Kinetics and Mechanism of the Cyclisation of 2',6'-Dihydroxy-4,4'-dimethoxychalcone; Influence of the 6'-Hydroxy Group on the Rate of Cyclisation under Neutral Conditions

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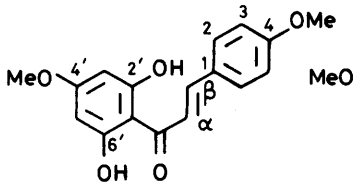
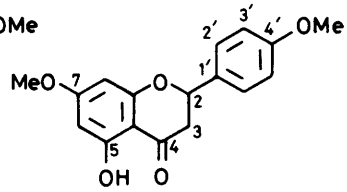
The pH–rate profile for the cyclisation of the title compound has been established under aqueous conditions and is accounted for in terms of contributions from uncatalysed cyclisation of neutral, monoanionic, and dianionic chalcone species, together with an acid-catalysed cyclisation. At high pH the reaction does not go to completion and a kinetic term representing hydroxide promotion of the reverse ring-opening reaction of the flavanone anion intervenes. Rate coefficients for all contributing reactions and dissociation constants for the chalcone and its monoanion are established. The chalcone monoanion cyclises about 440 times faster than the neutral chalcone and about 3 times faster than the dianion. The known ease of cyclisation under neutral conditions of 2',6'-dihydroxychalcones as compared with other 2'-hydroxychalcones is considered to be associated with two contributing factors: (i) a much larger first dissociation constant of the chalcone which results in a higher proportion of the chalcone being present as the reactive monoanion at neutral pH; (ii) specially high reactivity of the chalcone monoanion associated with intramolecular general acid catalysis by the 6'-OH group. The latter is implicated through the observation of a large kinetic isotope effect for monoanion cyclisation, which is 5.7 times slower in D_2O than in H_2O. This isotope effect also establishes for the monoanion that the s-*cis* ⟶ s-*trans* conformational isomerisation of the monoanion must be a pre-equilibrium rather than the rate-determining stage in the cyclisation.

2',6'-Dihydroxy-4,4'-dimethoxychalcone (**1**), which has been isolated from the orange exudate on the reverse side of the fronds of the gold ferns *Pityrogramma austroamericana*[1] and *P. chrysophalla*,[1,2] is one example of the 2',6'-dihydroxychalcones now well established as natural products. For many years only the isomeric 5-hydroxyflavanones [*e.g.* (**2**)], the products of chalcone cyclisation, were recognised in nature. The ease of such cyclisation under neutral conditions distinguishes 2',6'-dihydroxychalcones from other groups of 2'-hydroxychalcones, as has been noted in early synthetic,[3] natural product,[4] and kinetic[5,6] studies. With the role of the 6'-hydroxy group in promoting cyclisation in question, we studied the kinetics of cyclisation of (**1**) and established the full pH–rate profile, the first to be reported for a 2',6'-dihydroxychalcone. It reveals significant differences from the corresponding reactivity of simple 2'-hydroxychalcones, and accounts for the ease of cyclisation under neutral conditions.



(**1**)



(**2**)

Experimental

Materials.—Naringenin (Sigma) was methylated as previously described[7] to give 5-hydroxy-4',7-dimethoxyflavanone (**2**), m.p. 118 °C (lit.,[7] 118 °C). The standard[5,8] ring-opening reaction of the flavanone by refluxing in aqueous ethanolic KOH, followed by pouring into an excess of aqueous hydrochloric acid, gave an orange solid which, after recrystallisation from chloroform–light petroleum (b.p. 40—60 °C) (1:1), gave 2',6'-dihydroxy-4,4'-dimethoxychalcone (**1**), m.p. 156—158 °C (lit.,[1] 157 °C), u.v. and mass spectra as described[1] previously, δ_H [90 MHz; solvent $(CD_3)_2CO$; standard CD_3COCHD_2, δ_H 2.20] 3.96 (3 H, s, OCH_3), 4.00 (3 H, s, OCH_3), 6.18 (2 H, s, 3'-H, 5'-H), 7.14 (2 H, d, *J* 9 Hz, 3-H, 5-H), 7.83 (2 H, d, *J* 9 Hz, 2-H, 6-H), 7.92 (1 H, d, *J* 16 Hz, β-H), 8.31 (1 H, d, *J* 16 Hz, α-H), and 12.23 (2 H, br, s, OH); δ_C [90 MHz; solvent $(CD_3)_2CO$; standard $(CD_3)_2CO$, δ_C 29.85] 55.8 (4- and 4'-OCH_3), 94.6 (3'-C, 5'-C), 106.3 (1'-C), 115.3 (3-C, 5-C), 125.9 (α-C), 129.1 (1-C), 131.0 (2-C, 6-C), 143.2 (β-C), 162.6 (4-C), 165.3 (2'-C, 6'-C), 167.1 (4'-C), and 193.5 (C=O) (Found: C, 67.6; H, 5.4. Calc. for $C_{17}H_{16}O_5$: C, 68.0; H, 5.35%).

Kinetic Measurements.—The cyclisation reaction in unbuffered ($>10^{-2}$ mol dm^{-3} HCl or KOH) and buffered (10^{-2} mol dm^{-3} phthalate, acetate, phosphate, bicine [*NN*-bis-(2-hydroxyethyl)glycine], and carbonate) aqueous solutions (μ 1.0 mol dm^{-3} with KCl) containing 4% v/v added ethanol at 30 °C was monitored spectrophotometrically (Unicam SP 8500) at 365 nm (pH < 13) or at 322 nm (pH > 13). Identical conditions were employed for reactions in D_2O (98.5% isotopic purity after allowance for added EtOH). Values of pH or pD (given[9a] by adding 0.40 to the pH meter reading) were checked on individual reaction solutions after completion of runs, except for values at pH < 2 and pH > 12, which were calculated from known HCl and KOH concentrations. Observed first-order rate coefficients (k_{obs}), calculated as the gradients of plots of $\ln(A_t - A_\infty)$ *versus* time, or for very slow reactions by the Guggenheim method, were accurate and reproducible to within 2—3%. As in a previous study,[10] allowance is made in the kinetic analysis for contributions to k_{obs} from the reverse reaction [(**2**) ⟶ (**1**)] at high pH.

Results

Reaction in Water.—The experimental rate data are given by the points in the pH–rate profile (Figure). For comparison, the

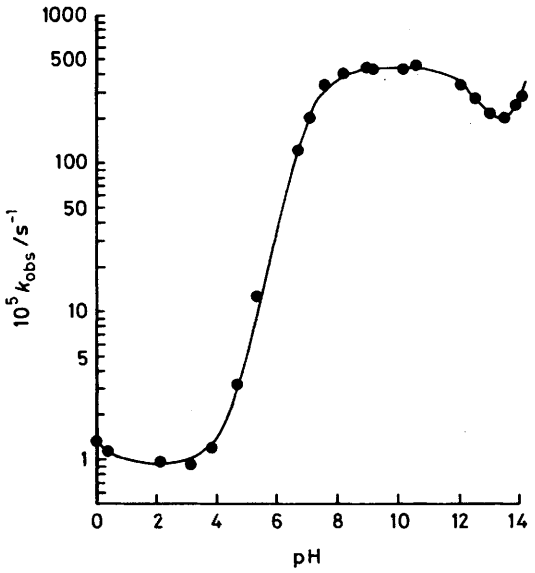
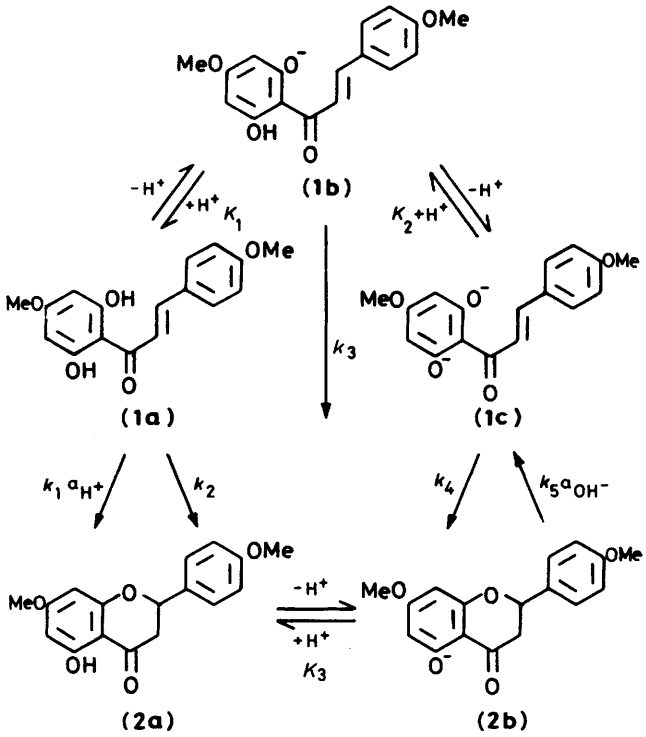


Figure. Semilogarithmic plot of k_{obs} *versus* pH. Points are experimental. The curve is theoretical, being based on equation (4) and the following rate and equilibrium constant values: $k_1 = 3.7 \times 10^{-6}$ dm^3 mol^{-1} s^{-1}; $k_2 = 9.7 \times 10^{-6}$ s^{-1}; $k_3 = 4.25 \times 10^{-3}$ s^{-1}; $k_4 = 1.37 \times 10^{-3}$ s^{-1}; $k_5 = 1.5 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}; pK_1 = 6.90; pK_2 = 12.30



Scheme.

theoretical rate profile (the line in the Figure) was derived as follows. If initially it is assumed that at the infinity stage of the reaction there is no residual chalcone, *i.e.* that the reaction has gone to completion rather than to equilibrium, the observed first-order rate coefficient (k_{obs}) measures only the forward reaction. Allowing for all likely contributions to this reaction {*i.e.* acid-catalysed cyclisation of neutral chalcone [(**1a**); second order rate coefficient k_1; Scheme], unimolecular cyclisations of neutral chalcone [(**1a**); first-order rate coefficient k_2], of chalcone monoanion [(**1b**); k_3], and of chalcone dianion [(**1c**); k_4]} leads to equations (1) and (2) for the rate in the forward

$$\text{Rate} = k_{obs}[\text{total chalcone}] \quad (1)$$

$$= k_1[(\mathbf{1a})]a_{H^+} + k_2[(\mathbf{1a})] + k_3[(\mathbf{1b})] + k_4[(\mathbf{1c})] \quad (2)$$

direction. Dividing by [total chalcone] and employing the symbol f to define fractions of total chalcone present in the form indicated by the superscript leads to equation (3) for k_{obs}. Values

$$k_{obs} = k_1 f^A a_{H^+} + k_2 f^A + k_3 f^B + k_4 f^C \quad (3)$$

of f at any pH can be calculated from the acid dissociation constants (K_1 and K_2; Scheme) for the chalcone, f^B being given by $K_1/(K_1 + a_{H^+})$ and f^C by $K_2/(K_2 + a_{H^+})$.

Trial values of K_1 and K_2 were varied to obtain a good fit of equation (3) to experimental data, the rate coefficients and equilibrium constants being those given in the legend to the Figure. Up to pH 12, there is good agreement with experiment of the rate profile defined by equation (3) (*cf.* the line in the Figure with points up to pH 12). Above pH 12, however, equation (3) predicts k_{obs} values which are too low (not shown in the Figure), the discrepancy increasing with increasing pH. This results from the initial assumption that only flavanone is present at the infinity stage of the reaction. In fact, at high pH, repetitive scans revealed significant amounts of chalcone at the infinity stage of the reaction; the final equilibrium composition and the measured rate coefficient for its attainment (k_{obs}) were the same whether the initial reactant was the chalcone or the flavanone, *e.g.* k_{obs} values at pH 13.68 were 2.52×10^{-3} s^{-1} for chalcone and 2.53×10^{-3} s^{-1} for flavanone, the latter being determined by monitoring an increase in chalcone absorbance. Thus, above pH 12, hydroxide ion is of sufficient activity partially to reverse the reaction and, importantly, the measured k_{obs} values include an additional contribution from the reverse reaction: for a first-order reaction going to equilibrium the measured rate coefficient turns out to be the sum of those for forward and reverse reactions.[9b] Under the pseudo-first-order conditions (constant pH) of individual reactions in the present study, the contribution from the reverse reaction based on the assumption of a first-order dependence on both hydroxide ion and flavanone anion is $k_5 a_{OH^-}$ where k_5 is the second order rate coefficient. This correcting term leads to equation (4) for k_{obs},

$$k_{obs} = k_1 f^A a_{H^+} + k_2 f^A + k_3 f^B + k_4 f^C + k_5 a_{OH^-} \quad (4)$$

the absence of an f factor (*i.e.* $f = 1$) in the last term resulting from the fact that the reverse reaction only intervenes at pH values at which essentially all the flavanone is ionised; that the neutral flavanone (**2a**) (Scheme) makes no detectable contribution to the reverse reaction is rationalised in the Discussion section.

With an appropriate k_5 value (see Figure legend), equation (4) now accurately follows experiment at all pH values, including those above pH 12, as shown by the complete line in the Figure.

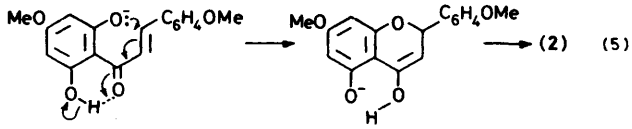
Reaction in Deuterium Oxide.—The cyclisation kinetics were determined at three pD values in the alkaline plateau region in solutions of 98.5% isotopic purity but with conditions otherwise as for reactions in water. Values of 10^4 k_{obs}/s^{-1} (pD in parentheses) were 7.7 (9.91), 7.5 (10.47), and 7.4 (10.93). The reaction represented by the plateau, the cyclisation of the chalcone monoanion, is therefore 5.7 times slower in D_2O than in H_2O.

Discussion

Form of Rate Profile.—The chalcone is most stable at pH 1—3, the uncatalysed cyclisation here being that of neutral chalcone. At lower pH, acid-catalysis intervenes, and at higher pH the rate increases sharply as the chalcone ionises (K_1), reaching a maximum at the plateau (pH 8—10) which represents cyclisation of the chalcone monoanion. Further ionisation (K_2) to the dianion leads to a rate decrease, in spite of increased nucleophilic potential in the dianion. At still higher pH, k_{obs} increases again because hydroxide ion at high activity promotes the reverse ring-opening reaction of flavanone anion (**2b**) ⟶ (**1**). The corresponding reaction of the neutral flavanone (**2a**) makes no detectable contribution, presumably because hydroxide prefers to abstract the phenolic proton to form (**2b**) or, if it abstracts an α-proton (3-position), it forms the enol of (**2b**) [rather than the enolate of (**2a**)], neither of these species leading to eliminative ring-opening without the participation of a second hydroxide ion. The rate coefficient (k_5) calculated for the reverse reaction is *ca.* 10^2—10^3 times smaller than values established[10] in previous studies of simple 2′-hydroxychalcones, for which the reverse reaction involves OH^- and neutral flavanone rather than, as here, a less reactive anionic flavanone. It should also be noted that for the simple 2′-hydroxychalcones previously studied,[10] the hydroxide-promoted ring opening of neutral flavanones did not become apparent until pH > 10. At such a pH the additional phenol group (5-OH) of the flavanone in the present study would already be significantly ionised (pK_3 estimated as *ca.* 10), and as hydroxide activity increases the fraction of neutral flavanone decreases, in direct proportion at higher pH, so that any minor contribution from such a reaction would be difficult to detect. For the same reason, the neutral flavanone clearly cannot be associated with the rate increase at pH > 13 which is assigned to flavanone anion reaction.

Effect of pK_1 *Value.*—A consequence of the absence of a contribution from the reverse reaction of neutral flavanone is that, unlike the case of 2′-hydroxychalcones previously studied,[10] the unimolecular cyclisation of the chalcone monoanion (**1b**) manifests itself as a clear plateau extending as high as pH 11. The plateau is extended also to lower pH, the rate decrease associated with the reduced degree of chalcone ionisation (K_1) starting to show up only below pH 8. This is a direct consequence of the decrease in pK_1 of *ca.* 2 units in the case of a 2′,6′-dihydroxychalcone (pK_1 6.90) as compared with a simple 2′-hydroxychalcone (*e.g.* pK_1 8.95 for 2′-hydroxy-6′-methoxychalcone[10]). Such a difference is in accord with that reported[11] between 2′-hydroxyacetophenone (pK_1 10.27) and 2′,6′-dihydroxyacetophenone (pK_1 8.12), which has been accounted for by the lack of hydrogen bonding to carbonyl oxygen of the first ionising phenolic proton in the latter. A substantial reduction of *ca.* 2 in pK_1 of (**1a**) over that of simple 2′-hydroxychalcones will clearly markedly increase the fraction [f^B; equation (4)] of reactive chalcone monoanion at neutral pH. The factor would be about 50 in the present case. Undoubtedly, then, the extent of ionisation at neutral pH is a major factor in the comparative ease of cyclisation of 2′,6′-dihydroxychalcones.

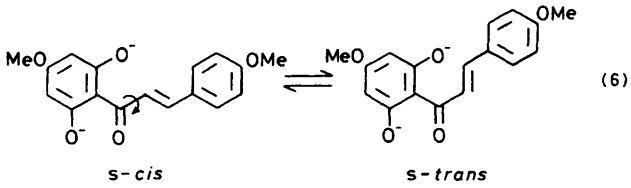
Intramolecular Catalysis by the 6′-OH *Neighbouring Group.*—The question remains whether the rate of cyclisation in neutral solution is high relative to that of 2′-hydroxychalcones, not only because of the high proportion of chalcone anion [factor f^B in term $k_3 f^B$; equation (4)] but also because of a specially high rate of cyclisation of the chalcone anion (factor k_3). In particular, we were interested in the possibility that the 6′-OH group might provide intramolecular general acid catalysis of conjugate



addition [equation (5)], which is not available for simple 2′-hydroxychalcone anions.

What might be taken as support for such catalysis is apparent from the dip in the pH–rate profile about pH 11: the chalcone dianion (**1c**) is about three times less reactive than the monanion (**1b**) in spite of a statistical factor of two in favour of the dianion. Normally a rate decrease for a potentially highly nucleophilic dianion over a monoanion could be taken to imply specially high reactivity of the monoanion. However, for this intramolecular reaction, dianion formation not only increases the power of the nucleophile but also decreases the power of the electrophilic centre, the β-carbon of the enone. It is electron withdrawal to the carbonyl oxygen which activates enones to nucleophilic addition, but this would be diminished in the dianion in the face of competition from charge delocalisation to the carbonyl oxygen from the phenolate oxygens in the aromatic ring. How these effects might balance out in the transition state is difficult to predict.

There is also the question of the effect of ionisation on the position of the equilibrium between the unreactive s-*cis* and reactive s-*trans* conformations [equation (6) for (**1c**)]. The

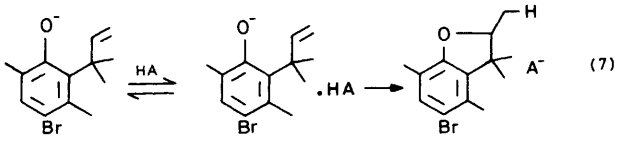


energy barrier to rotation about the C_α–CO bond would be lower for the dianion than for the monoanion in accord with decreased bond order resulting from more substantial competing delocalisation from the two phenolate oxygens to the carbonyl group as already discussed. However, assuming the conformational change from a kinetic viewpoint to be a pre-equilibrium, as appears to apply at least for the monoanion (see later), it is the relative free energy of the two conformations which determines the concentration of chalcone in the reactive s-*trans* form, and again this is not easily assessable. A third point of uncertainty concerns the possibility that the carbonyl group is forced out of plane with the aromatic ring of the dianion to the extent that cyclisation in the s-*trans* form is made difficult because the β-carbon becomes remote from the 2′-O^- group. The lack of hydrogen bonding in the dianion and existence of electrostatic repulsion between the carbonyl oxygen and the 6′-O^- group might have such an effect. Thus, although the higher reactivity of the monoanion over the dianion is interesting, it does not provide adequate evidence that the monoanion is unexpectedly reactive, as might result from the intramolecular general acid catalysis.

Better evidence comes, however, from an alternative approach, which was to study the cyclisation in D_2O. We reasoned that if cyclisation is assisted by intramolecular general acid catalysis by the 6′-OH group [equation (5)], the plateau k_{obs} value should be depressed in D_2O (catalysis by 6′-OD). Measurements showed k_{obs} to be almost six times less in D_2O than H_2O. This factor corresponds to a substantial primary isotope effect, considering that the calculated[12] maximum based on total loss of zero point energy of the O–H stretching

frequency in the transition state is 7.9. The present reaction is atypical of many previously studied in that the cyclisation involves bonding changes at sites remote from that of proton transfer, and also in that the geometry associated with the O ··· H ··· O proton transfer is not linear, bending vibrations possibly [9b] having an influence on the isotope effect. Nevertheless, the magnitude of the isotope effect represents a large loss of zero point energy, consistent [9c] with a transition state approaching the symmetrical type in which the proton is not strongly bonded to either the donor (6′-O) or the acceptor (C=O). In other words, the isotope effect points to a truly concerted general acid-catalysed cyclisation, rather than reactions involving proton transfer prior to or subsequent to cyclisation. We feel therefore that intramolecular general acid catalysis for the monoanion also plays its part in determining the instability of 2′,6′-dihydroxychalcones towards cyclisation in neutral conditions.

Intermolecular general acid catalysis of the closely related intramolecular nucleophilic addition of phenolate oxygen to *unactivated* double bonds [*e.g.* equation (7); HA = piperidine-



(7)

H^+) has been observed by Evans and Kirby,[13] and is consistent with pre-association of the catalyst (HA) with the phenolate ion as shown. This reaction is characterised by only a small isotope effect [$k(H_2O)/k(D_2O) = 1.6$] as is consistent with a small degree of proton transfer to the carbanionic carbon in the transition state. By comparison in the present study, in which acid catalysis is intramolecular and no pre-association is required, conjugate addition of phenolate [*cf.* equation (5)] to the activated double bond of the enone gives what would be a much more stabilised carbanionic transition state, and yet (intramolecular) acid catalysis as reflected in the isotope effect [$k(H_2O)/k(D_2O) = 5.7$] indicates a major gain from proton transfer. This difference between the data for the two systems can be accounted for by noting that in the encounter complex for intermolecular catalysis [equation (7)] it is unlikely that the acid (HA) would be hydrogen bonded to the very weakly basic site (terminal alkenyl carbon), proton transfer to which could subsequently stabilise the transition state. Hydrogen bonding to the nucleophile, phenolate oxygen, would be more likely. By contrast, geometry determines for the intramolecular catalysis of the present study [equation (5)] that the acid (6′-OH) cannot be hydrogen bonded to the nucleophile (2′-O^-) but that it is ideally situated for prior hydrogen bonding to the centre (C=O) at which negative charge will develop in the transition state. The magnitude of the associated catalytic effect as a rate factor remains to be assessed, in this case by further study using analogues of the chalcone.

Finally, we note that the isotope effect also establishes that the s-*cis* $\rightleftharpoons$ s-*trans* reaction [equation (6)] is a pre-equilibrium one for the chalcone monoanion. Were this isomerisation rate-limiting, with the s-*trans* conformer being trapped by 2′-O^- in a subsequent fast reaction, there would be little potential for an isotope effect of the magnitude observed. Since the isomerisation is likely to be even faster for the dianion, as already discussed, we suspect that isomerisation is a pre-equilibrium in dianion cyclisation too.

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