Kinetics and Mechanism of the Cyclisation of 2′,6′-Dihydroxychalcone and Derivatives

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pH–Rate profiles are reported for the cyclisation in water to 5-hydroxyflavanones of 2′,6′-dihydroxychalcone (**1**) and its 4-methoxy (**2**), 3,4-dimethoxy (**3**), 3,4,5-trimethoxy (**4**), 2,4,6-trimethoxy (**5**), 4-chloro (**6**), and 3,4,4′-trimethoxy (**8**) derivatives. As for the previously studied 2′,6′-dihydroxy-4,4′-dimethoxychalcone (**7**), rate coefficients are established for acid-catalysed cyclisation of neutral chalcone, for unimolecular cyclisation of the neutral, monoanionic, and dianionic chalcone, and for the base-catalysed reverse ring-opening reaction. Cyclisation of the monoanion of 2′,6′-dihydroxychalcone is almost 40 times faster than that of the monoanion of the 2′-hydroxy-6′-methoxychalcone (**10**) and is also estimated to be about ten times faster than that of the reactive monoanion of 2′,4′-dihydroxychalcone. These are the first calculations of the enhancement of rate of monoanion cyclisation by the 6′-OH group. The effect is only small, and is suggested to arise largely from stabilisation of the transition state for ketonisation by hydrogen bonding to enolate oxygen. Other reactivity differences amongst the chalcone monoanions are also discussed. Enthalpy and entropy of activation data are reported for monoanion cyclisation of (**1**), (**2**), and (**4**)–(**6**). Rate coefficients for the cyclisation of the chalcone monoanions are almost identical for (**1**)–(**4**) and (**6**) in water but not in deuterium oxide: kinetic hydrogen isotope effect (KIE) values are 3.4 (**1**), 5.7 (**2**), 4.9 (**3**), 3.0 (**4**), 7.5 (**5**), 2.9 (**6**), and 5.0 (**8**). For chalcones (**2**) and (**7**), the KIE values of which are both 5.7, the amounts of H *versus* D incorporation at the flavanone 3-carbon for monoanion cyclisation in H_2O/D_2O mixtures were established by mass spectroscopy. This gave product (or 'discrimination') isotope effect (PIE) values of 7.9 for (**2**) and 3.8 for (**7**), suggesting for (**2**) but not (**7**) an inverse isotope effect contribution to KIE from sources other than rate-limiting proton transfer to carbon. Monoanion cyclisation of (**1**) in D_2O was established by ^{1}H n.m.r. as involving almost equal amounts of *anti* and *syn* addition of 2′-O^- to the enone double bond. Reactivity differences amongst the chalcones for reactions other than monoanion cyclisation are only briefly considered.

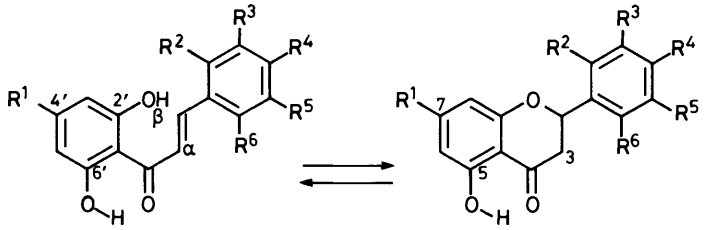
2′,6′-Dihydroxychalcones occur in plants along with their cyclisation products, the 5-hydroxyflavanones (Scheme 1). The first full pH–rate profile for such cyclisation was reported[1] by us for 2′,6′-dihydroxy-4,4′-dimethoxychalcone (**7**), and the possibility of an intramolecular catalytic role for the 6′-OH proton adjacent to the carbonyl group was suggested. The magnitude of any rate enhancement by 6′-OH was then unknown but we foreshadowed kinetic measurements on analogues with and without a proton on 6′-O to determine it. This was one aim of the present study in which the rate coefficient for cyclisation of the monoanion of 2′,6′-dihydroxychalcone (**1**) is measured and compared with the previously determined[2] value for the monoanion of 2′-hydroxy-6′-methoxychalcone (**10**). An additional comparison is made with the rate coefficient, now calculated from earlier[2] data, for cyclisation of the monoanion of 2′,4′-dihydroxychalcone (**9**) in which the additional 4′-OH group is remote from the carbonyl group involved in activating the nucleophilic addition step of the cyclisation.

The earlier study on 2′,6′-dihydroxy-4,4′-dimethoxychalcone (**7**) has also been extended across a series of 2′,6′-dihydroxychalcones [(**1**)–(**6**), (**8**)] with the aim of better understanding the mechanism of monoanion cyclisation, and the role in it of the 6′-OH group: the effects of other substituents on rate coefficients, activation parameters, and kinetic and product isotope effects are reported. The stereochemistry of the addition reaction was also of interest given that it has been established in one case[3] for cyclisation catalysed by a chalcone–flavanone isomerase enzyme.

Experimental

Materials.—The synthesis of chalcones (**1**)–(**6**) has been reported,[4] as has that[1] of (**7**). The method employed[1] for the latter was applied to (**8**): hesperetin (Sigma) was methylated and then ring-opened to the chalcone as follows. To a suspension of 3′,5,7-trihydroxy-4′-methoxyflavanone (hesperetin; 5.0 g) in methanol (25 cm^3) containing dimethyl sulphate (10 cm^3) was added dropwise methanolic potassium hydroxide (6 g KOH in 30 cm^3 MeOH) over a period of 6 h. The precipitated solid was collected and extracted with chloroform–water. The chloroform layer was separated, dried over magnesium sulphate, filtered, and the solvent removed under vacuum. Recrystallisation from methanol gave 5-hydroxy-3′,4′,7-trimethoxyflavanone (2.8 g, 49%) as white needles, m.p. 132–134 °C (lit.,[5,6] 136, 156 °C); λ_{max} 287 nm (log ε 4.28) (lit.,[6] 288 nm); δ_H(90 MHz; $CDCl_3$) 2.77 (1 H, dd, J_{3ax} 17.2 Hz, J_2 4.0 Hz, 3-H_{eq}), 3.14 (1 H, dd, J_{3eq} 17.2 Hz, J_2 12.6 Hz, 3-H_{ax}), 3.80, 3.90, 3.92 (all 3 H, s, OCH_3), 5.36 (1 H, dd, J_{3ax} 12.6 Hz, J_{3eq} 4.0 Hz, 2-H), 6.06 (2 H, s, 6-H and 8-H coincident), 6.9–7.0 (3 H, m, 2′-, 5′-, 6′-H), and 12.0 (1 H, br s, 5-OH); δ_C(22 MHz; $CDCl_3$) 43.3 (t, C-3), 55.5 and 55.9 (each q, OCH_3), 79.1 (d, C-2), 94.2 and 95.1 (each d, C-6, C-8), 103.1 (s, C-10), 109.4 and 111.2 (each d, C-2′, C-5′), 118.8 (d, C-6′), 130.8 (s, C-1′), 149.2 and 149.5 (each s, C-3′, C-4′), 162.8, 164.1, and 167.9 (each s, C-5, C-7, C-9), and 195.9 (s, C=O): *m/z* 330 (M^+) with fragmentation identical with that of the chalcone (**8**) below (Found: C, 65.3; H, 5.6. Calc. for $C_{18}H_{18}O_6$: C, 65.4; H, 5.5%).

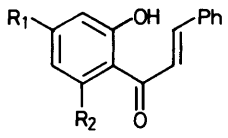
This flavanone (0.5 g) was refluxed for 5 min in methanolic potassium hydroxide [KOH (1 g) in MeOH (5 cm^3)] and the



All R = H except as indicated

(1) All R = H
(2) R^4 = OMe
(3) R^3 = R^4 = OMe
(4) R^3 = R^4 = R^5 = OMe
(5) R^2 = R^4 = R^6 = OMe
(6) R^4 = Cl
(7) R^1 = R^4 = OMe
(8) R^1 = R^3 = R^4 = OMe

Scheme 1.



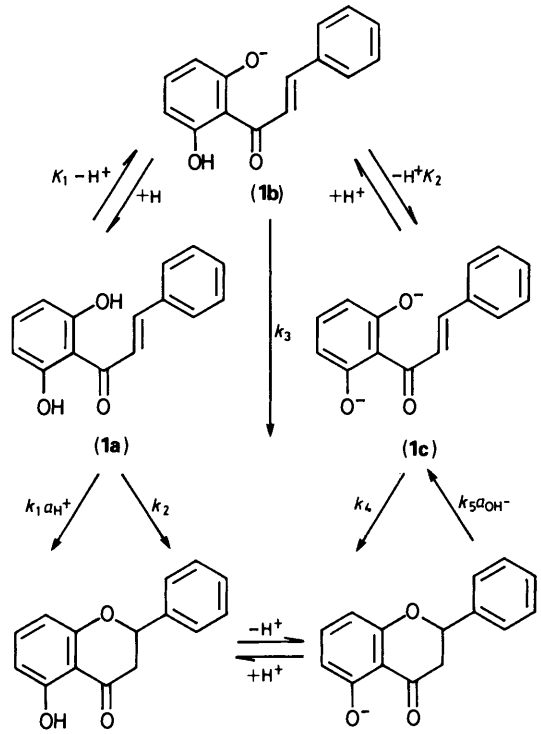
(9) R^1 = OH; R^2 = H
(10) R^1 = H; R^2 = OMe
(11) R^1 = OMe; R^2 = H

red solution was then added slowly with stirring to an excess of ice-cold hydrochloric acid (2 mol dm^{-3}). The precipitated solid was extracted into ether (2 × 50 cm^3), the solution washed, dried ($MgSO_4$), and evaporated under vacuum. The orange solid was purified by p.l.c. on silica gel (Merck 60 PF) using ethyl acetate–chloroform (4:6) as the eluant. The solid extracted from the bright yellow band (R_F 0.6) was recrystallised from light petroleum to which just sufficient toluene had been added to ensure solubility at 60 °C and gave 2′,6′-dihydroxy-3,4,4′-trimethoxychalcone (**8**) as an orange powder, m.p. 140–150 °C; λ_{max}(acidic methanol) 372 nm (log ε 4.35); δ_H(90 MHz; CD_3COCD_3) 3.96, 4.00, and 4.01 (all 3 H, s, OCH_3), 6.19 (2 H, s, 3′-, 5′-H), 7.14 (1 H, d, *J* 8.8 Hz, 5-H), 7.3–7.5 (2 H, m, 2-, 6-H), 7.92 (1 H, d, *J* 15.5 Hz, α-H), 8.28 (1 H, d, *J* 15.5 Hz, β-H), and 12.3 (2 H, br s, 2′-, 6′-OH); δ_C(22 MHz; CD_3COCD_3) 55.9 and 56.1 (3-, 4-, 4′-OCH_3 with coincidence), 94.6 (C-3′, -5′), 106.2 (C-1′), 111.9 and 112.6 (C-2, C-5), 123.6 (C-6), 126.0 (C-α), 129.3 (C-1), 143.5 (C-β), 150.5 and 152.6 (C-3, C-4), 165.4 (C-2′, -6′), 167.0 (C-4′), and 193.4 (C=O); *m/z* (EI) 330 (M^+, 40%); 329 (14), 193 (11), 164 (53), 151 (100), and 149 (26), which is identical with that of the flavanone above (Found: C, 65.2; H, 5.3. $C_{18}H_{18}O_6$ requires C, 65.4; H, 5.5%).

Kinetic Measurements.—Except where mentioned, the cyclisation reactions were carried out under the conditions previously used[1] for (**7**): aqueous solutions containing 4% v/v ethanol, buffered (10^{-2} mol dm^{-3}) as required, μ 1.0 mol dm^{-3} (KCl), 30 °C. Details can be obtained by referring to the earlier paper,[1] which should also be consulted for methods of spectrophotometric monitoring of reactions, of pH (pD) measurement, and of kinetic analysis of the time dependence of absorbance.

Results

Reaction Kinetics in Water.—Also detailed in the previous paper[1] is the derivation of equation (1), which gives the theoretical rate profile in terms of the rate coefficients (k_1–k_5)



Scheme 2.

of Scheme 2 and the fractions (f^A, f^B, f^C) of neutral (**1a**), monoanionic (**1b**), and dianionic (**1c**) chalcone, which vary with pH as a function of the dissociation constants (K_1 and K_2).

$$k_{obs} = k_1 f^A a_{H+} + k_2 f^A + k_3 f^B + k_4 f^C + k_5 a_{OH^-} \quad (1)$$

The origin of the last (k_5) term in the equation, which represents (Scheme 2) a contribution to the measured rate coefficient of the reverse ring-opening of flavanone anion to chalcone at high hydroxide activity (a_{OH^-}), was also explained in the previous paper.[1] The rate coefficients and equilibrium constants which gave the best fit (lines in rate profiles; see the Figure) of equation (1) to the experimental data (points in the rate profile figures) are collected in Table 1. Included for comparison in Table 1 are the corresponding rate coefficients and equilibrium constants (reported earlier[2] for slightly

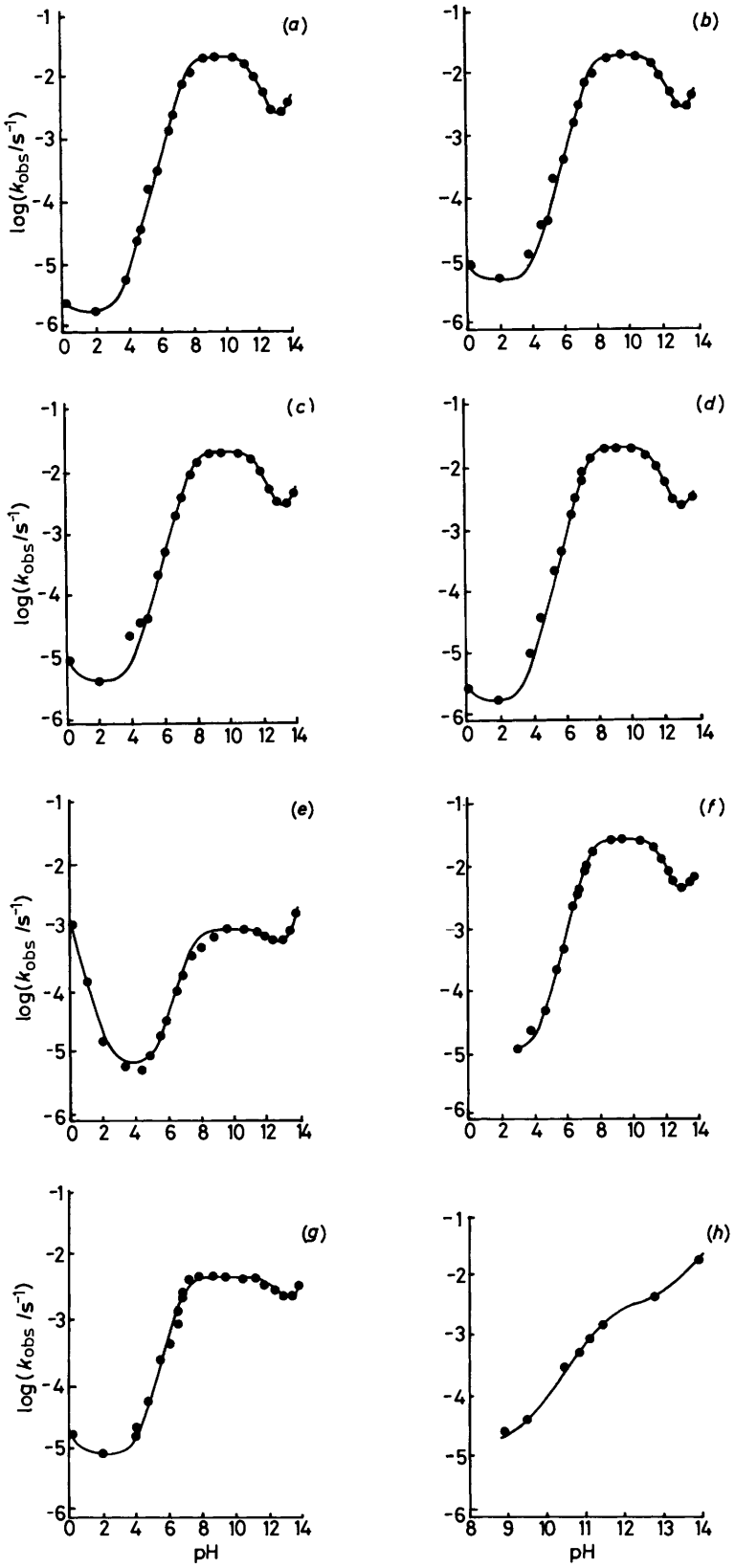


Figure. Plots of log k_{obs} *versus* pH for chalcones (**1**)–(**6**) (*a*)–(*f*) and (**8**) (*g*), and (from ref. 2) for (**9**) (*h*), as labelled by numbers on the plots. Points are experimental. Lines are theoretical and are based on equation (1) and the rate and the equilibrium constants listed in Table 1.

different reaction conditions; see footnote *b* to Table 1) for 2′-hydroxy-6′-methoxychalcone (**10**) and 2′-hydroxy-4′-methoxychalcone (**11**). Also included is 2′,4′-dihydroxychalcone (**9**) for which the raw kinetic data previously reported[2] have now been analysed by the method used for 2′,6′-dihydroxychalcones to give the rate coefficients for contributing reactions. Comparison

Table 1. Rate coefficients of contributing cyclisation reactions and dissociation constants for 2′,6′-dihydroxychalcones and other 2′-hydroxychalcones. (Units: k_2, k_3, k_4 in s^{-1}; k_1, k_5 in $dm^3\ mol^{-1}\ s^{-1}$); T = 30 °C; μ 1.0 mol dm^{-3} (KCl).

Chalcone	10^7k_1[a]	10^6k_2[a]	10^4k_3	10^4k_4	10^3k_5	pK_1	pK_2	$k_3^{H_2O}/k_3^{D_2O}$ (KIE)
(1)	9.6	1.6	210 (200)[b]	13	2.6	7.6	11.7	3.4
(2)	39	4.9	205	13	4.0	7.6	11.7	5.7
(3)	43	3.9	204	11	4.1	7.6	11.7	4.9
(4)	14	1.6	210	12	2.5	7.6	11.7	3.0
(5)	15 000	6.5	9.8	3.5	1.5	7.4	12.1	7.5
(6)	—	—	210	20	2.4	7.6	11.7	2.9
(7)[c]	37	9.7	42.5	13.7	1.5	6.9	12.3	5.7
(8)	78	7.6	44	13	2.0	6.8	12.3	5.0
(9)[b]	—	—	0.18 (18)[d]	34	14	—	11.7	—
(10)[b]	—	—	5.3	—	630	8.95	—	—
(11)[b]	—	—	27	—	1 170	9.55	—	—

[a] These k values have higher experimental error owing to the small number of experimental data used in their estimation. [b] Values for water containing 5 vol% dioxane (μ 0.5 mol dm^{-3} with KCl) reported previously[2] for (9)–(11) and for the first time for (1). [c] From ref. 1. [d] Corrected for approximately estimated fraction of the monoanion of (9) in the reactive (2′-O^-, 4′-OH) form (see the last section in the Results).

Table 2. Activation parameters for the monoanion cyclisation reactions (k_3) of 2′,6′-dihydroxychalcones (E_a, $\Delta H^‡$, $\Delta G^‡$ in kJ mol^{-1}; $\Delta S^‡$ in J $mol^{-1}\ K^{-1}$; standard deviations in brackets).

Chalcone	10^5A	E_a	$\Delta G^‡$	$\Delta H^‡$	$-\Delta S^‡$
(1)	7(3)	61.3(0.9)	84(2)	58.8(0.9)	84(3)
(2)	2.4(1)	58.6(0.9)	84(2)	56.1(0.9)	93(3)
(4)	2.2(0.7)	58.4(0.7)	84(1.4)	55.9(0.7)	94(2)
(5)	36(8)	72.9(0.5)	91.7(1)	70.4(0.5)	70(2)
(6)	3.2(2)	59.4(1.0)	84.3(2.3)	56.9(1.0)	91(4)

of these data allow the accelerative effect on monoanion cyclisation of 6′-OH over 6′-OMe and over 4′-OH to be quantified (see the last section in the Results).

Reaction Kinetics in Deuterium Oxide.—The full profiles were not determined but as the rate coefficient for the unimolecular cyclisation (k_3) of the chalcone monoanion (**1b**) is given by the measured k_{obs} anywhere on the rate plateau (pH 8–11 in H_2O), measurement of k_{obs} anywhere on the corresponding plateau in D_2O provides the corresponding $k_3^{D_2O}$ value. The plateau may be somewhat displaced for D_2O because of changes in K_1 and K_2 (a pK shift of +0.5 is typical for phenols[7a]) but routinely, agreement was found between k_{obs} values measured in D_2O (98.5% isotopic purity after allowance for added EtO*H* and *H*CO_3^-) at pD 10.3 and 10.9, which must therefore be on the plateau for D_2O. These k_{obs} values are recorded in Table 1 only in the form of the ratio of $k_3^{H_2O}/k_3^{D_2O}$, *i.e.* as the kinetic isotope effect (KIE) values.

Effect of Temperature on the Rate of Monoanion Cyclisation.—The shape of the pH–rate profile would be expected to change with temperature not only as a result of increasing rate coefficients but also of shifting dissociation constants. Such latter shifts with temperature are never substantial, however, and the effect of temperature on the rate coefficient for cyclisation of the monoanion (k_3) was therefore obtained (as k_{obs}) at a single mid-plateau pH using carbonate buffer solutions (0.01 mol dm^{-3}) with a ratio HCO_3^-:CO_3^{2-} of 3:7 (pH *ca.* 10.1 at 30 °C). Values of k_{obs} at 8 or 9 temperatures over the range 15–45 °C were determined for each chalcone in water. All data gave excellent linear plots of log k_{obs} *versus* T^{-1}. Activation parameters, calculated from gradients and intercepts by the standard method,[7b] are listed with their standard deviations in Table 2.

Buffer Effects.—Buffer dilution checks showed that buffers at the concentration (0.01 mol dm^{-3}) employed had a negligible effect on the rate coefficients. In the case of carbonate buffer, the effect at higher buffer concentration (up to 0.1 mol dm^{-3}) was checked because study of the stereochemistry of cyclisation in D_2O (see below) involved the use of much higher concentrations of the chalcone in solution than used in kinetic work and the higher buffer concentration was needed to ensure pH control. The possibility existed that a buffer-catalysed pathway might exist and have a different *syn*:*anti* ratio from that of the uncatalysed reaction (to which the kinetic data pertains). It was necessary to determine, then, the maximum possible contribution from any buffer catalysis in rate terms so that any uncertainty associated with whether the measured *syn*:*anti* ratio applies to the uncatalysed reaction could be quantified. Therefore the rate coefficient k_3 for chalcone (**1**) was measured (as k_{obs} in the plateau region) as a function of buffer concentration. Ten kinetic runs were carried out with a range of total buffer concentration up to 0.1 mol dm^{-3} for a HCO_3^-:CO_3^{2-} ratio of 2:8, and a similar set of runs with a 7:3 ratio. In both cases the effect of buffer concentration was so small that, although there was a general increase in rate evident, the experimental uncertainty in rate data left it unclear as to whether the increase was linear and prevented any catalytic rate coefficient being accurately calculated from the gradient. Maximum values can be put on the catalytic effect. For the 2:8 ratio buffer, this value is *ca.* 10% because k_{obs} increased from 2.0×10^{-2} to *ca.* $2.2 \times 10^{-2}\ s^{-1}$ at 0.1 mol dm^{-3}. For the 7:3 ratio buffer, the increase was about twice this much to *ca.* $2.4 \times 10^{-2}\ s^{-1}$ at 0.1 mol dm^{-3}. Therefore under the conditions of the stereochemistry experiment (see below) in which a 1:1 buffer ratio and total buffer concentration of 0.10 mol dm^{-3} were used, the maximum possible contribution from any catalysed reaction would be *ca.* 15% and thus it would be unable to mask significantly the *syn*:*anti* ratio of the uncatalysed reaction. The higher catalytic effect of HCO_3^- over CO_3^{2-}, implicit in the above results, suggests the possibility of very weak general acid catalysis but it is much too poorly defined to be reliably assigned to such an effect.

Measurement of the Product Hydrogen Isotope Effect (PIE) for Incorporation of H(D) at the 3-Position of the 5-Hydroxyflavanone Products from the Cyclisation of (**2**) *and* (**7**) *in* H_2O/D_2O *Mixtures.*—Irrespective of the origins of the overall KIE obtained as the rate coefficient ratio for monoanion

Table 3. Relative intensities (s.d.) of mass spectrum peaks (mu).

(*a*) For the flavanone from cyclisation of chalcone (**2**)

	269	270	271
Pure FlHH	73.0(1.9) $[M-1]^+$	100.0 $[M]^+$	17.4(0.5) $[M+1]^+$
Pure FlHD (assumed; *cf.* FlHH)		73.0 $[M-1]^+$	100.0 $[M]^+$
Cyclisation mixture [FlHH (fraction f^{FlHH}); FlHD (fraction f^{FlHD})]	64.3(1.4)	100.0	30.4(0.6)
FlHH after exchange test	70.4(1.2)	100.0	17.7(0.4)

(*b*) For the flavanone from cyclisation of chalcone (**7**)

	283	284	285
Pure FlHH	62.6(1.9) $[M-1]^+$	100.0 $[M]^+$	17.4(0.5) $[M+1]^+$
Pure FlHD (assumed; *cf.* FlHH)		62.6 $[M-1]^+$	100.0 $[M]^+$
Cyclisation mixture [FlHH (fraction f^{FlHH}); FlHD (fraction f^{FlHD})]	50.3(1.1)	100.0	47.1(1.3)
FlHH after exchange test	60.9(1.1)	100.0	18.9(0.5)

cyclisation in H_2O relative to D_2O, the kinetic isotope effect for just the (final) step in which a proton is transferred to the α-carbon (C-3) of the flavanone can be independently established simply by determining the ratio of all-protio flavanone [FlHH; (**12**)] to monodeuterio flavanone [FlHD; H_{ax} and/or H_{eq} in (**12**) replaced by D: see structures (**13**) and (**14**) below] when the cyclisation is conducted in H_2O/D_2O mixtures so as to allow discrimination between solvent H and D. The ratio of the latter must be taken into account in determining this isotope effect which is here termed the product isotope effect (PIE) to distinguish it from the kinetically measured KIE. Mass spectral measurements provide an accurate method for its determination as the ratio of FlHH to FlHD. The cyclisation reactions were carried out as follows. The chalcone (3 mg) in dry dioxane (0.5 cm^3) was added to a buffered H_2O/D_2O mixture (4 cm^3; $[HCO_3^-] = [CO_3^{2-}] = 0.01$ mol dm^{-3}; μ 1.0 mol dm^{-3} with KCl) in contact with hexane (4 cm^3). The mixture was shaken until colourless [4 h for (**2**) and overnight for (**7**)]. The hexane prevents precipitation of (partially deuteriated) flavanone by extracting it as it is formed. It was hoped that any base-catalysed H or D exchange in the flavanone subsequent to cyclisation which might change the ratio of FlHH to FlHD away from that associated just with chalcone cyclisation (kinetically controlled deuteriation) would be minimised in the non-polar environment of the extracting solvent. This was clearly the case because independent checks in which pure flavanone was subjected to the same reaction conditions as those described above for chalcone followed by the same work up procedure (below) showed that deuterium incorporation was negligible (see mass spectral results for the exchange test in Table 3). Prevention of exchange is achieved however only at the cost of altering the reaction conditions somewhat from those of the kinetic runs. After completion of reaction, the hexane layer was separated, added to chloroform (10 cm^3) and shaken vigorously with water (2 × 200 cm^3) to ensure exchange of any phenolic OD to OH. The solution was separated, dried ($MgSO_4$), and solvent removed under vacuum.

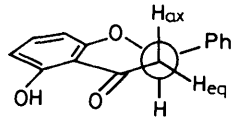
The flavanone samples obtained from chalcone cyclisation as well as samples obtained by subjecting pure flavanone to the same reaction and work up conditions as used in the cyclisation (as a check for any deuterium incorporation resulting from exchange subsequent to, rather than in, the final step of the chalcone cyclisation) were analysed on a Kratos MS30 spectrometer. Also analysed were samples of the pure flavanones (FlHH) to provide the comparative data needed to analyse the extent of incorporation of deuterium. For each sample, the relative intensities of the peaks in the molecular-ion region were averaged for 50 scans. The data are reported in Table 3 along with standard deviations.

In the absence of samples of the 3-deuterio flavanones (FlHD) we assume in analysing the data that the ratio of the $[M]^+$ and $[M-1]^+$ peak intensities will be the same for these as it is for the corresponding all-protio flavanones (FlHH). The (natural) isotopic composition of the ions should not differ between FlHH and FlHD, apart from the presence of absence of D incorporated in the reaction, but there is also the assumption that proton loss from the 5-OH of a parent molecular ion to give an ion reduced by 1 mass number ($[M]^+ - H^+ \longrightarrow [M-H]^+$) is not subject to a secondary isotope effect associated with the presence or absence of deuterium at the 3-position of the flavanone. Any such effect could change the relative intensities of the $[M-1]^+$ and $[M]^+$ peaks in FlHD away from the values (Table 3, line 2) assumed to be the same as those measured for FlHH. It is doubtful that a long-range isotope effect of this sort would be significant, but the analysis is subject to slight uncertainty for this reason.

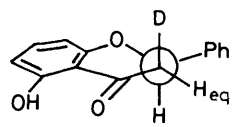
For the cyclisation mixture from chalcone (**2**), the ratio of the intensities of the mass 271 and 270 peaks is 0.304 (Table 3, line 3) and this value is determined by the fractions f^{FlHH} and f^{FlHD} of the all-H and monodeuterio flavanone in the sample, the peak intensities of which are given in Table 3 (lines 1 and 2). Thus the intensity ratio of 0.304 equates to $(17.4\ f^{FlHH} + 100.0\ f^{FlHD})/(100.0\ f^{FlHH} + 73.0\ f^{FlHD})$ from which the value of f^{FlHH}/f^{FlHD} is obtained as 6.0. For chalcone (**2**), the ratio of D to H in the water used in the cyclisation was 1.32, and when this correction factor is applied, the ratio of protiated to deuteriated product for equal competition is 7.9 (s 0.7). This product isotope effect value, therefore, differs from the KIE of 5.7.

For chalcone (**7**), the corresponding intensity ratio (0.471) equates to $(17.4f^{FlHH} + 100.0f^{FlHD})/(100.0f^{FlHH} + 62.6f^{FlHD})$ based on the data in Table 3. This leads to a ratio value of f^{FlHH}/f^{FlHD} of 2.37, which when corrected for the employed solvent D to H ratio, gives a PIE value of 3.8 (s 0.3) which is in this case smaller than the KIE.

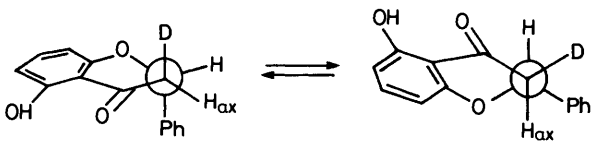
Stereochemistry of Addition in the Cyclisation of the Monoanion of 2′,6′-Dihydroxychalcone (**1**).—The reaction conditions and extraction were exactly as for the product isotope effect experiments described above for chalcones (**2**) and (**7**), except that the bicarbonate buffer ($[HCO_3^-] = [CO_3^{2-}] = 0.05$ mol dm^{-3}) was prepared in deuterium oxide (99.5%). When the ^1H n.m.r. spectrum of the product flavanone was recorded at 200 MHz in C_6D_6, the signals at δ 2.48 [3-H_{ax}; *cf.* (**12**)] and 2.25 (3-H_{eq}) were completely separated (in spite of *J* values of *ca.* 12 and 4 Hz, respectively, for coupling with the 2-H proton at δ



(**12**) FlHH



(**13**) *syn* adduct FlHD



(14a) *anti* adduct FIHD (14b)

4.65). Assignment of an integral value of unity to the signal of the latter (non-exchangeable) proton and comparison with the integral values for protons at the two non-equivalent 3-positions established the product to contain 0.445 H atoms per molecule at the 3-H_{ax} position, representing the *anti* addition product (**14a**, **b**) and 0.55 H atoms per molecule at the 3-H_{eq} position, representing the *syn* addition product (**13**). Deuterium incorporation during cyclisation was therefore to the extent of 0.555 atoms per molecule at 3-H_{ax} [product (**13**)] and 0.45 atoms per molecule at 3-H_{eq} [product (**14**)]: the ratio of *syn* to *anti* addition is *ca.* 55:45.

This ratio is subject to some uncertainty for two reasons. First, there is some all-protio flavanone product present in the largely mono-deuteriated product, the 3-H signals of which are not separated from those integrated as representing the deuterio compounds. This product arises from the presence of small amounts of H in the deuteriated solvent, the extent of which will become amplified in the product if a large product isotope effect, expected by comparison with the results for chalcones (**2**) and (**7**) above, applies. The isotope effect is not expected to be the same for the *anti* and *syn* additions so a distortion could result in the calculated *anti*:*syn* ratio as a result. The amount of the all-H flavanone in the product can be roughly gauged because its 3-H signals are superimposed on those of the monodeuterio compounds as sharp dd signals; the 3-H signals in the monodeuterio compounds are broadened through coupling to deuterium. We estimate there could be up to 5% of the all-H flavanone present. Another possible source of distortion is a contribution from a buffer-catalysed pathway which (see buffer catalysis section above) could account for as much as 15% reaction under the conditions employed. This would doubtless have a different *anti*:*syn* ratio from that of the uncatalysed (H_2O) reaction which we wish to establish. However, as for the presence of a little all-H flavanone, this effect can not change the ratio of *anti* to *syn* addition significantly from the calculated value. Irrespective of the minor uncertainties, it is clear that the uncatalysed cyclisation reaction gives nearly equal amounts of the *anti* and *syn* products.

In an earlier experiment on chalcone (**2**) in which the hexane extraction technique was not employed, ^{1}H n.m.r. analysis of the product flavanone revealed, by a deficiency in the net integral of the 3-H signals as compared with that of the 2-H signal, that about 13% dideuterio flavanone must have been present. The estimated *anti* to *syn* ratio under these circumstances is therefore subject to uncertainty because of the lack of knowledge of the stereochemical preference of the exchange reaction. Nevertheless the calculated *anti* to *syn* ratio of about 1:1 was similar to that reported for chalcone (**1**).

Estimation of the Magnitude of the Accelerative Effect of the 6′-OH Group in Monoanion Cyclisation: Comparison with 6′-OMe and 4′-OH Derivatives.—Irrespective of mechanism, the possibility[1] of the 6′-OH group having a specific catalytic (as opposed to a simple steric) effect on cyclisation led us to try and assess quantitatively the magnitude of any such effect. The 6′-OMe group of 2′-hydroxy-6′-methoxychalcone was chosen as being electronically similar* to the 6′-OH group but without the ability to hydrogen bond to or to protonate the carbonyl oxygen. The known[2] rate of monoanion cyclisation for 2′-hydroxy-6′-methoxychalcone was available for comparison, as was also[2] the (proximity) effect of 6′-OMe *versus* 4′-OMe on cyclisation rates. It was recognised nevertheless that different through-space (steric; electrostatic) effects of 6′-OMe *versus* 6′-OH might invalidate comparison. In particular, the different directional effect of the oxygen lone pairs (towards the carbonyl oxygen for 6′-OMe; away from it for 6′-OH) was seen to be a limitation. The comparison was therefore extended to include one with 4′-OH using earlier data for 2′,4′-dihydroxychalcone.

For the comparison of rates of chalcone monoanion cyclisation of 2′,6′-dihydroxychalcone (**1**) and 2′-hydroxy-6′-methoxychalcone (**10**) monoanions, it was chosen to repeat measurements on (**1**) just in the plateau region under the conditions of the previous study on (**10**). This avoided the need for the full pH–rate profile measurement and the detailed analysis required to extract the monoanion rate coefficient of (**10**) if it were to be restudied under the present conditions; there is[2] no plateau representing monoanion cyclisation for 2′-hydroxychalcones because of the intervention of the reverse ring-opening reaction of (neutral) flavanone at lower pH. The result was that monoanion cyclisation (k_3; Table 1) was found to be approximately 40 times faster for (**1**) than for (**10**) under identical conditions (footnote *b*; Table 1).

The separate estimate of the magnitude of the accelerative effect of the 6′-OH group based on comparison with the 4′-OH group was made as follows. The kinetic data[2] for 2′,4′-dihydroxychalcone [**9**; see Figure (*b*)] now accounted for quantitatively (Table 1) show the monoanion to cyclise with a rate coefficient (k_3) of 1.8×10^{-5} s^{-1}; *cf.* 2.0×10^{-2} s^{-1} for (**1**). The rate coefficient for (**9**) is expected to be low because the thermodynamically favoured monoanion (4′-O^-, 2-OH) is unreactive in cyclisation as compared with the unstable but reactive (2′-O^-, 4′-OH) monoanion. The rate coefficient for the latter is required and it can be calculated if the fraction of total monoanion present in the reactive form is known. This fraction is given by the ratio of the two chalcone first dissociation constants K_a(2′-OH)/K_a(4′-OH). These K_a values can be estimated, but only roughly, by assuming the same electronic effects on dissociation constants by OMe as by non-hydrogen-bonded OH groups. Thus, pK_a for 2′-OH of 2′,4′-dihydroxychalcone (**9**) is taken to be *ca.* 9.6 (the pK_a of 2′-hydroxy-4′-methoxychalcone[2]) and pK_a for 4′-OH to be *ca.* 7.6 (the first pK_a of the non-hydrogen-bonded OH of 2′,6′-dihydroxychalcone). The fraction of the 2′,4′-dihydroxychalcone monoanion in the reactive (2-O^-,4-OH) form is therefore *ca.* 0.01, so that the corrected k_3 value for cyclisation of this species is *ca.* 1.8×10^{-3} s^{-1}, *i.e.* about an order of magnitude smaller than that for the monoanion of 2′,6′-dihydroxychalcone under the same conditions. This suggests that the 6′-OH group has about a tenfold accelerative effect of non-steric origin which is associated with its proximity to the carbonyl group. The additional effect of the 6′-OMe (another fourfold factor to give a total factor of 40: see above) might then be assigned to unfavourable steric or electrostatic (lone pair) influences, including possibly ones on conformation, as compared with 6′-OH. By comparison can be noted (Table 1) a similar small (fivefold) rate depression of chalcone monoanion cyclisation of 2′-hydroxy-6′-methoxychalcone (**10**) over 2′-hydroxy-4′-

* The difference in the pK_a values of 2′,6′-dihydroxychalcone (**1**) (pK_a 7.6) and 2′-hydroxy-6′-methoxychalcone (**10**) (pK_a 8.95) might superficially appear to invalidate the assignment of electronic equivalence of 6′-OH and 6′-OMe but it is accounted for by the (first) ionisable proton being hydrogen-bonded in (**1**) but not in (**10**) as we discussed[1] in our earlier paper. The corresponding difference in basicity of the 2′-O^- groups would not be reflected in nucleophilicity because hydrogen bonding is irrelevant to products of *O*-alkylation.

methoxychalcone (**11**) assignable to some equivalent effect of the 6′-OMe group.

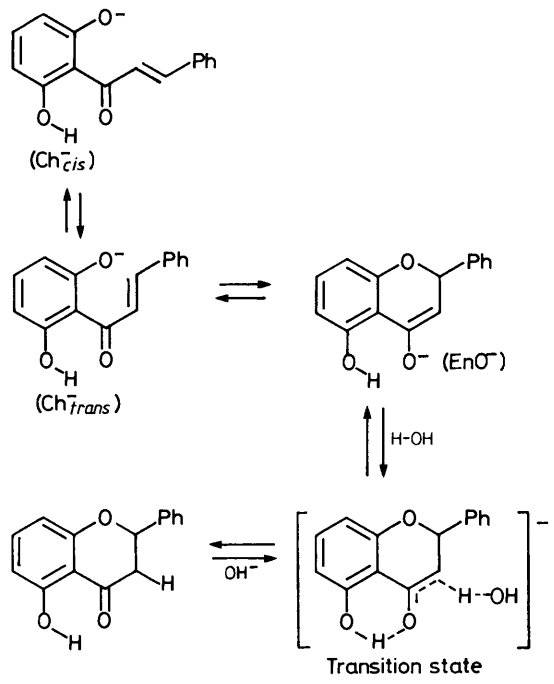
In summary, the 6′-OH group appears to have a definite accelerative effect of non-steric origin on 2′,6′-dihydroxychalcone monoanion cyclisation, but it is probably no larger than an order of magnitude.

Discussion

Mechanism Terminology.—Most kinetic studies on conjugate addition–elimination equilibria have been made for convenience in the reverse (elimination) direction on systems which lack the 2′-hydroxychalcones' intramolecular efficiency in the forward (addition) direction. It is therefore sensible for coherence of mechanistic analogy to base the present mechanistic discussion on the well-established elimination terminology, even though transition states are here being approached in the addition direction. Of particular note is that the primary isotope effect observed here as the PIE for proton transfer to carbon in the transition state for the final step in the addition direction has as its counterpart the primary kinetic isotope effects observed as criteria for rate-limiting proton abstraction from carbon in the first step of elimination reactions with E2 or irreversible E1cB mechanisms.

Origin of the Accelerative Effect of 6′-OH *on Flavanone Formation.*—In a recent paper,[8] an E1cB type of mechanism is rejected in favour of a concerted (E2) type of mechanism for 2′-hydroxychalcone monoanion cyclisation, but for no apparent reason; the evidence reported is equally consistent with a stepwise reaction involving rate-limiting ketonisation of enolate intermediate. Support for the E1cB mechanism comes by analogy with the very closely related elimination reactions of *para*-substituted 4-phenoxybutan-2-ones, as studied by Fedor,[9] for which there is unequivocal evidence of partitioning of an enolate intermediate. The E1cB mechanism is thus taken to provide the basis for the discussion which follows on the role of the 6′-OH group in accelerating cyclisation of 2′,6′-dihydroxychalcones, though the arguments need little modification to accommodate an E2-type mechanism with an enolate-like transition state.

The simplest E1cB mechanism is that of Scheme 3 with rate-limiting ketonisation represented by the transition state shown. It should be understood that the enolate (EnO^-) would be in equilibrium with an enol (EnOH; not shown, but see Scheme 4) in which the 6′-OH proton is transferred to enolate O^- but that this would be kinetically irrelevant since the enol is not on the reaction pathway to product. If the ketonisation is the rate-limiting step, an explanation is required as to how the 6′-OH group stabilises the transition state for ketonisation more so than it stabilises the reactant chalcone anion (Ch_{cis}^-; Scheme 3). The most likely explanation is that hydrogen bonding stabilises a larger partial negative change on oxygen in the transition state than exists on carbonyl oxygen in Ch_{cis}^- (though delocalisation from 2′-O^-). The degree of stabilisation of the transition state (*i.e.* the accelerative effect of the 6′-OH group) would then be expected to be largely a function of the residual charge on the enolate oxygen in (*i.e.* lateness of) the transition state for ketonisation although, apart from its direct electrostatic effect, hydrogen bonding may well also provide favourable conformational influences in the transition state. Hydrogen bonding by 6′-OH would of course also stabilise the enolate intermediate (EnO^-) with its almost full negative charge on oxygen and stabilise it more so than either the reactant chalcone monoanion (Ch_{cis}^-) or the transition state for ketonisation. Kinetically, however, this is irrelevant if ketonisation is rate-limiting because if the transition state for

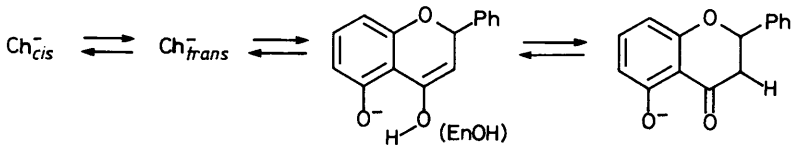


Scheme 3.

ketonisation had turned out to be sufficiently late that the residual charge on the enolate oxygen were smaller than the partial charge on the carbonyl oxygen of the ground-state reactant Ch_{cis}^-, net retardation by 6′-OH, not catalysis, would be expected, *in spite* of a more favourable equilibrium constant for formation of the enolate.

In terms of the above mechanism, the large overall kinetic isotope effect value ($k_3^{H_2O}/k_3^{D_2O}$) would be largely derived from rate-limiting proton transfer to carbon in the ketonisation step, the primary kinetic isotope effect values of which have been determined from the extent of deuterium incorporation into product as product isotope effect values in two cases [(**2**) and (**7**)]. The PIE values will of course not be the same as the KIE values determined kinetically by comparing rates in D_2O *versus* H_2O because the latter incorporate general solvent effects (H_2O *versus* D_2O) as well as the effect of hydrogen-bonded OH *versus* OD on stabilities of intermediates and on the rate-limiting step transition state (Scheme 3: second H-bonded proton only, not the one being transferred to C). If there is ultimately a full explanation for the variation in KIEs as a function of substituent effects on equilibrium isotope effects, predictions about which would be unreliable,[7a,c] it will have to accommodate our observation that for one chalcone (**2**) the PIE of 7.9 is reduced by such contributing isotope effects to a KIE of 5.7 (*i.e.* there is an inverse isotope effect contribution of about 0.7), whereas for another chalcone (**7**) which differs only in that it has an extra 4′-OMe group, the PIE of 3.8 is *increased* by a normal isotope effect contribution of about 1.5 to a KIE of 5.7. The puzzle is whether or how the very slightly increased acidity of the 6′-OH group of (**7**) relative to (**2**) (*m*-OMe reduces the pK_a of phenol by *ca.* 0.5) could transform an inverse isotope effect contribution into a normal one. In terms of free energy, however, the effects are small and any explanation may be subtle.

Also to be considered is Scheme 4 in which there is full proton transfer from 6′-OH to the carbonyl oxygen to form an enol (EnOH) directly as the product of cyclisation of Ch_{trans}^-. There seems, however, to be no kinetic advantage in formation of such an enol as a reactant for rate-limiting ketonisation because the enol seems certain to be less reactive than the H-bonded enolate



Scheme 4.

(EnO^-) of Scheme 3; free enolates undergo ketonisation very much faster than their enols. The availability of the 6′-O^- to remove the enol proton has little (thermodynamic) advantage given the similarity of pK_as of enol and phenol, and, moreover, it would imply that for the reverse enolisation reaction of flavanone with OH^- there is intramolecular general acid catalysis by 6′-OH of enol formation. This is unlikely for the same reason, obviously, and further because, when general acid catalysis has been observed[10] for enolisation of a ketone, it has been in the form of bifunctional catalysis by buffer species of $pK_a \leq 5$, *e.g.* acetic acid in tandem with acetate ion as a general base, giving[10] a termolecular term for kinetics of enolisation of acetone. It is therefore doubtful whether the strong base OH^- would require such assistance, and even more doubtful whether the relatively weak acid 6′-OH would be called upon to provide it along the chalcone–flavanone pathway, in spite of its enforced proximity and prior hydrogen bonding to the carbonyl oxygen. This doubt is reinforced somewhat by the observation[11] that intramolecular general acid catalysis by protonated amine does not occur[11] in enolisation of *N*-protonated aminoalkyl phenyl ketones by OH^-, even when the group is ideally placed, as in the present 5-hydroxyflavanone case, to transfer a proton to the carbonyl oxygen in a 5- or 6-membered ring; the catalytic effect found is very similar to that for cationic *N*-methylated analogues and is assigned to[11] a simple electrostatic effect of the cationic charge. However, the net effect of 6′-OH is only small in the present study, so the possibility of weak catalysis of this type can not be entirely excluded.

A modification of Scheme 3 is one with EnO^- as an additional intermediate between EnOH and flavanone product, *i.e.* Ch_{trans}^- is first trapped as EnOH as a result of prior hydrogen bonding of 6′-OH to the carbonyl oxygen, the enol only then yielding an enolate (EnO^-) as a reactant for ketonisation. This is distinct from Scheme 2 only if EnO^- is formed after the transition state for the rate-limiting step; otherwise EnO^- is in equilibrium with reactant Ch_{trans}^- and this is kinetically equivalent to Scheme 2 with rate-limiting ketonisation. To meet this limitation, the observed primary KIE values would have to be assigned to rate-limiting proton transfer in enol formation, *i.e.* proton transfer between 6′-OH and carbonyl O in the transition state for the Ch_{trans}^- to EnOH step. Certainly the pK_a values of 6′-OH and EnOH (both *ca.* 10) would put such a proton transfer in the (narrow) ΔpK_a region known[12] to be essential for (intermolecular) proton transfer from oxygen to become kinetically significant (in the face of diffusion control at larger ΔpK_a values[12]). However, this criterion is hardly likely to be relevant in an intramolecular situation if the acid (as here for 6′-OH) is already hydrogen-bonded to the carbonyl oxygen; rate-limiting diffusion is not available to mask an isotope effect associated with proton transfer at oxygen. More to the point, then, is that previously observed large primary kinetic isotope effects for proton transfer at oxygen have been limited to intermolecular general acid trapping of intermediates of much shorter lifetime than is likely for the enolate of the present study, *e.g.* zwitterions formed by addition of amines to carbonyl groups.[13] Therefore, although Scheme 4 with rate-limiting formation of an enol cannot be excluded with certainty, the mechanism of Scheme 2 with a solvent-equilibrated enolate intermediate is preferred.

Effect of Other Substituents on Rate of Monoanion Cyclisation.—The 4′-OMe (A ring) substituents of (**7**) and (**8**) depress the rate of monoanion cyclisation by a factor of 5 over the 4′-H counterparts (**2**) and (**3**). This is accounted for by more effective ground-state Ch_{cis}^-) stabilisation through delocalisation from 4′-OMe to the (hydrogen-bonded) carbonyl oxygen than stabilisation through any such partial delocalisation in the transition state for ketonisation. A similar effect of the 4′-OMe group was reported[2] for 2′-hydroxychalcones in the absence of hydrogen bonding.

Similar ground-state stabilisation effects unfavourable to the rate would be expected for electron-donating groups at the 2-, 4-, or 6-positions, since the B ring probably retains no conjugation with the enone in the transition state for the ketonisation step. For the compounds studied, the effect should be maximum for the 2,4,6-trimethoxy derivative (**5**), assuming planarity of the monoanion, as applies for the neutral compound (*s-cis* conformation) in the solid state.[4] This is consistent with its twenty-fold rate depression. The latter depression results from a higher $\Delta H^{\ddagger}$ (Table 2) as expected, but this is partly offset by a more favourable entropy factor [$\Delta S^{\ddagger}$ -70 J mol^{-1} K^{-1}; *cf.* -84 J mol^{-1} K^{-1} for (**1**)] which is probably partly associated with the reduced requirement for solvation by polar water molecules of the 2,4,6-trimethoxyphenyl ring in the transition state for ketonisation when delocalisation from, and associated charge development on, the methoxy groups is much reduced as compared with the ground state (Ch_{cis}^-) in which full conjugation through the enone system is available.

Such an entropy factor is not apparent, however, for a single (4-) OMe group as in (**2**). The negligible effect on the rate of monoanion cyclisation in H_2O (k_3; Table 1) of the 4-OMe and other B-ring groups [chalcones (**1**), (**4**), and (**6**)] is not (Table 2) associated with any balance of entropy and enthalpy factors. The rates do vary in D_2O but the small variation (twofold) across the series [(**1**)–(**4**), (**6**)] is a reminder of the very small differences in activation free energy responsible. It is therefore surprising that when converted into KIE values [2.9 to 5.7 for these compounds, and higher (7.5) for the 2,4,6-trimethoxychalcone (**5**)] there is some pattern: in-plane electron-donating methoxy groups [(**2**), (**3**), and (**5**), but not (**4**) which has an out-of-plane 4-OMe] give the higher KIE values. Initial indications are that this trend may be independent of the A-ring substituents because the additional 4′-OMe groups of (**7**) and (**8**), as compared with (**2**) and (**3**), respectively, lead to no change in KIE [5.7 for (**2**) and (**7**); 4.9 and 5.0 for (**3**) and (**8**), respectively]. We have no explanation for this pattern.

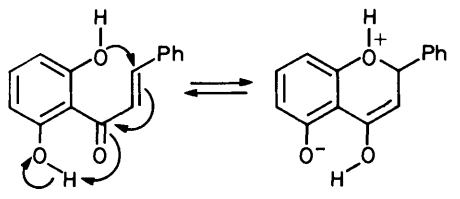
Acid-catalysed and Uncatalysed Cyclisation of Neutral Chalcones.—The calculated rate coefficients (k_1 and k_2; Table 1) are subject to more uncertainty than k_3 values because of the limited number of data points at low pH (see the Figure). Furthermore, no mechanisms are established as a basis for discussion. Nevertheless, some of the notable trends deserve comment.

Methoxy groups in the 4-position of the B ring accelerate the specific acid-catalysed reaction (k_1) presumably by increasing the concentration of enone-protonated conjugate acid. The effect disappears for (**4**) in which the 4-methoxy group is unable to attain the required coplanarity with the B ring. The

delocalisation effect would be maximised for the 2,4,6-trimethoxy chalcone (**5**) and it reacts *ca.* 380 times faster than 4-OMe chalcone (**2**) and 1 500 times faster than 4-H chalcone (**1**). At pH 0, (**5**) cyclises at about the same rate as it does in the monoanionic form in the alkaline pH plateau region (Figure). By contrast, rates for the other chalcones at pH 0 are 3 to 4 orders of magnitude slower than on the corresponding alkaline plateau.

For the isomerisation of *cis*-chalcones in acid solution, exceptionally high reactivity of the 2,4,6-trimethoxychalcone has been reported.[14] A mechanism was proposed involving rate-limiting rotation around the C_α–C_β bond through the non-planar carbocation transition state (or intermediate) in which the carbocation is conjugated to the 2,4,6-trimethoxyphenyl ring but not to the enol. This mechanism applies also to the 4-OMe chalcone, but for the 4-H compound it is too inefficient, in the absence of sufficient carbocation stabilisation, to compete with an alternative mechanism involving rotation after addition of water to the protonated enone. The relative rates for the series 2,4,6-$(OMe)_3$:4-OMe:4-H were $3 \times 10^8 : 10^5 : 1$, which contrast sharply with the present results, in which the single (4-) methoxy group has relatively very little effect (k_1; Table 1). This suggests that if the non-planar carbocation is involved in the 2,4,6-trimethoxychalcone cyclisation, it may not be for the 4-methoxy one. A difference between the chalcone cyclisation and *cis*-chalcone isomerisation is not unexpected, however. In the latter case the carbocation is essentially the transition state for a unimolecular rotation; for chalcone cyclisations the carbocation would have to exist long enough to be trapped by 2′-OH. Perhaps the 4-OMe provides insufficient stabilisation to increase the lifetime of the carbocation such that trapping can occur, whereas the 2,4,6-trimethoxy case is beyond that stability threshold. An alternative explanation for the high reactivity of the 2,4,6-trimethoxy chalcone is that competing C_α protonation of the enone, rather than the thermodynamically favoured *O*-protonation, is kinetically important. *C*-Protonation would avoid the rigidity of the *O*-protonated enone system and possibly provide flexibility in achieving a more favourable conformation for intramolecular cyclisation by 2′-OH; the carbocation is, of course, a more reactive electrophile as well.

For neutral cyclisation (k_2), 2,4,6-trimethoxychalcone (**5**) is only four times more reactive than (**1**) and less reactive than (**7**) and (**8**) [*cf.* 200 and 400 times more reactive than (**7**) and (**8**), respectively, in acid-catalysed cyclisation (k_1)]. Possibly intramolecular proton transfer (Scheme 5) assists this reaction.



Scheme 5.

Geometry excludes *C*-protonation concerted with cyclisation and this might explain the now relatively low reactivity of the 2,4,6-trimethoxychalcone (**5**). The slightly higher acidity of 6′-OH in the 4′-OMe compounds (**7**) and (**8**) may be responsible for their relatively higher reactivity than in the specific acid-catalysed (k_1) cyclisation.

Dianion Cyclisation and the Reverse Ring-opening of 5-Hydroxyflavanone Monoanion.—For 2′,6′-dihydroxychalcone (**1**) the rate of both forward (k_4; Table 1) and reverse (k_5) reactions are reduced compared with 2′, 4′-dihydroxychalcone (**9**), presumably as a result of the proximity of the 6′-O^- group to the carbonyl oxygen, which is itself required to accommodate negative charge in the transition state. The kinetic effect is larger for the reverse reaction. Other substituent effects in the reverse reaction are very small. In the forward direction (k_4) the additional 4′-OMe group of (**7**) and (**8**) has no effect [*cf.* (**2**) and (**3**)] presumably because its deactivating effect through electron donation to the carbonyl oxygen in the monoanion (k_3) is swamped out when both the 2′- and 6′-OH groups are ionised.

Stereochemistry of Cyclisation of the Monoanion of 2′,6′-Dihydroxychalcone (**1**).—The monoanion cyclises in D_2O with little stereoselectivity in deuteriation at C-α (C-3 of 5-hydroxyflavanone product): the *syn*:*anti* ratio for the addition is *ca.* 55:45. Such relatively high fractions of *syn* addition or elimination are not unusual for reactions likely to involve enolate intermediates, including reactions of chalcone derivatives, *e.g.* the elimination of HBr from 2′-hydroxychalcone dibromide[15] (*ca.* 64% *syn* to 36% *anti*) and similar findings in synthetic work reported much earlier by Donnelly and co-workers.[16] If, as is suggested above to be most likely, the cyclisation reaction involves rate-limiting ketonisation, the *syn*:*anti* ratio is determined in that step, and the following stereoelectronic and steric factors are relevant. Structures (**13**) and (**14a**) (one of the enantiomers each of the *syn* and *anti* products, respectively) indicate the conformational requirements for maximum overlap between the C–D bond (partly formed in the transition state for ketonisation) and the C=O π-system (partly formed from the coplanar C=C–O π-system in the transition state). The overlap is the stereoelectronic factor responsible[17] for the known preference for perpendicular attack of the proton on the enone. Conformation (**14b**) for the *anti* compound would be the thermodynamically favoured conformation for this product in which there is reduced steric interaction of Ph with the chromanone ring, but its direct formation in ketonisation would not meet the stereoelectronic preference of the proton transfer. This requirement is met in (**14a**) in the formation of which there would clearly be less steric hindrance by Ph to protonation (by D_2O) than for the *syn* case (**13**). However, this is only at the expense of a less favourable steric interaction of Ph with the chromanone ring in the transition state as is evidenced from the Newman projection [(**14a**); *cf.* (**14b**) and (**13**)]. The balance of stereoelectronic and steric effects is apparently such that there is little preference between *syn* and *anti* addition.

In a study[3] of the stereochemistry of chalcone cyclisation catalysed by a chalcone–flavanone isomerase enzyme in D_2O, 2′,4,4′-trihydroxychalcone was found to show a preference for *syn* over *anti* addition of 7:3 though this ratio is subject to some uncertainty because of the apparent lack of full stereochemical control in bond-formation at the β-carbon: the (2*S*)-4′,7-dihydroxyflavanone enantiomer, the known natural product, was formed in excess, but not exclusively. However, for the biosynthesis (from a chalcone-like precursor) of 2-methyl-5,7-dihydroxy-8-(2-hydroxyethyl)chromanone, which occurs with full asymmetric control at C-2, equal amounts of *syn* and *anti* addition were found,[18] indicating a lack of enzyme control in proton donation to carbon.

Acknowledgements

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