Mechanism of Cyclization of Substituted 2'-Hydroxychalcones to Flavanones†

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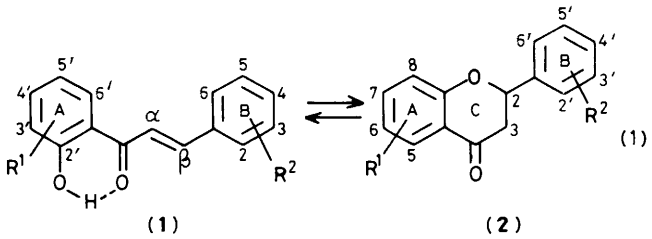
The conversion of 2'-hydroxy-(**3**), 2',4'-dihydroxy- (**4**), 2',3-dihydroxy- (**5**), and 2',4-dihydroxychalcone (**6**) in aqueous alkali; and of (**3**) and 2'-hydroxy-4-nitrochalcone (**7**) in alkaline methanolic medium into the corresponding substituted flavanones has been studied kinetically and spectroscopically. It has been found that results from both methods are consistent with the existence of chalcones in the *trans-s-cis* conformation and indicate a mechanism that involves general acid attack to the ionized form of the 2'-hydroxychalcones, rotation through the CO–C_α bond and annelation to the flavanone.

The 2'-hydroxychalcones (**1**) form an important group of organic compounds (see ref. 1) not only because of their pharmacological properties[2] but also because they are intermediates in the biosynthesis of flavonoids.[3] Chalcones (**1**) can be easily converted into flavanones (**2**), by chemical or enzymatic processes [equation (1)].



(1)

(**1**) (**2**)

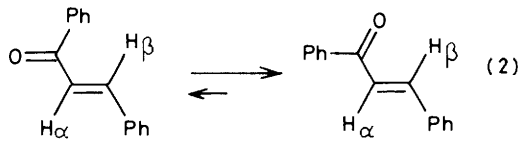
(**3**) $R^1 = R^2 = H$
(**4**) R^1 = 4'-OH, $R^2 = H$
(**5**) $R^1 = H$, R^2 = 3-OH
(**6**) $R^1 = H$, R^2 = 4-OH
(**7**) $R^1 = H$, R^2 = 4-NO_2

Closely related compounds have been isolated from the aerial parts of *Zuccagnia punctata* Cav.,[4] some of them in the form of chalcones and others as flavanones. It was therefore of interest to study the rate of interconversion (**1**) ⇌ (**2**). The present paper reports a kinetic and spectroscopic study of the conversion of 2'-hydroxy- (**3**) ($R^1 = R^2 = H$), 2',4'-dihydroxy- (**4**) (R' = 4'-OH, $R^2 = H$), 2'3-dihydroxy- (**5**) ($R^1 = H$, R^2 = 3-OH), and 2',4-dihydroxy-chalcone (**6**) ($R^1 = H$, R^2 = 4-OH) in aqueous alkali; and of (**3**) and 2'-hydroxy-4-nitrochalcone (**7**) ($R^1 = H$, R^2 = 4-NO_2) in alkaline methanolic medium into the corresponding flavanones.

Results and Discussion

Chalcone Conformations.—There are no experimental studies on the conformation 2'-hydroxychalcones and in most of the kinetic studies on its conversion into flavanone[5–7] the conformation is not defined.

Conformational investigation of some related α,β-unsaturated ketones have been performed using the method of ^1H n.m.r. aromatic-solvent-induced shifts (ASIS).[8] Application of



trans-s-trans (**8**) *trans-s-cis*

(2)

this method to compound (**8**) [equation (2)] indicates that it exists almost exclusively in the *s-cis* conformation in chloroform solution.[9] These results are in agreement with some previous i.r. determinations[10] which show that compound (**8**) exists in both conformations but that the equilibrium lies largely towards the *s-cis* conformer.

The n.m.r. data of Table 1 all suggest a *trans*-conformation for the 2'-hydroxychalcones. Unfortunately the ASIS method is not applicable to the present 2'-hydroxychalcones because it is based on the solvent-induced shifts of protons α and β, which in the spectra of these compounds appear together as a complex signal (δ 7.5—8.3) in the aromatic proton zone (Table 1). Nevertheless, taking into account the results for compound (**8**), it can be expected that the present chalcones are in the thermodynamically more stable *s-cis* conformation, which is in agreement with some theoretical calculations carried out on compound (**3**) by the Pariser–Parr–Pople method, the CNDO/S approximation,[11] and some recent INDO calculations.†,‡[12]

The following n.m.r. results have been helpful in elucidating the structural changes that occur in the isomerization process and give support to the proposed initial conformation.

As can be observed in Table 1 the ^1H n.m.r. spectra of substituted chalcones are very sensitive to the sodium hydroxide concentration of the medium. There are very few studies of the ^1H n.m.r. spectra of these compounds; isolated spectra of (**4**),[13a] 2',5'-dihydroxychalcone,[13b] and of some flavanones[14] have been published as well as a systematic study of the variation of the chemical shift of the 2'-phenolic proton as a function of the substituent.[15]

The 2'-OH signal. 2',4'-Dihydroxy-3'-methoxychalcone (larrein), (**9**), was selected to study this signal due to its high solubility. The ^1H n.m.r. spectrum of compound (**9**) in $CDCl_3$ exhibits a wide low-field signal (δ 13.65), which does not change

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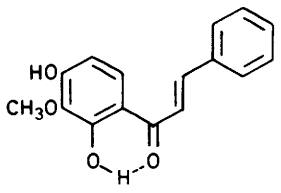
‡ Although it is known that INDO methods do not reproduce absolute values they are good in showing relative stabilities. INDO[12] calculations show an important difference between the energies of the *s-trans* and *s-cis* conformers, the *s-cis* being the more stable.

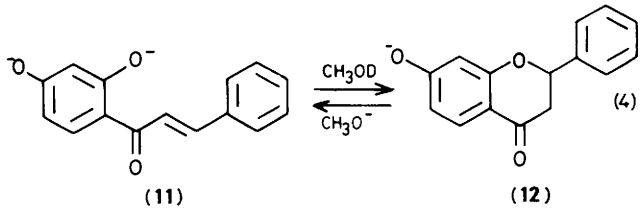
Table 1. ^{1}H N.m.r. data of chalcones in CD_3OD–D_2O–NaOD solutions[a]

Compound	R[b]	3′-H	5′-H	4′-H	6′-H	H_α	H_β	Ring B protons	
(3)[c]	0	7.15—6.65(m)		7.75—7.60(m)	8.05(dd)[d]	7.85(s)		7.45—7.40—7.35(m)[e]	
	1.6	6.85—6.15		7.25—6.90	7.50	8.20(d)[f]	7.55(d)[f]	7.40—7.30—7.25	
(4)	0	6.30(d)[g]	6.45(dd)[d]		7.95(d)[h]	7.80(s)		7.45—7.40—7.35(m)[e]	
	0.6	6.15	6.30		7.85	7.75		7.45—7.40—7.35	
	1.6	6.00	6.15		7.70	7.90(d)[f]	7.60(d)[f]	7.45—7.35—7.30	
	4.2	5.90	6.05		7.60	8.15	7.55	7.40—7.30—7.25	
(9)	0	3.85(s)[i]	6.45(d)[h]		7.70(d)[h]	7.75(s)		7.45—7.35—7.30(m)[e]	
	0.8	3.80	6.35		7.60	7.70		7.40—7.35—7.30	
	2	3.75	6.10		7.50	8.00(d)[f]	7.55(d)[f]	7.40—7.35—7.30	
	3	3.75	6.00		7.40	8.15	7.50	7.35—7.30—7.25	
(5)[c]	0	7.35—6.75(m)[j]		7.70—7.35(m)[k]	8.10(dd)[d]	7.85(s)		7.35—6.75(m)[j]	
	0.5	7.35—6.65		7.65—7.35	8.10	7.85		7.35—6.65	
	2.1	7.15—6.35		7.40—7.05	7.70	8.00(d)[f]	7.55(d)[f]	7.15—6.35	
								2-, 6-H	3-, 5-H
(6)	0	7.15—6.55(m)		*l*	8.05(dd)[d]	7.60(d)[f]	7.90(d)[f]	7.60(d)[h]	6.85(d)[h]
	0.4	7.15—6.65			8.05	7.50	7.90	7.55	6.70
	0.75	7.10—6.65			7.95	7.45	7.90	7.50	6.60
	1.3	6.90—6.35			*l*	7.55	7.80	7.45	6.60
	1.7	6.85—6.25		7.10(dd)[d]		7.65(s)		7.45	6.60
	2.6	6.85—6.15		7.10		7.60		7.40	6.60

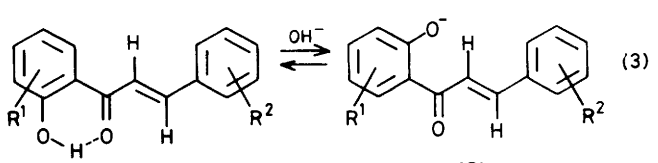
[a] Data in the order: chemical shift [multiplicity and coupling constant (Hz), unless otherwise stated, are the same for all R]. Precessional frequencies were obtained by first-order treatment of the signals. [b] R = [NaOD]: [Chalcone]. [c] A small amount of DMSO was added to dissolve the sample. [d] J_1 9, J_2 2. [e] The three most important signals of the multiplet are reported. [f] J 15. [g] J 2. [h] J 9. [i] Signal corresponds to OCH_3. [j] Multiplet: 3′-, 5-H and ring B protons. [k] dt: $J_1 = J_2 = 9$; J_3 2. [l] Difficult assignment due to mixing of the signals.



(9)



(11) (12)



(1) (10)

on dilution (2′-OH) and another signal at δ 6.55 that shifts to δ 6.45 on three-fold dilution of the sample. The insensitivity of 2′-OH to dilution is a clear indication of its intramolecular interaction with the carbonyl group.

Effect of NaOD *addition.* The effect of successive additions of NaOD was studied to determine the conformation in alkaline solution. Table 1 shows the results at different [NaOD]: [chalcone] ratios, R. It can be observed that ring A proton signals of compound (**4**) are shifted upfield. For R = 1.6, 4′-OH is completely ionized, the aromatic protons of ring A exhibit an upfield shift (20 Hz for the 3′-H, with respect to the spectrum in neutral solution), and a doublet centred at δ 7.90 appears, which can be tentatively assigned to one of the olefin protons. When R = 4.2, the doublet is now easily distinguishable from the aromatic proton multiplet and is assigned to H_α of the olefin chain: it is evident that ring A has rotated 180° about the C(4′)–C(1′) axis [equation (3)] and a strong interaction between the C(2′) oxyanion and H_α exists [compound (**10**)]. The signal for H_β is less shifted and merges into the aromatic proton signals.

The change of the signals observed at R = 4.2, as a function of time, is also very illustrative. The downfield absorption assigned to H_α (Figure 1a) gradually diminishes as the solution stands at room temperature (Figure 1b), and finally disappears (Figure 1c). This confirms the assignment, since H_α becomes deuteriated in alkali solution. By observing other changes in the spectrum it is reasonable to assume that isotopic interchange occurs *via* the flavanone structure. In fact, the signals for 3′- and 5′-H also change on standing of the solution, and once equilibrium is reached two doublets [one due to the 5′-H of flavanone (5′-H-F) and the other to the 5′-H of chalcone (5′-H-C)] are observed. The signal for 3′-H has disappeared and also the signal due to the 8-H of the flavanone is missing as well as the respective coupling with 5′-H-F and 5′-H-C. That this is due to the deuteriation of C-3′ in (**4**) [equilibrium (**11**) ⇌ (**12**)], was confirmed by n.m.r. and mass spectral determinations of resorcinol in MeOD–D_2O–NaOD mixtures.[16]

The simplification of the spectrum as time proceeds (Figure 1c) allows a quantitative determination of the amount of flavanone in the equilibrium: from Figure 1c compound (**12**) was estimated to form 68% of the equilibrium mixture. The spectrum of isolated (**12**) is consistent with the above considerations.

Table 2. Observed rate constants for the isomerization of 2′-hydroxychalcones[a]

Water								Methanol			
2′-OH (3)		2′,4′-(OH)$_2$ (4)		2′,3-(OH)$_2$ (5)		2′,4-(OH)$_2$ (6)		2′-OH (3)		2′-OH-4-NO$_2$ (7)	
T/ °C	pH 11.0	T/ °C	pH 12.5	T/ °C	pH 11.4	T/ °C	pH 11.2	T/ °C	6.3×10^{-3}[b]	T/ °C	6.3×10^{-3}[b]
6.1	0.70	7.4	0.415	11.6	0.945	17.0	0.135	17.4	0.665	15.9	0.473
10.0	1.125	11.4	0.60	16.3	1.455	19.8	0.193	20.4	0.965	19.9	0.756
13.65	1.65	15.0	0.84	18.15	1.73	24.0	0.300	23.2	1.305	22.65	1.019
16.6	2.16	19.6	1.31	21.25	2.325	27.8	0.421	25.8	1.775	26.2	1.454
19.5	2.67	22.7	1.78	24.4	2.99	30.6	0.580	28.1	2.305	28.2	1.788
23.3	3.69	25.4	2.24	28.1	4.18	34.1	0.729	30.2	2.95	30.2	2.262

[a] The rates ($10^3 k$/s^{-1}) were measured in the pH-independent zone at the pH value indicated in each case. [b] [CH_3O^-]/M.

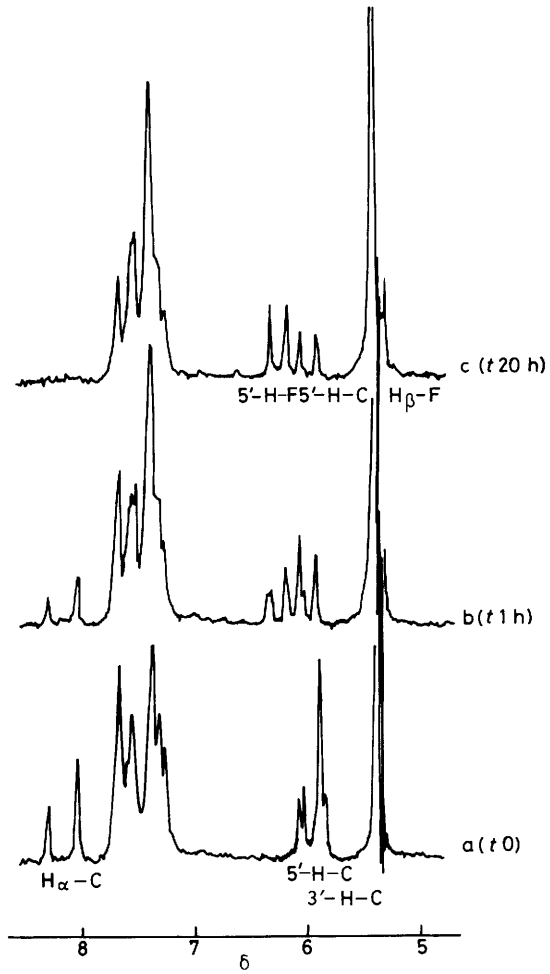


Figure 1. Time-dependent n.m.r. spectra resulting from additions of NaOD to chalcone (**4**). [NaOH]:([**14**)] 4.2

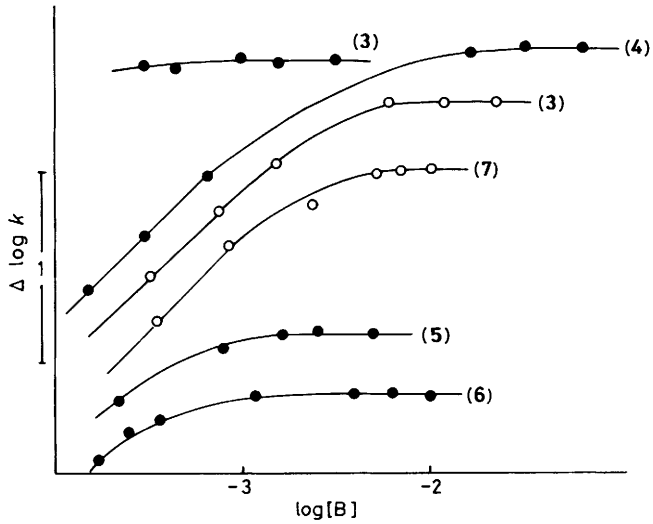


Figure 2. Kinetic parameters for isomerization of chalcones. Effect of pH. ●, In water: ○, in methanol

A detailed examination of the n.m.r. spectra of the other compounds reveals that they exhibit similar behaviour, and some results are condensed in Table 1. The amounts of other flavanones in the equilibrium, at $R = 2$, are: 70% for 2′,4′-dihydroxy-3′-methoxychalcone (**9**), 40% for compound (**5**), and very low for compound (**6**).

Kinetic Studies.—The sensitivity of 2′-hydroxychalcones to the pH of the solution has been known for some time,[17,18] but no kinetic studies of the influence of base concentration on the rate of conversion have been reported, apart from the work by Panasenko *et al.*[7] These authors studied the kinetics of cyclization of (**4**) in aqueous ethanol solution at pH 13—14, and found that the rate of cyclization is 7.74×10^{-2} min^{-1} at 20 °C, the activation parameters being $\Delta H^{\ddagger}$ 81.2 kJ mol^{-1} and $\Delta S^{\ddagger}$ −23.4 J mol^{-1} K^{-1}.

In previous work, the rate of isomerization of (**4**) was studied as a function of pH, temperature, ionic strength, and dielectric constant of the solvent.[19] It was found that the rate is pH-dependent for pH < 12, being independent at higher pH values. We now report similar kinetic determinations for the other chalcones. It can be observed in Figure 2 that the pH-independent region occurs at lower pH values for compound (**3**) than for the other dihydroxylated compounds, indicating that the isomerization reaction takes place on the ionized form.

The first step would then imply base-catalysed breakdown of the strong hydrogen-bond between 2′-OH and the oxygen of the carbonyl group [equation (3)]. There is u.v.[20,21] and i.r.[21] evidence for the existence of that intramolecular hydrogen-bond, and for the case of compound (**3**) an energy of *ca.* 42 kJ mol^{-1} has been estimated from i.r. measurements. This compares satisfactorily with INDO calculations of the energy of the hydrogen bond.[12]

Next, the rate constants for the isomerization of (**3**)—(**6**) in aqueous solution, and of (**3**) and (**7**) (insoluble in water) in methanol, were measured at several temperatures in the pH-independent zone for each compound, and the results are

Table 3. Activation parameters for the rate of conversion (1) ⟶ (2)[a]

Solvent	Water				Methanol	
Compound	(3)	(4)	(5)	(6)	(3)	(7)
$10^5 k_{25°C}$/l mol^{-1} s^{-1}	8.22 ± 0.3	3.89 ± 0.05	5.68 ± 0.09	0.58 ± 0.02	6.61 ± 0.06	5.29 ± 0.09
$\Delta G^{\ddagger}_{25°C}$/kJ mol^{-1}	96.3	98.2	97.2	102.9	96.9	97.4
$\Delta H^{\ddagger}$/kJ mol^{-1}	62.8	64.0	61.1	70.0	81.6	74.9
$\Delta S^{\ddagger}_{25°C}$/J mol^{-1} K^{-1}	−112.5	−114.6	−121.2	−110.7	−51.2	−75.5

[a] Error in $\Delta H^{\ddagger}$ ±2.5 kJ mol^{-1}, $\Delta S^{\ddagger}$ ±9 J mol^{-1} K^{-1}.

Table 4. Catalysis by glycine in the isomerization of 2′-hydroxychalcone at 26.1 °C

10^3[Glycine]/M	10^3[Glycine anion]/M	pH[a]	$10^3 k_{obs}$/ s^{-1} [b]
0.0	5.0	11.14	5.01
0.55	4.95	10.62	5.31
2.75	24.75	10.62	5.64
5.5	49.5	10.62	6.17

[a] Buffer glycine–NaCl–NaOH. [b] Each value is the average of three runs (error ≤3%). Total ionic strength was held constant at 0.1M.

gathered in Table 2. The calculated rates at 25 °C, as well as the free energies of activation at the same temperature (Table 3), indicate that a change in substituent has little effect on the rates, except for compound (**6**) for which the rate is slower. This compound has a noticeably larger activation energy.

When the reaction of (**3**) was studied in glycine–NaOH buffer at 26.1 °C the dependence of the reaction rate on the buffer concentration revealed general acid catalysis (k_{BH^+} 0.19 l mol^{-1} s^{-1}). As can be observed in Table 4, although the reaction is carried out in the pH zone where the 2′-OH group is fully ionized, an increase in rate is observed with a decrease in pH, and at pH 10.62 the rate increases with increasing buffer concentration. This effect could in principle be ascribed to proton transfer to the carbonyl group, but in that case an activating effect of the 4′-OH group should have been found, while the contrary is observed (Table 3). This also indicates that the previously proposed mechanism[7] where the slow step is formation of the flavanone enolate no longer holds.

A similar objection applies to the mechanism suggested by Tirouflet and Corvaisier[5] which involves slow base-catalysed formation of the flavanone enolate from the un-ionized chalcone since it has been shown that ionization is a fast reaction. In addition, both mechanisms do not take into account the preferred conformation of the substrate and of its anion, shown by the n.m.r. determinations.

In addition to general acid catalysis a large isotope effect (k_H/k_D 5.3) has been observed in the isomerization of (**3**). Both findings, the acid-catalysis and the isotope effect, are now being studied for other substituted chalcones.

All the above facts are consistent with the mechanism shown in the Scheme for the isomerization of compound (**3**).

In the short time the rate is measured the conversion of (**1**) into (**2**) may be considered as irreversible, then the whole reaction can be treated as a fast ionization of the substrate followed by a slow conversion of the anion into the flavanone. At low pH the rate will be dependent on the base concentration as was found [equation (5) where k_2 is the overall rate of the path (**13**) ⟶ (**15**)].

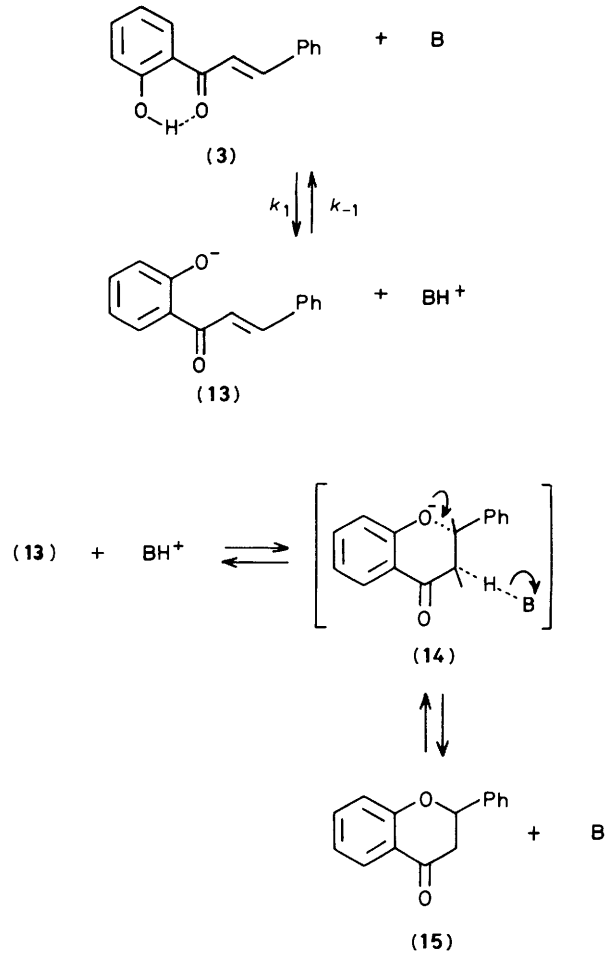
$$d[(15)]/dt = k_2K_1[(3)][B] \quad (5)$$

At pH > 11 the chalcone (**3**) is almost completely in the anionic form; the overall rate of reaction is determined by the slow conversion of the oxyanion into the flavanone and general acid catalysis could be observed [equation (6) where k_2 measures the slow conversion of (**13**) into (**15**)].

$$d[(15)]/dt = k_2[(13)][BH^+] = -d[(13)]/dt \quad (6)$$

The existence of compound (**13**) as a stable intermediate has been well demonstrated by ^{1}H n.m.r. determinations at different hydroxide concentrations and several times of reaction. Slow conversion of (**13**) into (**15**) could involve steps (**13**) ⟶ (**14**) ⟶ (**15**) as suggested by the following evidence.

Substituent effects. The observed general acid catalysis can be interpreted as due to water (BH^+ in the general case) attack on C_α. Such an attack should assist the required rotation about the



Scheme.

$CO–C_\alpha$ bond and it could occur through the cyclic transition state (**14**).

Nucleophilic displacement of the hydroxy anion (B in the general case) from (**14**) forms the final product (**15**). On the basis of the weak substituent effects, steps (**13**) ⟶ (**14**) and (**14**) ⟶ (**15**) are proposed to have similar rates or constitute a concerted process.

For compound (**6**) step (**14**) ⟶ (**15**) seems to be rate determining, after the addition of water. The electron-releasing effect of the 4-hydroxy group (the oxyanion) would reduce the nucleophilic displacement rate of the hydroxy anion and the slow rupture of the B • • • H bond would be responsible for the observed isotope effect.

Alternative Mechanism.—When this work was finished, a paper on the mechanism of cyclization of some 2′-hydroxychalcones was published by Old and Main.[22] No n.m.r. measurements nor a search for acid catalysis are reported. The proposed mechanism is a stepwise conjugative addition in the chalcone anion. This mechanism does not account for the general acid catalysis and isotope effects reported here, it does not take into account the preferred conformation of the chalcone anion shown by the n.m.r. determinations, and finally it requires a greater substrate reorganization than the mechanism proposed in the Scheme.

Experimental

M.p.s are uncorrected. U.v. and visible spectra of the chalcones and flavanones were recorded with a Beckman DK-2A spectrophotometer. Spectroscopic properties are gathered in Table 5. ^{1}H N.m.r. spectra were run with a Varian EM 360 A spectrometer.

2′-Hydroxychalcone (**3**).—Benzaldehyde (2.0 g, 18.8 mmol) and 2′-hydroxyacetophenone (2.0 g, 14.7 mmol) were dissolved in 10% NaOH in ethanol (40 ml). After stirring for 5 h at room temperature, a light yellow material precipitated, the reaction mixture was acidified (using 10% aqueous hydrochloric acid), and the ethyl acetate extract was washed thrice with water, dried (Na_2SO_4), and evaporated. Chromatographic purification of the residue on a Sephadex LH-20 column using methanol as eluant gave pure (**3**) (1.5 g, 45%), m.p. 88.5—89 °C (lit., 87—88 °C;[23] 88—89 °C[24]).

Flavanone (**15**).—A solution of compound (**3**) (100 mg) in methanol (30 ml) was made alkaline (pH 10) with NaOH pellets and was allowed to react for 2—3 days at room temperature. The reaction mixture was worked up in a similar way to that described for (**3**), and chromatographed on Kieselgel 40 silica gel (70—230 mesh) using benzene as eluant to remove the unchanged (**3**). Further elution with ethyl acetate–chloroform (1:4) give needles of pure (**15**) (0.02 g, 20%), m.p. 76 °C (lit., 75—76 °C;[23] 76 °C[25]); δ (CD_3OD) 7.90 (dd, 1 H, $J_{5,6}$ 8 $J_{5,7}$ 2 Hz, 5-H), 5.50 (q, 1 H, 2-H), 3.40—2.50 (m, 2 H, 3-H), and 7.75—6.85 (m, 8 H, 6-, 7-, 8-H and protons of the B ring).

4′-Nitroflavanone.—4-Nitrobenzaldehyde (3.0 g, 19.9 mmol) and 2′-hydroxyacetophenone (2.0 g, 14.7 mmol) were dissolved in ethanol (20 ml). A buffer of K_2HPO_4 (pH 9; 20 ml) with a little glycine as catalyst was then added and the solution was stirred for 5 h at room temperature. The mixture was kept at 4 °C for 2 days and a yellowish precipitate appeared which was filtered off, dissolved in acetone, partly evaporated, and filtered again, giving crystals of 4′-nitroflavanone. The filtrate was evaporated and crystallised from acetone–methanol (4:1). The total yield was 2.8 g (70%), m.p. 158.5—159.5 °C; δ ($CDCl_3$–Me_2S) 5.55 (q, 1 H, 2-H), 3.0—3.85 (m, 2 H, 3-H), 6.65—7.15 (m, 2 H, 6- and 8-H), and 7.35—8.35 (m, 6 H, 5-, 7-H and protons of ring B).

2′-Hydroxy-4-nitrochalcone (**7**).—4′-Nitroflavanone (100 mg) was dissolved in acetone (5 ml). 5% NaOH (30 ml) was then added (pH 14) and the mixture was stirred for 2 min until the mixture turned red. The mixture was acidified and worked up in the way described for compound (**3**). The residue was chromatographed on a Kieselgel 40 silica gel (70—230 mesh) column with benzene–chloroform (2:1) as eluant. The eluate was further chromatographed on a Sephadex LH-20 column with methanol as eluant affording yellow crystals of pure (**7**) (0.01 g, 10%), m.p. 207—209 °C; δ (Me_2S) 12.25 (s, 1 H, 2′-OH), 7.35—6.85 (m, 2 H, 3′- and 5′-H′), and 8.5—7.35 (m, 8 H, 4′- and 6′-H H_α, H_β, and protons of the ring B).

Table 5. Spectroscopic properties of chalcones and flavanones[a]

Compound	Solvent	pH	$\lambda_{max.}$/nm	240	260	290	320	350	380	410	440	500
(**3**)	Water	7	318	0.38	0.30	0.62	1.00	0.70	0.32	0.10	0.06	0.03
		11.2	309	0.71	0.32	0.80	0.89	0.24	0.18	0.24	0.18	0.02
	Methanol	0[b]	315	0.30	0.22	0.58	0.97	0.51	0.27	0.05	0.01	0
		0.048[b]	303	0.72	0.35	0.90	0.75	0.13	0.19	0.27	0.22	0.04
(**15**)	Water	7	254	0.51	0.86	0.09	0.33	0.08	0			
	Methanol	0[b]	251	0.64	0.59	0.11	0.38	0.03	0			
(**4**)	Water	7	345	0.27	0.21	0.54	0.91	0.99	0.50	0.09	0.01	0
		12.7	404	0.86	0.51	0.72	0.87	0.63	0.90	0.98	0.71	0.07
7-Hydroxyflavanone		7	276	0.61	0.52	0.66	0.47	0.02	0			
		10.8	333	0.20	0.19	0.21	0.76	0.38	0			
(**5**)		7	313	0.39	0.39	0.71	0.98	0.84	0.46	0.08	0.01	0
		12	235	0.91	0.56	0.58	0.75	0.35	0.34	0.36	0.23	0.02
3-Hydroxyflavanone		7	254	0.50	0.88	0.12	0.32	0.07	0			
		10.8	239	1.00	0.68	0.27	0.29	0.07	0			
(**6**)		7	365	0.47	0.33	0.23	0.47	0.88	0.90	0.29	0.04	0
		12.3	392	0.72	0.52	0.28	0.22	0.54	0.96	0.95	0.80	0.10
4′-Hydroxyflavanone		7	254	0.59	0.86	0.12	0.31	0.08	0			
		10.8	245	0.90	0.54	0.19	0.19	0.06	0			
(**7**)	Methanol	0[b]	317	0.18	0.22	0.59	0.99	0.44	0.28	0.08	0.02	0
		0.048[b]	320	0.64	0.28	0.58	1.00	0.43	0.14	0.16	0.19	0.09
4′-Nitroflavanone		0[b]	257	0.48	0.97	0.43	0.28	0.13	0.01	0		

[a] Values of extinction coefficients relation, $\varepsilon/\varepsilon_{max.}$, evaluated by extrapolation to $t = 0$. [b] $[CH_3O^-]$/M.

2′,4′-*Dihydroxychalcone* (**4**).—A mixture of benzaldehyde (1.5 g, 14.1 mmol) and 2′,4′-dihydroxyacetophenone (2.0 g, 13.1 mmol) in 10% NaOH in methanol (30 ml) and water (10 ml) was allowed to react for 2 days at room temperature. The mixture was worked up as already described and chromatographed on a Kieselgel 40 silica gel (70—230 mesh) column with benzene–ethyl acetate (3:2) as eluant, and the eluate was further chromatographed on a Sephadex LH-20 column using methanol as eluant, affording yellow needles of (**4**) (1.2 g, 40%), m.p. 146—150.5 °C (lit., 147.2 °C;[4] 151 °C;[26] 150 °C[27]).

7-*Hydroxyflavanone*.—A natural compound was isolated from the aerial parts of *Zuccagnia punctata* Cav.[4] as a light yellow product. By submitting this to chromatographic separation on a Sephadex LH-20 column using methanol as eluant, it was possible to obtain needles of the flavanone, m.p. 184—186 °C (lit., 179.4 °C;[4] 182.4 °C;[4] 188—190 °C[27]); δ ($CDCl_3$–Me_2S) 7.75 (d, 1 H, *J* 8.5 Hz, 5-H), 7.65—7.15 (m, 5 H, protons of ring B), 6.65—6.35 (m, 2 H, 6- and 8-H), 5.55—5.25 (q, 1 H, 2-H), and 3.4—2.5 (m, 2 H, 3-H).

2′,3-*Dihydroxychalcone* (**5**).—To a mixture of 3-hydroxybenzaldehyde (3.5 g, 28.7 mmol) and 2′-hydroxyacetophenone (3 g, 22 mmol) dissolved in ethanol (50 ml), 60% KOH (40 ml) was added slowly at 0 °C. The mixture was allowed to react for 3 days at 4 °C, and was worked up in the same way as for compound (**4**), affording yellow needles of (**5**) (1.8 g, 35%), m.p. 165—166.5 °C (lit., 161 °C;[28] 167—168 °C[29]).

3′-*Hydroxyflavanone*.—2′,3-Dihydroxychalcone (**5**) (0.2 g, 0.8 mmol) was dissolved in methanol (100 ml) and made alkaline with NaOH pellets to pH 10. The mixture was allowed to react for 3 days at room temperature and the mixture, worked up as described for compound (**3**), provided crystals of 3′-hydroxyflavanone (0.04 g, 20%), m.p. 141—144 °C.

2′,4-*Dihydroxychalcone* (**6**).—4-Hydroxybenzaldehyde (3.5 g, 28.7 mmol) and 2′-hydroxyacetophenone (3.0 g, 22 mmol) were dissolved in ethanol (30 ml); 10% aqueous NaOH (30 ml) was then added and the mixture was allowed to react for 2 days at room temperature. The usual work-up with the double chromatography [Kieselgel 40 silica gel, benzene–ethyl acetate (3:2) as eluant, followed by Sephadex LH-20 with an eluant of methanol] afforded yellow crystals of (**6**) (1.6 g, 30%), m.p. 157—159.5 °C (lit., 157—159 °C;[23] 166—167 °C;[29] 162—162.5 °C[30]).

4′-*Hydroxyflavanone*.—A similar method to that described for 3′-hydroxyflavanone was used, using (**6**) as starting material. White crystals of 4′-hydroxyflavanone were obtained (5% yield), m.p. 180—184 °C (lit., 186—188 °C;[23] 186—187 °C[30]).

2′,4′-*Dihydroxy*-3′-*methoxychalcone* (*Larrein*) (**9**).—The natural compound was isolated from the aerial parts of *Zuccagnia punctata* Cav.[4] The extraction and separation methods provided a yellow-orange product which was purified by chromatography on a Sephadex LH-20 column using methanol as eluant, giving intense yellow crystals of pure (**9**), m.p. 126.5—128 °C (lit., 124.3 °C[4]).

Kinetic Measurements.—Kinetic determinations were performed spectrophotometrically[31] using 1.00 cm thermostatted silica cells. In all cases pseudo-first-order kinetics were observed. Standard solutions of the chalcone (*ca.* 10^{-2}M) in methanol and of sodium hydroxide (0.1M) in water were prepared. A typical reaction was performed by adding the necessary amount of NaOH solution by a microsyringe to water (3.3 g) contained in the cell at the desired working temperature. The chalcone solution was added with shaking, the cell closed, and the optical density was recorded at a fixed wavelength (depending on the substrate) as a function of time. The pseudo-first-order rate coefficients k_ψ were calculated as the slope of the linear regression analysis of ln A_t *versus* t for the cases in which the reaction product does not absorb at the measuring wavelength, and of ln $(A_t - A_\infty)$ *versus* t for compound (**7**). Since the reaction is reversible the value of the 'infinity' optical density A_∞ could not be obtained experimentally but was evaluated by measuring the variation of A_0/A_t at several wavelengths as a function of the time and extrapolating to total conversion.

A rapid equilibrium (K_1) is established between the substrate and its anion and the overall rate of the substrate disappearance has the form (7) for compound (**3**). The limiting conditions of

$$\frac{d[(3)]}{dt} = \frac{K_1 k_2 [(3)][B]}{1 + K_1 \dfrac{[B]}{[BH^+]}} \qquad (7)$$

equation (7) are equations (5) and (6) at low and high pH respectively, which account for the experimental results.

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