The Kinetics and Mechanism of the Cyclisation of Some 2'-Hydroxychalcones to Flavanones in Basic Aqueous Solution

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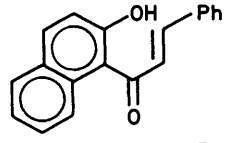
Rate coefficients for the chalcone–flavanone equilibration reaction have been established for some 2'-hydroxychalcones over the pH range (*ca.* 8—11) in which their 2'-hydroxy-groups undergo ionisation. The chalcones studied were the parent 2'-hydroxychalcone (I) and its following derivatives: 4'-OMe (II), 6'-OMe (III), 4'-OH (IV), 4',6'-Me_2 (V), and 5',6'-benzo (VI). Pseudo-first-order rate coefficients (k_{obs}) which, for the reversible reactions concerned, are the sum of the forward and reverse rate coefficients, were fitted to the kinetic form $k_{obs} = kf_A + k'f_B + k''a_{OH^-}$ in which k and k' are the rate coefficients for the unimolecular cyclisation of neutral and of ionised chalcones respectively (f_A and f_B are the fractions of total chalcone present in the neutral and ionised forms at the pH concerned) and where k'' is the second-order rate coefficient for the reverse reaction of flavanone which involves hydroxide ion (activity a_{OH^-}). Data analysis gave rate coefficients and pK_a values for all but chalcone (IV) which has a different kinetic form. A conjugate addition–elimination mechanism is proposed to account for the pH–rate profiles, one of which [chalcone (I)] differs from that previously reported. Possible effects on rate coefficients of non-bonded interactions between 6'-substituents and carbonyl oxygen are briefly considered but the differences between chalcones with and without 6'-substituents are sufficiently small to deter prolonged discussion. There seems to be little serious hindrance of cyclisation for any of the 6'-substituted chalcone anions.

CYCLISATIONS of 2'-hydroxychalcones to flavanones (Scheme 1), which are reactions catalysed in plants by chalcone–flavanone isomerase enzymes,[1] have been the subject of little mechanistic investigation. Few studies of substituent effects favouring cyclisation as measured by equilibrium position have been made,[2,3] and kinetic measurements are even sparser: Panasenko and his co-workers have studied[4] the cyclisation of 2',4'-dihydroxychalcone (IV) in strongly alkaline solutions,

(I) $R^1 = R^2 = H$
(II) $R^1 = OMe$, $R^2 = H$
(III) $R^1 = H$, $R^2 = OMe$
(IV) $R^1 = OH$, $R^2 = H$
(V) $R^1 = R^2 = Me$



SCHEME 1

and Litkei and his co-workers[5] the kinetics of cyclisation of the parent 2'-hydroxychalcone (I). The latter authors reported a 100-fold increase in rate of attainment of equilibrium between pH 9.5 and 10, with only a slight further increase in rate between pH 10 and 12. This suggested a dependence of rate on ionisation of chalcone, and in the present study we set out to establish pH–rate profiles for cyclisation of the chalcones (I)—(VI) with a view to properly establishing the mechanism as well as to tentatively exploring substituent effects, especially those of 6'-substituents which, by analogy with their effect on the course of reactions of 2'-hydroxychalcone epoxides[6] and 2'-hydroxychalcone dibromides,[7] might be expected to modify reactivity by non-bonded interactions affecting the degree of coplanarity of the carbonyl group with the attached benzene ring.

EXPERIMENTAL

^1H N.m.r. spectra (60 MHz; $CDCl_3$) were recorded on a JEOL C-60HL spectrometer and mass spectra on a Varian MAT CH5 instrument. U.v. spectral and kinetic measurements were carried out using a Cary 17 spectrophotometer equipped with a 1 729 programmer and a 1 718 digital repetitive scan unit, the cell block being maintained at 30 °C by circulation of water from an external thermostatted bath.

Preparations of 2'-Hydroxychalcones.—The standard method of condensation of 2'-hydroxyacetophenones with benzaldehyde was used but because methods previously described in the literature were not generally successful in providing products of the purity we required, we describe our preparations in detail. The general method was as follows. The appropriate 2'-hydroxyacetophenone (1 mol) was added as a solution in ethanol to a concentrated (50%) aqueous solution of sodium hydroxide (1.5 mol). The solution was stirred vigorously while benzaldehyde (1 mol) in ethanol was added dropwise. The resulting mixture was allowed to stand. It was then poured into excess of concentrated hydrochloric acid containing ice. The product was collected and recrystallised from ethanol. In this way were obtained the following (period of standing and temperature prior to work-up given in parentheses): 2'-hydroxychalcone (I) (24 h, 0 °C), m.p. 88 °C (lit.,[8] 89 °C); 2'-hydroxy-4'-methoxychalcone (II) (48 h, 20 °C), m.p. 108 °C (lit.,[9] 106—108 °C); 2'-hydroxy-6'-methoxychalcone (III) (350 h, 20 °C), m.p. 65 °C (lit.,[10] 65 °C). For 2',4'-dihydroxychalcone (IV) the amount of sodium hydroxide was increased to 10 mol, and during the addition of benzaldehyde and for a further period (18 h) until being worked up, the reaction solution was maintained at 55 °C. The product had m.p. 148 °C (lit.,[11] 147—149 °C).

For the remaining two chalcones, products were obtained which contained so much flavanone that they could not successfully be purified by recrystallisation. In each case, it was found best to obtain pure flavanone, before converting it back to the chalcone. For chalcone (V), the general method was used but after stirring (2 h, 20 °C) water (ca. 2 volumes) was stirred in. After standing (12 h, 20 °C) the precipitated flavanone was filtered, recrystallised, and dissolved in ethanol. Aqueous sodium hydroxide solution (10%; *ca.* 5 volumes) was added. The solution was acidified (HCl) to pH 3. Ether extraction and work-up gave the chalcone which was recrystallised without cyclisation by dissolving in the minimum volume of light petroleum (b.p. 40—60 °C) at room temperature and cooling to −15 °C. Thus obtained were orange crystals, m.p. 47 °C, of 2′-*hydroxy-4′,6′-dimethylchalcone* (V), *m/e* 252, 175, 161, 149, and 148; τ − 1.38 (1 H, s, OH), 2.19—2.85 (7 H, m, ArH), 3.32 also 3.40 (1 H, s, α- and β-H), and 7.47 also 7.71 (3 H, s, CH_3) (Found: C, 80.65; H, 6.4. $C_{17}H_{16}O_2$ requires C, 80.95; H, 6.4%). Similarly prepared *via* 5,6-benzoflavanone, m.p. 115 °C (lit.,[12] 117 °C), were yellow needles of 2′-hydroxy-5′,6′-benzochalcone (VI), m.p. 79 °C, *m/e* 274, 197, and 171, τ −2.60 (1 H, s, OH), 1.9—2.9 (13 H, m, Ar-, α-, and β-H) (Found: C, 83.7; H, 5.25. Calc. for $C_{19}H_{14}O_2$: C, 83.2; H, 5.15%). Previously reported m.p.s are as follows: 88 °C,[13] 112—113 °C,[14] and 109 °C.[15] We deduce that the last two values may represent impure 5,6-benzoflavanone.

Buffer Solutions.—Analytical grade potassium salts were used in the preparation of phosphate and carbonate buffer solutions. The total buffer concentration was maintained at 0.02 mol l^{-1} and ionic strength, with potassium chloride, at 0.50 mol l^{-1}. The pH was measured after the completion of each kinetic run, *i.e.* in the presence of some dioxan, in which solvent the reactant was added to the aqueous buffer. Quite large volumes of dioxan were essential to prevent precipitation of chalcone or flavanone product. The ratios of mixed volumes of aqueous buffer and dioxan were uniformly 19 : 1 except for chalcone (VI) for which a ratio of 9 : 1 was necessary. Values of the activity of hydroxide ion, a_{OH^-}, were calculated from pH using the pK_w value [16] of 13.83 at 30 °C.

Kinetic Methods.—The aqueous buffer solution in a stoppered cuvette was equilibrated at 30 °C in the cell block of the spectrophotometer. The reaction was started by the addition of the chalcone solution in dioxan, also at 30 °C. Absorbance decrease was recorded at a wavelength appropriate to the chalcone under study. The reaction was normally run for at least ten half-lives to obtain an accurate infinity reading (A_∞), and the first-order rate coefficient was calculated as the gradient of a plot of ln $(A_t - A_\infty)$ *versus* time using a linear least squares fit computer program. Duplicate measurements were always carried out and reproducibility of rate coefficients was within 2%. The observed rate coefficients (k_{obs}) for these reversible equilibration reactions are the sums of the forward and reverse rate coefficients.[17] Repetitive scans during the course of the equilibration reactions invariably showed sharp isosbestic points right up to the infinity stage, indicating the absence of any complicating secondary reactions and of any solubility problems. Buffer dilution studies showed that buffer catalysis, if any, is very small and at the buffer concentrations (0.02 mol l^{-1}) used it is negligible.

Kinetic Analysis.—Values of k_{obs} have been fitted to the kinetic form of equation (1), in which the first two terms

$$k_{obs} = kf_A + k'f_B + k''a_{OH^-} \quad (1)$$

represent the forward reaction, k and k' being respectively the rate coefficients for unimolecular cyclisation to flavanone of the neutral (acid) and anionic (base) chalcone species. The fractions of the acid and base species [f_A and f_B in equation (1)] are determined at any pH by the pK_a value of the 2′-hydroxy-group of the chalcone. The third term in equation (1) represents the reverse reaction, a pseudo-first-order process at constant pH, k'' being the second-order rate coefficient for reaction of flavanone with hydroxide ion of activity a_{OH^-}.

The aid of computer-assisted least squares analysis allowed the determination of a pH–rate profile based on theory [equation (1)] which fitted very closely the experimental data, thus providing support for the validity of the rate equation. The fit of data was found to be quite sensitive to values of k', k'', and pK_a, but the first term in equation (1) is so small in relation to the others that the calculated values of k are subject to very large experimental error.

Independent pK_a *Determinations.*—A check on the validity of apparent pK_a values obtained from pH–rate profile fitting by comparison with pK_a values directly determined by spectrophotometric measurement of degrees of chalcone ionisation as a function of pH was desirable, but it was only for the two chalcones without 6′-substituents that the long wavelength band of the chalcone anion was sufficiently intense for such measurements to be made with accuracy. At each pH, absorbance was recorded to the infinity stage of cyclisation and the zero (mixing) time absorbance due to chalcone species alone was obtained by extrapolating the linear infinity plot.

Using the same concentration (*ca.* 10^{-4} mol l^{-1}) of (I) in a series of runs, the initial chalcone anion absorbance at 460 nm (A_i) at a series of pH values was determined. The absorbance due to chalcone when *completely* converted into chalcone anion (A_∞) was also determined, using the same chalcone concentration in potassium hydroxide solution (0.50 mol l^{-1}; there is negligible cyclisation to flavanone under these conditions). Because the neutral chalcone species has negligible absorbance at 460 nm (established separately at pH 5.3), the ratio A_i/A_∞ gives for each pH the fraction [f_B; *cf.* equation (1)] of the chalcone present as the anion (base), and from this value the ratio f_B/f_A required for plotting the data according to equation (2) can

$$pH = \log (f_B/f_A) + pK_a \quad (2)$$

be calculated. In Figure 1 the points are experimental and the line is that of best fit; least squares analysis gave for

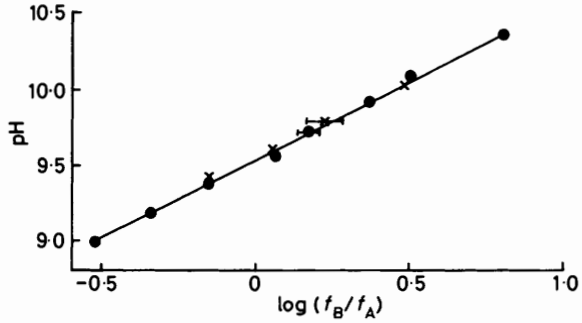


FIGURE 1 pH *versus* log f_B/f_A for chalcones (I) (●) and (II) (×). The line is from least squares analysis for (I)

(I) an intercept value [*i.e.* pK_a from equation (2)] of 9.54 (gradient, 1.04; correlation coefficient, 0.999) which agrees well with that (9.55; Table) obtained from analysis of the kinetic data.

For the less soluble 4′-methoxychalcone (II) reduced (*ca.* 2×10^{-5} mol l^{-1}) chalcone concentrations and the taking of measurements at 430 nm, with corrections for neutral chalcone absorbance, resulted in larger experimental error (see error bar in Figure 1, in which, for clarity, no best fit line is drawn). The pK_a value of 9.57 (gradient, 0.97; correlation coefficient, 0.998) is in agreement with that (9.55) from kinetic analysis.

RESULTS

The kinetic data are shown as points in the pH–rate profiles in Figure 2 in which the lines are theoretical, being based on equation (1) and the appropriate values of k, k', k'', and pK_a (Table). No analysis was made of the data for 2′,4′-dihydroxychalcone (IV) which can form mono- and dianionic chalcone species as well as an anionic flavanone, so that a complex kinetic form for equilibration is likely.

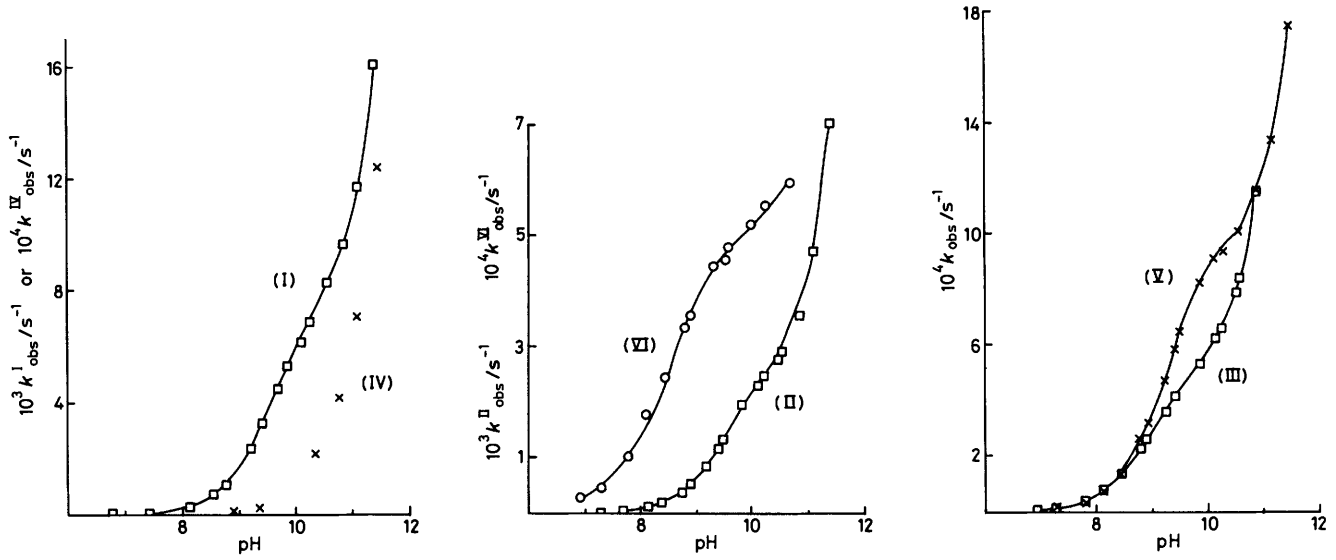


FIGURE 2 k_{obs} *versus* pH for chalcones (I)—(VI). Points are experimental. Curves are theoretical, being based on equation (1) and the rate and equilibrium constant values in the Table

Ionisation and rate coefficients based on equation (1)

Chalcone	pK_a	$10^4 k'/s^{-1}$	$10k''/dm^3$ mol^{-1} s^{-1}	$10^6 k/s^{-1}$ [a]
(I)	9.55	75	22.5	10
(II)	9.55	27	11.7	7
(III)	8.95	5.3	6.3	8
(V)	9.25	10.0	1.9	4
(VI) [b]	8.50	5.0	1.7	16

[a] Subject to large experimental error (see text). [b] Measured in 9 : 1 water–dioxan, *cf.* 19 : 1 for remaining chalcones.

DISCUSSION

pH Rate Profile and Mechanism.—The agreement between experimental rate coefficients and theoretical rate profiles supports the validity of equation (1). The simplest mechanism to account for this kinetic form, is stepwise conjugate addition, as shown in Scheme 2 for the chalcone anion, with ring closure rate-determining in the forward direction. For the reverse reaction, equilibration of flavanone and enolate ion is followed by rate-limiting ring opening, a mechanism which is consistent with the absence of general base catalysis by buffer bases.

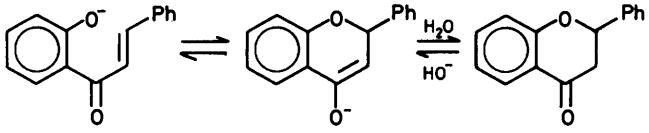
The pH–rate profile for equilibration of (I) (Figure 2) is not in accord with that given by Litkei and his co-workers [5] particularly at high pH where, in contrast to the steep dependence on pH we observed, they reported a near plateau. Theirs would be the type of rate profile expected for cyclisation alone (*i.e.* the forward reaction) when the product flavanone is immediately trapped (for instance by an oxidant; *cf.* ref. 5), but in the absence of any such traps the reverse ring opening reaction of flavanone must make an unavoidable contribution to the overall reaction rate.

Effect of 6′-Substituents.—The most noteable feature of the results (Table) is the small variations in rate coefficients for forward and reverse reactions. There is certainly no marked effect associated with the presence of 6′-substituents.

We find no clearcut trends in or explanations for our data and our discussion is consequently brief. For simplicity it is restricted mainly to a comparison of the electronically similar 4′- and 6′-methoxychalcones (II) and (III) and it is based on consideration of non-bonded interactions associated with the 6′-methoxy-group.

Cyclisation.—The rate coefficients for cyclisation of chalcone anions (k', Table) are lower for the 6′-substituted chalcones but not by large factors considering that their pK_a values are lower anyway. It might, of course, reasonably be argued that correlation of rate with pK_a may be unrealistic for the following reason: comparison of the electronically similar 4′- and 6′-methoxychalcones suggests that the pK_a of the former may be raised by hydrogen-bonding of the phenolic

hydrogen to the carbonyl oxygen, which hydrogen-bonding, as a result of non-bonded interactions, may be weakened in the (more acidic) 6'-methoxychalcone. If such a view is taken, a close correlation of basicity and nucleophilicity of the 2'-O^- groups would not be expected and the rate coefficient differences between 6'-substituted and 6'-unsubstituted chalcones could take on a little more significance. Nevertheless we are forced to the conclusion that there seems to be little serious hindrance of cyclisation in any of the 6'-substituted cases.



SCHEME 2

Ring Openings.—The rate of the reverse reaction, according to Scheme 2, is dependent on both the concentration of enolate ion in equilibrium with flavanone and the rate coefficient for opening of the enolate ion. Maximum stabilisation of the enolate ion requires coplanarity of the enolate oxygen with the methoxy-substituted benzene ring, so that the non-bonded interaction with the 6'-methoxy group might be expected to increase the free energy of the enolate ion more so than would the corresponding interaction in the (uncharged) flavanone itself, whose stability would probably not be so dependent on coplanarity of carbonyl oxygen with the benzene ring. Consequently, it seems likely that the 6'-methoxy enolate ion would be present in lower equilibrium concentration than the 4'-methoxy enolate ion under the same conditions. However, the non-bonded interaction in the former should be considerably relieved in the transition state for its ring opening and, as a result, the free energy of activation for this step would probably be lower than that for the 4'-methoxy enolate ion. One can envisage again, therefore, compensating effects associated with the 6'-methoxy-group, in this case a reduced concentration of enolate ion being compensated for by a faster rate of its ring opening. The result, once again, may be a rather similar rate for the overall ring-opening of flavanone in the 6'-methoxy as compared with the 4'-methoxy case.

Relevance to the Algar–Flynn–Oyamada Reaction.—We note the relevance of our data to previous suggestions [6] that 2'-hydroxy-6'-methoxychalcone is unable, because of steric effects associated with the 6'-methoxy group, to undergo cyclisation under the conditions (ethanolic alkaline H_2O_2) of the Algar–Flynn–Oyamada reaction at room temperature, whereas such cyclisation becomes possible at high temperatures when steric effects are more easily overcome. We feel that our results are more in accord with the alternative proposition of Ferreira [18] and his co-workers that there should be little significant steric hindrance to cyclisation of 6'-methoxychalcones. Nevertheless, it is possible that our results may not strictly be applicable under the conditions of the Algar–Flynn–Oyamada reaction.

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