as presented in Scheme 1.

2′-Hydroxyflavanone was obtained from the flavylium ion, by equilibrating a water–ethanol (1:1) solution at pH ca. 9 followed by isolation of the compound as a precipitate. Full assignment of the NMR spectra was possible on the basis of COSY, HMQC and HMBC spectra. In particular, 6′-H was identified in the COSY spectra through a ^{4}J constant with 2-H, and 5-H is recognized in the HMBC spectra by its connectivity with 4-C of the carbonyl group.

When dissolved in aqueous solution, 2′-hydroxyflavylium tetrafluoroborate gives rise to the network of chemical reactions shown in Scheme 1. Most of the network of 2′-hydroxyflavylium is similar to those of other flavylium compounds.[1a] The flavylium ion, $\mathbf{AH}^{+}$, can either loose a proton to the solvent to form the quinoidal base **A** or be hydrated to form hemiketal **B**; the hemiketal can tautomerize to *cis*-chalcone, **Cc** and the latter can isomerize to the *trans*-chalcone, **Ct**. At basic pH values, all species can deprotonate to form anionic species like the ionized chalcones in Scheme 1. The novelty of this flavylium cation is conferred by the position of a hydroxyl in position 2′, which in a narrow pH range gives rise to the formation of 2′-hydroxyflavanone, **F**.

In acidic media, the network of chemical reactions reported in Scheme 1 of the manuscript can be accounted for by the following set of equations:

$$\mathbf{AH}^{+} + \mathrm{H_2O} \rightleftharpoons \mathbf{A} + \mathrm{H_3O}^{+} \quad K_a \tag{1}$$

$$\mathbf{AH}^{+} + 2\mathrm{H_2O} \rightleftharpoons \mathbf{B} + \mathrm{H_3O}^{+} \quad K_h \tag{2}$$

$$\mathbf{B} \rightleftharpoons \mathbf{Cc} \quad K_t \tag{3}$$

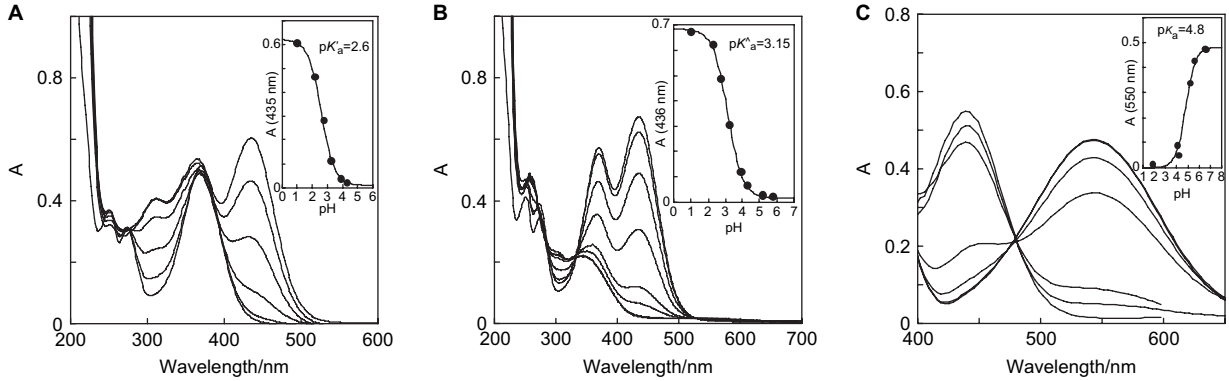


Figure 1. (A) Equilibrated aqueous solutions of compound 2′-hydroxyflavylium tetrafluoroborate (after 1 day) as a function of pH; (B) the same after 1 min; (C) stopped flow data taken after 20 ms.

$$\mathbf{Cc} \rightleftharpoons \mathbf{Ct} \quad K_i \tag{4}$$

Eqs. 1–4 can be substituted by a single acid–base equilibrium,[2a] as shown in Eq. 5:

$$\mathbf{AH}^+ + 2H_2O \rightleftharpoons \mathbf{CB} + H_3O^+ \quad K_a' \tag{5}$$

where

$$[\mathbf{CB}] = [\mathbf{A}] + [\mathbf{B}] + [\mathbf{Cc}] + [\mathbf{Ct}] \tag{6}$$

and

$$K_a' = K_a + K_h + K_hK_t + K_hK_tK_i \tag{7}$$

The pH dependent spectral variations of equilibrated solutions of compound 2′-hydroxyflavylium are reported in Figure 1A. The flavylium cation, $\mathbf{AH}^+$, is the dominant species at low pH values, while at higher pH values, **Ct** dominates the equilibrium composition. From the data reported in Figure 1A, the global constant $K_a'=K_a+K_h+K_hK_t+K_hK_tK_i=10^{-2.6}$ is obtained.

In the cases, as in the present compound, where an activation barrier exists for the interconversion of *cis*- to *trans*-chalcone, a pseudo-equilibrium can also be defined:

$$\mathbf{AH}^+ + 2H_2O \rightleftharpoons (\mathbf{A} + \mathbf{Cc} + \mathbf{B}) + H_3O^+ \quad K_a^{\wedge} \tag{8}$$

As in Eq. 5, the pseudo-equilibrium can be accounted for by an acid–base reaction, Eq. 8, where

$$K_a^{\wedge} = K_a + K_h + K_hK_t \tag{9}$$

There is experimental evidence (see below) that the pseudo-equilibrium of the present compound is constituted by **Cc** and **B** (major forms) together with a very low percentage of **A**. Figure 1B represents the pseudo-equilibrium, from which an observed constant, $K_a^{\wedge}=K_a+K_h+K_hK_t=10^{-3.15}$ can be calculated.

When a pH jump from the stock solutions at pH=1.0 to higher pH values is carried out, the first reaction to occur is the formation of the base, **A**, followed by the appearance of the pseudo-equilibrium and finally the 'real' equilibrium. The disappearance of the base, **A**, was monitored by stopped flow, and its rate constant is slightly pH dependent (1.5 s^{-1} at pH=3; 1.0 s^{-1} at pH=9). In Figure 1C, the absorption spectra as a function of pH taken after 20 ms are presented, allowing to calculate $K_a=10^{-4.8}$. The pK_as of the chalcones could also be determined upon pH jumps from acidic solutions of $\mathbf{AH}^+$ to the neutral–basic region (*cis*-chalcones) and from basic solutions of $\mathbf{Ct}^{2-}$ to the neutral–basic region (*trans*-chalcones), see Supplementary data.

Taking profit from the fact that the cis–trans isomerization is very slow (several hours), making the pseudo-equilibrium long lived, the following experiment was carried out: after a pH jump from 1 to 6.3 to form the pseudo-equilibrium, the solution was introduced in the stopped flow and a 'reverse' pH jump back to acidic values was monitored, see Figure 2.

Figure 2 is compatible with two consecutive kinetic processes. The first one is very fast and occurs within the mixing time of the stopped flow experiments. At pH=6.3, the system

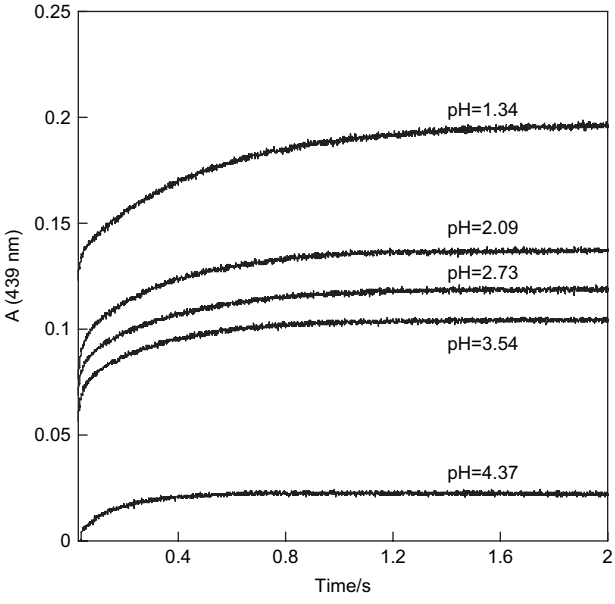


Figure 2. Stopped flow traces corresponding to reverse pH jumps carried out from pH=6.3 to the indicated pH (upon previous pH jump from pH=1 to pH=6.3).

is mainly constituted by **B** and **Cc** in the pseudo-equilibrium, and by consequence the first kinetic process can only be attributed to the (very fast) formation of $\mathbf{AH}^+$ at the expenses of **B**, through the hydration–dehydration mechanism. The slowest second kinetic process (responsible for the traces in Fig. 2) exhibits a rate constant of $3.0\pm0.2\ s^{-1}$ and is practically pH independent. It can be attributed to the formation of $\mathbf{AH}^+$ from **Cc**, involving a tautomerization process through **B**. Moreover, the amplitudes of the two processes should be proportional to the concentrations of **Cc** and **B** at the pseudo-equilibrium, allowing to calculate K_t=0.46. Using Eqs. 7 and 9 and the values of K_a and K_t, $K_h=4.8\times10^{-4}$ and K_i=8.2 can be obtained.

2.1. Flavanone equilibrium

When an aqueous solution of flavylium cation at pH=1.0 is subjected to a pH jump to 8.25, Figure 3, the observed spectral changes are compatible with two consecutive first order kinetic processes. The first step is the trivial formation of ionized *trans*-chalcones (at this pH, $\mathbf{Ct}^-$ is in equilibrium with $\mathbf{Ct}^{2-}$; see Supplementary data) with $k_{obs1}=0.1\ min^{-1}$; the second one corresponds to the formation of flavanone **F** in equilibrium with $\mathbf{Ct}^-/\mathbf{Ct}^{2-}$, occurring with $k_{obs2}=0.011\ min^{-1}$.

Pure 2′-hydroxyflavanone was isolated by precipitation from saturated solutions of this mixture and characterized by NMR, elemental analysis and MS, allowing to confirm the spectral evolution of *trans*-chalconate towards flavanone formation. The species $\mathbf{Ct}^-$ is clearly the immediate precursor for the formation of flavanone, which is neither formed at lower pH values (from **Ct**) nor at higher pH values (from $\mathbf{Ct}^{2-}$). On the other hand, flavanone is stable at low pH values and only at pH>10 leads to $\mathbf{Ct}^{2-}$, suggesting the involvement of mono-ionized flavanone, $\mathbf{F}^-$, in this reaction.

The *trans*-chalcone–flavanone equilibrium has received large attention.[9] It has been established that (i) both species are stable in the acidic and neutral region; (ii) they start to interconvert under basic conditions; (iii) as the pH increases, the intermediate of the cyclization and ring-opening reactions shifts progressively from an enolate to a carbanion intermediate mechanism.[9] Our observations clearly support the need of ionized species for the interconversion to occur. The chalcone starts to form flavanone only when $\mathbf{Ct}^-$ is present (pK_{Ct1}=8.3) while conversion of flavanone to chalcone starts at pH≈10, a value expected for the $\mathbf{F}/\mathbf{F}^-$ p*K*a.[9d] This peculiar situation, where flavanone is formed from $\mathbf{Ct}^-$ at lower pH values (from ca. pH>7) and leads to $\mathbf{Ct}^{2-}$ at higher pH values, is responsible for the formation of a pH dependent equilibrium involving **F** and $\mathbf{Ct}^{2-}$ whose inflection point occurs at pH=11.7, see Figure 4.

The thermodynamic data reported above allows to calculate the mole fraction distribution of the different species as a function of pH. A thermodynamic equilibrium can be reached starting either from $\mathbf{AH}^+$ at pH=1 or from $\mathbf{Ct}^{2-}$ at pH=12. In the first case, the quinoidal base **A** is the first species to be formed, Figure 5A, but rapidly leads to the pseudo-equilibrium described in Figure 5B; the final equilibrium involves $\mathbf{AH}^+$ and **Ct** in the acidic region and **F** and $\mathbf{Ct}^{2-}$ in the basic region, Figure 5C. Starting from $\mathbf{Ct}^{2-}$ at pH=12, the final equilibrium is the same (Fig. 5C) but the pseudo-equilibrium is different (Fig. 5D), containing unprotonated *trans*-chalcones instead of their cis-isomers.

On the other hand, starting from flavanone at neutral pH leads to a different thermodynamic state, where only **F** and $\mathbf{Ct}^{2-}$ are present at the final equilibrium (Fig. 5E), since the flavanone is stable in the acidic region.

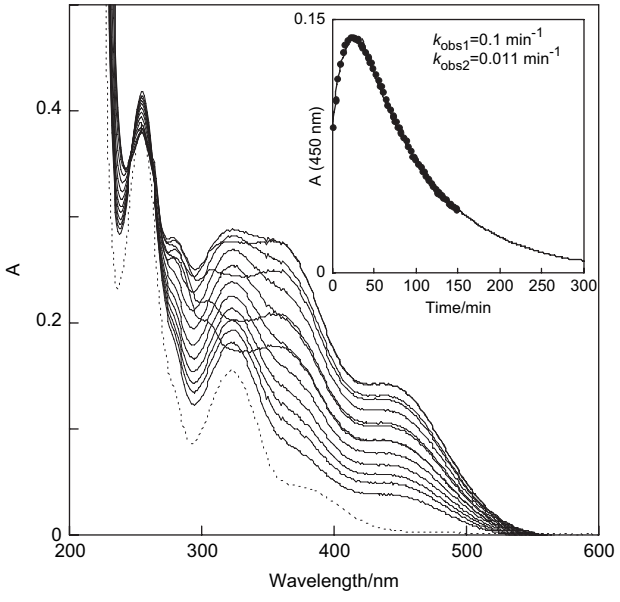


Figure 3. Spectral modifications upon a pH jump of 4.0×10^{-5} M 2′-hydroxyflavylium, from 1 to 8.25, showing formation of ionized *trans*-chalcones ($\mathbf{Ct}^-/\mathbf{Ct}^{2-}$) followed by the appearance of flavanone (full lines); spectrum of pure flavanone (traced line).

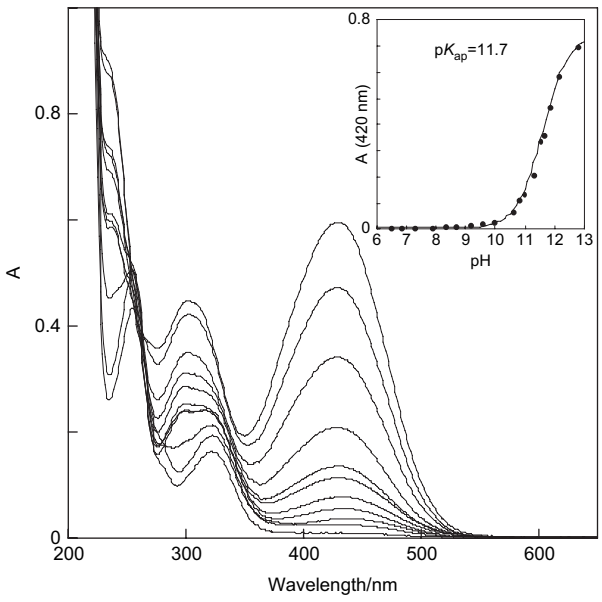


Figure 4. Titration of 4.4×10^{-5} M flavanone towards basic pH values, with formation of $\mathbf{Ct}^{2-}$.

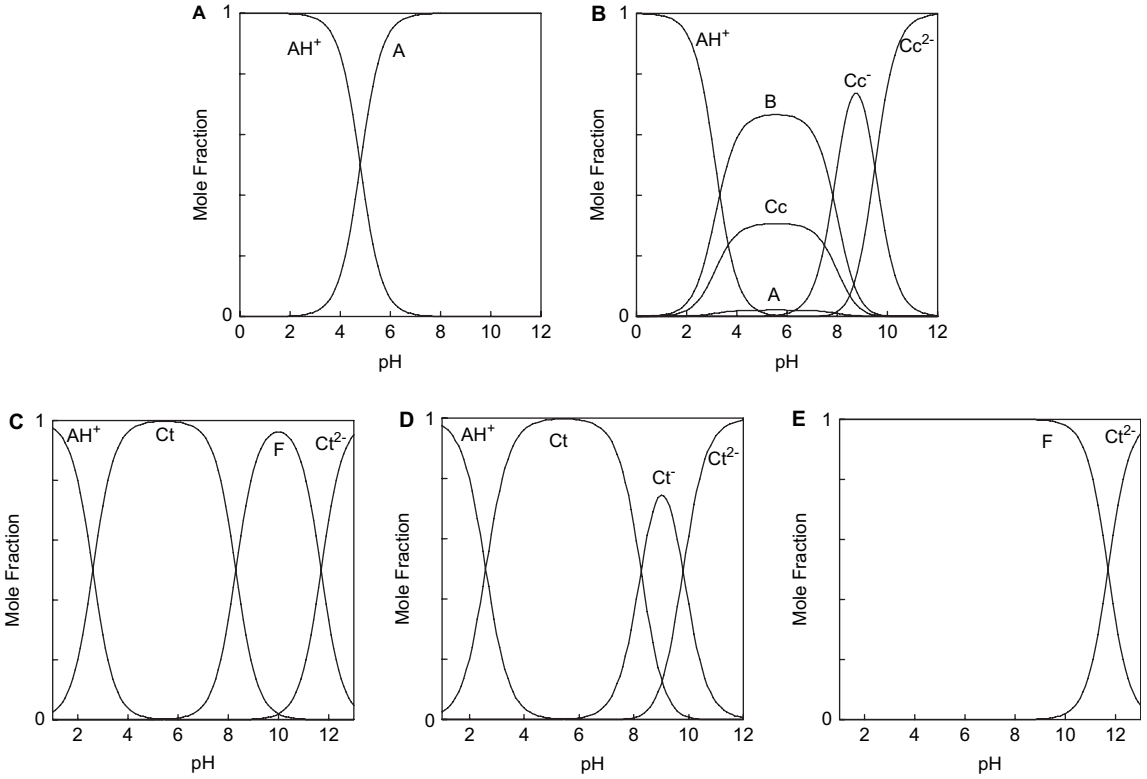


Figure 5. Mole fraction distribution of the species upon pH jumps from $\mathbf{AH}^{+}$ at pH=1 to higher pH values (A—after 25 ms, B—pseudo-equilibrium after 1 min, C—final equilibrium after 24 h), from $\mathbf{Ct}^{2-}$ at pH=12 to lower pH values (D—pseudo-equilibrium after 1 min) and from **F** at neutral pH values (E—final equilibrium after ca. 1 min).

Figure 6 illustrates the integration of the flavanone into the flavylium network of chemical reactions. Starting from $\mathbf{AH}^{+}$ at pH=1 and carrying out a pH jump to 12, the ionized *cis*-chalcone in equilibrium with $\mathbf{B/B}^{-}$ is immediately formed. This reaction was followed by stopped flow and occurs with a rate constant of 1.8 s^{-1}. The thermodynamic equilibrium, with only $\mathbf{Ct}^{2-}$, is reached upon ca. 3 h at pH=12, Figure 6A. As can be seen in Figure 6B, a second pH jump to 8.5 gives rise to the formation of flavanone. The flavanone at acidic pH values is stable, but at pH=12 leads to $\mathbf{Ct}^{2-}$ in the time scale of seconds. The recovery of the initial $\mathbf{Ct}^{2-}$ species is practically complete, see Figure 6C.

The chalcone–flavanone equilibrium in the present system does not exhibit photochemistry. However, some flavanones can be photochemically converted into chalcones in organic solvents.[10] This possibility is being explored in the framework of multistate flavylium networks.

3. Conclusions

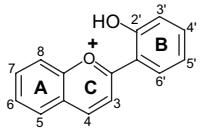
The introduction of two hydroxyl groups in positions 2 and 2′ of *trans*-chalcone defines a multistate system that responds to pH changes by evolving to a *cis*-chalcone through an isomerization or to a flavanone through a cyclization process. The multiequilibria were fully characterized and show that flavanones may be used to enlarge the number of states in flavylium based multistate–multifunctional systems.

4. Experimental

4.1. Synthesis

All reagents and solvents used were of analytical grade. NMR spectra were run on a Bruker AMX 400 instrument operating at 400.13 MHz (^{1}H) and 100.00 MHz (^{13}C). COSY, HMQC, HMBC and eventually NOESY spectra were run on each sample to allow full assignment of the NMR peaks. Field Desorption MS spectra were run on a Micromass GCT machine and elemental analysis was obtained on a Thermofinnigan Flash EA 1112 Series instrument.

4.1.1. 2′-Hydroxyflavylium tetrafluoroborate



2′-Hydroxyflavylium tetrafluoroborate was prepared according to a procedure adapted from Katritzky et al.[7] Salicylaldehyde (1.1 ml, 10 mmol) and 2′-hydroxyacetophenone (1.2 ml, 10 mmol) were dissolved in 10 ml of acetic acid and 2 ml of HBF_4. Acetic anhydride (8.5 ml) was then added dropwise and the temperature of the reaction mixture rose until 75 °C. The reaction mixture became red and was stirred overnight. A yellow precipitate had formed that was filtered off, washed with water and then carefully with diethyl ether and dried

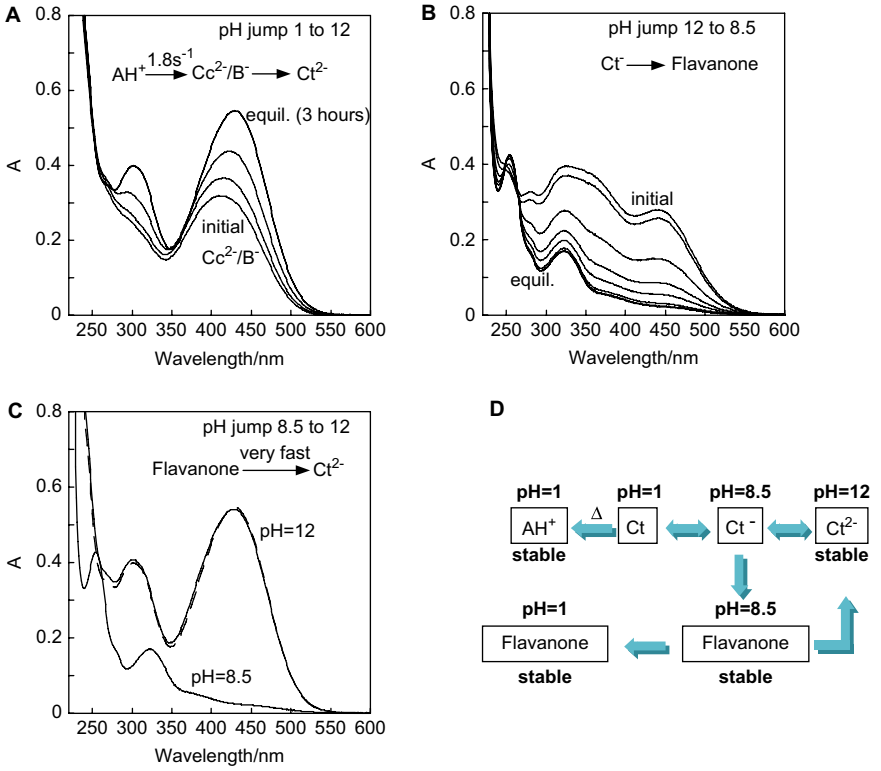
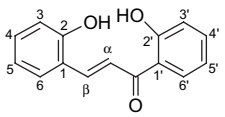


Figure 6. (A) Spectral modifications upon a pH jump of 4.0×10^{-5} M 2′-hydroxyflavylium, from 1 to 12; (B) the same upon a pH jump form equilibrated solutions at pH=12 to 8.5. The flavanone is formed during this step; (C) the spectrum obtained from the flavanone upon a pH jump to 12 (traced line) is the same of the initial species (full line); (D) diagram showing how the flavanone can be integrated in the network of the flavylium.

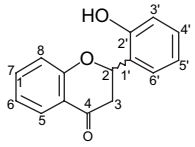
(0.48 g, 16.3%). ^{1}H NMR[†] (400.13 MHz, $CDCl_3$) δ_H 9.31 (1H, d, *J* 9.21, 4-H), 9.13 (1H, d, *J* 9.2, 3-H), 8.27 (1H, m, 6′-H), 8.23 (1H, m, 5-H), 8.19 (1H, m, 7-H), 8.16 (1H, m, 8-H), 7.94 (1H, t, *J* 7.4, 6-H), 7.71 (1H, td, *J* 7.6, ^4J 1.5, 4′-H), 7.33 (1H, d, *J* 8.5, 3′-H), 7.16 (1H, t, *J* 7.6, 5′-H). ^{13}C NMR (100.00 MHz, $CDCl_3$) δ_C 175.0 (2-C), 163.7 (2′-C), 155.4 (8a-C), 141.4 (4′-C), 138.8 (7-C), 130.4 (6′-C), 130.2 (5-C and 6-C), 123.7 (4a-C), 122.3 (4′-C), 122.1 (5′-C), 119.9 (3′-C), 118.9 (8-C), 114.6 (1′-C), 113.2 (3-C). FD-MS *m/z*: 222.06 $[M-H]^+$ (100%). EA Found: C, 58.52; H, 3.79. Calcd for $C_{15}H_{11}BF_4O_2$: C, 58.11; H, 3.58.

4.1.2. *trans*-2,2′-Dihydroxychalcone



2′-Hydroxyflavylium tetrafluoroborate was dissolved in water at pH=13 and allowed to equilibrate. The solution was then carefully acidified to pH=7. After a few moments in an ice bath, *trans*-2,2′-dihydroxychalcone precipitated. The yellow solid was filtered and washed with cold water. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ_H 12.92 (1H, s, 2′-OH), 8.22 (1H, d, *J* 15.6, β-H), 8.06 (1H, d, *J* 8.0, 6′-H), 7.98 (1H, d, *J* 15.6, α-H), 7.69 (1H, d, *J* 7.6, 6-H), 7.50 (1H, t, *J* 8.0, 4-H), 7.30 (1H, t, *J* 7.7, 4′-H), 7.03 (1H, d, *J* 8.4, 3-H), 7.00 (1H, t, *J* 7.7, 5-H), 6.94 (1H, t, *J* 7.7, 5′-H), 6.87 (1H, d, *J* 8.1, 3′-H), 5.89 (1H, br s, 2-OH). ^{13}C NMR (100.00 MHz, $CDCl_3$) δ_C 194.5 (C carbonyl), 163.6 (2′-C), 155.6 (2-C), 141.5 (β-C), 136.3 (4-C), 132.1 (4′-C), 130.1 (6-C), 130.0 (1′-C), 129.9 (6′-C), 121.3 (1-C), 121.2 (5-C or α-C), 118.9 (5-C or α-C), 118.6 (5′-C), 116.8 (3-C), 116.6 (3′-C). FD-MS *m/z*: 240.08 $[M]^+$ (100%). EA Found: C, 74.46; H, 5.03. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03.

4.1.3. 2′-Hydroxyflavanone



2′-Hydroxyflavylium tetrafluoroborate was dissolved in a mixture of water and ethanol (1:1) and the pH was adjusted to 9. By the following day, ethanol had evaporated and a white solid precipitated from the aqueous solution. 2′-Hydroxyflavanone was then filtered off, carefully washed with water and dried. It was recrystallized from a solvent mixture of chloroform and hexane. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ_H 7.97 (1H, dd, *J* 7.8, ^4J 1.6, 5-H), 7.54 (1H, td, *J* 7.5, ^4J 1.6, 7-H), 7.32 (1H, d, *J* 7.6, 6′-H), 7.27 (1H, t, *J* 7.6, 4′-H), 7.10 (1H, t, *J* 7.6, 6-H), 7.09 (1H, d, *J* 7.5, 8-H), 6.99 (1H, t, *J* 7.6, 5′-H), 6.92 (1H, d, *J* 8.1, 3′-H), 6.62 (1H, br s, 2′-OH), 5.78 (1H, dd, *J* 13.0, 3.0, 2-H), 3.16 (1H, dd, *J* 13.0, ^2J 17.1, 3a-H),

[†] Values for chemical shifts are presented in parts per million and coupling constants in Hertz. Unless otherwise noted, coupling constants are vicinal.

3.01 (1H, dd, J 3.0, ^2J 17.1, 3b-H). ^{13}C NMR (100.00 MHz, $CDCl_3$) δ_C 192.9 (4-C), 161.3 (8a-C), 153.5 (2′-C), 136.4 (7-C), 129.8 (4′-C), 127.3 (5-C), 126.8 (6′-C), 124.6 (1′-C), 122.1 (6-C), 121.1 (4a-C), 120.8 (5′-C), 118.1 (8-C), 116.6 (3′-C), 77.2 (2-C), 43.0 (3-C). FD-MS m/z: 240.08 $[M]^+$ (100%). EA Found: C, 73.53; H, 5.36. Calcd for $C_{15}H_{12}O_3 \cdot 1/3H_2O$: C, 73.16; H, 5.18.

4.2. General

All experiments were carried out in aqueous solution. The pH was adjusted by addition of HCl and NaOH, or buffer, and was measured in a Meterlab pHM240 pH meter from Radiometer Copenhagen. UV/vis absorption spectra were recorded in a Shimadzu UV2501-PC spectrophotometer.

pH jumps were done by adding a certain volume of a stock solution of the flavylium salt in 0.1 M HCl to a 10 cm^3 flask containing an equivalent amount of NaOH to neutralize HCl and 1 cm^3 of 0.1 M universal buffer of Theorell and Stenhagen[11] at the desired final pH followed by the addition of water to the final volume. The spectra were measured immediately (ca. 1 min) and the solutions kept in the dark to follow the establishment of the final equilibrium. Reverse pH jumps involved a second pH jump to a lower pH value, by addition of HCl.

The titrations of flavanone and of *trans*-chalcone were made in a batch process due to their reactivity. An aqueous solution containing 0.1 M buffer was actually titrated, when the desired pH was reached, 2 ml of the solution was transferred to a dry cuvette and an aliquot of the respective stock solution added (25 μl of flavanone in EtOH, 5 μl of $\mathbf{Ct}^{2-}$ at pH=12). The spectrum was run immediately and the pH controlled at the end.

Flash photolysis experiments were carried out as described previously.[12]

The stopped flow experiments were conducted in an SFM-300 spectrophotometer, controlled by an MPS-60 unit (Bio-Logic) and the data were collected by a TIDAS diode array (J&M), with wavelength range between 300 and 1100 nm, all connected to a computer. The standard cuvette has an observation path length of 1 cm. For these experiments, the dead time of each shot was previously determined to be 5.6 ms with a 8 ml/s flow rate.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.11.007.

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