Practical and Theoretical Aspects of Flavanone–Chalcone Isomerisations

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Isomerisation equilibria of the flavanone/2'-hydroxychalcone, 7-hydroxyflavanone/2',4'-dihydroxychalcone and 4',5,7-trihydroxyflavanone (naringenin)/2',4',6',4-tetrahydroxychalcone systems have been studied in dilute and saturated solutions – the latter in equilibrium with the solid precipitated form. Seemingly inconsistent results found in the literature are rationalized in terms of proton transfer reactions and solubility effects. The results indicate that at least two different isomerisation mechanisms operate, one in the acidic and weakly alkaline region, the second in strongly alkaline solutions. Practical conclusions may ease the preparation of pure chalcones or flavanones in which contamination with the other isomer is minimized.

The cyclisation reaction of chalcones to flavanones has been studied, albeit intermittently, since the fundamental papers by Kostanecki *et al.* were published.[1,2] The general chemistry of flavanoid compounds is discussed in the treatise by Geissman.[3] Kinetic studies have been described, together with several different mechanisms of isomerisation.[4–13]

The synthesis of chalcones was conducted (from the appropriate benzaldehyde and acetophenone) in concentrated, 10–50%, solutions of strong alkali; flavanones could be obtained by boiling of the respective chalcone in mineral acids.[1,2] In fairly concentrated mineral acids only flavanones are stable; in concentrated alkali only the chalcones are formed. Inconsistent results can be found in the literature as to the pH ranges in which the two isomers coexist. Equilibria between flavanone and 2'-hydroxychalcone have been reported in neutral and weakly alkaline dilute solutions[6,9] but there were also indications that at pH 8[7] or 10[5] the equilibrium is shifted 100% toward flavanone. However, a partial opposite reaction of ring-opening at pH 9.57 has been mentioned.[9]

At pH 10.9 a mixture of flavanone and 2'-hydroxychalcone, or pure 2'-hydroxychalcone has been reported,[4] but at pH 13 (0.4% NaOH)[4] or even in 1.5% NaOH (pH 13.6)[14] pure flavanone has been prepared. Determinations of the ionisation constant of 2'-hydroxychalcone have shown its negligible cyclisation to flavanone in 0.50 mol dm^{-3} KOH (*i.e.* at pH 13.7).[10]

We extended our earlier study on naringenin[15] (in which we determined three protonation constants for naringenin, log $K_1 = 11.6$; log $K_2 = 9.5$; log $K_3 = 6.9$ and noted that only its trianion Ng^{3-} isomerises at significant rate) to investigate the proton-transfer and final isomerisation equilibria in buffered solutions over a wide range of concentrations and pH values including the primary flavanone/ 2'-hydroxychalcone and the 7-hydroxyflavanone/2',4'-dihydroxychalcone systems.

Experimental

The protonation constants K_A and their logarithms, log K_A were determined by the spectrophotometric method for dilute solutions containing 10^{-4}–10^{-5} mol dm^{-3} of flavanone or chalcone, by the potentiometric titrations of *ca.* 10^{-2} mol dm^{-3} of the respective anions of chalcones, and by the solubility method of Krebs and Speakman which permits the evaluation of protonation constants from concentration profiles of saturated aqueous solutions *vs.* pH of sparingly soluble chalcones and flavanones. All those methods are described in an excellent manual[16] and details are given in our preceding

paper.[15a] We now use the solubility method to study the isomerization equilibria. Solubility determinations of S_i and S_H-values were made simultaneously to avoid temperature effects. Precision was *ca.* $\pm 10\%$, but these values appear in calculations as a quotient under the logarithm so the errors are less significant. The precision of spectrophotometric measurements was ± 2–3%.

Reagents.—Pure 2'-hydroxychalcone (m.p. 87–89 °C), flavanone (m.p. 76–78 °C), 7-hydroxyflavanone (m.p. 188–190 °C) and 2',4'-dihydroxychalcone (m.p. 144–146 °C) were synthesised and kindly offered to us by the Laboratory of Organic Chemistry, School of Medicine, Łódź. Naringenin was obtained from Sigma without further purification. The tetrasodium salt of 2',4',6',4-tetrahydroxychalcone ($TChNa_4$) was prepared by trituration and gentle heating of naringenin with an excess of carbonate-free aqueous 50 w/w % NaOH. The neutral chalcone ($TChH_4$)–m.p. 170–176 °C was obtained from $TChNa_4$ by rapid treatment with an excess of 1 mol dm^{-3} of cold orthophosphoric acid.

UV–VIS spectra were recorded with a C. Zeiss (Jena) M-40 spectrometer. IR spectra were recorded with a C. Zeiss (Jena) M-80 spectrophotometer. ^1H NMR spectra were obtained with a Varian EM 360 (60 MHz) spectrometer in solutions of [$^2H_6$]DMSO or CD_3OD.

Isomerisation equilibria were measured both in undersaturated dilute solutions and in concentrated saturated solutions equilibriated with an excess of solid flavonoid.

The composition of solutions of flavanoids was determined by means of UV–VIS spectra as each form has a distinct characteristic spectrum, when necessary after dilution with the neat solution used. Independently, the alkaline solutions of flavonoid anions were rapidly acidified and the composition of the precipitate containing neutral flavanones and/or chalcones was determined by NMR in [$^2H_6$]DMSO and UV–VIS spectroscopy in neat methanol.

Fair agreement between the two techniques was found, and there was no indication of enol formation (by the ^1H NMR method). The composition of the residual solid substance in equilibrium with the supernatant solution was tested (after filtration through sintered-glass crucibles, Schott Jena G-4, and washing with the neat solution) by NMR and UV–VIS spectroscopy.

Saturated solutions at several pH values and in buffers containing different cations, namely K^+, Na^+ and Li^+, were in each case prepared starting with flavanone and, separately, with its chalcone using solvent of the same composition. Concordant results indicated true equilibria (Table 1).

Table 1 Composition of the phases in equilibrium at indicated pH. Concentrations in the supernatant aqueous solution saturated with the 2'-hydroxychalconate of lithium (ChOLi), sodium (ChONa), potassium (ChOK) are expressed in mol dm^{-3}.

Solution pH (H_-)	Solid phase	Saturated solution		
7.00	Fl	1.0×10^{-4} Fl (stable)		
7.00	ChOH	6×10^{-6} ChOH (metastable)		
		ChOLi	Fl	pH$_{\frac{1}{2}}^s$ (see text)
11.80	Fl	1.7×10^{-4}	1.2×10^{-4}	11.65
13.00	Fl	2.3×10^{-3}	1×10^{-4a}	11.64
13.20	ChOLi	2.6×10^{-3}	—	—
13.80	ChOLi	1.7×10^{-3}	—	—
		ChONa		
11.30	Fl	2.4×10^{-5}	1×10^{-4}	11.92
12.00	Fl	2.5×10^{-4}	1×10^{-4}	11.60
12.70	Fl	5.5×10^{-4}	1×10^{-4}	11.96
14.00	Fl	1.7×10^{-2}	1×10^{-4a}	11.77
14.30 (14.37)	ChONa	1.5×10^{-2}	—	—
15.00 (16.20)	ChONa	$ca.\ 1 \times 10^{-2}$	—	—
		ChOK		
13.40	Fl	6×10^{-3}	1×10^{-4a}	11.62
13.70 (13.75)	Fl	7×10^{-3}	1×10^{-4a}	11.86 (11.91)
13.90 (14.00)	Fl	1.9×10^{-2}	1×10^{-4a}	11.62 (11.72)
14.18 (14.33)	Fl	3×10^{-2}	1×10^{-4a}	11.7 (11.85)
14.50 (14.85)	ChOK	2.4×10^{-2}	—	—
14.70 (15.44)	ChOK	$ca.\ 2 \times 10^{-2}$	—	—

[a] Extrapolated value (S_i). In parentheses are the values obtained with the appropriate H_- function.[17b]

Distilled water and chemically-pure reagents were used throughout this work. Solutions of sodium hydroxide were prepared by dilution of saturated NaOH free of carbonates with freshly boiled water. 30% NaOD and 36% DCl (Sigma) were used for deuteriations. Lithium hydroxide was prepared from analytical grade lithium carbonate (POCh, Gliwice) by decomposition at 1100 °C. Potassium hydroxide solutions were obtained with pellets of KOH thoroughly washed with distilled water before dissolution. The concentration of each hydroxide was then determined by titration vs. phenolphthalein and Methyl Orange with standardized hydrochloric acid. The carbonate content thus determined was less than 1% in NaOH, ca. 2% in LiOH and KOH.

The pH values above 13 were calculated from the concentration of NaOH, KOH or LiOH, respectively pcH values) according to the suggestion by Rochester[17a] who recommended that the tabulations should indicate concentrations at which half protonation in strong acids occurs. We extended this to protonations in strong bases and added the H_- values[17b] where these differ markedly from pH.

Up to pH 13 measurements were made with glass electrodes and Mera-Elmat N-152 pH-meters. The glass electrode was calibrated with Merck buffers of pH = 6.86 and 12.0. Separate rapid pH determinations were made in chalcone solutions containing 150%, 200%, ..., 350%, 400% (for naringenin chalconate) of NaOH equivalents, i.e. at $a = 1.5$–4.0 (cf. ref. 15a) to minimize the effect of the isomerisation reaction.

Kinetic measurements were made by UV–VIS spectroscopy using 2×10^{-5} mol dm^{-3} and 4×10^{-5} mol dm^{-3} of pure forms of each flavanoid in buffered solutions, with 5 vol% of methanol added in the case of 2'-hydroxychalcone to increase its solubility.

Data from early stages of isomerisation were used to calculate

the approximate rate constants obviating the corrections for the backward reaction. Then after several half-lives of the (pseudo-first-order) reaction the final composition of the mixture was evaluated from absorbances measured at two different wavelengths at which the spectra of two forms present at equilibrium differ largely.

Reproducibility of Results.—Measurements of pH were reproducible within ± 0.02 pH. The final isomerisation equilibria do not depend on temperature between 15 and 35 °C, so most experiments were performed at 21 ± 1 °C (without thermostatting). The coefficient of variation for solubility and concentration determination of each form in mixtures was ca. 5% by spectrophotometry and 7–8% by NMR integration (solutions containing significant amounts of more than two forms of flavonoids were not considered).

When logarithms of the mole fractions (or concentrations) are used and pH errors are added a standard deviation s of 0.06 log unit is obtained in determinations of log K_A, pS_H, pS_i and pH$_{\frac{1}{2}}$. The error for pH$_{\frac{1}{2}}^s$ is larger $s = 0.132$ (Table 1), but the mean pH$_{\frac{1}{2}}^s = 11.76$ is remarkably near the mean pH$_{\frac{1}{2}} = 11.79$ (16 determinations) found for dilute solutions of Fl and ChO$^-$ in the pH range 10.50–12.70. A normal Gaussian distribution was found both for pH$_{\frac{1}{2}}$ and for pH$_{\frac{1}{2}}^s$ by the D-test, but with $F = 0.132^2/0.06^2 = 4.85$ a disparity appears between the two estimates indicating some additional source of random errors in the evaluation of pH$_{\frac{1}{2}}^s$. The FlOH/ChH$_2$ system is described elsewhere in greater detail.[15b]

Results

For solutions in the acidic, neutral and weakly alkaline pH range our experimental data confirmed those published results which stated that flavanones are the only stable species between pH 0 and 10, and that the chalcones, when present, are quantitatively transformed into flavanones, albeit very slowly, in acidic solutions where the rate of cyclisation is ca. 10^{-5} min^{-1} and depends little on pH. Neutral flavanones and chalcones are very sparingly soluble in aqueous solutions not containing other solvents.

In the alkaline region the cyclisation rate increases first rapidly, then quite slowly as indicated by others.[12] The C3-protons are easily deuteriated[7,26] but the C2-hydrogen remains firmly bound even during ring-opening in 30% NaOD and subsequent cyclisation with DCl in D$_2$O (see Scheme 1).
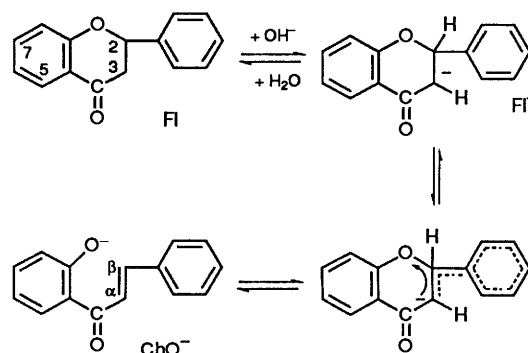
Scheme 1

For the system flavanone (Fl) and 2'-hydroxychalcone (ChOH) measurable quantities of both isomers in final equilibrium are present in solution only between pH 10.5 and 13, i.e., where the chalcone anion (ChO$^-$) prevails. Isomerisations with pseudo-first-order rates ca. 10^{-2} min^{-1} proceed between species of unequal charge, i.e. between Fl^0 and
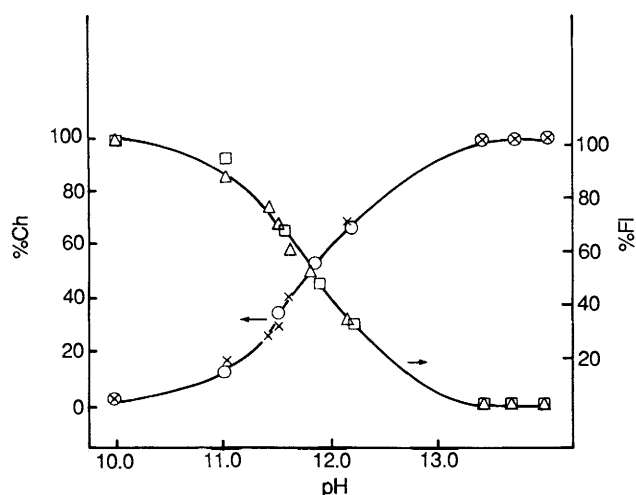
Fig. 1 Relative composition of the flavanone/2'-hydroxychalcone system in dilute undersaturated solutions near equilibrium (after at least six half-lives calculated from the rate constants at each pH) as a function of pH. Independent determinations of flavanone and 2'-hydroxychalcone were made by UV spectrophotometry for each indicated pH: (a) with flavanone (Fl) as the substrate; ×, relative % content of chalcone as chalconate; △, relative % content of flavanone; (b) with chalcone (Ch) as the substrate; ○, relative % content of chalcone as chalconate; □, relative % content of flavanone.

ChO^-, which therefore appears as a proton-transfer reaction: $Fl^0 + OH^- \rightleftharpoons ChO^- + H_2O$.

When the % content of flavanone or chalcone was recorded vs. pH after equilibrium was reached, sigmoidal curves were obtained, Fig. 1. That one particular pH value at which relative concentrations of the two isomers present in solution were equal to 50% is the $pH_{\frac{1}{2}}$-value; it corresponds to pK_{BH^+} at half protonation.[17a,18] Such equilibria ought to be described by a Henderson equation used for calculations of acid–base equilibria, titration curves and protonation constants K_A (or dissociation constants K_D) but in the case of isomerisation equilibria we use the $pH_{\frac{1}{2}}$ value in place of log K_A (or pK_A) and concentrations instead of activities.[18] The Henderson equation then takes the form given in eqn. (1).

$$pH = pH_{\frac{1}{2}} - \log\frac{[Fl]}{[ChO^-]} \text{ and}$$

$$pH_{\frac{1}{2}} = pH + \log\frac{[Fl]}{[ChO^-]} \quad (1)$$

Square brackets indicate analytical concentrations of the respective isomers. If the total concentration of both isomers is constant (it was 2×10^{-5} mol dm^{-3} in this part of our work) then the mole fractions of isomers Φ are obtained, and these follow an acid–base titration curve with $\Phi_{Fl} + \Phi_{ChO^-} = 1$.

This equation implies that one constant value of $pH_{\frac{1}{2}}$ ought to be obtained from measurements made at different pH values. Spectrophotometric results obtained in the pH interval from 10.5–12.7 show that this is indeed the case, and a value of $pH_{\frac{1}{2}} = 11.8 \pm 0.1$ was found. It corresponds to the intersection point of the two sigmoidal curves in Fig. 1.

In alkaline solutions the solubility of the ionized forms increases rapidly with increasing pH. For ChO^- it reaches a plateau at constant (room) temperature. Such behaviour was not unexpected,[19] but 7-hydroxyflavanone is an exception. Table 1 indicates that one-component solid phases were always observed in equilibrium with the saturated solution, even if the latter contained the other isomer in virtually pure form, e.g., in NaOH at pH 14. The solution was always saturated with the

isomer present in the precipitate; if it was flavanone, the solution contained 1×10^{-4} mol dm^{-3} flavanone. The concentration of chalconate in equilibrium with the precipitated chalconate was not quite constant (salting-out effect in concentrated alkalis, Table 1). In LiOH solution only lithium chalconate is stable in the precipitate at pH 13.2. At pH 13.0, however, in the same system (aqueous LiOH) neat flavanone is found in the solid phase after equilibration. The change in composition of the precipitate with changing pH is abrupt (Table 1), while in solution the ratio of chalconate to flavanone (always after the equilibrium has been reached) changes smoothly along a sigmoidal curve (Fig. 1) in accordance with the Henderson equation (1).

Thus, in saturated systems another equilibrium intervenes, the one between a solid phase and its saturated solution, described by the Krebs and Speakman equation.[16] Here a three-component system is present at equilibrium: flavanone (Fl) $\rightleftharpoons$ 2'-hydroxychalconate (ChO$^-$) $\rightleftharpoons$ 2'-hydroxychalcone (ChOH), the reversibility of which is effected via these forms in solution.

In the pH region below pK_D only undissociated Fl and ChOH are dissolved in small concentrations given by their respective S values. The low solubility combined with very slow cyclisation rates make ChOH metastable in acidic and neutral solutions. At pH $> pK_D$ neutralisation of the weak acid ChOH leads to an increase of ChO$^-$. The solubilities of the uncharged forms Fl and ChOH do not depend on pH or the ionic strength of solutions, so both will be treated as constants if the solution is in equilibrium with solid Fl (or ChOH). The two equilibria under consideration are described by the following equations which apply to saturated solutions.

(a) The Krebs and Speakman equation (2) for protonation

$$pH = pK_D + \log(S_H/S_{ChOH}) - 1 \quad (2)$$

equilibria of the ChOH/ChO$^-$ pair, where K_D is the dissociation constant, which is the reciprocal of the protonation constant K_A; $K_D = 1/K_A$. Hence the log K_A value has exactly the same numerical value as pK_D; log $K_A = pK_D$.[20] The value of pK_D (ChOH $\rightleftharpoons$ ChO$^-$) is 9.6 (from ref. 10) and $pH_{\frac{1}{2}}$ (Fl/ChO$^-$) = 11.8. The solubility S of different forms (in mol dm^{-3}) is as follows: $S_{Fl} = 1 \times 10^{-4}$; $pS_{Fl} = 4$; $S_{ChOH} = 6 \times 10^{-6}$; $pS_{ChOH} = 5.2$; S_{ChO^-} at the indicated pH was calculated from the Krebs and Speakman equation for solutions in equilibrium with solid flavanone, or measured for solutions in equilibrium with solid chalconate salt ChOM (M = Li$^+$, Na$^+$, K$^+$), i.e. eqn. (3)

$$\frac{S_H}{S_{ChOH}} - 1 = \frac{S_{ChOH} + S_{ChO^-}}{S_{ChOH}} - \frac{S_{ChOH}}{S_{ChOH}} = \frac{S_{ChO^-}}{S_{ChOH}} \quad (3)$$

$$[S_H = S_{ChOH} + S_{ChO^-}]$$

We then obtain eqn. (4)

$$pH = pK_D + \log(S_{ChO^-}/S_{ChOH}) =$$

$$pK_D - \log(S_{ChOH}/S_{ChO^-}) \quad (4)$$

and eqn. (5), where c is the concentration of the indicated species.

$$c_{H^+} = K_D S_{ChOH}/S_{ChO^-} \quad (5)$$

Since $K_D S_{ChOH} = S_{ChO^-} c_{H^+}$, i.e. $pK_D + pS_{ChOH} = pS_{ChO^-} + pH$, then $pS_{ChO^-} = pK_D + pS_{ChOH} - pH$. The sum $pK_D + pS_{ChOH} = 9.6 + 5.2 = 14.8$ is a constant $pK_{Ch} = 14.8$, then $pS_{ChO^-} = 14.8 - pH$. For example, at pH 11.8, $pS_{ChO^-} = 14.8 - 11.8 = 3$ and $S_{ChO^-} = 1 \times 10^{-3}$ (calculated value as cyclisation interferes). S_{ChO^-} increases in step with rising c_{OH^-} in accordance with the equilibrium which defines K_D, but only to the saturation concentration of the chalcone salt.

(b) The equation for isomerisation equilibria $Fl \rightleftharpoons ChO^- + H^+$ which is based on the Henderson equation with an equilibrium constant for isomerisation $K_i = c_{ChO^-} c_{H^+}/c_{Fl}$. Then, $c_{H^+} = K_i c_{Fl}/c_{ChO^-}$, and $pH = pK_i - \log(c_{Fl}/c_{ChO^-})$. If $c_{Fl} = c_{ChO^-}$ then $pH = pK_i = pH_\ddagger$; here $pH_\ddagger = 11.8$, so $pc_{Fl} = pc_{ChO^-} + pH - pH_\ddagger$.

In solutions saturated with flavanone its concentration is constant, $c_{Fl} = S_{Fl} = 1 \times 10^{-4}$ mol dm^{-3}, $pc_{Fl} = pS_{Fl} = 4$. Then the sum $pH_\ddagger + pS_{Fl} = 15.8$ is another constant, pK_{Fl}, eqn. (6)

$$pc_{ChO^-} = pH_\ddagger + pc_{Fl} - pH =$$
$$11.8 + 4 - pH = 15.8 - pH \quad (6)$$

The concentration of the chalconate anion, c_{ChO^-}, which is in equilibrium with solid (precipitated) flavanone thus depends on pH: $pc_{ChO^-} + pH = 15.8$, e.g., at pH 11.8, $pc_{ChO^-} = 11.8 + 4 - 11.8 = 4$, $c_{ChO^-} = 1 \times 10^{-4}$ mol dm^{-3}, and at pH 11.8 it is the maximum concentration of ChO^- which may result from this equilibrium, because here $c_{ChO^-} = S_{Fl}$. A general inequality appears: $pK_{Fl} - pH > pK_{Ch} - pH$ and $S_{ChO^-} > c_{ChO^-}$ for the same pH.

The two equilibria are connected via the chalconate which appears in both, and its concentration defines the position of the equilibria. The solution which is saturated with ChO^- vs. chalcone is oversaturated vs. flavanone, which in accordance with the law of mass action forces the equilibrium towards flavanone, which precipitates out. Simultaneously, the solution becomes more alkaline (if not well buffered): $ChO^- + H_2O \rightleftharpoons Fl(S) + OH^-$, and the concentration of ChO^- drops below the S_{ChO^-} value. Then, however, the (solid) chalcone reacts with OH^- to restore the equilibrium.

This process continues until all of the chalcone is dissolved and reprecipitated as flavanone. Only then does the chalconate concentration drop from the S value to the c value, and the weaker acid (Fl) replaces the stronger one (ChOH). The case is similar to the titration of a mixture containing two acids of unequal strength. If only flavanone is present from the start, the S_{ChO^-} value will not be reached, and only the c_{ChO^-} value will be attained at equilibrium.

The inequality holds as long as the chalcone solubility increases in step with c_{OH^-}. Eventually the maximum solubility, S_{ChOM}, of chalconate salt with the cation M^+ present in solution will be attained at a $pH = pH_M$; further increase of pH above pH_M will not bring an increase of solubility and the Krebs and Speakman equation will no longer hold; only the Henderson equation will still be obeyed.

For the weaker acid couple (flavanone/chalconate), the c_{ChO^-} at that pH (viz. pH_M) is smaller than S_{ChOM}, so with further increase of pH, c_{ChO^-} will reach the latter value. When $c_{ChO^-} = S_{ChOM}$ three phases may coexist at that particular pH: solid flavanone, solid chalconate salt and the supernatant saturated with the chalconate and flavanone; this is the pH of the triple point, pH_{TP}.

In the interval between pH_M and pH_{TP} the solid chalcone, if present, will be rapidly transformed into chalconate and reprecipitated as flavanone; the latter will form the single stable solid phase in the full pH range below pH_{TP}.

pH_M is the highest pH value at which the Krebs and Speakman equation holds; it can still be used to calculate pH_M if the S_{ChOM} values are available. Thus, $pS_{ChOM} = pK_D + pS_{ChOH} - pH_M$, i.e. $pH_M = pK_D + pS_{ChOH} - pS_{ChOM} = 9.6 + 5.2 - pS_{ChOM} = 14.8 - pS_{ChOM}$.

Taking our experimental data we obtain the following relations:

With $S_{ChOLi} = 2 \times 10^{-3}$ mol dm^{-3}, $pS_{ChOLi} = 2.7$,
$$pH_M = pH_{ChOLi} = 12.1$$

With $S_{ChONa} = 2 \times 10^{-2}$ mol dm^{-3}, $pS_{ChONa} = 1.7$,
$$pH_M = pH_{ChONa} = 13.1$$

With $S_{ChOK} = 3 \times 10^{-2}$ mol dm^{-3}, $pS_{ChOK} = 1.5$,
$$pH_M = pH_{ChOK} = 13.3$$

The pH_{TP} value is characterized by the equality $c_{ChOM} = S_{ChOM}$ and $c_{Fl} = S_{Fl}$ so it can be calculated by means of the Henderson equation for the flavanone/chalconate pair, i.e. $pc_{ChOM} = pK_{Fl} - pH_{TP}$; $pH_{TP} = 15.8 - pS_{ChOM}$. The same data as before will give: $pH_{TP(ChOLi)} = 15.8 - 2.7 = 13.1$; $pH_{TP(ChONa)} = 15.8 - 1.7 = 14.1$; $pH_{TP(ChOK)} = 15.8 - 1.5 = 14.3$.

The analyses of the solid phases as shown in Table 1 corroborate the above calculations; also, $pH_{TP} + pS_{ChOM} = 15.8$ and if pH > pH_{TP}, $c_{ChOM} > S_{ChOM}$ and $c_{Fl} < S_{Fl}$.

When the pH of the solution increases beyond pH_{TP} solid flavanone if present dissolves to reprecipitate as chalconate. Again only one solid phase is present but now it is the chalconate. For example, in LiOH of pH 13.8 > $pH_{TP(ChOLi)}$, the Henderson equation holds, but $pc_{ChOLi} = pS_{ChOLi}$. Then: $pc_{Fl} = pS_{ChOLi} + pH - pH_\ddagger = 2.7 + 13.8 - 11.8 = 4.7$; $c_{Fl} = 2 \times 10^{-5}$ mol dm^{-3} and flavanone dissolves. If c_{Fl} increases to S_{Fl}, then $pc_{ChOLi} = 11.8 + 4 - 13.8 = 2$; $c_{ChOLi} = 1 \times 10^{-2}$ mol dm^{-3}. Here, $c_{ChOLi} > S_{ChOLi}$ and solid lithium chalconate precipitates out. With NaOH solution at pH 13.8 < $pH_{TP(ChONa)}$, $c_{ChONa} < S_{ChONa}$ and $c_{Fl} = S_{Fl}$; $pc_{ChONa} = pH_\ddagger + pc_{Fl} - pH = 11.8 + 4 - 13.8 = 2$; $c_{ChONa} = 1 \times 10^{-2}$ mol dm^{-3} in the presence of solid flavanone. Sodium chalconate, if added, will dissolve, and neat flavanone will be precipitated.

With solubilities of chalconate salts determined at known pH (or H_-) it was possible to evaluate $pH_\ddagger^s$, the $pH_\ddagger$ for solutions in equilibrium with flavanone in the solid phase, from $pH_\ddagger^s = pH - \log(c_{ChOM}/S_{Fl})$, Table 1. This is a different approach from the one described above for dilute solutions when the Φ_{ChO^-}/Φ_{Fl} ratio was used.

The results collected in Table 1 clearly indicate that the values of $pH_\ddagger$ and $pH_\ddagger^s$ describe the same quantity, K_i, which is constant over a wide range of pH: $pH_\ddagger = pH_\ddagger^s$. This implies that the solubility of flavanone, S_{Fl}, remains virtually constant even in strongly alkaline solutions, as the rearrangement of the equation for $pH_\ddagger^s$ yields $S_{Fl} = S_{ChOM}/10^{(pH-pH_\ddagger)}$. For example, with $S_{ChOK} = 0.03$ mol dm^{-3} at pH 14.2, one obtains $S_{Fl} = 1.2 \times 10^{-4}$ mol dm^{-3}.

In the 7-hydroxyflavanone (FlOH) and 2',4'-dihydroxychalcone (ChH$_2$) system the neutral species are sparingly soluble with 6×10^{-5} and 2×10^{-5} mol dm^{-3} for FlOH and ChH$_2$, respectvely. Their solubility increases in accordance with the Krebs and Speakman equation which makes possible independent determinations of their protonation constants[15b]: $\log K_1(FlO^-) = 7.3, \log K_2(ChH^-) = 7.2, \log K_1(Ch^{2-})$ ca. 11.4.

Rather unexpectedly, the solubility of FlONa at pH 12 (i.e., well before the ring-opening reaction becomes important) reaches a value of ca. 5 mol dm^{-3}. A viscous, amber-coloured but translucent solution containing ca. 52 wt % of flavanone is obtained. It appears to be something like a liquid, hydrated salt.

The solubility of ChNa in equilibrium with precipitated FlOH reaches 4×10^{-2} mol dm^{-3} at pH 10.4 but at higher pH ChNa$_2$ is formed, accompanied by further solubility increase, with a maximum of ca. 1 mol dm^{-3} for ChNa$_2$ in 30% NaOH (H_- ca. 16.2).

For 7-hydroxyflavanone (FlOH) and 2',4'-dihydroxychalcone (ChH$_2$) the kinetics of isomerisation have been studied[8,10,11] but the final equilibrium has not been discussed. High solubilities of the ionized forms impeded the study of solid phases in equilibrium with saturated solutions above pH 12 so we could determine the isomerisation equilibria only for solutions, but a constant $pH_\ddagger = 13.4 \pm 0.1$ was obtained with the Henderson equation for pH from 12.5–14.0. Again, a

sigmoidal titration curve appears for the reaction $FlO^- + OH^- \rightleftharpoons Ch^{2-} + H_2O$, which even at $H_- > 15$ is a relatively slow reaction at room temperature with a steady rate constant of $ca.$ 1 min^{-1} in 10–30% NaOH.

The system naringenin (Ng)/2′,4′,6′,4-tetrahydroxychalcone (TChH$_4$) is complex as it may contain several species of different degrees of protonation.[15a] We have now determined the log K_1(TCh) = 12.1 value for the equilibrium $TCh^{4-} + H_2O \rightleftharpoons TChH^{3-} + OH^-$ and the pH$_{\frac{1}{2}}$ = 14.0 value for the Ng^{3-}/ TCh^{4-} system.

Discussion

In this study seemingly contradictory results found in the literature have been reconciled. Now it is clear why from 0.5 mol dm^{-3} NaOH solutions containing only a chalcone[10] pure flavanone can be obtained.[14] This also explains why the 'chalcone sodium salt' yields flavanone;[21] it is not valid to assume it to be a sodium complex of 2′-hydroxychalcone and enolized flavanone which on treatment with water decomposes. In NaOH solution the 2′-hydroxychalcone simply isomerises to flavanone at any pH less than 14. We were not able to find evidence for the presence of enolized flavanone for which the pK_A values ought not to be much different from phenols in the range 8–11;[22] a pK_A value of $ca.$ 10 for EnOH has been mentioned (ref. 12c, p. 1630). That by itself strongly indicates that the pH$_{\frac{1}{2}}$ values found (as described above) in highly alkaline solutions between $ca.$ 12 and 14 are not related to enolization equilibria. There is also the relatively slow cyclisation rate not encountered in enol-protonation equilibria which are very rapid.[23] Our results show that proton transfer is the driving force for cyclisation and ring-opening reaction and point to a carbanion mechanism via carbon acid (or pseudoacid) equilibrium characterized by pH$_{\frac{1}{2}}$. This mechanism operates above pH $ca.$ 12. In less alkaline solutions enolization may provide a principal reaction path.[12] Indirect proof that two distinct isomerisation mechanisms operate in the alkaline region (a third via enone-protonated chalcones may appear in acidic solutions)[12c] is provided by the experimental fact that the kinetics of most flavanone–chalcone isomerisations reach a more or less pronounced plateau in the pH 10–12 interval after a large rate increase in weakly alkaline solutions. This plateau is frequently followed by a shallow dip at $ca.$ pH 13 after which another rate increase takes place;[12] the latter levels off at pH > 14. A plateau has also been described for some sulfonation reactions[24] in which one mechanism recedes but a second takes over.

The mechanism via a distinct carbanion intermediate (Fl$^-$) begins to intervene at pH > 10 supporting the isomerisation rate when the mechanism based on protonations involving the enol equilibria declines at high pH values. This carbanion mechanism would be insignificant at pH < 10, prevailing only above pH 12 or 13 when a strong base (such as OH$^-$) efficiently scavenges the labile C3 hydrogen from the chromone ring before it can settle on the carbonyl oxygen and stabilize the enol tautomer. With increasing pH the concentration of the enol (or enolate) intermediate decreases exponentially, but at the same time increases the concentration [related to the $k_5a_{OH^-}$ term in eqn. (4) of ref. 12a] of the carbanion; once formed the negative charge remaining on C3 is shifted towards the ether oxygen which is reduced to phenolate anion. Similar reductive ring-opening of cyclic ethers is known.[25] In other words, the free electron pair of the C3–carbanion will directly form the C2–C3 double bond as part of a more extensively conjugated (hence stable) mesomer. It is a transition state with a partial negative charge on C2. This charge stabilizes the C2 hydrogen so that it is not exchanged by deuterium. In the next step the ether oxygen–C2 bond dissociates, the pyrone ring opens while the final stable products, the basic 2′-phenolate oxygen anion and a α,β-double bond of the chalcone appear. This, now phenolic,

oxygen may attack the olefinic double bond, as a strong base resulting in the formation of a carbanion which reacts with any proton donor to form the respective flavanone; the reversibility of isomerisation is secured by a nucleophilic addition to alkene.

The sigmoidal titration curve and a sharp pH$_{\frac{1}{2}}$ value observed for naringin with evaluated pH$_{\frac{1}{2}}$ $ca.$ 13.2 (from Fig. 2, ref. 12b) support this carbon acid, or carbanion mechanism, which for the ring-opening reaction could be labelled as an (El)$_{anion}$ mechanism. Accordingly, if the flavanone is present only as its conjugate carbanion (at $H_- > 15$) the elimination (ring-opening) kinetics become independent of c_{OH^-} but remain first order $vs.$ the flavanone as observed in our work and in the case of naringin.[12b] The pH$_{\frac{1}{2}}$ acts not only as an isomerisation constant but also as a protonation constant if the flavanone–chalcone isomerisations are vizualized as belonging also to the class of neutralisation reactions.

The preparation of flavanones from the respective chalcone obtained in strongly alkaline solutions can be facilitated by dilution or neutralization to a pH less alkaline than pH$_{\frac{1}{2}}$. After cyclization the neutral flavanone can be precipitated with an ice-cold acid such as orthophosphoric acid.

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