SYNTHESIS
AND
KINETICS OF ISOMERISATION
OF
2',6'-DIHYDROXYCHALCONES

A thesis
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ABSTRACT

The first-order rate constant, k_{obs}, for the chalcone – flavanone isomerisation has been determined over the pH range 0-14 in water (μ = 1.0 mol l^{-1} with KCl) at 30°C for the following 2',6'-dihydroxychalcones: 2',6'-dihydroxychalcone (*34*); 2',6'-dihydroxy-4-methoxychalcone (*38*); 2',6'-dihydroxy-3,4-dimethoxychalcone (*40*); 2',6'-dihydroxy-3,4,5-trimethoxychalcone (*42*); 2',6'-dihydroxy-2,4,6-trimethoxychalcone (*44*); 4-chloro-2',6'-dihydroxychalcone (*46*); 2',6'-dihydroxy-4,4'-dimethoxychalcone (*32*); 2',6'-dihydroxy-3,4,4'-trimethoxychalcone (*36*); and 2',4,6'-trihydroxy-4'-rhamnoglucosyloxychalcone (naringin-chalcone) (*48*). The pH-rate profiles have been analysed in terms of contributions to cyclisation and ring opening of the various ionised chalcone and flavanone species, and individual rate and equilibrium constants for all the contributing reactions have been determined. Rate measurements with D_2O as the solvent (for *32*, *34*, *36*, *38*, *40*, *42*, *44*, *46*) revealed a large (presumably primary) hydrogen kinetic isotope effect for one of the contributing reactions, the cyclisation of the chalcone mono-anion. Enthalpies and entropies of activation for the mono-anion cyclisation have also been determined for chalcones *34*, *36*, *38*, *42*, *44*, and *46*.

^1H n.m.r. studies of the product from the cyclisation of the mono-anion in D_2O for two chalcones (*34*, *38*) showed little preference in protonation at the 3-position of the flavanone.

Mass spectrometry allowed measurement of the preference for protonation over deuteriation at the 3-position of the flavanone during cyclisation of the mono-anions of *32* and *38*, under kinetically-controlled conditions in H_2O – D_2O mixtures. The data obtained were not in accord with the those from kinetic isotope effect measurements.

The above results were considered in terms of possible mechanisms for the various reactions involved in the isomerisation. Factors contributing to the specially high rate of cyclisation of 2′,6′-dihydroxychalcones as compared to simple 2′-hydroxychalcones, and to the high stability of the 5-hydroxyflavanones as compared to simple flavanones (relative to their isomeric chalcones) were also considered. The low stability of the 2′,6′-dihydroxychalcones, compared to the 2′-hydroxychalcones (relative to their flavanones) was found to be due to a highly reactive mono-anion form combined with a lower first pK_a, and a greatly reduced susceptibility of the flavanone anions to base-catalysed ring opening.

By utilising the above kinetic results, new and improved syntheses of 2′,6′-dihydroxychalcones have been developed in the face of what are shown to be unreliable literature reports. The following compounds have been synthesised and characterised for the first time: (***32***); (***34***); (***36***); (***38***); 5-hydroxy-4′-methoxyflavanone (***39***); (***40***); 5-hydroxy-3′,4′-dimethoxyflavanone (***41***); (***42***); 5-hydroxy-3′,4′,5′-trimethoxyflavanone (***43***); (***44***); 5-hydroxy-2′,4′,6′-trimethoxyflavanone (***45***); (***46***); 5-hydroxy-4′-chloroflavanone (***47***); 6-benzyl-7-benzyloxy-5-hydroxyflavanone (***68***); 6-benzyl-5,7-dihydroxyflavanone (***69***); 2′-hydroxy-6′-tetrahydropyranyloxy-3,4,5-trimethoxychalcone (***70***); 3′-benzyl-4′,6′-dibenzyloxy-2′-hydroxychalcone (***72***); 2′,3,4′,6′-tetrahydroxy-4-methoxychalcone (***86***); 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (***93***).

Two new routes to 2′,6′-dihydroxychalcones were developed. The first involved base-catalysed condensation of acetophenones and benzaldehydes with free phenolic groups protected as their tetrahydropyranyl ethers, followed by deprotection at pH 2. The second involved reaction of flavanones with chlorosilanes, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene, in which ring opening of the *O*-silylated flavanone was immediately followed by silylation of the resultant phenolate oxygen, thereby preventing recyclisation.

The crystal structures of 5-hydroxy-4',7-dimethoxyflavanone (***33***) and 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***) were determined by X-ray crystallography, ***44*** being the first 2',6'-dihydroxychalcone for which a crystal structure has been obtained.

A computer programme was developed for the analysis of pH-rate data for 2'-hydroxy- and 2',6'-dihydroxy-chalcones, but which is suitable for the analysis of pH-rate data for most mono- or di-acids in which the contributing reactions are (pseudo) first-order. The programme can also include second-order H^+-catalysed reaction of the di-acid, and one second-order OH^--catalysed reaction, and calculates the concentrations of the various ionised species present on the basis that the two pK_a values may not be well-separated. This programme has been used to successfully analyse the 2',4'-dihydroxychalcone – 7-hydroxyflavanone pH-rate profile previously reported, but not analysed, in the same way as for the 2',6'-dihydroxychalcones.

ACKNOWLEDGEMENTS

My especial thanks go to my supervisor, Dr L. Main, for his guidance, interest and assistance throughout both the research and writing-up of this work. I would also like to thank Dr B. K. Nicholson for his assistance with the X-ray crystallography, and his helpful suggestions for chapter 4.

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ABBREVIATIONS

LIST OF ABBREVIATIONS

b.p.	boiling point
br	broad
chalcone	1,3-diphenylprop-2-ene-1-one
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMF	*N*,*N*-dimethylformamide
ether	diethyl ether
flavanone	2,3-dihydro-2-phenyl-4H-benzo[*b*]pyran-4-one
k.i.e	kinetic isotope effect
q	quartet
m	multiplet
Me	methyl
m.p.	melting point
n.m.r.	nuclear magnetic resonance
OMe	methoxy
PAL	phenylalanine ammonium lyase
p.i.e.	product isotope effect
p.l.c.	preparative layer chromatography
q	quartet
THP	tetrahydropyranyl
s	singlet
t	triplet
t.l.c.	thin layer chromatography

u.v.	ultraviolet
σ	(general) estimated standard deviation
σ	(n.m.r.) chemical shift in Hz

CHAPTER 1

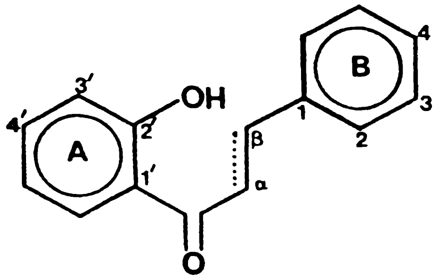
1.1.1 Introduction

The flavonoid compounds are a group of biosynthetically related pigments which are widespread in the plant kingdom, especially in the higher plants. The term flavonoid is usually used to include the isoflavonoid compounds (see figure 1-1). The flavonoids are usually highly oxygenated, resulting in a myriad of natural derivatives of the structures in figure 1-1, with various patterns of hydroxylation, *C*- and *O*-alkylation and glycosylation; in addition, quinone and dimer formation also occur.

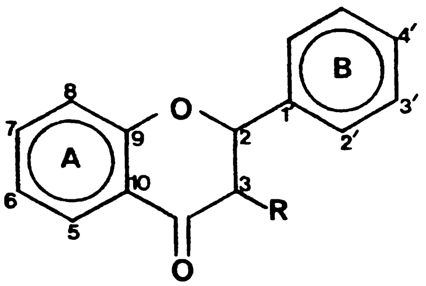
Flavonoids are important to mankind for many reasons, quite apart from their properties as pigments. They contribute to the flavour[1-5] and colour[6,7] of many foods, and some act as wood preservatives,[8] antioxidants,[9] sweeteners,[9] insecticides[10] or ultraviolet absorption filters.[9] The chemical pulping of wood is adversely affected by the presence of certain flavonoids.[8] Many flavonoids possess pharmacological,[10,11] antibacterial[11] or antifungal[11] properties, or affect plant growth.[11] Studies of the flavonoid distribution in plants[12-15] are widely used for the purpose of taxonomic classification of plant species.

1.1.2 Flavonoid Biosynthesis

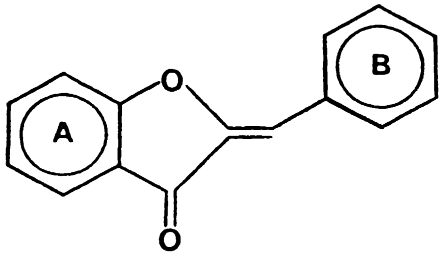
It has been well established[16-18] by radioactive labelling experiments that the flavonoids are derived *in vivo* from the addition of three acetate units linked head to tail, to a phenylpropanoid unit



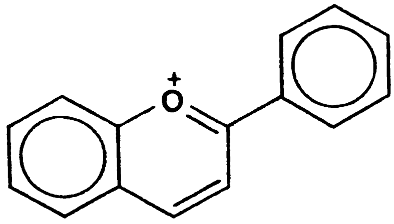
2'-HYDROXYDIHYDROCHALCONE
Δα,β 2'-HYDROXYCHALCONE



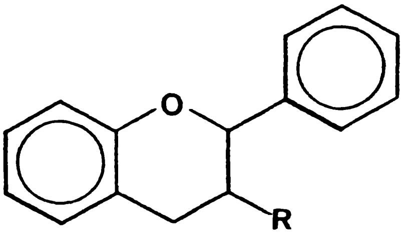
R = H FLAVANONE
R = OH FLAVANOL
R = H, Δ2,3 FLAVONE
R = OH, Δ2,3 FLAVONOL



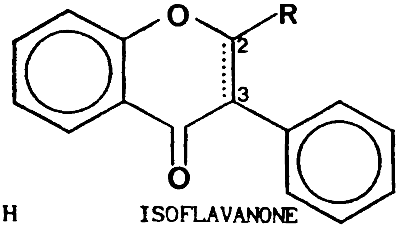
AURONE



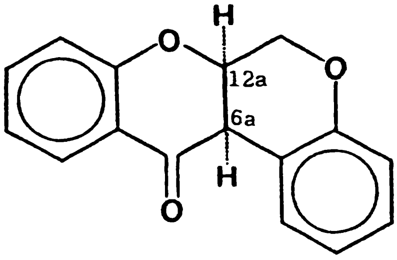
ANTHOCYANIN



R = H FLAVAN
R = OH CATECHIN



R = H ISOFLAVANONE
R = OH ISOFLAVANOL
R = H, Δ2,3 ISOFLAVONE
R = OH, Δ2,3 ISOFLAVANOL



ROTENOIDS

FIGURE 1-1 Major Classes of the Flavonoids (see figure 1-2) which is a product of the shikimic acid metabolic pathway (see scheme 1-1) in plants. The acetate units form the A-ring, while the B-ring and the three carbon unit (C2, C3 and C4 in the flavanone nomenclature) are derived from the phenylpropanoid unit (in this case a cinnamic acid derivative). This is the reason for the preponderance in nature of the phloroglucinol (1,3,5-trihydroxybenzene) A-ring substitution pattern (see figure 1-2 and the section on chalcone synthase).

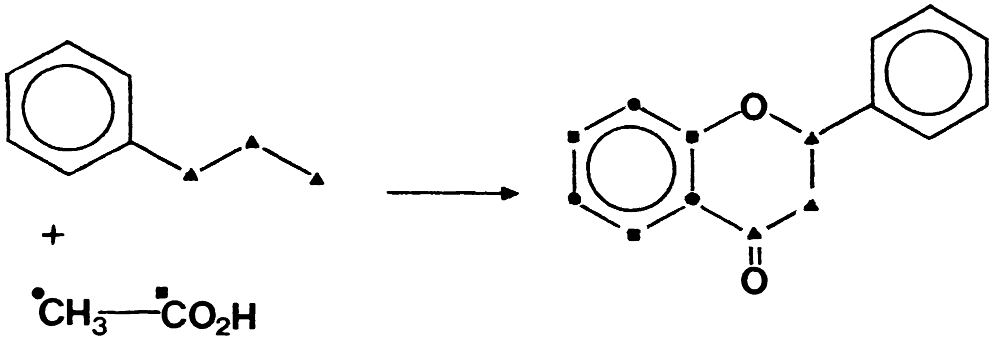
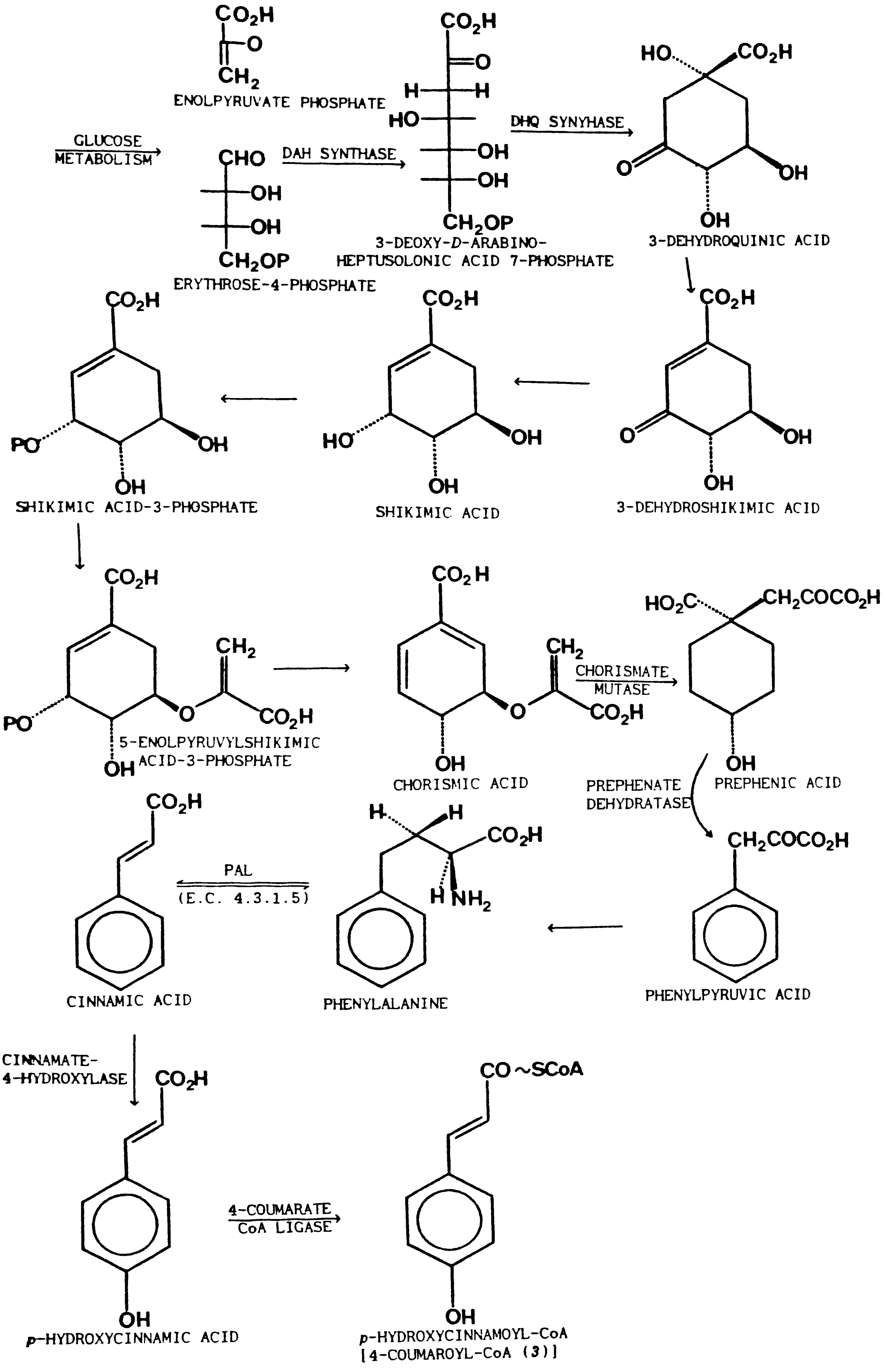


FIGURE 1-2 Origin[16] of the Pyrone and A-Ring Carbon Atoms in the Flavonoids

The origin of the B-ring substitution pattern is less obvious, and has been the centre of some controversy. The substitution pattern may be determined at the cinnamic acid stage (*i.e.* prior to condensation with the acetate units) as suggested by Hess.[19] Thus the synthase enzyme might select the appropriately substituted cinnamic acid derivative from the cell's cinnamic acid pool, and subsequently synthesise the substituted flavonoid from it. Alternatively, chemical modifications may take place after initial formation of the flavonoid. There is support for both these pathways *in vivo*, although work with isolated synthase enzymes (see below) suggests the latter pathway may be the more important of the two, at least in most of the plants from



SCHEME 1-1 Biosynthetic Route to 4-Coumaroyl-CoA, via the Shikimic Acid Pathway[17,18,23]

which the enzymes studied have been obtained.

All the flavonoids studied appear to be derived from an initial chalcone/flavanone precursor, via various enzyme-mediated chemical modifications.[16,17,20,21] Thus the key step in flavonoid biosynthesis would appear to be chalcone/flavanone synthesis.[19,24]

1.1.*3* Chalcone Synthase

The overall reaction resulting in the formation of the chalcone/flavanone intermediate is the enzyme-catalysed addition of three acetate units derived from malonyl-CoA, to the CoA ester of a cinnamic acid, a product of phenylpropanoid metabolism, followed by cyclisation and aromatisation of the side chain. The enzyme catalysing this reaction is known as chalcone synthase.

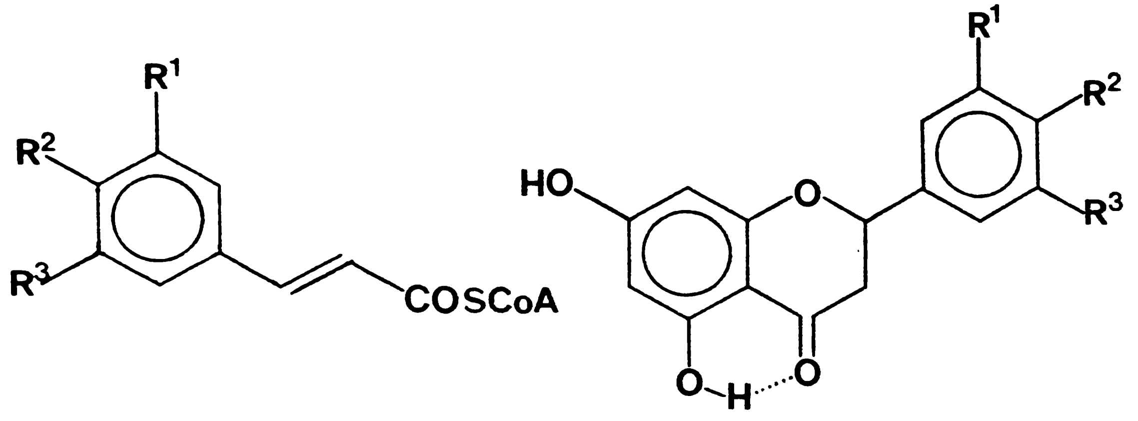
Study of this reaction *in vivo* is complicated by the presence of the enzymes within the cell for further metabolism of the initial product, including various oxidases, isomerases and methyl- and glycosyl-transferases. In particular the presence of isomerase enzymes catalysing the chalcone – flavanone equilibrium makes it impossible to be sure whether the chalcone or the flavanone is the first product of biosynthesis.

Studies *in vitro* using cell extracts also suffer from this difficulty. Contributing to the problem is the fact that chalcones possessing 2′,6′-dihydroxy substitution (in this case the expected substitution in the A-ring is 2′,4′,6′-trihydroxy) are subject to rapid base-catalysed cyclisation to flavanones. The half-life for the chemical cyclisation of naringin-chalcone (*48*) at pH 7 can be calculated to be 4.6 minutes from the data in the literature.[25]

Not surprisingly, early *in vitro* studies[26] identified only the flavanone as the immediate product of flavonoid biosynthesis, even when isolated synthase was used in the assay,[24,27-31] and the enzyme responsible for the reaction was referred to as a flavanone synthase until 1980.

The difficulties with enzymatic cyclisation have now been avoided, either by using enzyme extracts from plant strains lacking chalcone – flavanone isomerase activity,[32] or by careful purification of the synthase free from the isomerase.[33] Then conditions to minimise non-enzymatic chalcone cyclisation were used (*e.g.* low pH in the accumulation phase of a bi-phasic assay[34]), or the relative amounts of chalcone and flavanone were compared at long and short reaction times.[32,35] For example, Heller and Hahlbrock[35] were able to show that in the absence of isomerase, flavanone formation was solely due to non-enzymatic cyclisation with a rate constant of 2.0×10^{-3} s^{-1} ($t_{0.5}$ = 5.75 minutes) at pH 6.8, so that the ratio of flavanone to chalcone increased with time even though the rate of formation of flavanone plus chalcone was constant. They also found[35] that when isomerase was added to the assay, the flavanone was the only product. These experiments clearly demonstrated that the chalcone, and not the flavanone, was the first product of flavonoid biosynthesis, and that under the usual experimental conditions (the pH optimum of chalcone synthases is usually 7-8) the chalcone rapidly cyclises to its isomeric flavanone. Chemo-genetic studies[36-38] and labelling experiments[39] using $^{13}CH_3{}^{13}COOH$ provide strong independent support for chalcones as the first intermediate of flavonoid biosynthesis.

Several chalcone synthases have been isolated from various plant sources, and characterised. They appear to be dimers with subunit molecular weights[31,40,41] of approximately 42×10^3 g mol^{-1} and pH optima[30,32] of 6.5-8, and have no cofactor requirements. The substrates are malonyl-CoA and the CoA-ester of a cinnamic acid derivative. In all cases studied[29,30,32,33,42] 4-coumaroyl-CoA (*3*) is a better substrate than either caffeoyl- (*5*) or feruloyl-CoA (*9*).



R^1	R^2	R^3	CoA Ester	Flavanone
H	H	H	*1* Cinnamoyl-CoA	*2* Pinocembrin
H	OH	H	*3* 4-Coumaroyl-CoA	*4* Naringenin
OH	OH	H	*5* Caffeoyl-CoA	*6* Eriodictyol
OH	OMe	H	*7* Isoferuloyl-CoA	*8* Hesperetin
OMe	OH	H	*9* Feruloyl-CoA	*10* Homoeriodictyol
OMe	OH	OMe	*11* Sinapoyl-CoA	

FIGURE 1-3 Common Cinnamic CoA Ester Substrates for Chalcone Synthase and their Flavanone Derivatives (and their Trivial Names)

The synthase enzymes of *P. crispum* and *H. gracilis* both show pH optima of about 8 for formation of naringenin (*4*), and of about 6-7 for eriodictyol (*6*) formation, with eriodictyol formation being somewhat slower than naringenin formation at their respective pH optima.[30] This phenomenon may merely reflect the differing acidity constants of the substrates (and hence their state of ionisation) since the pK_a of caffeoyl-CoA (*5*) would be expected to be lower than that of 4-coumaroyl-CoA (*3*). Interestingly the pH optimum of PAL (EC 4.3.1.5) for phenylalanine and tyrosine were 8.7 and 7.7 respectively. Spribille and Forkman[42] found the *A. majus* synthase would accept only 4-coumaroyl-CoA (*3*) even though 3',4'-dioxygenated flavonoids are common in this species (which possesses a flavonoid

3'-hydroxylase enzyme). This and other evidence suggests that the cinnamic starter hypothesis may in many species be incorrect, or only partially correct. It is surprising in view of the generally high substrate specificity of chalcone synthases towards cinnamic acids, that the *P. hortense* synthase has been shown to catalyse the addition of malonyl-CoA to a number of aliphatic CoA esters, forming 2',4',6'-trihydroxyphenyl ketones.[43]

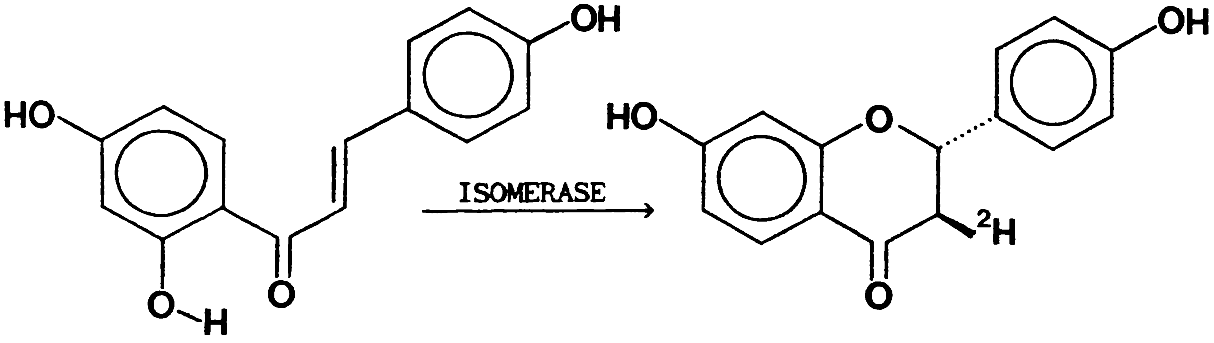
In studies using partially purified chalcone synthases it has been found that under certain assay conditions,[28] a reduced number of acetate units (one or two instead of the usual three) are added to the cinnamic acid substrate, leading to short-chain release products.[29] The structure of these release products suggests the sequence of reactions shown in figure 1-4. Release products of this type are not produced *in vivo*. In one study[29] when 4-coumaroyl-CoA (**3**) was used as the substrate for the chalcone synthase of parsley (*P. hortense*), release products were only minor products, while with caffeoyl-CoA (**5**) they were the major products and only minor amounts of the flavanone were present. In the case of feruloyl-CoA (**9**), only release products were formed, and none of the expected flavanone [homoeriodictyol (**10**)] was detectable. This suggests that *in vivo* the normal sustrate of the synthase may be 4-coumaroyl-CoA, and that the first product of flavonoid biosynthesis may be 2',4,4',6'-tetrahydroxychalcone, at least for this particular plant.

Chalcone synthase in some plants (along with PAL) has been strongly implicated in the phytoalexin response to infection.[41,44,45]

1.1.4 Chalcone - Flavanone Isomerase (EC 5.5.1.6)

Historically, the isolation of optically active flavanones from plant sources provided confirmation that flavanones were indeed natural products, and not merely the result of over-vigorous isolation techniques on the naturally occurring isomeric chalcones. Flavanones and their isomeric 2'-hydroxychalcones exist in equilibrium with each other in aqueous solutions, interconversion being acid- or base-catalysed. Although the chalcone – flavanone equilibrium lies heavily in favour of the flavanone (the position of the equilibrium can be calculated quantitatively from the data of Old and Main[46]) at physiological pH, the rate at which equilibrium is approached is rather slow, at least in the case where the chalcone lacks an additional hydroxy group in the 6'-position. In the case of 2'-hydroxychalcone[46] the half-life for the attainment of equilibrium is about 6¼ hours at 30°C and pH 7, even though an equilibrium mixture can be calculated to consist of 99% flavanone under these conditions.

The presence of optically active flavanones in plants implies enzyme-mediated flavanone formation, either by a flavanone synthase or by a chalcone – flavanone isomerase. In 1957 Shimokoriyama[25] isolated a crude enzyme extract that catalysed the isomerisation of several chalcone glycosides to flavanones. Moustafa and Wong[47,48] isolated the soybean (*S. hispida*) chalcone – flavanone isomerase in 1967, the first enzyme of flavonoid metabolism to be isolated. This enzyme catalysed the cyclisation of several chalcones to flavanones, with some degree of substrate specificity. It was found that 2',4,4'-trihydroxychalcone (*12*) was cyclised to (-)(2*S*)-4',7-dihydroxyflavanone, although the specific rotation of the product[47] was considerably lower than (1/3) that reported[49] for the same flavanone isolated from natural sources. When enzymatic cyclisation was carried out in buffered deuterium oxide[50] (scheme 1-3), the deuteron added to the double bond with a small degree of stereo-specificity; the deuterium was found to enter the flavanone preferentially in the axial position (at C-3 of the flavanone). However the ratio of axial to equatorial was only 7:3, and circular dichroism measurements indicated the 2*S* isomer to be present in an enantiomeric excess of only 57 to 74%. This indicates either a surprising lack of stereoselectivity on the part of the enzyme, or that some other factor was involved (the authors showed that non-enzymatic cyclisation was sufficiently slow under the conditions used to be negligible). These anomalies are often glossed over by authors in discussing the stereochemical aspects of this enzyme.



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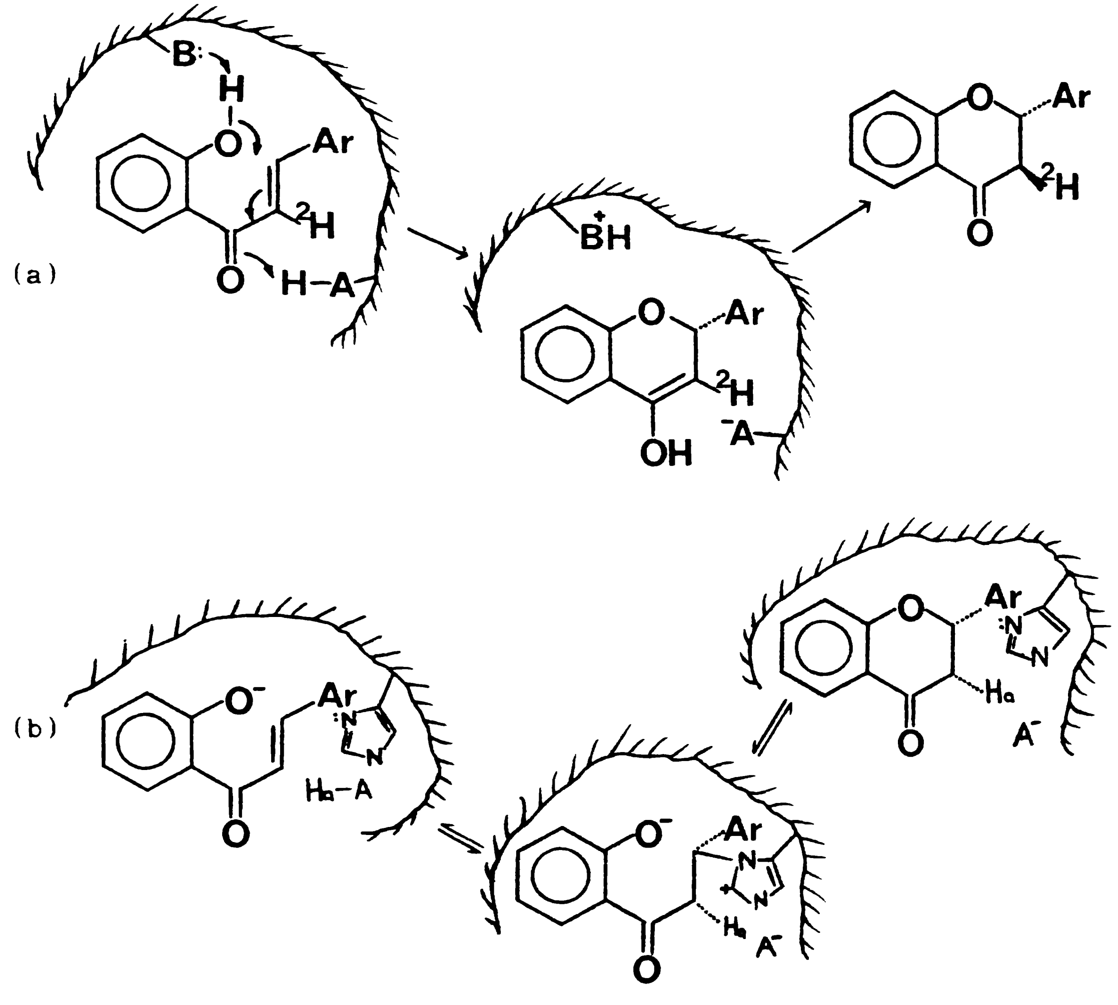
SCHEME 1-3 Isomerase-Catalysed Incorporation of ^{2}H into 4',7-dihydroxyflavanone[50]

The chalcone – flavanone isomerases isolated from plants can often be separated into isoenzymes,[50-52] with differing molecular weight, pH optima and substrate specificity. The pattern of substrate specificity of a plants isomerase enzymes tends to reflect the substitution patterns of the flavonoids found in the plant tissue from which the enzyme was isolated.[37,51,53] For instance, the parsley isomerase is specific for 2',4,4',6'-tetrahydroxychalcone, and only 5,7-dihydroxy flavonoids have been found in this plant.[51]

Moustafa and Wong[48] found that replacement of a hydroxy group with a methoxy group in the chalcones being used to study the substrate specificity of the soybean isomerase resulted in a dramatic reduction of the rate of enzymatic cyclisation , even when the methoxy group was introduced in the B-ring (*cf.* ref. 54). In no case studied[51,52] so far have chalcone glycosides been shown to be substrates for a chalcone - flavanone isomerase (in contrast with the crude extract of Shimokoriyama[25]). This and other work suggests that, at least for the plants studied so far, methylation and glycosylation occur during later stages of flavonoid biosynthesis.

Estimates for the molecular weights of the isomerases from various plants vary considerably, being 15600,[55] 28000,[54,56] 31000[53] and 50000,[27] and the pH optima range from 7.3-8.6.[37,38,48,51,53-55] Examination of the pH-rate profiles for the enzyme-substrate complex implicates an imidazole group[54,55,57] (possibly of a histidine residue) in the rate determining step, and a sulphydryl group does not appear to be involved.[57] Boland and Wong[55,57] also suggest that the 2'-hydroxy group needs to be ionised for enzymatic cyclisation to take place. The rate constant for the decomposition of the isomerase-chalcone complex to the isomerase-flavanone complex is given as 10 min^{-1} (0.17 s^{-1}) by Boland and Wong[55] at pH 7.6. In contrast a figure of 5 s^{-1} can be calculated using the isomerase V_{max} from the work of Dixon *et al.*,[54] assuming the isomerase to be totally pure, at a pH of 8. The specific activity of Dixon's isomerase was 73 times that of Boland's,[55] and the rate constant calculated from the maximum specific activity attained by Dixon's group[54] is actually 21.6 s^{-1}. The activation energy was found[54] to be 17.6 kJ mol^{-1}. Thus $\Delta H^{\ddagger} = 15.0$ kJ mol^{-1}, and assuming a rate constant of 5.0 s^{-1}, $\Delta S^{\ddagger} = -177$ J K^{-1} mol^{-1}, although the value of $\Delta S^{\ddagger}$ will be strongly affected by either inaccuracies in the measured rate constant, or by the purity of the isomerase. Two mechanisms[50,57] have so far been put forward to explain the enzyme-catalysed isomerisation of chalcones to flavanones (scheme 1-4).



SCHEME 1-4 Proposed Mechanisms of Action for Chalcone – Flavanone Isomerase: (a)[50]; (b)[57]

The enzyme-catalysed isomerisation is reversible, with an equilibrium constant[55] of 37 in favour of the flavanone at pH 7.6 in the case of 2',4,4'-trihydroxychalcone. This equilibrium constant, which must be the same as that for the uncatalysed isomerisation, is of the same order of magnitude as that for other 2'-hydroxychalcones (*e.g.* $K_{eq.}$ = 54 for 2'-hydroxy-4'-methoxychalcone at this pH when calculated from the data of Old[46]).

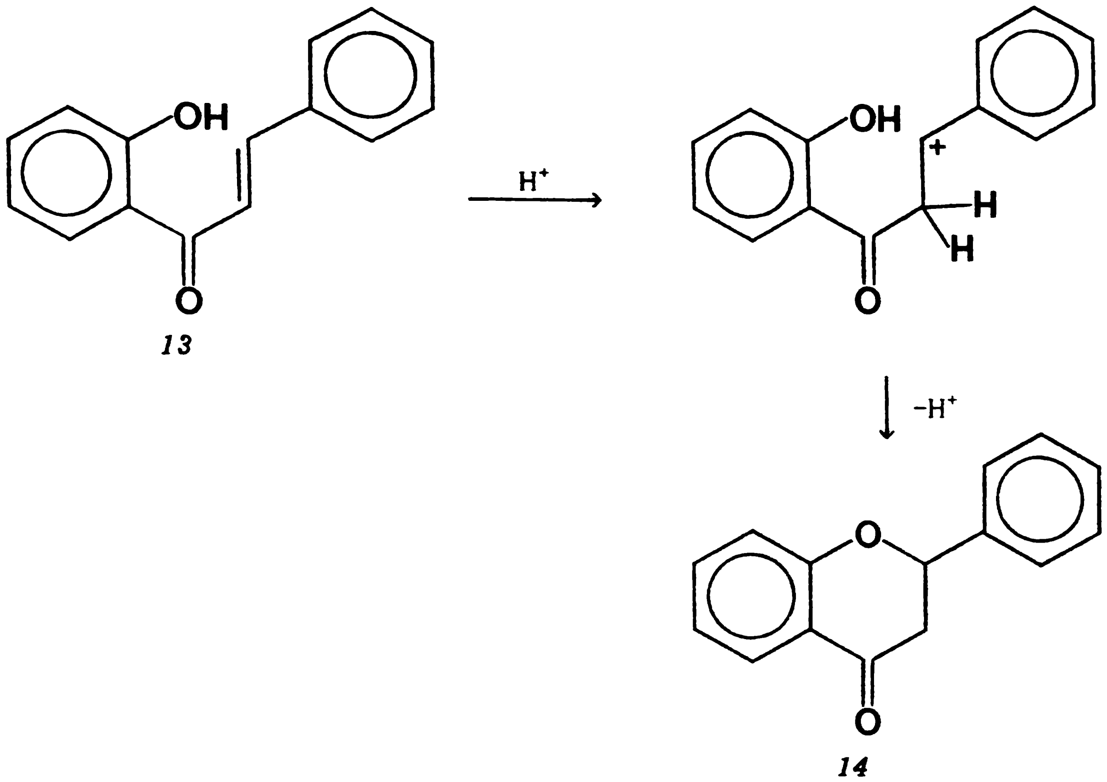
The reversibility of this reaction calls into question the role of flavanones as the direct precursors of the majority of the flavonoids, since the flavanones could conceivably act as storage compounds from which the isomerase could generate chalcone precursors which could then be converted to other flavonoids[55]. That this is not the case, however, has been demonstrated with genetically defined plants which accumulate chalcones and chalcone glycosides. It was shown that this accumulation was due to a complete absence of chalcone – flavanone activity, resulting not only in chalcone accumulation, but also an almost complete absence of other flavonoids.[36-38,58]

1.2 The Chalcone – Flavanone Equilibrium

Although the chalcone – flavanone equilibrium is of considerable interest due to its biosynthetic importance, it is only relatively recently that it has become the subject of systematic investigations, while some of the early studies of it were rather crude in nature.

In 1941, Reichel and Burkhart[59] found that 2'-hydroxychalcone (***13***) (1.0 g) in ethanol (80 ml) and citrate–HCl buffer (10 ml, pH 4.5) on standing at 37°C, gave 40% flavanone (***14***) after 30 days and 65% flavanone after 60 days. The mechanism postulated by the authors is shown in scheme 1-5.

In 1947, Narasimhachari and Seshadri[60] studied the relative stabilities of flavanones with or without a 5-hydroxy group, relative to their isomeric chalcone forms. It was noted that when butrin (***15***) was hydrolysed under acid conditions, a mixture of butin (***16***) and

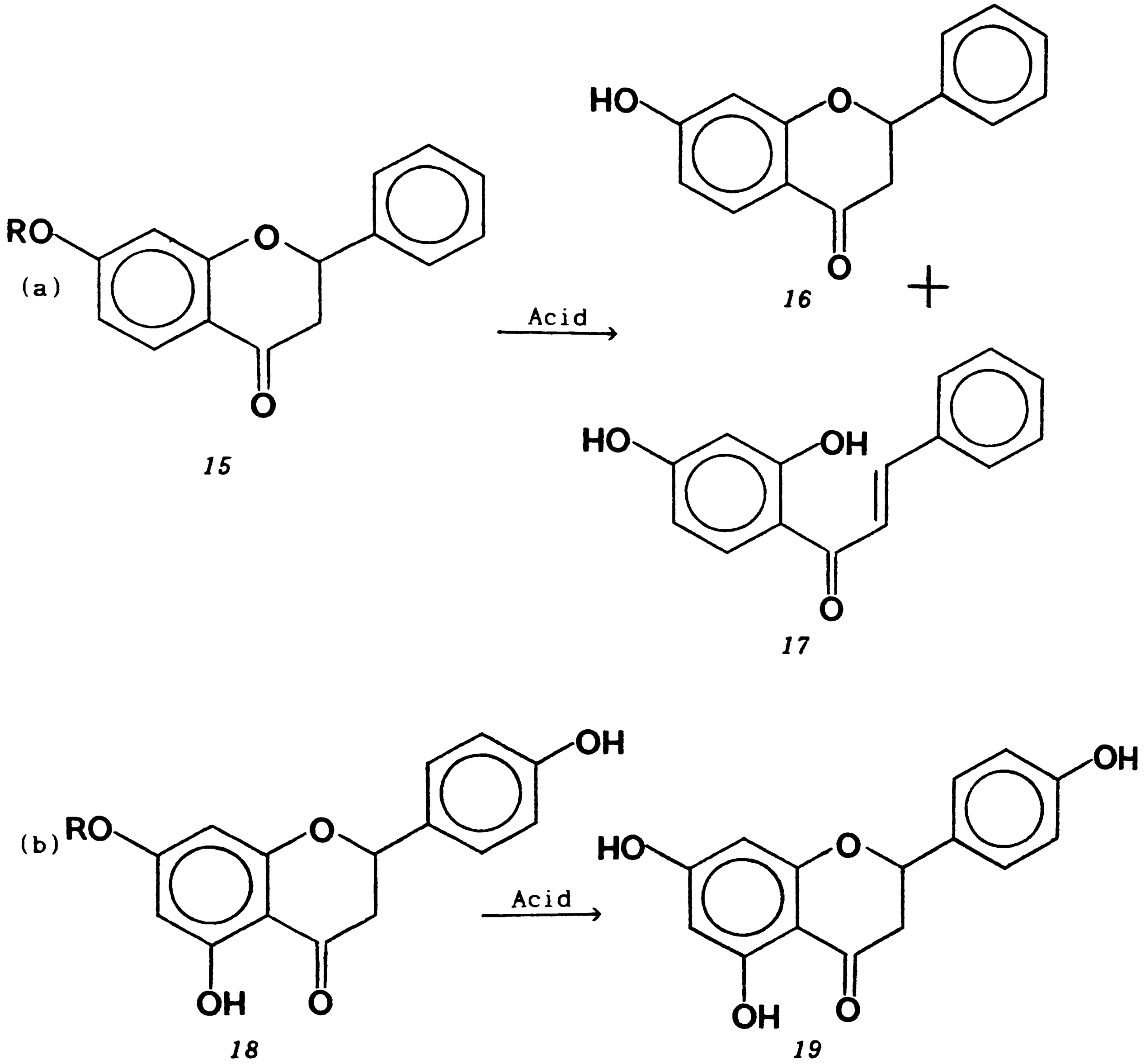


SCHEME 1-5 Proposed Mechanism of Cyclisation for 2'-hydroxychalcone[59]

butein (*17*) (the flavanone and chalcone aglycones of butrin respectively) was isolated, whereas a similar hydrolysis of naringin (*18*) afforded only the flavanone aglycone, naringenin (*19*), as shown in scheme 1-6.

Also, a series of flavanones were dissolved in a 10% NaOH solution, and after 30 minutes the solution was acidified. In the case of 5-hydroxyflavanones, the flavanone was recovered unchanged, while from the corresponding 5-methoxyflavanones, only the isomeric chalcones were isolable. The authors concluded that "the presence of a 5-hydroxy group adds more stability to the flavanone structure by the formation of an additional chelate ring", and that even if ring opening did occur, the groups involved in the ring closure would not be able to move far enough apart to yield a stable chalcone.

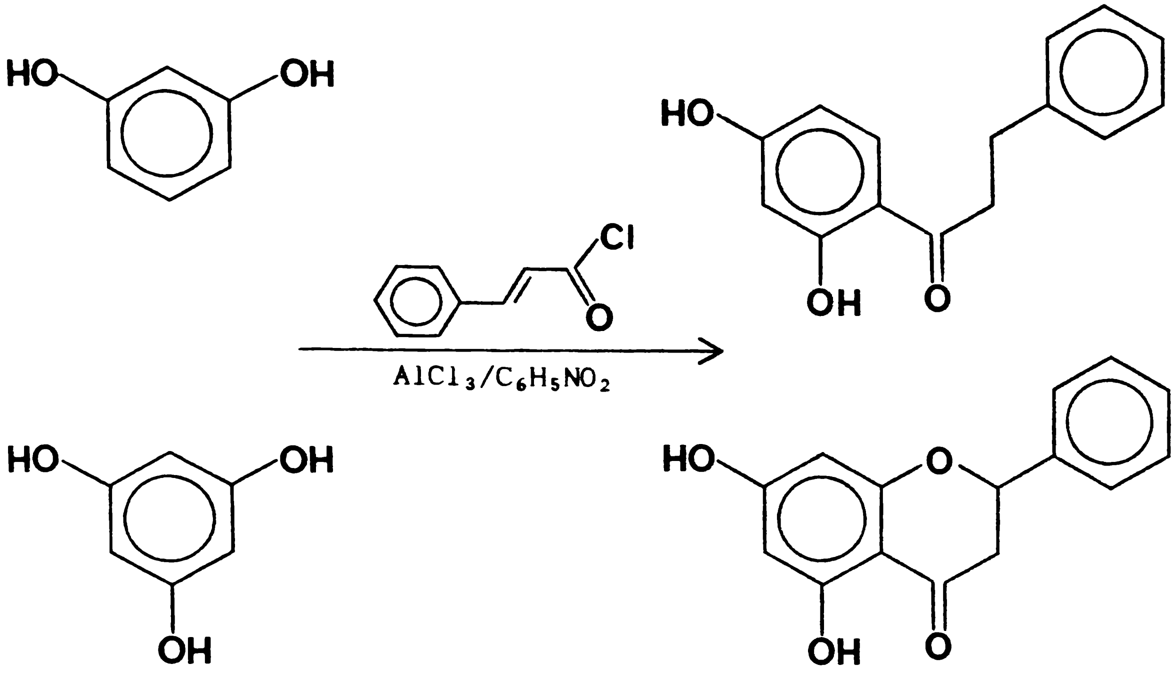
They also pointed out that a similar stability relationship is found in the synthesis of chalcones by Friedel-Crafts acylation of polyphenols with cinnamoyl chloride; when resorcinol is the phenol, the main product is the chalcone, while when the phenol used is



SCHEME 1-6 Hydrolysis Products of: (a) Butrin (*15*); and (b) Naringin (*18*), R = Rhamnoglucose

phloroglucinol, only the flavanone derivatives are obtained (Scheme 1-7).

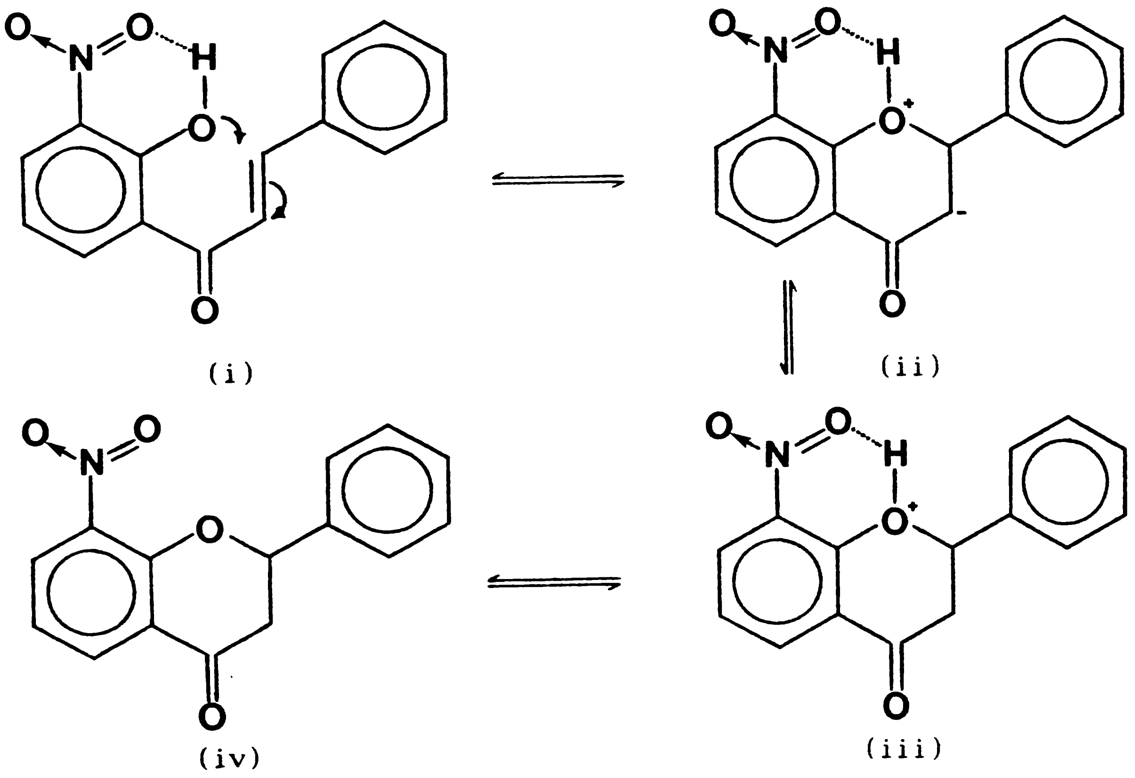
The explanation for the relative stability of 5-hydroxyflavanones relative to their isomeric chalcones given by these authors has not been seriously challenged, even though it is necessarily incorrect for the phenomenon under moderately basic conditions – for under these conditions the "additional chelate ring" cannot exist, because the 5-hydroxy group will be substantially ionised (estimated pK_a ≈ 10) under these conditions.



SCHEME 1-7 Friedel-Crafts Acylation of Polyphenols with Cinnamoyl Chloride

In 1954, Mentzer *et al.*[61] isolated 2',4,6'-trihydroxy-4'-methoxychalcone from the sodium hydroxide-soluble extracts of the heartwood of *Prunus avium*. This compound was probably generated by the base cleavage of the isomeric flavanone. They found this chalcone to cyclise rapidly upon heating to its melting point, to the isomeric flavanone.

In 1957 Seshadri and Trivedi[62] studied the cyclisation of ring-A nitro-substituted chalcones to flavanones in refluxing 3% ethanolic HCl. The 2',6'-dihydroxy-substituted chalcones cyclised rapidly (within 4 hours) to the flavanones, while the 2'-hydroxy- substituted chalcones cyclised very slowly, if at all. In fact B-ring substituted 7-hydroxy-8-nitroflavanones were completely converted to the chalcones by refluxing in 1:1 ethanol–conc. HCl within 15 minutes. The following mechanism (scheme 1-8) was put forward to account for the instability of the flavanone in acidic solution, along with the explanation that "the chelation of the *ortho* nitro group will prevent proton elimination at the last stage and retard the cyclisation. On the same basis, the tendency of (iv) to take up a proton will be much greater than the tendency of (iii) to lose one, and this accounts for the instability of the flavanone ring in acid medium".



SCHEME 1-8 Proposed[62] Mechanism to Account for the Stabilising Effect of the 3'-nitro group on 2'-hydroxychalcones

Shimokoriyama,[25] in 1957, prepared four 2',6'-dihydroxy-4'-glycosyloxychalcones from the naturally occurring

5-hydroxyflavanones, using strongly basic conditions to achieve ring opening of the flavanone, followed by acidification. All these chalcones isomerised rapidly to the racaemic flavanones under mild (weakly acidic to weakly basic) conditions. In addition, the rate of cyclisation of naringin-chalcone to naringin was determined spectrophotometrically in neutral to weakly acidic buffers at 22°C.

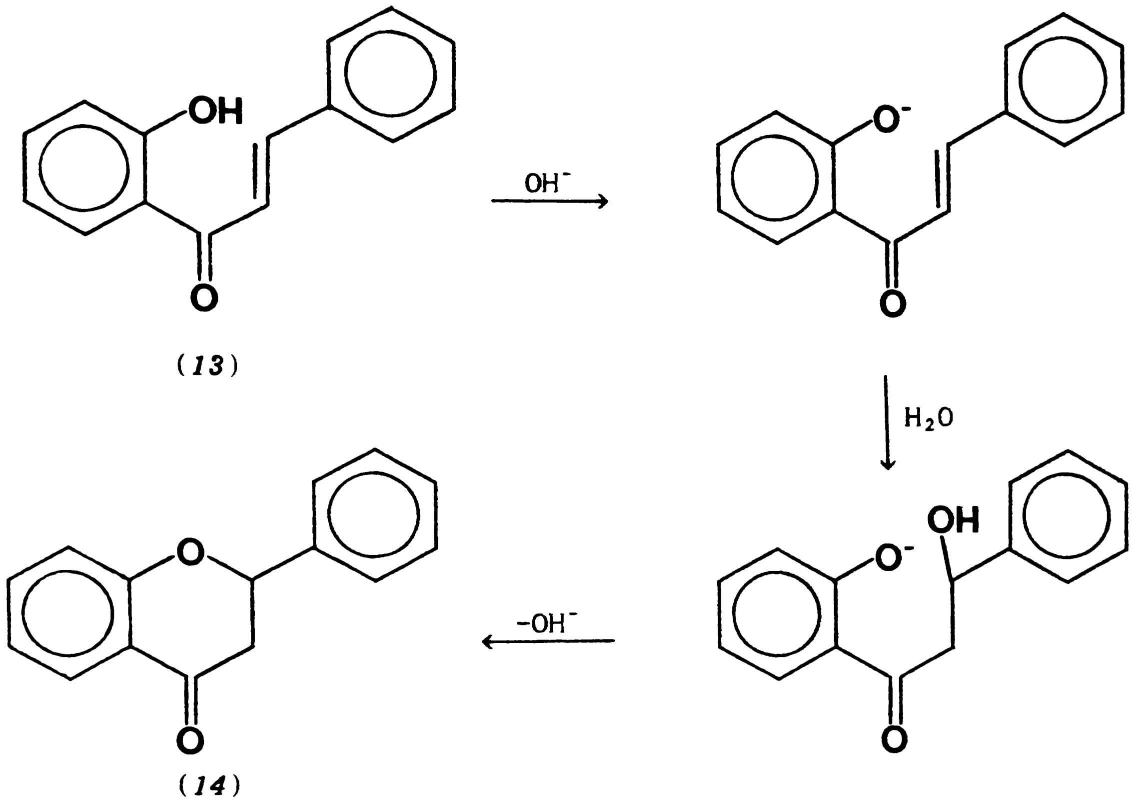
TABLE 1-1 Shimokoriyama's pH-Rate Data for Naringin-Chalcone[25]

pH	3	4	5	6	7
k (min.$^{-1}$)	0.000	0.001	0.018	0.055	0.150
$t_{0.5}$ (min.)	∞	693	38.5	12.6	4.6

He concluded that the reaction was monomolecular, and noted, as shown in table 1-1, that cyclisation was rapid at neutral pHs, while at pH 3 the chalcone was extremely stable.

David and Bognar[63] in 1961 studied the isomerisation of 2'-hydroxychalcone (*13*) to flavanone (*14*). They found the rate to increase with increasing temperature and pH, and found the reaction to be first order. The mechanism depicted in scheme 1-9 was given to account for the results.

In 1962, Tirouflet and Corvaisier[64] reported studies of the chalcone – flavanone equilibrium (where R = phenyl or a number of heterocyclic ring systems) using polarographic methods. Shown in figure 1-4 is their pH-rate profile for the cyclisation of 2'-hydroxychalcone (*13*) to flavanone (*14*). The authors proposed the mechanism given in scheme 1-10 for the acid-catalysed cyclisation.



SCHEME 1-9 Proposed Mechanism for 2'-hydroxychalcone Cyclisation[63]

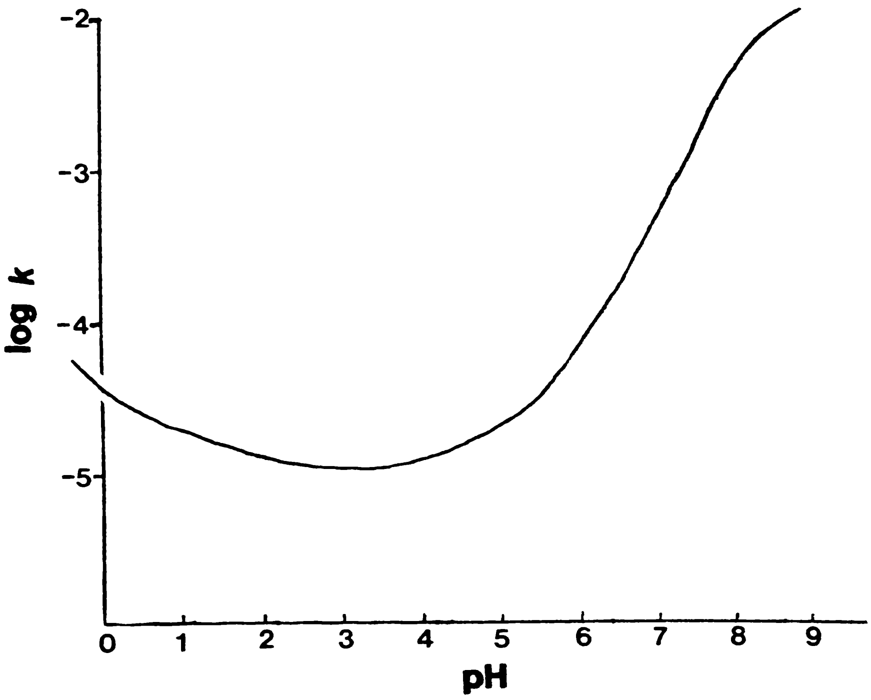
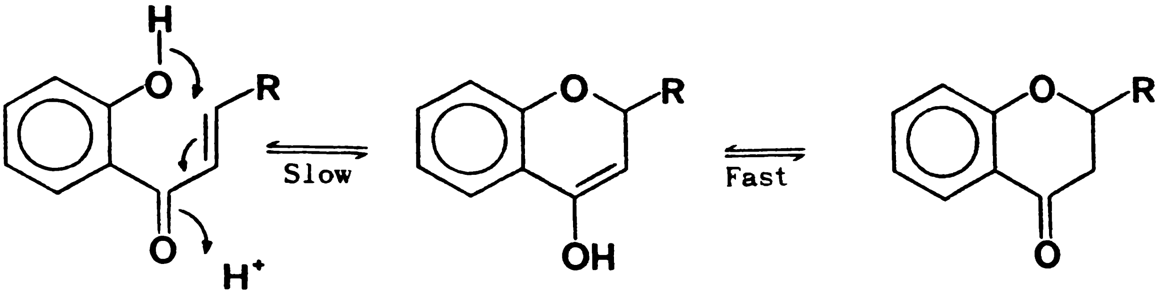
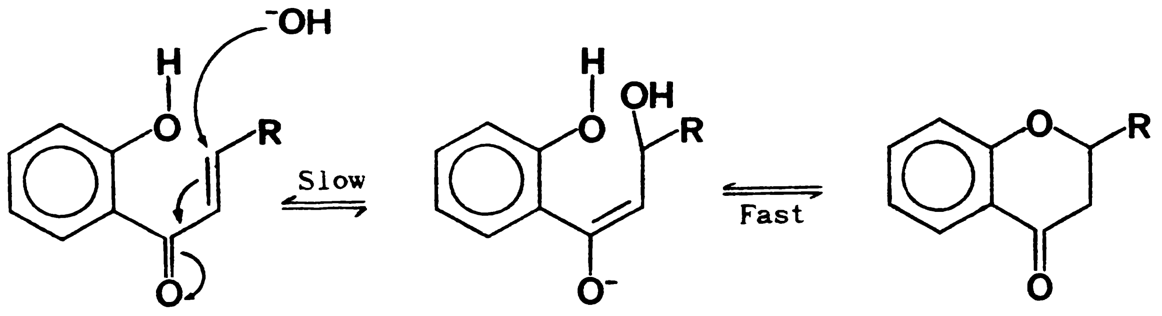


FIGURE 1-4 pH-Rate Profile for the 2'-Hydroxychalcone - Flavanone Isomerisation[64]



SCHEME 1-10 Proposed[64] Mechanism for the 2'-Hydroxychalcone - Flavanone Isomerisation (R = C_6H_5)

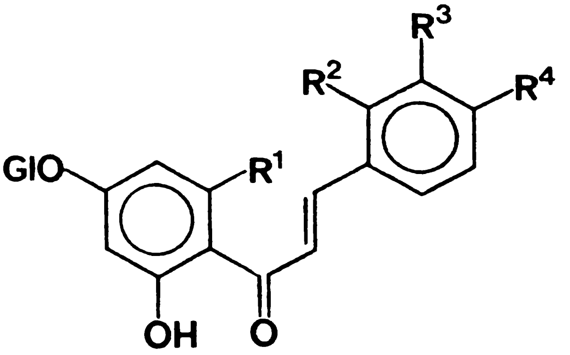
At higher pH values, two possible mechanisms for basic catalysis were considered, although the one shown below (scheme 1-11) involving a ketol intermediate was considered to be most likely.



SCHEME 1-11 Proposed[64] Base-Catalysed Mechanism for 2'-hydroxychalcone Isomerisation (R = C_6H_5)

In 1968 David *et al.*[65] studied spectrophotometrically the cyclisation of a number of glycosyloxychalcones (table 1-2) at several pH values, in water as well as in a number of other solvents. They found that at pH 7 in aqueous solution, the relative rates of cyclisation were 5 > 1 > 6 > 2 > 3 > 4.

TABLE 1-2 2′-Hydroxychalcones Studied by David *et al.*[65]



	R^1	R^2	R^3	R^4
1	glycosyloxy	H	H	OH
2	"	OH	H	H
3	"	H	OH	OH
4	"	H	OH	OCH_3
5	OH	H	H	OH
6	OH	H	OH	OCH_3

In 1973, Grouiller *et al.*[66] investigated the effect of substituents on the equilibrium constant of the chalcone – flavanone equilibrium. They measured the amount of residual chalcone at equilibrium for a number of chalcones at 80°C and pH 7 in ethanol–water (1:3). It was concluded that cyclisation, as measured by the equilibrium constant, was favoured by electron-withdrawing groups at the 4- and 4′-positions, and by electron-donating substituents at the 5′- position. It should be noted, however, that the equilibrium constant obtained by these authors for 2′-hydroxychalcone (3.50) is very different from that which can be calculated from the data of Old[46,71] for the same chalcone at 30°C in dioxane–water (1:19) at pH 7, of 98.

Also in 1973, Litkei *et al.*[67] reported that the decisive factor in the rate of oxidation of chalcones (and flavanones) in basic hydrogen peroxide was the rate of the chalcone – flavanone isomerisation. The pH-rate profile for the isomerisation of 2′-hydroxychalcone is shown in figure 1-5, and essentially the same plot was obtained in the presence of H_2O_2.

This pH-rate profile is markedly different in form to that obtained for the cyclisation reaction of the same compound by

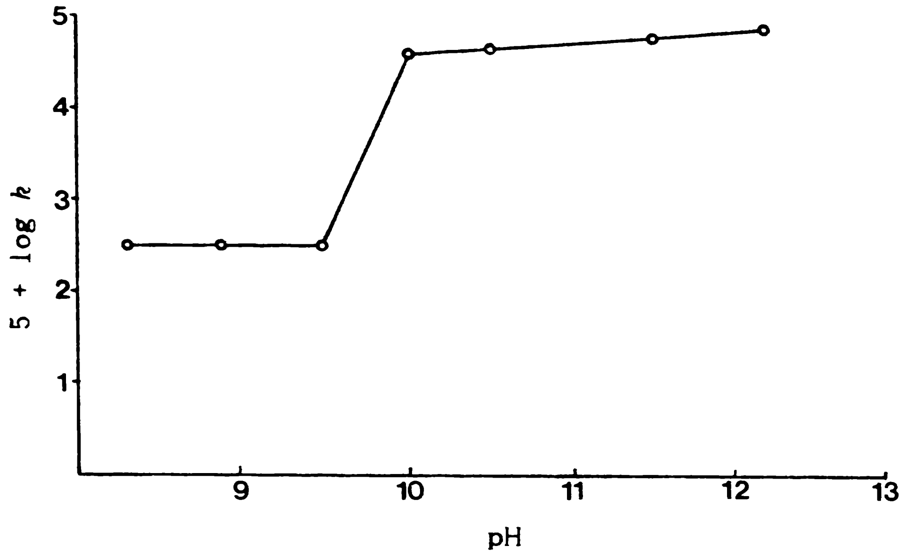


FIGURE 1-5 pH-Rate Profile for the 2'-Hydroxychalcone - Flavanone Isomerisation[67]

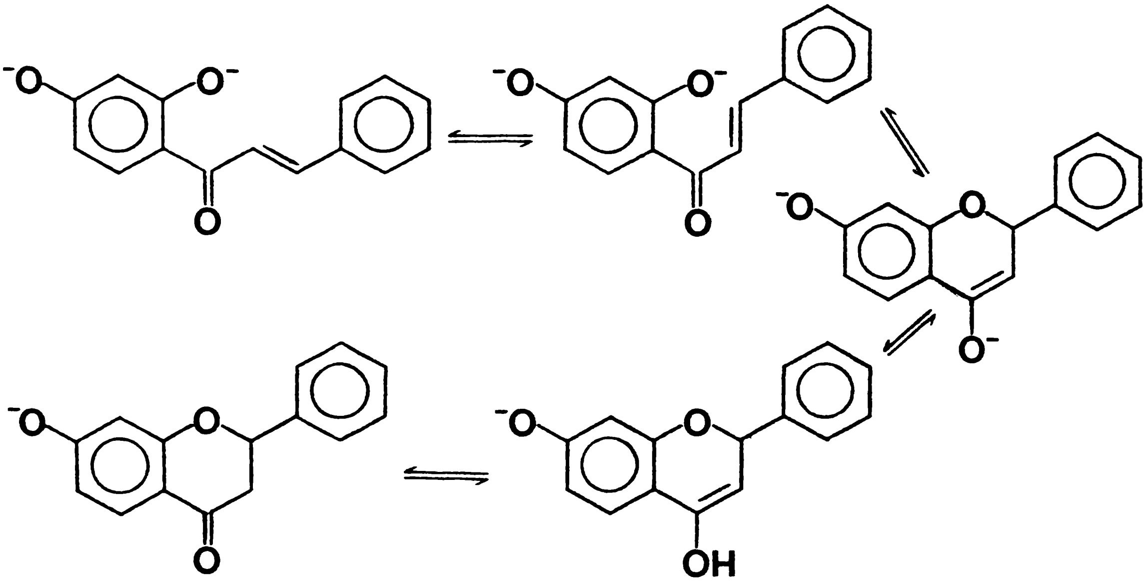
Old,[46,71] who found that the reverse ring-opening reaction intervened at higher pH so that a plateau in the pH-rate profile was not observed (*e.g.* see figure 1-6).

In 1975, Panasenko[68] reported the study of the chalcone - flavanone equilibrium for 2',4'-dihydroxychalcone in the high pH region (pH > 12), by measuring the ratio of chalcone to flavanone present at equilibrium in 0.0859-5.08 mol l^{-1} NaOH, as well as the rate of approach to equilibrium. They obtained the highest yields of the flavanone at the lowest pH, although the rate at which equilibrium was approached was found to decrease sharply with decreasing pH. In addition, they measured the acidity constants (pK_{a1} = 9.9, pK_{a2} = 12.3) of the chalcone and the activation parameters (E_a = 20.6 kCal mol^{-1}, $\Delta H^{\ddagger}$ = 19.4 kCal mol^{-1}, $\Delta S^{\ddagger}$ = -5.6 Cal mol^{-1} K^{-1}) of the isomerisation reaction.

Unfortunately we were unable to fit the %chalcone and %flavanone data from this paper to a curve generated by a computer programme and based on the relationship:

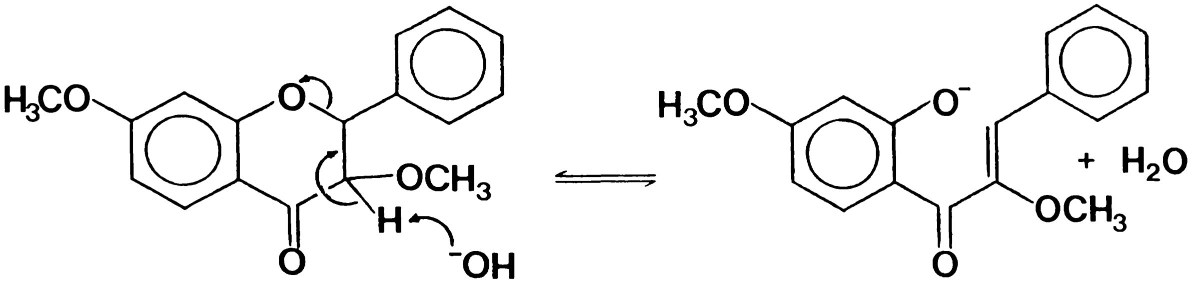
$$\% \text{ chalcone} = \frac{100k_r}{k_r + k_f}$$

(the derivation of which is performed in chapter 2) and their pK_a values. Possibly this was because the kinetic studies were not carried out at constant ionic strength, although subsequent work (see section 2.11) suggests that the isomerisation reaction of this compound has a low sensitivity to ionic strength effects. Given below (Scheme 1-12) is the mechanism that the authors proposed for the reaction.



SCHEME 1-12 Proposed[68] Mechanism for Base-Catalysed 2',4'-Dihydroxychalcone - 7-Hydroxyflavanone Isomerisation

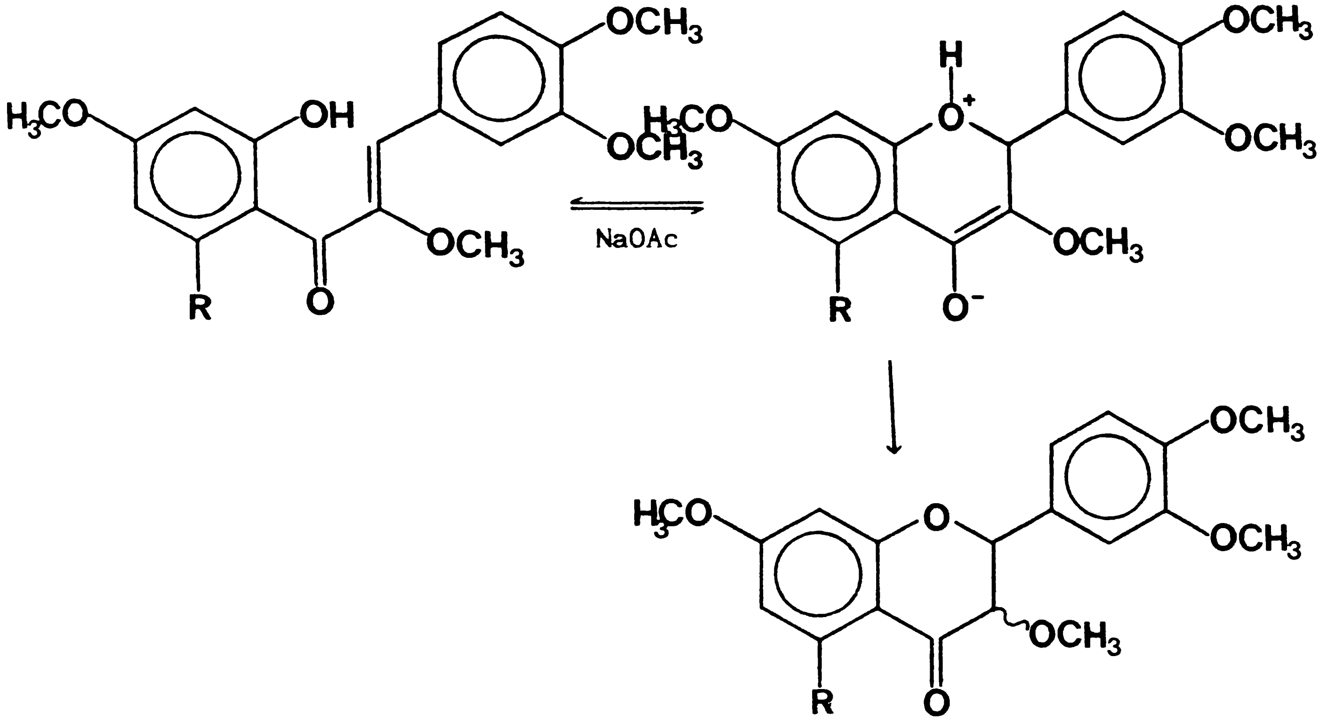
Studies of the products from the acid- and base-catalysed cyclisations of a number of 2'-hydroxy- and 2'-hydroxy-α-alkoxychalcones were reported in 1975 by Ferreira *et al.*[69] Under strongly basic conditions, they found the chalcone - flavanone equilibrium was shifted towards the chalcone form, particularly in the case of the α-alkoxy compounds. This behaviour was rationalised as resulting from the increased acidity of the α-hydrogen in the flavanone isomers containing a 3-alkoxy group, as shown in scheme 1-13.



SCHEME 1-13 Mechanism Accounting for the Low Stability of 3-Alkoxyflavanones in Basic Solution[69]

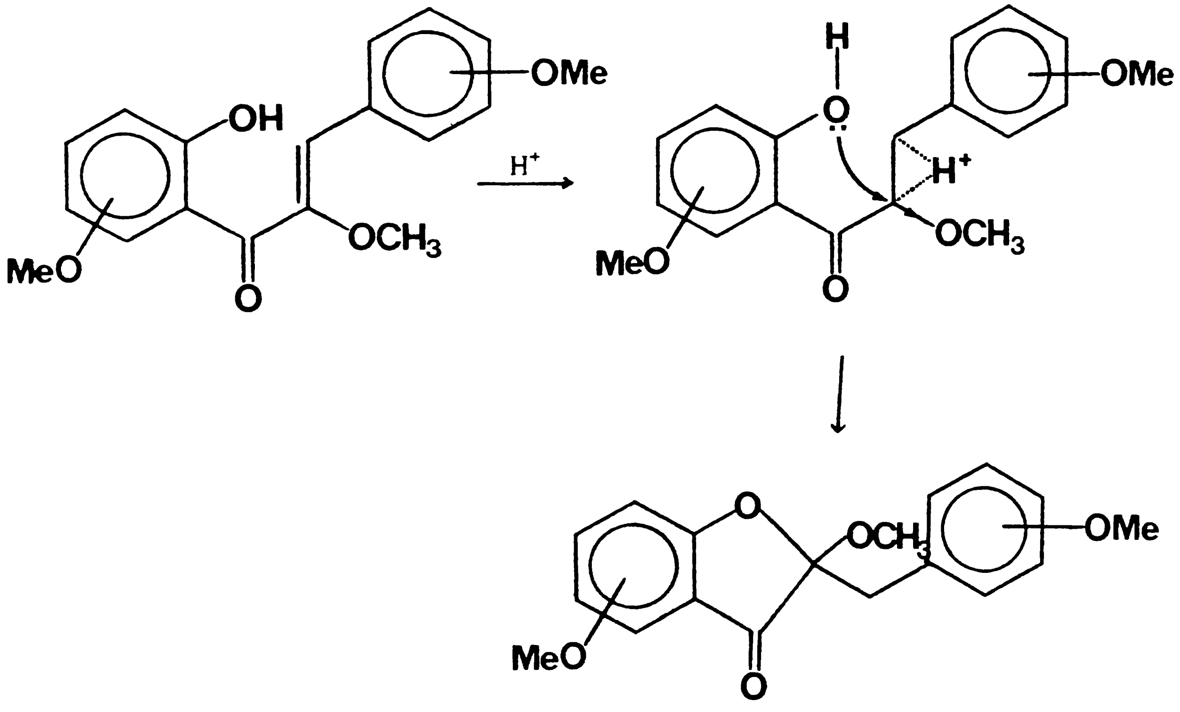
Weakly basic to weakly acidic conditions resulted in normal cyclisation to flavanones for both α-methoxy- and α-protio-chalcones. Because the authors did not detect ionisation of the 2'-hydroxy group of the chalcones under the weakly basic conditions used (dilute sodium acetate), the mechanism shown in scheme 1-14 was put forward for the cyclisation. The sodium acetate was assumed to push the equilibrium to the right by acting as a buffer, since the yield of 3-methoxyflavanones under near neutral conditions was much lower.

Under strongly acidic conditions, however, the α-methoxychalcones were found to cyclise to the α-position, to give 5-membered ring compounds. On the other hand, the other 2'-hydroxychalcones cyclised in the normal manner to the β-position to yield flavanones. The mechanism depicted in scheme 1-15 was put forward to explain this anomalous cyclisation, which apparently is a result of the inductive effect of the α-alkoxy substituent. The authors suggest that this strong inductive effect is also responsible for driving the reaction



2,3-*trans*:2,3-*cis* = 2:1

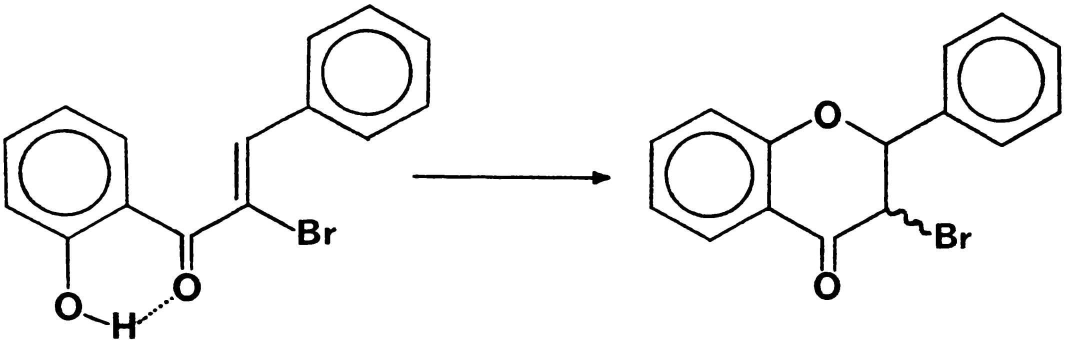
Scheme 1-14 Proposed Mechanism for the Cyclisation of α-Alkoxy-2'-hydroxychalcones in Weakly Basic Solution[69] (R = H, OCH$_3$)



SCHEME 1-15 Proposed[69] Mechanism for the Acid-Catalysed Cyclisation of α-Alkoxy-2'-hydroxychalcones to 5-Membered Ring Compounds

to completion.

David *et al.*[70] in 1981 reported the rate constants for cyclisation of (*E*)- and (*Z*)-α-bromo-2′-hydroxychalcones to 3-bromoflavanone at pH 7.21 and 7.88. From these rates it can be estimated (assuming the reverse reaction to be negligible at these pH values) that if the pK_a of the phenolic group is 9-10, then the specific rate coefficients for the cyclisation of the (*Z*)-chalcone is $k^Z_{CH} = 2.4\text{-}2.5\times10^{-3}\ s^{-1}$ for the un-ionised chalcone, and $k^Z_{C^-} = 0.28\text{-}2.6\ s^{-1}$ for the chalcone anion. Thus the (*Z*)-α-bromo-2′-hydroxychalcone anion appears to cyclise at least 40 times faster than the 2′-hydroxychalcone anion, while the un-ionised bromochalcone cyclises *ca.* 240 times faster than neutral 2′-hydroxychalcone (see below and ref. 46,71).

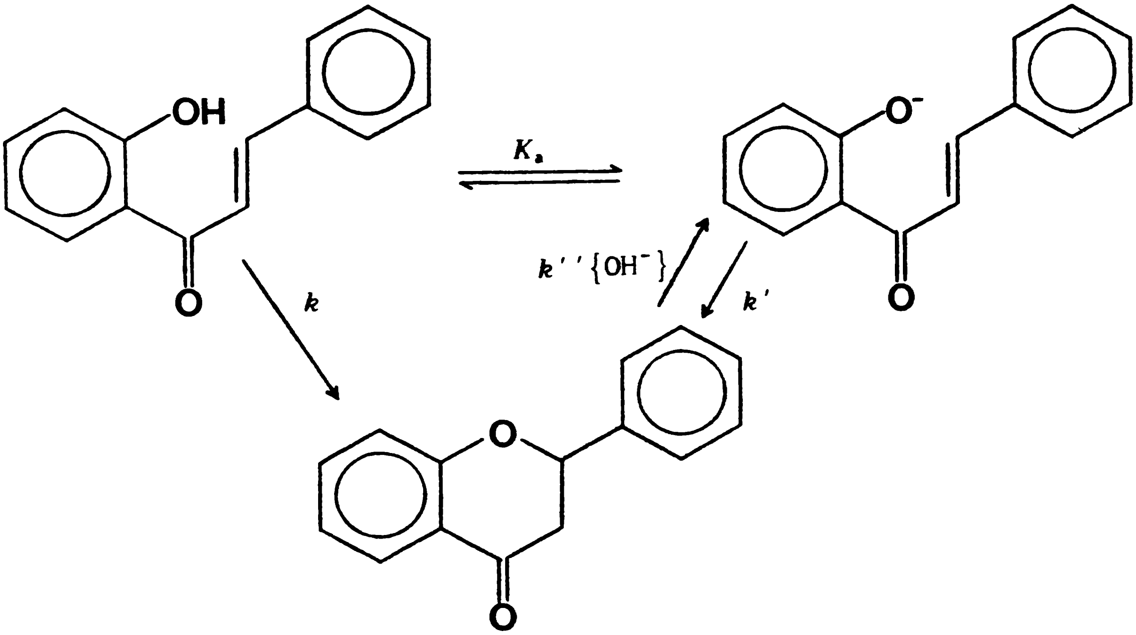


SCHEME 1-16 Mode of Cyclisation of (*Z*)-α-bromo-2′-hydroxychalcone to 3-bromoflavanone[70]

In 1982 came the first thorough study of the kinetics of the chalcone – flavanone equilibrium; Old and Main[46,71] reported the kinetics of cyclisation for a number of variously substituted chalcones to their isomeric flavanones over the pH range 7-11.5. They were able to fit the observed rate constants (k_{obs}) to equation 1-1, based on the reaction scheme shown in scheme 1-17;

$$k_{obs} = kf_A + k'f_B + k''\{OH^-\} \quad ....1\text{-}1$$

where *k* and *k*' were the rate coefficients for unimolecular cyclisation to the flavanone of the neutral and anionic forms of the chalcone respectively; f_A and f_B are the fractions of the chalcone present in the neutral and anionic forms respectively ($f_A + f_B = 1$), and depend only upon the pK_a of the chalcone and the pH. The third term was considered to represent the ring-opening reaction of the flavanone, a pseudo first-order process at any given constant pH, *k*'' being the second order rate coefficient for the reaction of the flavanone with hydroxide ion of activity $\{OH^-\}$. Figure 1-6 shows a plot of logk_{obs} versus pH for the 2'-hydroxy-6'-methoxychalcone – 5-methoxyflavanone isomerisation, as well as the individual contributions to k_{obs} due to kf_A. $k'f_B$ and $k''\{OH^-\}$.



SCHEME 1-17 Reaction Scheme for 2'-Hydroxychalcone – Flavanone Isomerisation[46,71]

The following mechanism (scheme 1-18) was put forward[46] as being the simplest one which accounted for the observed kinetics of the reaction of the chalcone anion, stepwise conjugate addition, with ring closure being rate limiting in the forward direction. For the reverse

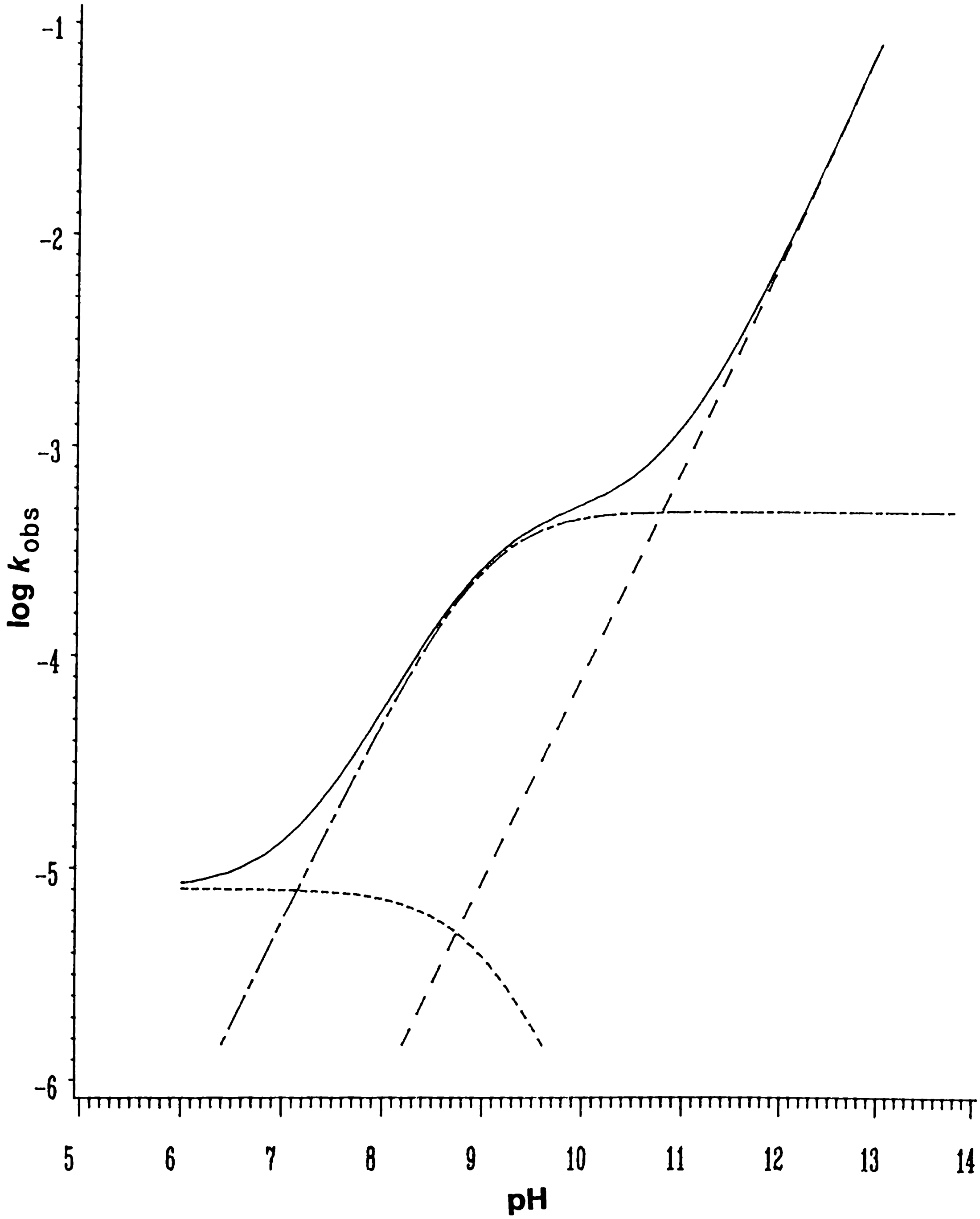
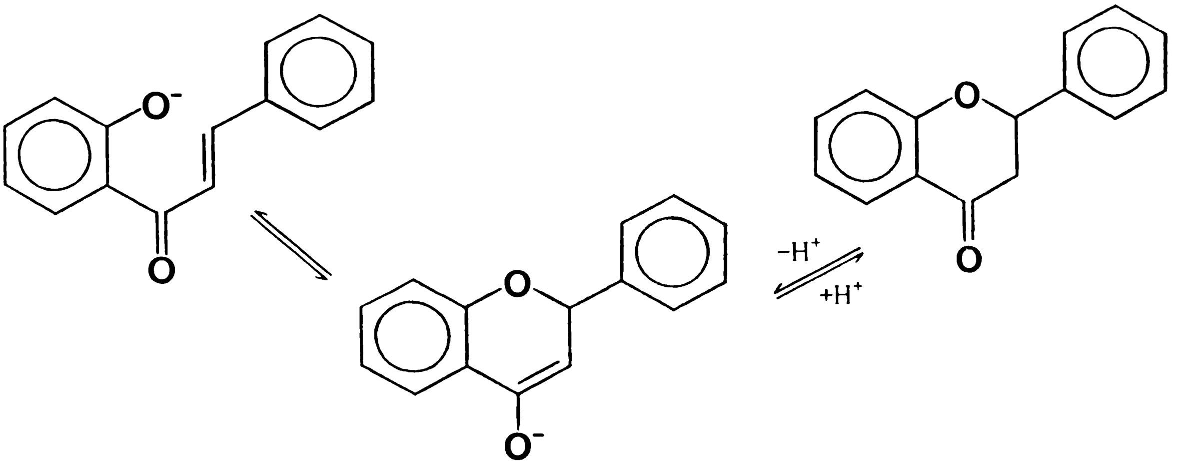


FIGURE 1-6 The pH-Rate Profile[46,71] for the 2'-Hydroxy-6'-methoxychalcone - 5-Methoxyflavanone Isomerisation (——), and the Individual Contributions to k_{obs} of kf_A (---), $k'f_B$ (— – —) and $k''\{OH^-\}$ (– – –)

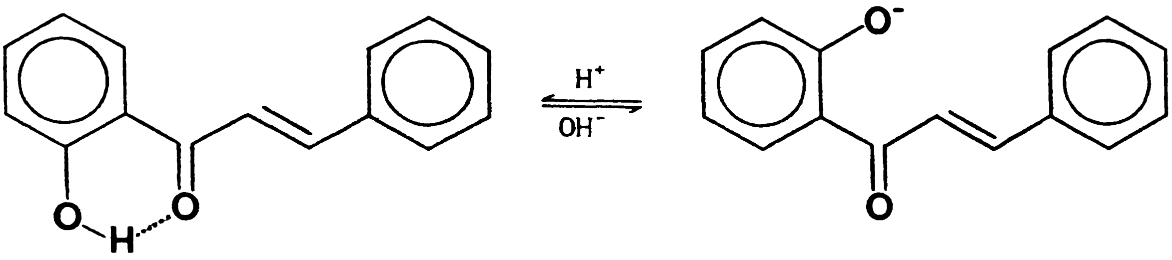
of this reaction, equilibration between the flavanone and its enolate anion is followed by rate-limiting ring-opening, a mechanism consistent with the absence of general base catalysis by the buffers used.



SCHEME 1-18 Proposed[46] Mechanism for the 2'-Hydroxychalcone anion - Flavanone Isomerisation

Recently (1985) Furlong and Nudelman[72] studied the mechanism of cyclisation of 2'-hydroxychalcones to flavanones. They attempted to use the ^{1}H n.m.r. spectra of the chalcone anions to obtain information about their solution conformation. They concluded that the chalcones exist mainly in the s-*cis* conformation, and that upon ionisation of the 2'-hydroxy group the A-ring rotates 180° around the (4'-C)—(1'-C) axis, giving rise to a strong interaction between the 2'-oxyanion and the α-H as shown in scheme 1-19.

In addition they studied the effect of sodium hydroxide concentration on the rate of ring closure of the chalcones. In what was presumably an attempt to circumvent the problem of the reverse reaction interfering with the measurements, the pseudo first-order rate coefficients were calculated from the slope of ln A versus time at a wavelength where the product does not absorb at the wavelength of measurement. Alternatively where this approach was not possible, the slope of $\ln(A_t - A_\infty)$ versus time was used, where A_∞ was calculated from the variation of A_0/A_t with time at several wavelengths.



SCHEME 1-19 Proposed[72] Effect of Ionisation on the Conformation of 2'-Hydroxychalcones

They found the rate of cyclisation to increase with increasing hydroxide concentration, until a plateau was reached which corresponded to the conversion of all the chalcone present into its anionic form. A plot of log$k$ versus log[NaOH] is shown in figure 1-7 for a number of chalcones.

The authors also observed weak general acid catalysis by glycine (k_{BH^+} = 0.19 l mol^{-1} s^{-1}) in the plateau region for 2'-hydroxychalcone. They calculated activation parameters for the cyclisation of all the chalcones, and the rates for ring closure of the chalcone anions are reported as second-order rate constants ($k_{plateau}/[H_2O]$). A large kinetic isotope effect was observed (k_H/k_D = 5.3) in the cyclisation of 2'-hydroxychalcone. Scheme 1-20 shows the mechanism given to account for these results, although it is unclear from the text whether the species in brackets is supposed to be an intermediate, or a transition state.

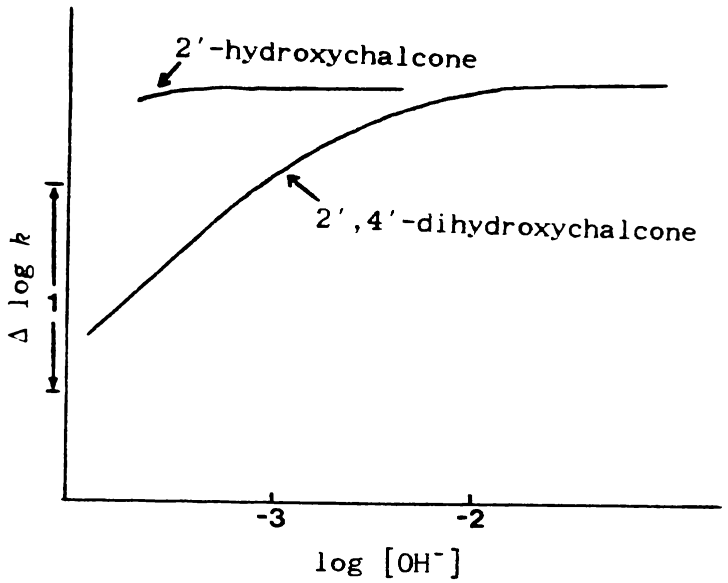
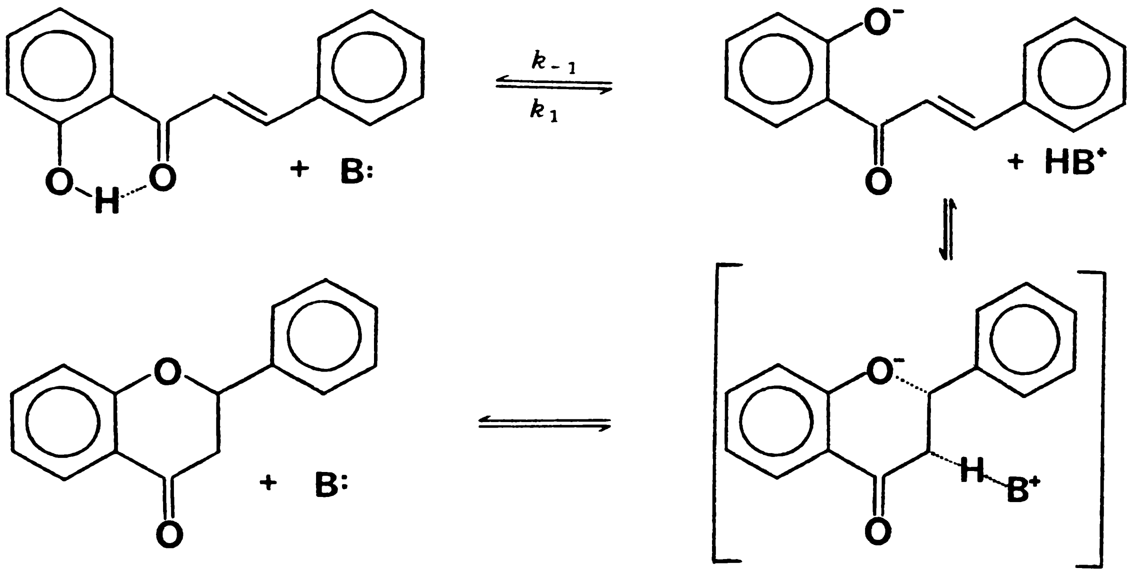
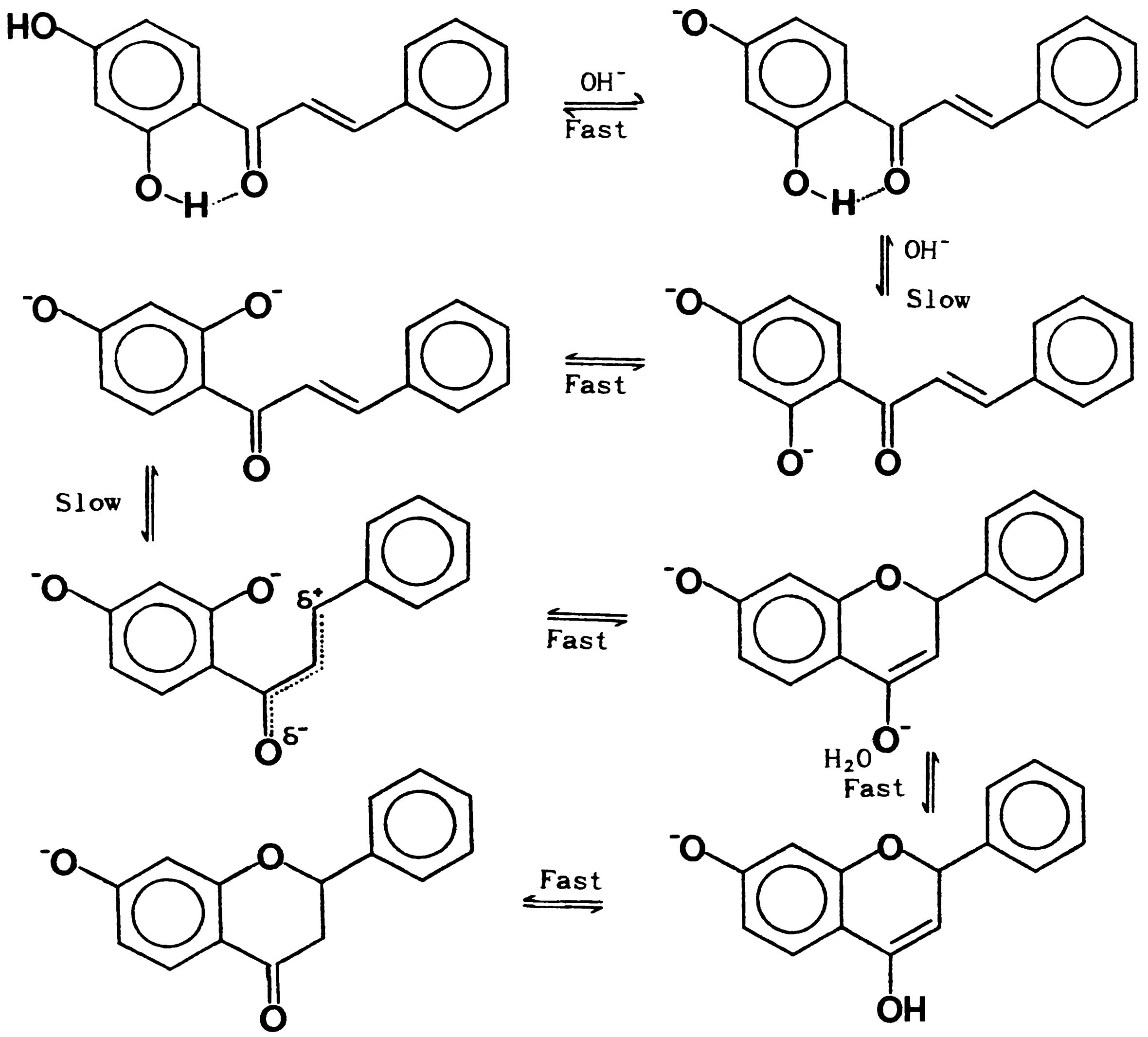


FIGURE 1-7 Plot of log$k$ versus log[OH^-] for some 2'-Hydroxychalcones[72]



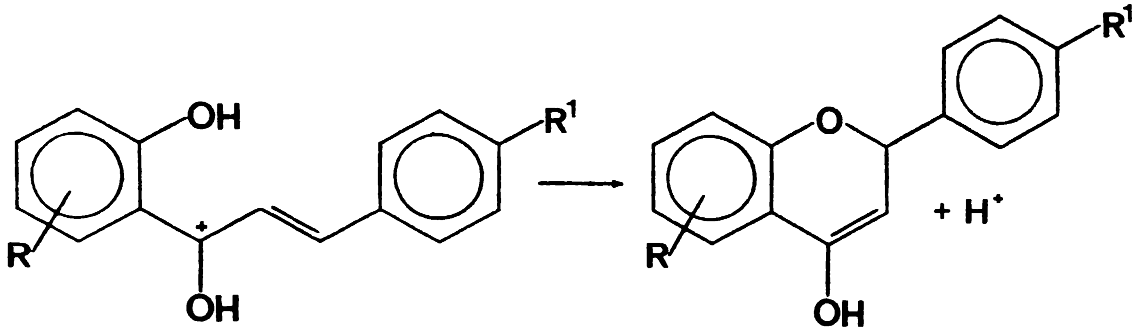
SCHEME 1-20 Proposed[72] Mechanism the Base-Catalysed 2'-Hydroxychalcone – Flavanone Isomerisation

Furlong *et al.*[73] published a more detailed study of the kinetics of cyclisation of 2',4'-dihydroxychalcone in alkaline solution soon after their initial chalcone cyclisation studies[72] (above). Here the authors concluded that cyclisation of the chalcone had two rate-determining steps: at pH 9-11, removal of the 2′- phenolic proton from the chalcone mono-anion was said to be rate-determining; while at pH > 12, the rate of the phenolic proton abstraction became more rapid than the internal rotation (*trans*-s-*cis* to *trans*-s-*trans*) that allows the close approach of the 2′-phenoxide to the β-carbon, so that this internal (pH-independent) rotation step becomes rate-limiting at high pH, accounting for the observed plateau in the pH-rate profile. The proposed[73] mechanism is given in scheme 1-21.



SCHEME 1-21 Proposed[73] Mechanism for the 2′,4′-Dihydroxychalcone - Flavanone Isomerisation in Basic Solution

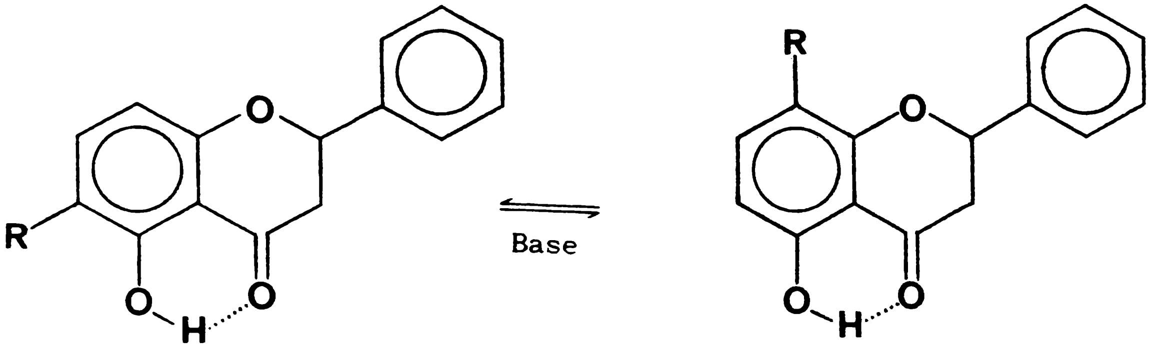
In 1986, as part of an effort to correlate the physico-chemical properties of some chalcones with indices derived from simple molecular orbital calculations, Debattista *et al.*[74] determined the rate constants for the acid-catalysed cyclisation of 2',4'-di- (0.26×10^{-2} l mol^{-1} s^{-1}), 2'- (2.99×10^{-2} l mol^{-1} s^{-1}), and 2',4-di- (47.6×10^{-2} l mol^{-1} s^{-1}) hydroxychalcones. A mechanism (scheme 1-22) involving the following rate-determining step was postulated.



SCHEME 1-22 Proposed[74] Rate-Determining Step in the Acid-Catalysed Cyclisation of 2'-Hydroxychalcones

From the foregoing discussion of the research into the chalcone – flavanone equilibrium. it is clear that the 2'-hydroxychalcone – flavanone isomerisation is becoming better understood. The situation in the case of 2',6'-dihydroxychalcones is less clear, with conflicting reports in the literature as to their stability. However a good deal of evidence exists which demonstrates that these chalcones exist, although not necessarily as stable compounds, both as synthetic and natural products.

For instance, a large number of reports are now present in the literature, of the synthesis of 2',6'-dihydroxychalcones, although many of the purported chalcones are of dubious constitution, as will be discussed in detail in chapter 6. More compelling evidence for their existence as reasonably stable compounds comes from the growing number of well characterised[75-91] and not so well characterised[7,92-99] 2',6'-dihydroxychalcones which have been isolated as natural products.



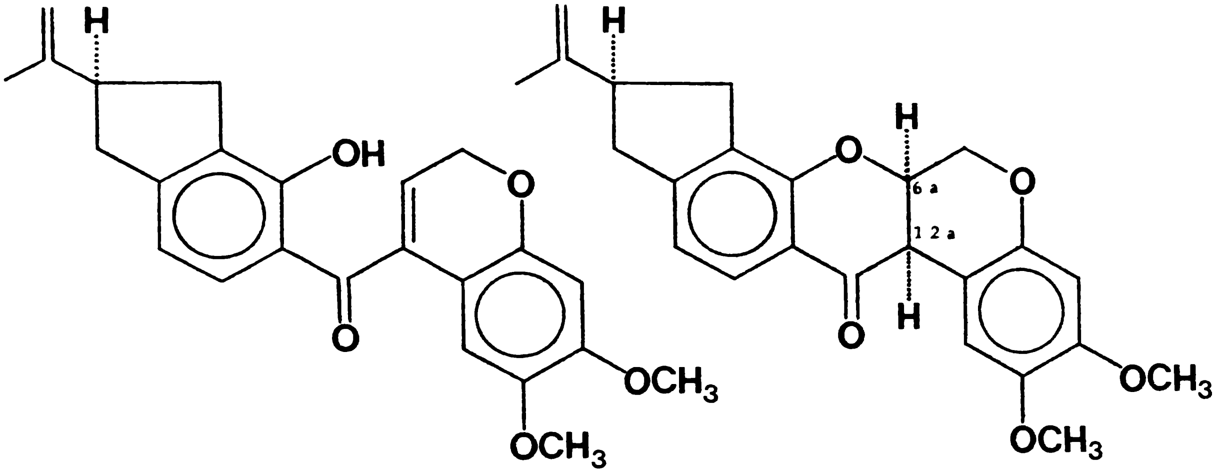
SCHEME 1-23 Ring Isomeric Change in 5-Hydroxyflavanones

Less direct evidence comes from the observation[100-104] of ring isomeric change in 5-hydroxyflavanones, where 6- or 8- substituted 5-hydroxyflavanones isomerise to varying degrees under acid or basic conditions into the 8- and 6- substituted isomers respectively (scheme 1-23). This phenomenon, which has been observed in quite a number of variously substituted 5-hydroxyflavanones, along with the several syntheses of this class of chalcone by ring-opening (to be discussed fully in chapter 6), proves that the chromanone ring of 5-hydroxyflavanones is susceptible to ring opening in most cases, and in several cases at least, the resultant 2',6'-dihydroxychalcone is a reasonably stable compound.

1.3 Related Reactions of Interest

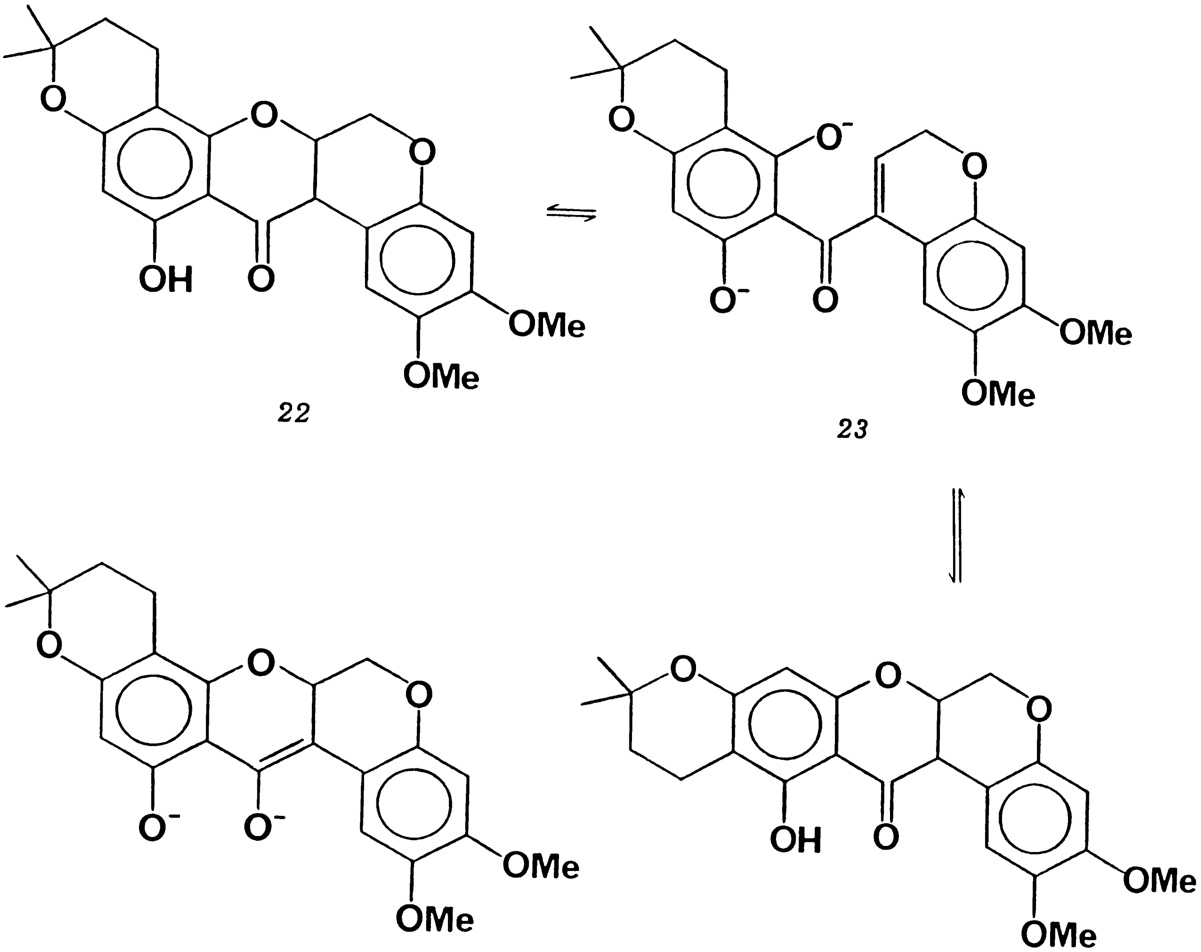
Ring closures of the chalcone to flavanone type are not at all rare. For instance in the synthesis of rotenone (*21*) and related compounds[105-109], the final step involves ring-closure of chalcone type intermediates (*20*) under weakly basic conditions.

Furthermore, basic conditions cause racaemisation[109] of the centres at the 6a- and 12a- positions of ***21***. With mild bases, such as sodium acetate in ethanol, the species involved is thought to be the anion of ***20***. The chalcone analogue (***20***) could not be synthesised directly by base-promoted ring opening of ***21***, but it has been obtained by an alternative method using sodium in benzene. With the 5-hydroxyflavanone analogue α-toxicarol (***22***), the chalcone-type anion and di-anion (***23***) are implicated in the ring isomeric change (analogous to that discussed earlier) to β-toxicarol (***24***) that is catalysed by potassium carbonate in wet acetone. The difference in behaviour of α-toxicarol and rotenone towards methylation with dimethyl sulphate in potassium carbonate and wet acetone was discussed[109] in terms of the relative stabilities of the enolate di-anion (***25***) and chalcone-type di-anion (***23***) forms of α-toxicarol.



20 ***21***

Interestingly, acid-catalysed ring closure of the rotenone-chalcone compound (***20***) yields a 5-membered ring spiroketone[109] (***26***) (scheme 1-24), a mode of ring closure identical to that discussed previously for α-methoxy substituted 2'-hydroxychalcones.



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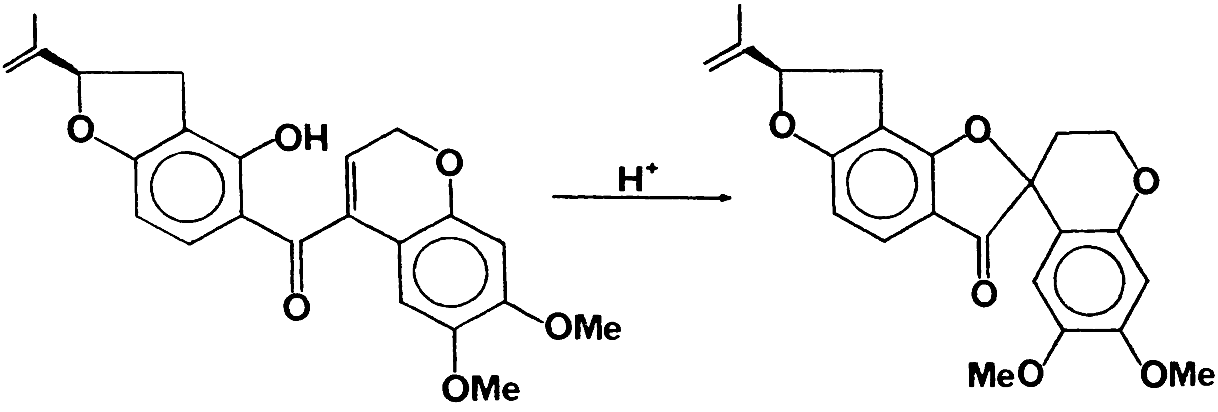
Radio-labelling studies[110] of the biosynthesis of the chromanone (*27*) showed that a proton was taken up at C-3, and although ring closure was stereospecific at C-2, the proton that appeared at C-3 was shown by ^{2}H n.m.r. to be present equally in the two possible positions. Thus protonation of the proposed intermediate enolate must occur with equal facility to each face, as shown in scheme 1-25. The author noted that this rather unexpected result was in direct contrast to the results obtained during enzyme-catalysed cyclisation of chalcones to flavanones (which was discussed earlier in this chapter).



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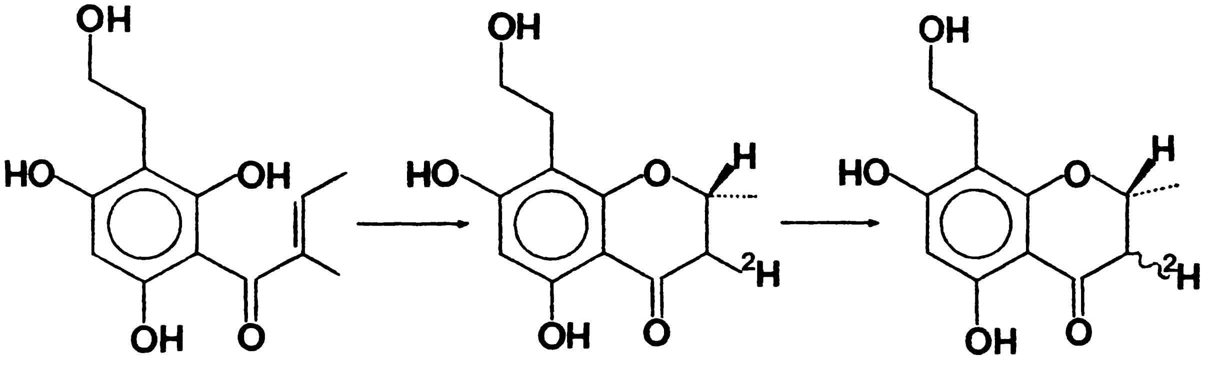
SCHEME 1-24 Mode[109] of Acid-Catalysed Cyclisation of (-)-6a,12a-Dehydrorotenol (*20*)



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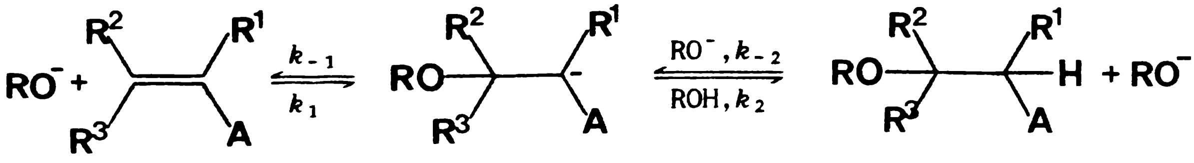
SCHEME 1-25 Proposed[110] Biosynthetic Pathway to LL-D253α (*27*)

The isomerisation of chalcones to flavanones, and the related reactions above, represent intramolecular attack by a nucleophile (ArOH or ArO$^-$) on a double bond activated by the electron withdrawing effect of a carbonyl group. The closely related intermolecular attack of nucleophiles on activated (not necessarily by a carbonyl group) double bonds has been the subject of considerable investigation, and some representative examples follow.

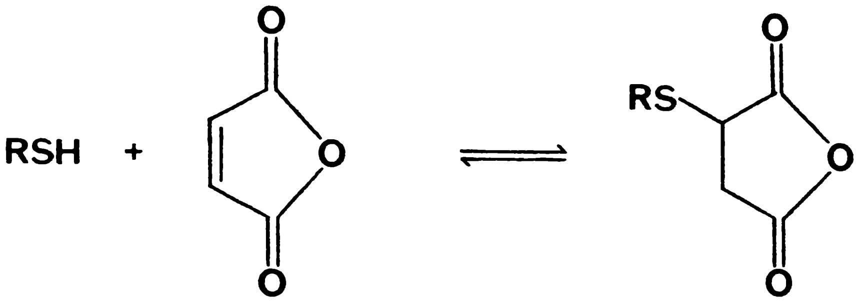
Toma[111] has studied the addition of ethyl α-cyanobutyrate to a series of A- and B-ring sustituted chalcones. The reaction was first order with respect to the chalcone, with electron withdrawing substituents in the A-ring and to a lesser extent the B-ring, accelerating the rate of addition. Logarithmic plots of the rate constant versus σ gave $\rho = 1.7$ for A-ring and $\rho = 1.1$ for B-ring substituted chalcones.

Ring *et al.*[112] have studied the base-catalysed addition of alcohols to activated double bonds of the type $R^3R^2C{=}CR^1A$, where A is a strongly electron-withdrawing group. The rate-enhancing effect of the activating group A increased in the order CONHR < $CONH_2$ < $CONR_2$ < COOR < SO_2NR_2 < CN < SO_2R < COR < $^+PR_3$. The most effective activating group gave a rate enhancement of *ca.* 10^4 over the rate for the least activating group. A methyl group on either vinyl carbon lowered the rate of reaction relative to the unsubstituted compound. The reaction was first-order with respect to the alkoxide and to the vinyl compound. The following mechanism (scheme 1-26) was given to account for the results.



SCHEME 1-26 Ring's Mechanism[112] for the Base-Catalysed Addition of Alcohols to Activated Double Bonds

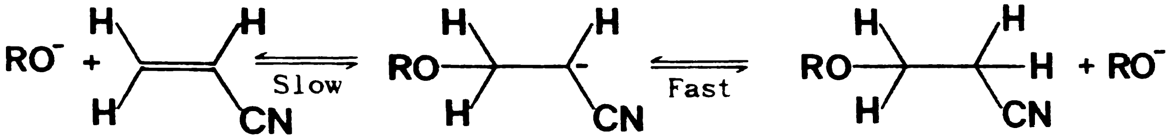
Zienty *et al.*[113] have studied the addition of a variety of thiols to maleic anhydride (scheme 1-27) in the presence of a basic catalyst under anhydrous conditions.



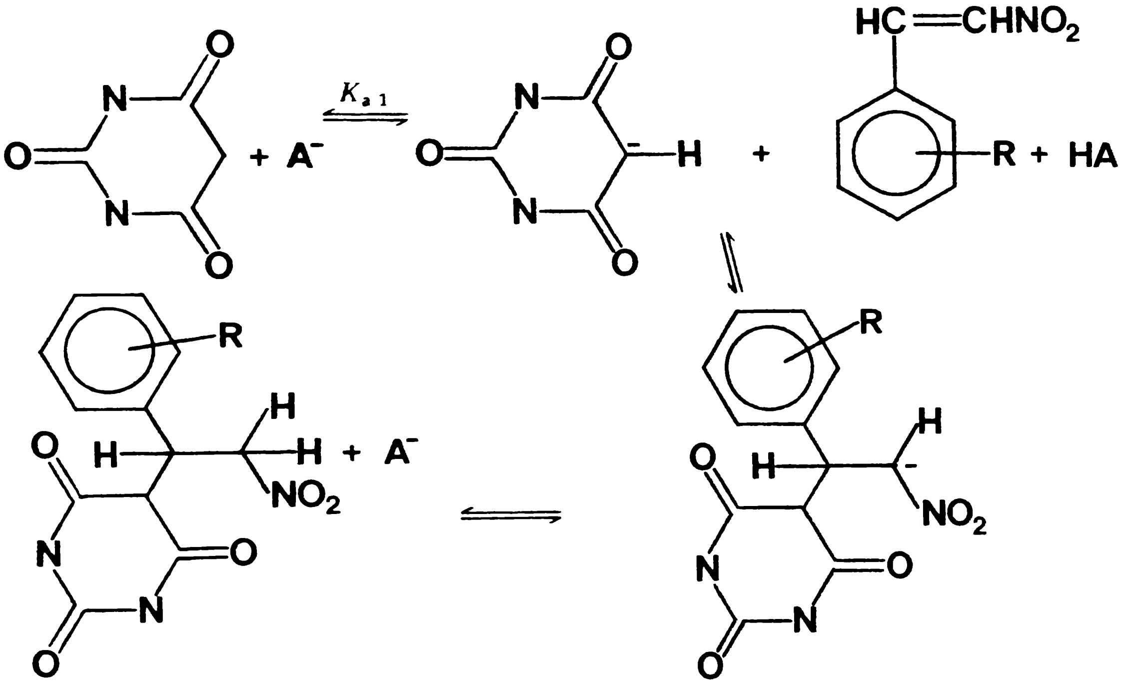
SCHEME 1-27 The Mode of Addition of Thiols to Maleic Anhydride

The rate of addition for aromatic thiols was 4-methoxy- < 4-chloro- < 4-nitro-thiophenol. It was concluded that these thiols were reacting via their anions, since the pK_as are in the reverse of the above order (while the nucleophilic power of the undissociated thiols ought to run in the reverse order to that given above, resulting in rates in the reverse of the order found). The actual kinetic data were not presented in the paper, however. The reverse reaction (β-elimination) was also observed, and was estimated to be at least 10^3 times slower than the forward reaction, under the reaction conditions used.

Feit and Zilka[114] investigated the base-catalysed addition of alcohols to acrylonitrile. The reaction was first-order in acrylonitrile and alkoxide ion, was independent of the alkali metal counter-ion, and the rate increased with increasing basicity of the alkoxide ion. The results were said to be consistent with slow addition of alkoxide ion to acrylonitrile followed by rapid abstraction of a proton from the solvent by a carbanion intermediate, as shown in scheme 1-28. The authors were able to show that the alcohol itself was not the nucleophile under the conditions used.



SCHEME 1-28 Proposed[114] Mechanism for the Addition of Alkoxides to Acrylonitrile



SCHEME 1-29 Proposed[115] Mechanism for the Addition of Barbituric Acid to β-Nitrostyrene

Kamlet and Glover[115] studied the addition of barbituric acid to β-nitrostyrene in weakly acidic media. The reaction was second-order, the rate depending on the concentrations of β-nitrostyrene and barbiturate anion. Electron-withdrawing groups on the phenyl group of the styrene increased the rate, with $\rho \approx 0.8$. The reverse reaction was also demonstrated to be present. The mechanism shown in scheme 1-29 was considered the most likely of those consistent with the experimental results.

Crowell *et al.*[116] studied the addition of methanol to dibenzoylethylenes (*28*) over the pH range 6-14, and for the parent compound obtained the pH-rate profile shown in figure 1-8.

They considered rate-controlling attack of methoxide ion competing with the much slower attack of methanol to be consistent with their results. A Hammett plot of $\sigma_X + \sigma_Y$ gave $\rho = 1.0$. That the reaction was reversible and ran to equilibrium was established by starting the experiment with the methanol adduct and following the return to equilibrium. The following mechanism (scheme 1-30) was given by the authors as being consistent with their results.

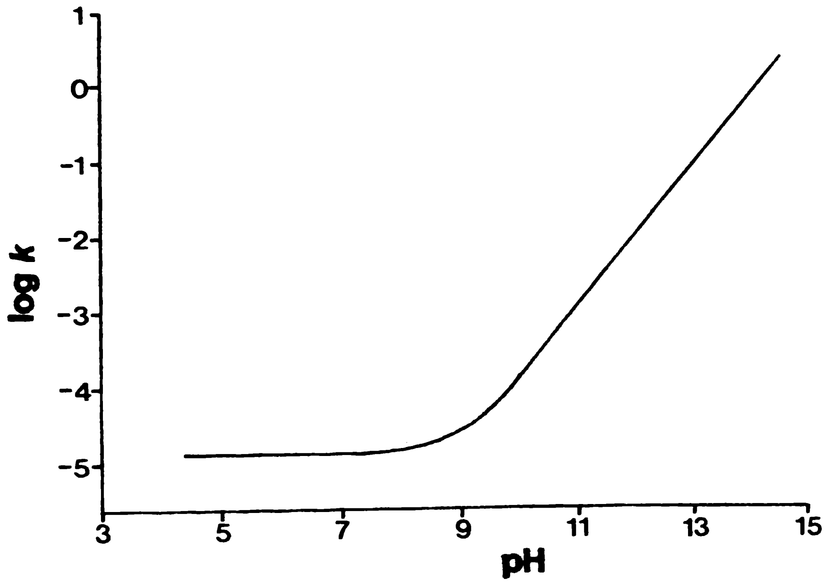
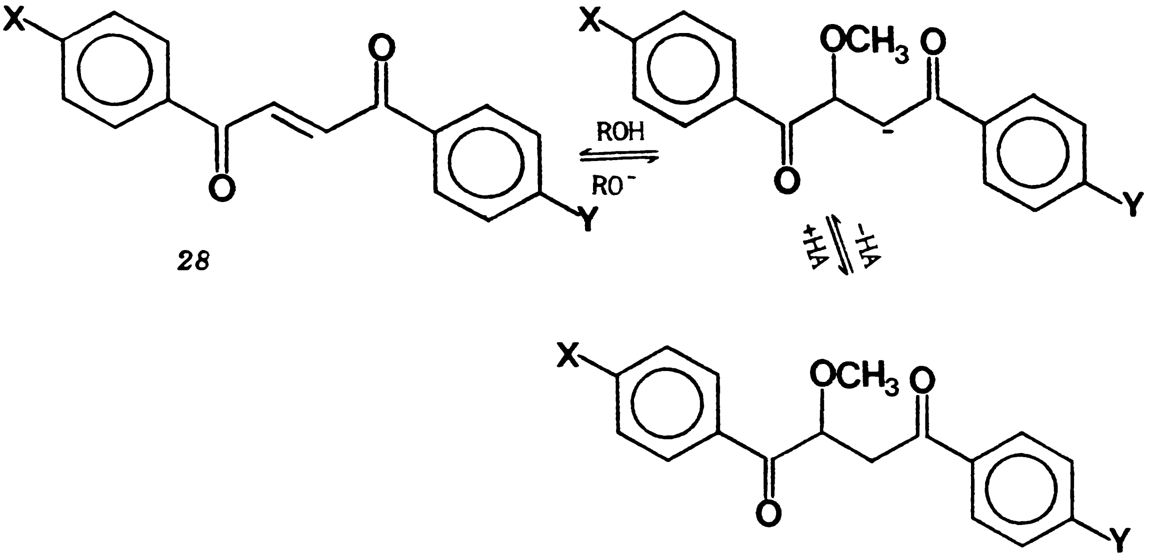


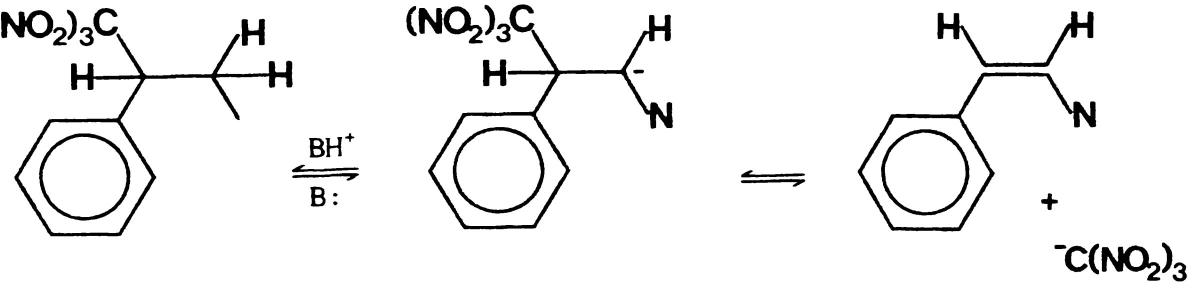
FIGURE 1-8 pH-Rate Profile for Addition of Methanol to Dibenzoylethylene[116]

Hine and Kaplan[117] have investigated the decomposition of 1,1,1,3-tetranitro-2-phenylpropane, and the reverse of the reaction,



SCHEME 1-30 Proposed[116] Mechanism for the Addition of Methanol to Dibenzoylethylenes

the addition of nitroform to β-nitrostyrene. The results were consistent with an ElcB-type mechanism (where B was one of a number of bases), but not with an E2-type mechanism.



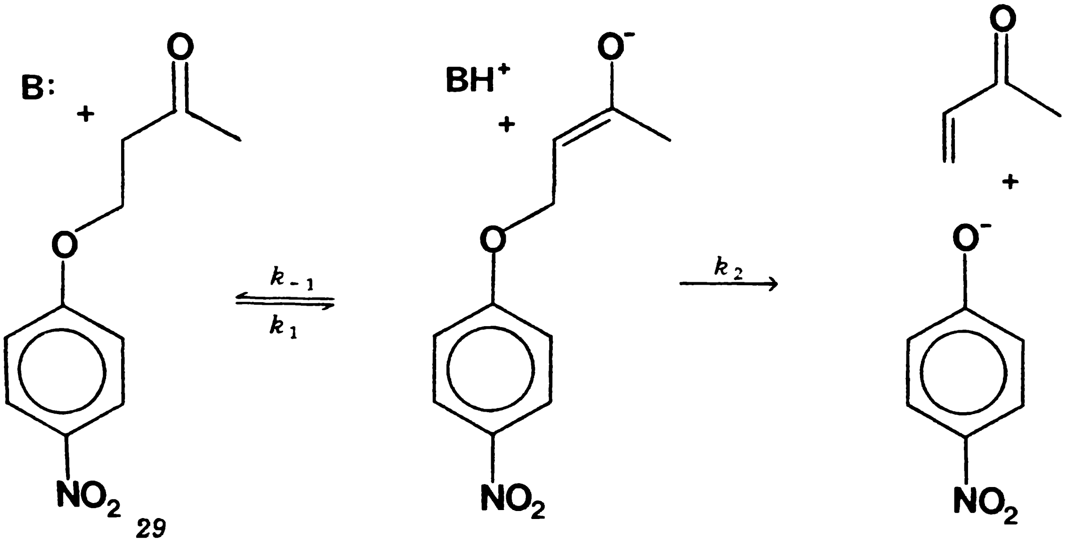
SCHEME 1-31 Proposed[117] Mechanism for the Base-Catalysed Decomposition of 1,1,1,3-Tetranitro-2-phenylpropane

Similarly Hupe and Wu[118] examined the base-catalysed decomposition of the adduct of 4-nitrophenol and methyl vinyl ketone (*29*). Under the conditions used the reverse (*i.e.* addition) reaction was negligible. The decomposition obeyed the rate law:

$$k_{obs} = k_{OH^-}[OH^-][adduct] + k_B[B][adduct]$$

where B was the basic form of the buffer used. The following E1cB mechanism (scheme 1-32) was given as consistent with the results, since kinetic work on a number of closely related systems strongly suggested that an E2 mechanism was not involved.

This type of reaction is directly analogous to the base-catalysed ring opening of flavanones to chalcones, except that here the reverse of the reaction (*i.e.* addition) would involve an inter- rather than an intra-molecular (as is the case for 2'-hydroxychalcones) addition.



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SCHEME 1-32 Proposed[118] Mechanism for the Base-Catalysed Decomposition of *29*

Although addition of nucleophiles to activated double bonds is a widespread phenomenon, the addition of nucleophiles to unactivated double bonds is less well known. Such additions do occur (disregarding enzyme-catalysed reactions) in cases where steric effects produce a high effective molarity for the nucleophile in its attack on the double bond (*e.g.* ref. 119-122), as was pointed out by Evans and Kirby.[123] These authors studied the kinetics of the intramolecular nucleophilic addition to double[123] (*30*) and triple[124] (*31*) bonds. Shown below (figure 1-9) are the pH-rate profiles for two representative reactions, along with other relevant data for the reactions.

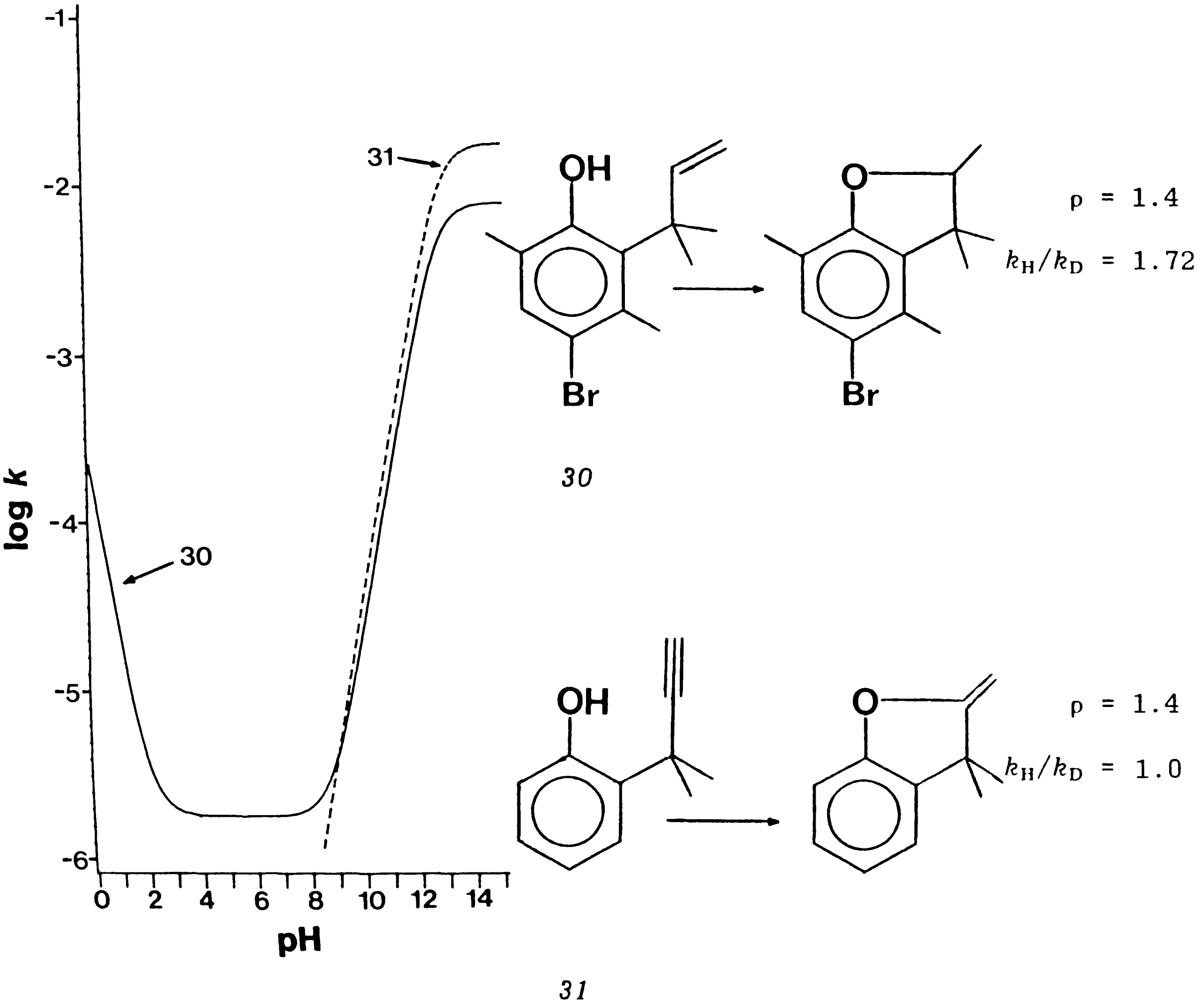
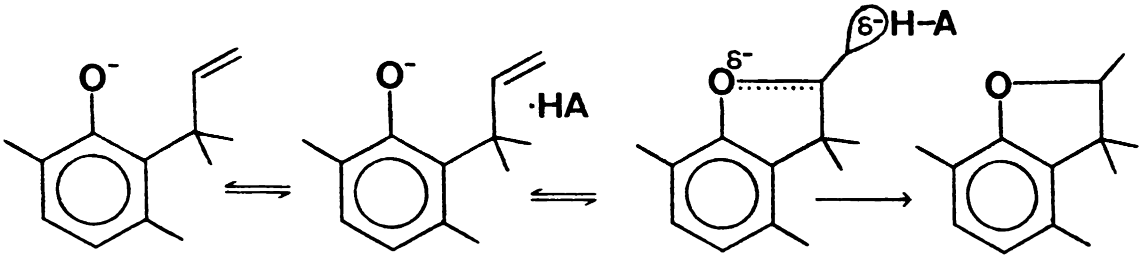


FIGURE 1-9 pH-Rate Profiles[123,124] for the Cyclisation of *30* and *31*

The cyclisation of *30* at high pH was found to be consistent with the mechanism depicted in scheme 1-33.

The pH-independent cyclisation, it was claimed, could be due to the H_2O-catalysed addition of the un-ionised phenol, while the acid-catalysed reaction could be due to rate-limiting attack by H^+ on the double bond, assisted by concerted addition of the phenol oxygen.



SCHEME 1-33 Proposed[123] Mechanism for the Cyclisation of *30* (X = H)

A rather different approach was adopted by Leska and Zahradnik.[125] They used molecular orbital calculations to examine several properties of substituted chalcones, including their susceptibility to nucleophilic attack. A plot of the logarithm of the observed rates of nucleophilic addition (from the data of Toma[111]) against the calculated electron density at the β-carbon gave straight lines for both A- and B-ring substituted chalcones. It should be noted, however, that while the rate of addition correlated negatively with increasing β-carbon electron density for A-ring substituted chalcones, as might be expected, the rate for B-ring substituted chalcones correlated positively with increasing β-carbon electron density. Thus either the electron density at the β-carbon is not a critical factor in the reaction, or the model used by the authors for their calculations was not completely appropriate for this reaction.

1.4 Aims of this Study

Although the isomerisation of 2'-hydroxychalcones to flavanones has been the subject of kinetic studies in recent years, no similar thorough studies have been conducted on 2',6'-dihydroxychalcones. In view of the importance of 5-hydroxyflavanones as natural products, of the intermediacy of 2',6'-dihydroxychalcones in the biosynthesis of most flavonoids, and of the unusual stability of the 5-hydroxyflavanones as compared to simple flavanones, kinetic and mechanistic information about the 2',6'-dihydroxychalcone – 5-hydroxyflavanone isomerisation would be of considerable interest. In addition, such information would also be invaluable in the studies of the chalcone isomerase enzyme. Furthermore, the effects of electron-withdrawing and -donating substituents on the isomerisation could also provide an insight into the nature of the mechanism of the reaction.

It was therefore envisaged that this study would involve first the synthesis of a series of variously substituted 2',6'-dihydroxychalcones, with careful authentication of the products in view of the unreliability of literature methods for their preparation (refer to chapter 6). It was hoped that some of the pitfalls in previous syntheses might be recognised, and that general and reliable synthetic routes would be established. Second, after synthesis of the chalcones, a kinetic investigation of their isomerisation to 5-hydroxyflavanones as a function of pH would be undertaken, with a view to elucidating the mechanism of the reaction, and perhaps to reveal the reason for the anomalous stability of the 5-hydroxyflavanones.

CHAPTER 2

The Rate of Attainment of Equilibrium Between 2',6'-Dihydroxychalcones and 5-Hydroxyflavanones

2.1.*1* Introduction

In order to ascertain the reason for the high stability of 5-hydroxyflavanones, relative to 2',6'-dihydroxychalcones, and to obtain information on the mechanism of the isomerisation, the rate constants for attainment of the chalcone – flavanone equilibrium were determined for the following nine chalcones in aqueous solution: 2',6'-dihydroxychalcone (***34***); 2',6'-dihydroxy-4-methoxychalcone (***38***); 2',6'-dihydroxy-3,4-dimethoxychalcone (***40***); 2',6'-dihydroxy-3,4,5-trimethoxychalcone (***42***); 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***); 4-chloro-2',6'-dihydroxychalcone (***46***); 2',6'-dihydroxy-4,4'-dimethoxychalcone (***32***); 2',6'-dihydroxy-3,4,4'-trimethoxychalcone (***36***); and 2',4,6'-trihydroxy-4'-rhamnoglucosyloxychalcone (naringin-chalcone) (***48***).

In all cases, the form of the pH-rate profile allowed calculation of the pK_a values and rate coefficients for the ring-closure (forward) and ring-opening (reverse) reactions. The form of the pH-rate profile was usually such that a plateau occurred in the region pH 9-11, representing the ring-closure of the chalcone mono-anion. It was therefore convenient to carry out a number of experiments in the pH range of this plateau, where the observed rate constant was almost independent of pH, and was independent of the values of dissociation constants (which would be solvent- and temperature-dependent).

The rate of cyclisation in deuterium oxide, in the plateau region, was determined for the following eight chalcones: 2',6'-dihydroxychalcone (***34***); 2',6'-dihydroxy-4-methoxychalcone (***38***); 2',6'-dihydroxy-3,4-dimethoxychalcone (***40***); 2',6'-dihydroxy-3,4,5-trimethoxychalcone (***42***); 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***); 4-chloro-2',6'-dihydroxychalcone (***46***); 2',6'-dihydroxy-4,4'-dimethoxychalcone (***32***); and 2',6'-dihydroxy-3,4,4'-trimethoxychalcone (***36***).

The effect of temperature on the rate of attainment of the chalcone – flavanone equilibrium in water was also investigated in the plateau region. For two of the chalcones, the effect of temperature on the rate of cyclisation was similarly studied in deuterium oxide solutions. The results of these studies were analysed to give activation parameters (E_a, A, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$) for the following six chalcones: 2',6'-dihydroxychalcone (***34***) (in H_2O and in D_2O); 2',6'-dihydroxy-4-methoxychalcone (***38***) (in H_2O and in D_2O); 2',6'-dihydroxy-3,4,5-trimethoxychalcone (***42***) (in H_2O); 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***) (in H_2O); 4-chloro-2',6'-dihydroxychalcone (***46***) (in H_2O); and 2',6'-dihydroxy-3,4,4'-trimethoxychalcone (***36***) (in H_2O).

The effect of buffer concentration on the rate of attainment of the chalcone – flavanone equilibrium was also studied in the plateau region for two different carbonate buffers, using 2',6'-dihydroxychalcone (***34***) and 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***).

In addition, the rate of attainment of the chalcone – flavanone equilibrium of 2',6'-dihydroxychalcone (***34***) was measured under conditions identical to those of a previous study,[46,71] in the plateau region, to provide a meaningful comparison of the rates of cyclisation of 2'-hydroxy-6'-methoxychalcone anion and 2',6'-dihydroxychalcone mono-anion, which are electronically similar but differ in the presence or absence of a 6'-OH group.

Because a kinetic isotope effect was observed, some of the chalcones were cyclised in approximately 1:1 H_2O – D_2O, and the products analysed by high resolution mass spectrometry. The level of incorporation of deuterium into the products was then compared with that expected from the measured kinetic isotope effect. If the two are significantly different, then it is likely that the proton being transferred in the rate determining step (and hence responsible for the kinetic isotope effect) is not the proton that ends up covalently bound in the product. This experiment is reported in detail in chapter 3.

The stereospecificity of the cyclisation reaction was also examined. Several chalcones were cyclised in D_2O in the plateau region, and the products were analysed by n.m.r. spectroscopy, to determine whether the deuteron added during cyclisation appeared preferentially in either the axial or equatorial position at C-3. This experiment is reported in detail in chapter 3.

2.1.2 General Experimental

In order to measure the rate of a reaction, it is necessary to measure the change in the concentration of one of the reactants, or products, with respect to time. Because chalcones possess a strong absorption band in the visible wavelengths, it proved convenient to follow the decrease or increase in the chalcone absorbance with time, at an appropriate wavelength.

The wavelength chosen to follow the reaction was not necessarily that of maximum absorbance of the chalcone (at the pH in question).

Rather, the wavelength which resulted in the highest accuracy in the measurement of the rate constant was that where the difference between the initial and the equilibrium (final) absorbances was greatest.

Although it proved possible to follow the course of the reaction by monitoring the change in the flavanone concentration, this proved to be less accurate than following the change in chalcone concentration, due to the (generally) lower absorption coefficients of the flavanones. In the case of 2',6'-dihydroxy-4,4'-dimethoxychalcone, at several (high) pH values the rate constants were determined by starting with chalcone and following the decrease in chalcone concentration, and independently, by starting with the flavanone and following the increase in chalcone concentration. Both methods gave the same result (within experimental error), as is expected in the case of a reversible reaction.

In addition, changes in the absorption spectra of the chalcones and flavanones with pH often gave important clues as to the approximate pK_a values of these compounds, as well as to the approximate equilibrium composition (and hence equilibrium constant) of the reaction.

Up to four chalcone/chalcone anion, and three flavanone/flavanone anion [in the case of naringin-chalcone (*48*)] species may be present in the reaction solution, each with its own absorption spectrum, but this will have no effect on the validity of spectrophotometric rate measurements. This is due to two factors. Firstly, the acidity constants of the species involved in the reaction will not change as long as the ionic strength and temperature remain constant, so that as long as the pH of the solution remains constant with time (*i.e.* is well buffered), the ratios of the different ionised forms of the chalcone (and also of the flavanone) will remain constant. Secondly, the rate of cyclisation is very much slower than the diffusion-controlled rate of proton transfer at oxygen expected for the chalcone and flavanone species present, even for the highly reactive chalcone mono-anions. Thus, at all stages of the reaction, the species present in solution will be present in their acid-base equilibrium proportions.

2.1.3 Buffer Solutions

The solutions used in this study were all of ionic strength $\mu = 1.0$ mol l^{-1}, and the buffer concentration was 0.01 mol l^{-1} in all cases where a buffer was used, except of course where the effect of buffer concentration on the rate of the reaction was being studied.

Buffer solutions were made up with doubly-glass-distilled water. Stock 0.10 mol l^{-1} solutions of the required weak acids and bases were made up to $\mu = 1.0$ mol l^{-1} with KCl. Then x ml of the weak acid and $10-x$ ml of its conjugate base ; or 10 ml of the weak acid and x ml $(1 < x < 9)$ of stock KOH (0.10 mol l^{-1}, $\mu = 1.0$ mol l^{-1} with KCl), were added to a 100 ml volumetric flask, and the volume made up with stock 1.0 mol l^{-1} KCl.

The high ionic strength was necessary not just to minimise problems due to different activity coefficients in different buffers, but also because it was hoped to extend the kinetic studies into very high and low pH regions (*i.e.* pH 0-14), where the acid or base concentration is necessarily *ca.* 1 mol l^{-1}.

In the regions $2 > \text{pH} > 12$, solutions of HCl, NaOH or KOH were used. With the low ($< 10^{-4}$ mol l^{-1}) concentration of chalcone used, a buffer is no longer required when the acid or base concentration is high. The HCl and NaOH solutions were made up from commercially available stock solutions to 1.00 mol l^{-1}, and diluted to the required concentration with 1.0 mol l^{-1} KCl. The KOH solutions were similarly prepared from a stock 1.00 mol l^{-1} solution made from fresh analytical grade KOH pellets.

Deuterium oxide – carbonate buffers were made up by the addition of the calculated amounts of potassium bicarbonate, potassium carbonate and potassium chloride to a 10 ml volumetric flask, so that when the volume was made up with D_2O (> 99% isotopic purity), the total carbonate concentration was 0.01 mol l^{-1} and μ = 1.0 mol l^{-1} (KCl). The buffer solutions also contained ethanol (*ca.* 4% by mixed volume) when used for kinetic runs (see kinetic methods, section 2.1.5). Calculations showed that the isotopic purity of the reaction solution (2.50 ml of buffer plus the 100 μl of ethanol used when adding the chalcone) to be *ca.* 98.5 atom % D, after allowing for the presence of $Na\mathit{H}CO_3$ and $CH_3CH_2O\mathit{H}$.

2.1.4 pH Measurements

At the completion of the reaction, the pH of the reaction solution was determined at 30°C, with a Radiometer model 26 or PHM 84 pH meter fitted with a GK2401B combination electrode. The pH meter was calibrated immediately prior to use, at 30°C, with the pH standards:[126] 0.01 mol l^{-1} sodium borate (pH 9.139 at 30°C); and 0.05 mol l^{-1} potassium hydrogen phthalate (pH 4.011 at 30°C).

Because the neutral to weakly basic reaction solutions were of small volume (*ca.* 2.6 ml), pH measurements were carried out as rapidly as possible in small test tubes to minimise contact with the atmosphere. The pH measurements were carried out very soon after the opening of the u.v. cell.

Due to the high ionic strength of the reaction solutions, and to the presence of a small amount of co-solvent, it would be a difficult matter to correlate the measured pH with the hydrogen ion concentration, and no attempt was made to do so.

Unfortunately, it is not possible to make accurate meaningful pH measurements at very high and low pH. In addition, problems with corrosion of glass components of the electrode are apt to occur at high hydroxide concentrations. Thus it was necessary to estimate the pH for the NaOH, KOH and HCl solutions. This was done simply from the known acid concentration in the case of HCl solutions. In the case of the hydroxide solutions, the pH was estimated from the the known hydroxide concentration and[127a] pK_W = 13.833 at 30°C.

Fortunately, the transition from measured to estimated pH usually took place close to the plateau regions in the pH-rate profile, and a significant break in the pH-rate profile is therefore not expected at this transition. However, any pK_a values estimated from data in the non-measured pH region may be different from the true pK_a, since they are not based on activity measurements. In addition, the second-order rate constants determined in HCl and NaOH solutions must be expressed in concentration rather than activity units.

The $pH_{meas.}$ values in deuterium oxide were obtained in exactly the same manner as the pH, as above, at 30°C. The relationship:[128a]

$$pD = pH_{meas.} + 0.40$$

was then used to calculate the value of the pD.

2.1.5 Kinetic Methods

Kinetic measurements were carried out using a Pye Unicam SP8-500 u.v.-visible spectrophotometer fitted with a C. Itoh Electronics model 8510B dot matrix printer. Rapid circulation of water from an external thermostatted water bath maintained the cell blocks at the required temperature (usually 30.0°C).

The buffer (2.50 ml) was added to the silica cells, and the cells were allowed to equilibrate in the cell blocks for approximately 15 minutes. For kinetic studies, a reference cell was not necessary, and was not usually used.

The reaction was initiated by addition of 100 μl of a stock solution of the required chalcone (or flavanone) in anhydrous ethanol, to the buffer, *via* a micropipette (the resulting solution composition will subsequently be referred to as 4% ethanolic H_2O). The change in absorbance was then followed at a suitable wavelength. The course of the reaction was usually followed for 8-10 half-lives to obtain a good infinity reading. Where this was not practical (due to the long half-life at some pH values), the reaction was followed for as long as possible (usually 4-6 half-lives), and the Guggenheim method was used to analyse the results. In every case, the infinity [$\log(A_t - A_\infty)$ versus time] plot and/or the Guggenheim [$\log(A_t - A_{t+\Delta})$ versus time] plot gave straight lines. In the numerous cases where both methods were applied to the same data, the same value for the rate constant was obtained (within experimental error) using either method.

Initially the rate constants were determined graphically. However for all but 2',6'-dihydroxy-4,4'-dimethoxychalcone (***32***), the first-order rate constants were determined from the absorbance versus time data using a computer programme. This computer programme gave not only the rate constant, but also its standard deviation, σ. When $3\sigma > 3\%$ of the observed rate constant, k_{obs} (about one run in fifty), the value of k_{obs} was not included in subsequent rate constant calculations. Usually 1% $k_{obs} < 3\sigma <$ 2% k_{obs}, and in those cases where rate constants were determined by both graphical and computer methods, the results were the same within experimental error.

The stock ethanolic solution of the chalcone was made up to a concentration such that addition of 100 μl of the solution to 2.50 ml of the buffer gave an initial absorbance of 0.2 to 0.8 at the wavelength at which rate measurements were being taken. Since the absorption coefficient for the chalcones was *ca.* 10^4, the chalcone concentrations present in the reaction solution were approximately 2 to 8×10^{-5} mol l^{-1}, so that negligible buffering effects apply. The chalcones cyclised rapidly in the ethanolic solution at room temperature, but at *ca.* -20°C, in the dark, they were sufficiently stable to last for periods up to 2-3 months. After this time it was necessary to prepare fresh solutions, as otherwise the chalcone concentration became too low to allow accurate rate determinations. At very high pH it was more accurate to measure the increase in chalcone absorbance, starting with the flavanone. The required stock solutions of the flavanones were prepared simply by allowing the stock chalcone solutions to stand at room temperature for several hours. The stock flavanone solutions were stable at room temperature over a period of several months. In contrast to the ethanolic solutions, a solution of 2′,6′-dihydroxychalcone in anhydrous dioxane was very stable. Over a period of three months, at room temperature, no detectable flavanone formation took place. However, it was felt desirable to use hydroxylic cosolvents in the kinetic studies, wherever this was practical.

For several of the chalcones, repetitive scans (500-250 nm) showed solubility at low pH to be a problem; the flavanones were precipitating to some extent during the reaction, and a clear isosbestic point was not obtained. Where necessary, this problem was overcome by using the lowest concentration of the chalcone consistent with the requirements for accuracy, and/or by using a 4.0 cm (rather than the usual 1.0 cm) silica cell. The procedure for using the 4.0 cm cells was the same as that outlined above, except that 10.0 ml of buffer and 300 μl of ethanol were placed in the cell, and allowed to equilibrate prior to addition of 100 μl of the stock solution of the chalcone.

2.1.6 Kinetic Form of a Reversible First-Order Reaction

It has previously been shown[71,128b] that the observed rate constant of a reversible first-order (or pseudo first-order) reaction is equal to the sum of the forward and reverse rate constants. The derivation of this fact is shown below.

For an equilibrium reaction between A and B:

$$\mathrm{A} \underset{k_f}{\overset{k_r}{\rightleftharpoons}} \mathrm{B}$$

where k_f is the forward rate constant, for the conversion of A into B, and k_r is the reverse rate constant, for the conversion of B into A, the rate expression is:

$$-\frac{d[A]}{dt} = k_f[A] - k_r[B] \quad \ldots\ldots\ldots\ldots\ldots\ldots 2\text{-}1$$

but since $[B] = A_0 - [A]$, where $A_0 = [A]$ at time $t = 0$, we get

$$-\frac{d[A]}{dt} = k_f[A] - k_r(A_0 - [A])$$

$$= (k_f + k_r)[A] - k_rA_0 \quad \ldots\ldots\ldots\ldots 2\text{-}2$$

but at equilibrium $k_f[A]_{eq} = k_r[B]_{eq}$ $\ldots\ldots\ldots\ldots$ 2-3

$$= k_r(A_0 - [A]_{eq})$$

$$= k_r A_0 - k_r[A]_{eq}$$

thus $$(k_f + k_r)[A]_{eq} = k_r A_0 \quad \ldots\ldots\ldots\ldots\ldots 2\text{-}4$$

Substituting equation 2-4 into equation 2-2 we get

$$-\frac{d[A]}{dt} = (k_f + k_r)[A] - (k_f + k_r)[A]_{eq}$$

$$= (k_f + k_r)([A] - [A]_{eq}) \quad \ldots\ldots\ldots\ldots 2\text{-}5$$

Thus, the approach to equilibrium is a first-order process, with the observed rate constant (k_{obs}) being equal to the sum of the forward and reverse reactions:

$$k_{obs} = k_f + k_r \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 2\text{-}6$$

Further important information about the reaction may be obtained from the above expressions. For instance the equilibrium constant, K_{eq}, is equal to k_r/k_f. This equation may be rearranged to give the fraction of the original chalcone which will be present in the chalcone form at equilibrium, which is equal to $k_r/(k_r+k_f)$. The derivation of these expressions is shown below.

By rearranging equation 2-4

$$(k_f + k_r)[A]_{eq} = k_r A_0$$

we have $$\frac{[A]_{eq}}{A_0} = \frac{k_r}{k_f + k_r} \quad \ldots\ldots\ldots\ldots\ldots 2\text{-}7$$

Thus, the fraction of A + B present in the form of A at equilibrium is equal to the rate constant of the reverse reaction divided by the sum of the forward and reverse rate constants (k_{obs}). In addition, we know from equation 2-3 that:

$$k_f[A]_{eq} = k_r[B]_{eq}$$

$$\frac{[A]_{eq}}{[B]_{eq}} = \frac{k_r}{k_f} = K_{eq} \quad \ldots\ldots\ldots\ldots\ldots 2\text{-}8$$

The equilibrium constant K_{eq}, then, is equal to the reverse rate constant (k_r) divided by the forward rate constant (k_f).

The significance of these equations seems not to have been realised in the study of chalcone – flavanone equilibria. It should be an easy matter to determine the ratio of chalcone to flavanone at equilibrium spectrophotometrically (*e.g.* ref. 66,68) in the pH region where the reverse reaction is significant. In the case of simple 2′-hydroxychalcones, with only one pK_a and three significant rate constants in this pH region, a minimum of four such measurements would suffice to define the four unknowns. In fact the method can be considerably streamlined; the pK_a of the chalcone can be determined spectrophotometrically,[46,129] while the observed rate constants for the approach to equilibrium can be determined prior to measurement of K_{eq}. Thus at each pH used, we know both K_{eq} (= k_r/k_f) and k_{obs} (= k_r+k_f), so that measurements at fewer pH values are required. Such a method should be much more convenient than the more tedious method used by Old.[46,71]

In addition to possible use in the determination of rate constants, the above equations also allow us to calculate the fraction of a chalcone/flavanone pair which will be present in the chalcone (or flavanone) form at equilibrium at a given pH, from its rate constants. Such information is useful from a synthetic viewpoint, as it allows one to calculate the optimum pH for formation of either the chalcone or flavanone forms, since we can now calculate both the percentage of either form present at equilibrium, as well as the rate at which that equilibrium will be approached.

In the present study, the approach of Old[46,71] was used. The alternative approach outlined above would not be practical for the study of 2′,6′-dihydroxychalcones, since only at very high pH values would $k_r \sim k_f$. The limited pH range over which measurements could be taken would preclude accurate determinations of the increased number of possible parameters contributing to the forward (two pK_a values and four separate forward rate constants) and reverse (one pK_a and two reverse rate constants) reactions.

2.1.7 Effect of Temperature on Rate

The Arrhenius activation parameters were determined from the dependence of k_{obs} (in the high pH plateau where $k_{obs} \approx k_{CH^-}$) upon temperature for each compound, by a least squares analysis of $\ln k_{obs}$ versus 1/T using a computer programme. These k_{obs} versus temperature data were obtained in carbonate buffers (at *ca.* pH 10) in the pH-rate plateau region (pH 9-11), thus circumventing any problem with the dependence of the buffer pH and the chalcone pK_a upon temperature affecting k_{obs}. In the plateau region, $k_{obs} \approx k_{CH^-}$ (see section 2.2), so that the activation parameters obtained pertain only to the cyclisation of the chalcone mono-anion. The slope of the line of such a plot is equal to $-E_a/R$, while the intercept is equal to ln A. In addition to the slope and intercept, the computer programme yielded the standard deviation (σ) of both these parameters. These σ values were then used to calculate the estimated standard deviations (σ) of the values of $\Delta G^\ddagger$, $\Delta H^\ddagger$ and $\Delta S^\ddagger$. The values of $\Delta G^\ddagger$, $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were derived from the Arrhenius activation parameters as follows.

Empirically, the relationship between reaction rate and temperature is well represented by the Arrhenius equation:

$$k = Ae^{-E_a/RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots 2\text{-}9$$

$$\text{thus} \quad \frac{d(\ln k)}{d(1/T)} = \frac{-E_a}{R} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2\text{-}10$$

and
$$\ln k = \ln A - E_a/RT \tag{2-11}$$

From transition state theory, the rate constant (k) of a reaction is given by the equation 2-12:

$$k = (\underline{k}T/h)K^{\ddagger} \tag{2-12}$$

where $K^{\ddagger}$ is the equilibrium constant for the formation of the transition state from the reactants, $\underline{k}$ is Boltzmann's constant and h is Planck's constant. Since thermodynamic equilibrium is assumed to exist between the reactants and the transition state, we can define the free energy, enthalpy and entropy of activation for such a reaction in terms of the equilibrium constant ($K^{\ddagger}$) in the usual manner.

thus
$$\Delta G^{\ddagger} = -RT\ln K^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \tag{2-13}$$

and
$$\Delta H^{\ddagger} = -R\,\frac{d(\ln K^{\ddagger})}{d(1/T)} \tag{2-14}$$

Substituting equation 2-12 into equation 2-14 we get

$$\Delta H^{\ddagger} = -RT - R\,\frac{d(\ln k)}{d(1/T)}$$

And from equation 2-10

$$= -RT - R\frac{-E_a}{R}$$

$$= E_a - RT \tag{2-15}$$

$$= E_a - 2.52 \ \text{kJ mol}^{-1} \text{ at } 30°\text{C}.$$

Substituting equation 2-9 into equation 2-13 gives

$$\Delta G^{\ddagger} = -RT\ln k + RT\ln(\underline{k}T/h) \tag{2-16}$$

$$= -RT(\ln A - E_a/RT) + RT\ln(\underline{k}T/h)$$

$$\Delta H^{\ddagger} - T\Delta S^{\ddagger} = -RT\ln A + E_a + RT\ln(\underline{k}T/h)$$

$$E_a - RT - T\Delta S^{\ddagger} = -RT\ln A + E_a + RT\ln(\underline{k}T/h)$$

$$-T\Delta S^{\ddagger} = -RT\ln A + RT\ln(\underline{k}T/h) + RT$$

$$= RT(\ln(\underline{k}T/h) - \ln A + 1)$$

$$\Delta S^{\ddagger} = -R(\ln(\underline{k}T/h) - \ln A + 1)$$

$$= R(\ln A - \ln(\underline{k}T/h) - 1) \quad \ldots.2\text{-}17$$

$$= 8.317(\ln A - 30.47) \text{ J mol}^{-1} \text{ K}^{-1} \text{ at } 30^{\circ}\text{C}.$$

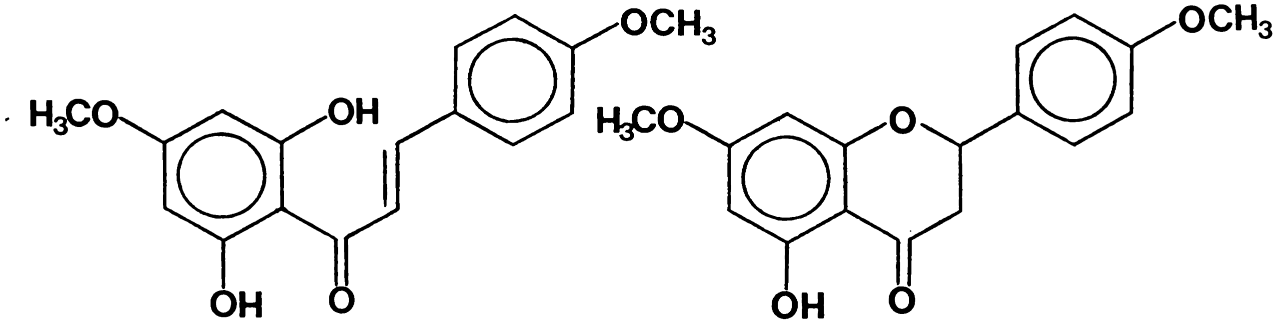
A plot of $\ln k_{obs}$, at 30°C, against the calculated value of $\Delta G^{\ddagger}$ for the chalcones in the present study gave the expected straight line, with a slope of -3.98 ($\sigma = 0.03$)$\times10^{-4}$ mol $J^{-1}$and intercept 29.6 ($\sigma = 0.3$). This is in close agreement with theoretical predictions from equation 2-16:

$$\Delta G^{\ddagger} = -RT\ln k + RT\ln(\underline{k}T/h)$$

thus $$\ln k = \frac{-1}{RT}\Delta G^{\ddagger} + \ln(\underline{k}T/h) \quad \ldots\ldots\ldots\ldots\ldots.2\text{-}18$$

So a plot of $\ln k_{obs}$, at any temperature T (in °K), versus $\Delta G^{\ddagger}$ should have a slope of $-1/RT$ (*i.e.* -3.97×10^{-4} mol J^{-1} at 30°C), and an intercept of $\ln(\underline{k}T/h)$ (*i.e.* 29.47 at 30°C). The close agreement between observed and theoretical values obtained confirms that the calculation of the activation parameters has been carried out correctly.

2.2 The 2′,6′-Dihydroxy-4,4′-dimethoxychalcone – 5-Hydroxy-4′,7-dimethoxyflavanone Equilibrium[130]



32 33

2.2.*1* The Effect of pH on the Rate of Equilibration

The rate of conversion of 2′,6′-dihydroxy-4,4′-dimethoxychalcone (*32*) or 5-hydroxy-4′,7-dimethoxyflavanone (*33*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0-14.3. Repetitive scans at a number of pH values (figure 2-1) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. By following changes in absorbance at an appropriate wavelength, first-order rate constants were determined graphically. The results are tabulated in table 2-1.

2.2.2 Analysis of the pH-Rate Data

In equation 2-6 we found that the observed rate constant is equal to the sum of the forward and reverse reaction rate constants.

$$k_{obs} = k_f + k_r$$

Old[46,71] proposed that the forward reaction, k_f, was due to a contribution from ring closure of the neutral chalcone and of the chalcone anion, in the case of the simple 2′-hydroxychalcones.

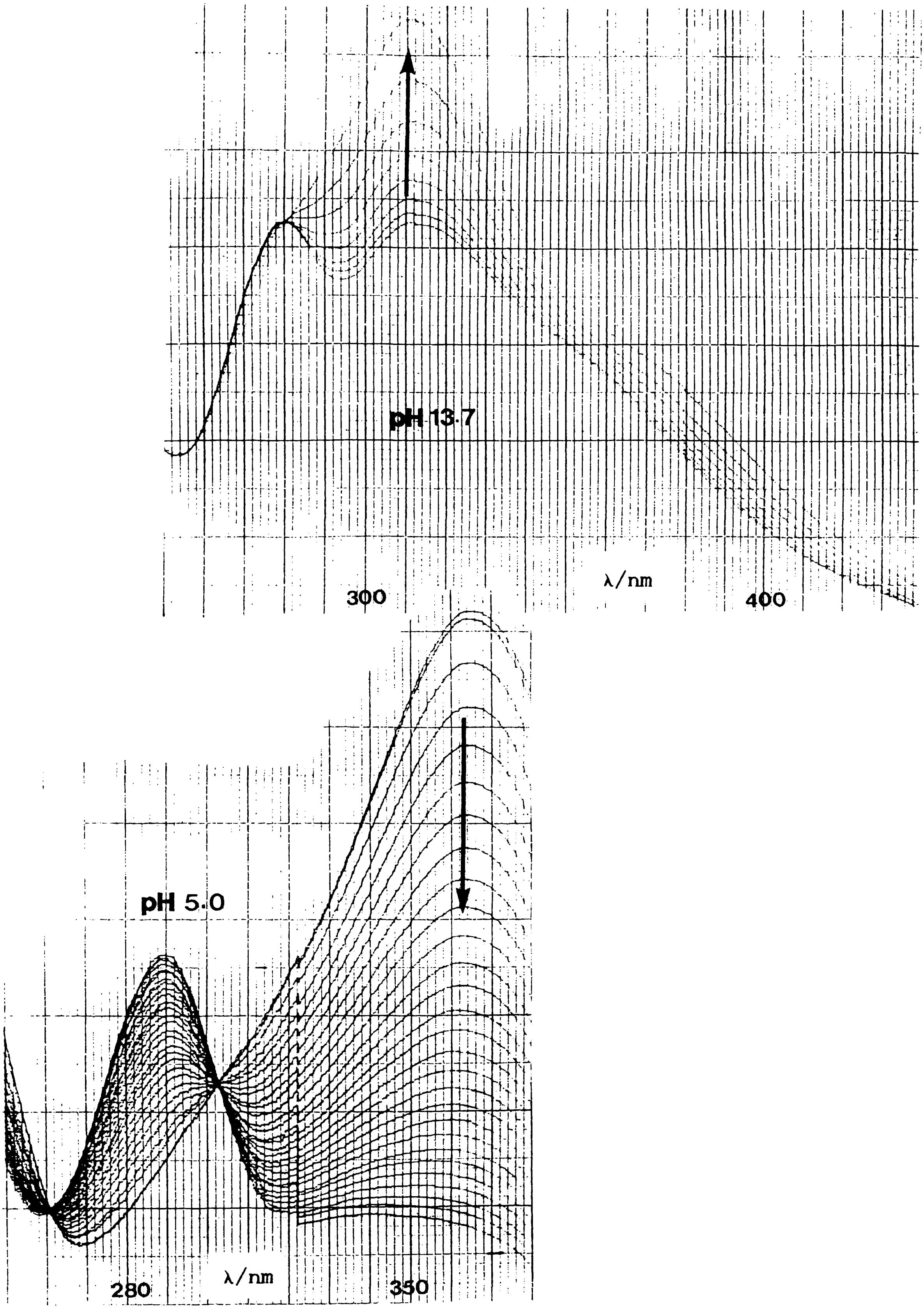


FIGURE 2-1 Repetitive Scans for the Equilibration of *32* and *33* at various pH Values

TABLE 2-1 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-4,4'-dimethoxychalcone (*32*) - 5-Hydroxy-4',7-dimethoxyflavanone (*33*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), total [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	k_{obs}/s^{-1}
0.00	HCl	365	1.33×10^{-5}
0.34	HCl	365	1.13×10^{-5}
2.05	HCl	365	9.83×10^{-6}
2.05	HCl	365	9.83×10^{-6}
3.03	PHTHALATE	365	9.58×10^{-6}
3.79	ACETATE	360	1.20×10^{-5}
3.79	ACETATE	360	1.20×10^{-5}
4.60	ACETATE	360	3.25×10^{-5}
5.26	ACETATE	360	1.25×10^{-4}
6.51	PHOSPHATE	360	1.28×10^{-3}
6.51	PHOSPHATE	360	1.29×10^{-3}
6.88	PHOSPHATE	360	2.03×10^{-3}
6.88	PHOSPHATE	360	2.08×10^{-3}
6.88	PHOSPHATE	360	2.08×10^{-3}
6.88	PHOSPHATE	360	2.13×10^{-3}
7.35	PHOSPHATE	360	3.50×10^{-3}
7.35	PHOSPHATE	360	3.45×10^{-3}
7.35	PHOSPHATE	360	3.40×10^{-3}
7.89	BICINE	360	4.08×10^{-3}
7.89	BICINE	360	4.00×10^{-3}
7.89	BICINE	360	4.17×10^{-3}
8.71	BICINE	360	4.27×10^{-3}
8.71	BICINE	360	4.25×10^{-3}
8.89	BICINE	360	4.25×10^{-3}
8.89	BICINE	360	4.12×10^{-3}
8.89	BICINE	360	4.17×10^{-3}
9.89	CARBONATE	360	4.20×10^{-3}
10.34	CARBONATE	360	4.32×10^{-3}
11.83	NaOH	365	3.47×10^{-3}
11.83	NaOH	365	3.46×10^{-3}
11.84	KOH	365	3.53×10^{-3}

TABLE 2-1 Continued

pH	BUFFER	λ/nm	k_{obs}/s^{-1}
11.84	KOH	365	3.57×10^{-3}
11.85	KOH	365	3.60×10^{-3}
12.31	NaOH	370	2.64×10^{-3}
12.31	NaOH	370	2.57×10^{-3}
12.33	KOH	365	2.98×10^{-3}
12.33	KOH	365	2.97×10^{-3}
12.34	KOH	365	2.82×10^{-3}
12.34	KOH	365	2.83×10^{-3}
12.83	NaOH	338	2.28×10^{-3}
12.83	NaOH	338	2.30×10^{-3}
12.84	KOH	324	2.22×10^{-3}
12.84	KOH	324	2.25×10^{-3}
12.85	KOH	324	2.42×10^{-3}
12.85	KOH	324	2.45×10^{-3}
13.31	NaOH	338	2.28×10^{-3}
13.31	NaOH	338	2.28×10^{-3}
13.31	KOH	322	2.03×10^{-3}
13.31	KOH	322	2.03×10^{-3}
13.68	KOH	322	2.52×10^{-3}
13.68	KOH	322	2.53×10^{-3} [†]
13.83	NaOH	338	3.47×10^{-3} [†]
13.83	NaOH	338	3.48×10^{-3} [†]
13.84	KOH	322	2.92×10^{-3} [†]
13.86	KOH	322	2.97×10^{-3} [†]
13.86	KOH	322	2.95×10^{-3} [†]
14.31	KOH	322	9.88×10^{-3} [†]
14.31	KOH	322	9.88×10^{-3} [†]

[†] Determined by ring opening of the isomeric flavanone.

the forward rate constant could be represented as:

$$k_f = k_{CH}f_{CH} + k_{C^-}f_{C^-}$$

where k_{CH} is the rate constant for cyclisation of the un-ionised chalcone, k_{C^-} is the rate constant for cyclisation of the chalcone anion, f_{CH} is the fraction of the chalcone which is present in the un-ionised form and f_{C^-} is the fraction of the chalcone present in the anionic form ($f_{CH} + f_{C^-} = 1$). The values of f_{CH} and f_{C^-} at any given pH are determined solely by the pK_a of the chalcone under the experimental conditions.

It was also proposed, to account for the pH-rate profile, that the rate of the reverse (pseudo first-order) reaction was proportional to the hydroxide ion activity.

$$k_r = k_{OH^-}\{OH^-\}$$

Overall, then, the observed rate constant was proposed to be:

$$k_{obs} = k_{CH}f_{CH} + k_{C^-}f_{C^-} + k_{OH^-}\{OH^-\}$$

Old[46,71] successfully fitted the pH-rate profiles for a number of 2'-hydroxychalcone – flavanone equilibria to this expression to obtain values for k_{CH}, k_{C^-}, k_{OH^-} and the pK_a (see section 1.2 and figure 1-6). In two cases he determined the pK_a spectrophotometrically, under the same conditions as used for the kinetic studies, and in both cases the pK_a values determined by the two methods were in very close agreement.

It was envisaged that the same approach could be used in the case of the 2',6'-dihydroxychalcones. Thus, a contribution to k_f would be expected from cyclisation of the neutral chalcone, its mono- and its di-anion. In addition, it was obvious from table 2-1 that some kind of acid catalysis was taking place at low pH. This represents acid-catalysed ring closure of the neutral chalcone. Therefore, the forward rate constant may be represented as:

$$k_f = k_{H^+}f_{CHH}[H^+] + k_{CHH}f_{CHH} + k_{CH^-}f_{CH^-} + k_{C2^-}f_{C2^-}$$

where k_{H^+} is the second-order rate constant for the acid-catalysed cyclisation of the neutral chalcone, k_{CHH} is the rate constant for the non acid-catalysed cyclisation of the neutral chalcone, k_{CH^-} is the rate constant for ring closure of the chalcone mono-anion and k_{C2^-} is the rate constant for cyclisation of the chalcone di-anion; while f_{CHH}, f_{CH^-} and f_{C2^-} represent the fraction of the chalcone present in the neutral, mono- and di-anionic forms respectively (f_{CHH} + f_{CH^-} +

$f_{C2-} = 1$).

The case of the reverse reaction is more complex than was the case in the work of Old,[46,71] since in the present work the flavanones are capable of ionisation. Thus, k_r may be represented as the sum of contributions due to ring opening of the neutral and of the ionised flavanone:

$$k_r = k_{FH}f_{FH}[OH^-] + k_{F-}f_{F-}[OH^-]$$

where k_{FH} and k_{F-} are respectively the second-order rate constants for ring opening of the neutral and ionised flavanones, and f_{FH} and f_{F-} are the fractions of the flavanone present in the neutral and ionised forms respectively ($f_{FH} + f_{F-} = 1$). Experimentally it was found that the contribution of k_r to k_{obs} was significant only at *ca.* pH > 12 (except in the case of naringin-chalcone). At such a high pH, essentially all the flavanone present could be expected to be present in the anionic form, since the pK_a of the 5-hydroxy group was estimated (from ref. 131) to be *ca.* 10; that the neutral flavanone makes no detectable contribution to the reverse reaction is rationalised in chapter 5 (section 5.4). So, at pH values where the reverse reaction is significant, $f_{F-} \approx 1$. Making this assumption, then:

$$k_r = k_{F-}[OH^-]$$

and

$$k_{obs} = k_{H+}f_{CHH}[H^+] + k_{CHH}f_{CHH} + k_{CH-}f_{CH-} + k_{C2-}f_{C2-} + k_{F-}[OH^-] \quad ....2\text{-}19$$

The pH-rate data were then analysed on the basis of this equation. Estimates of the values for the individual rate constants, and of pK_{a1} and pK_{a2}, were entered into a computer programme, which then calculated expected values of k_{obs} (based on the rate constants and

pK_a values supplied) for specified pH values. The calculated values of k_{obs} were then compared to the experimentally obtained values, and the values of the rate constants and pK_as were revised accordingly. This process was repeated until satisfactory agreement between calculated and experimental values of k_{obs} was obtained.

The computer programme used calculated the fraction of the chalcone in the various ionised forms on the assumption that the two pK_a values may not be sufficiently different for them to be treated as independent of each other. For the 4'-methoxy-substituted chalcones, this was not, in fact, the case. However, for some of the other chalcones studied, $pK_{a2} - pK_{a1} < 4$, and the two ionisations could not accurately be treated as independent. The derivation of the formulae expressing the fraction of the chalcone present in the various ionised forms as a function of pH and the two pK_a values is given in appendix 1, along with the computer programme itself.

In the case of 2',6'-dihydroxy-4,4'-dimethoxychalcone, the rate coefficients and the pK_a values obtained by the above procedure were:

$k_{H+} = 3.7\times10^{-6}$ l mol^{-1} s^{-1}

$k_{CHH} = 9.7\times10^{-6}$ s^{-1}

$k_{CH-} = 4.25\times10^{-3}$ s^{-1}

$k_{C2-} = 1.37\times10^{-3}$ s^{-1}

$k_{F-} = 1.5\times10^{-3}$ l mol^{-1} s^{-1}

$pK_{a1} = 6.9$

$pK_{a2} = 12.3$

In figure 2-2a, the experimentally determined values of k_{obs} are plotted as crosses, while the line is the calculated curve based on the above rate constants and pK_a values. The agreement between the calculated and experimental values of k_{obs} is excellent, and the pK_a values obtained are close to those expected (from ref. 131). This

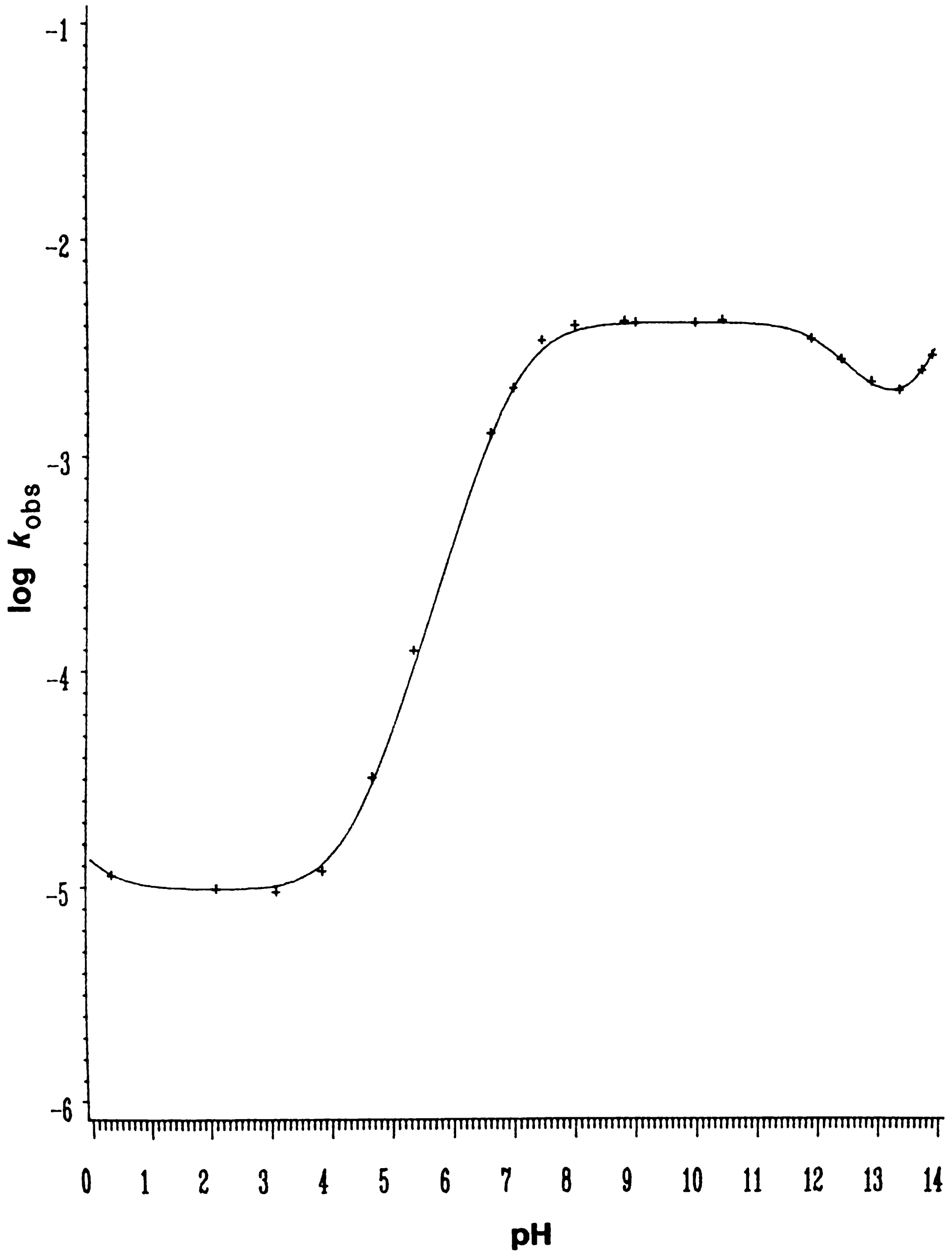


FIGURE 2-2a pH-Rate Profile for the Equilibration of *32* and *33*: Experimental Results (+); Curve (——) Calculated from Equation 2-19, and from the Rate Coefficients and pK_a Values Given in the Text (Page 68)

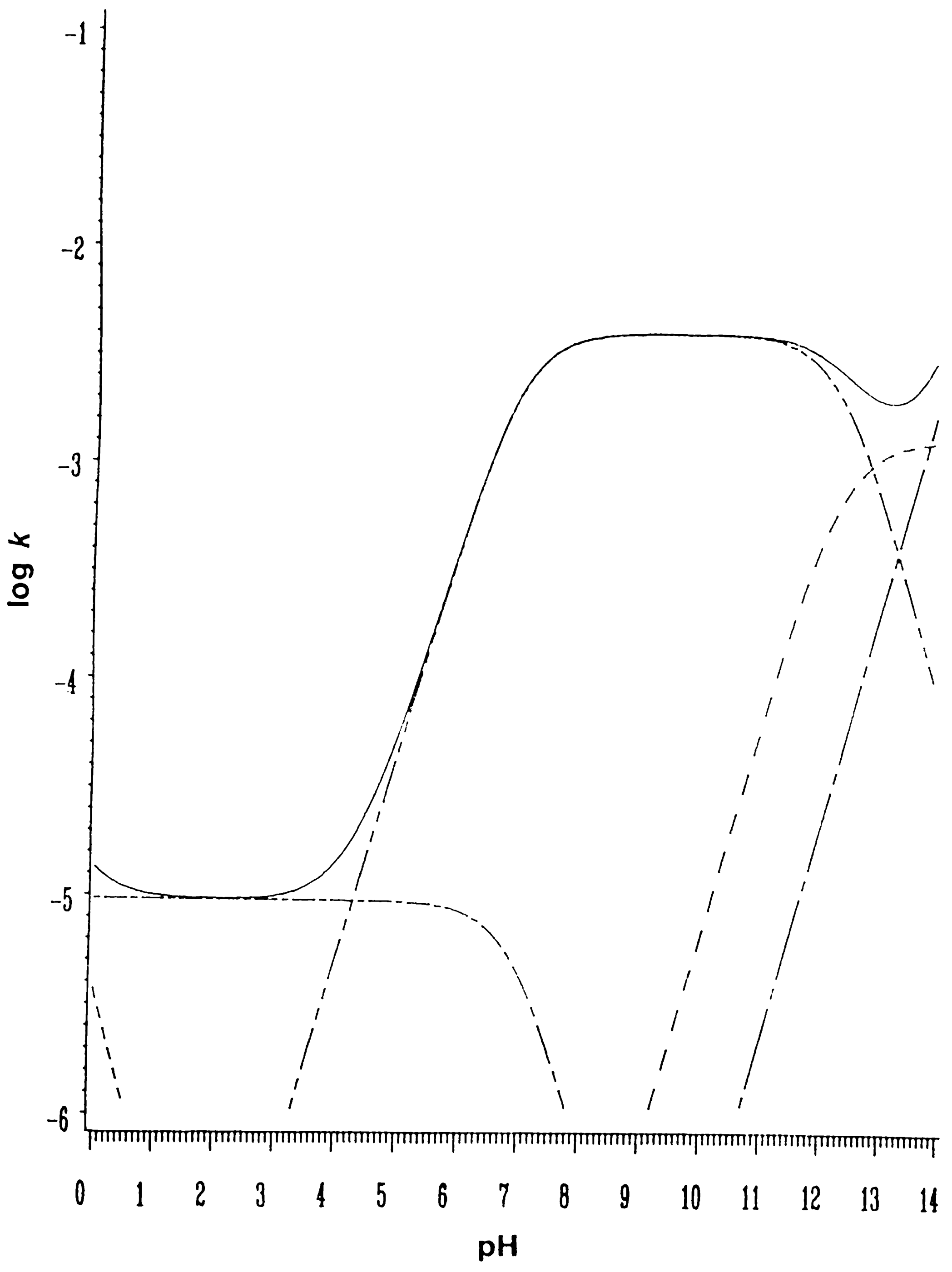
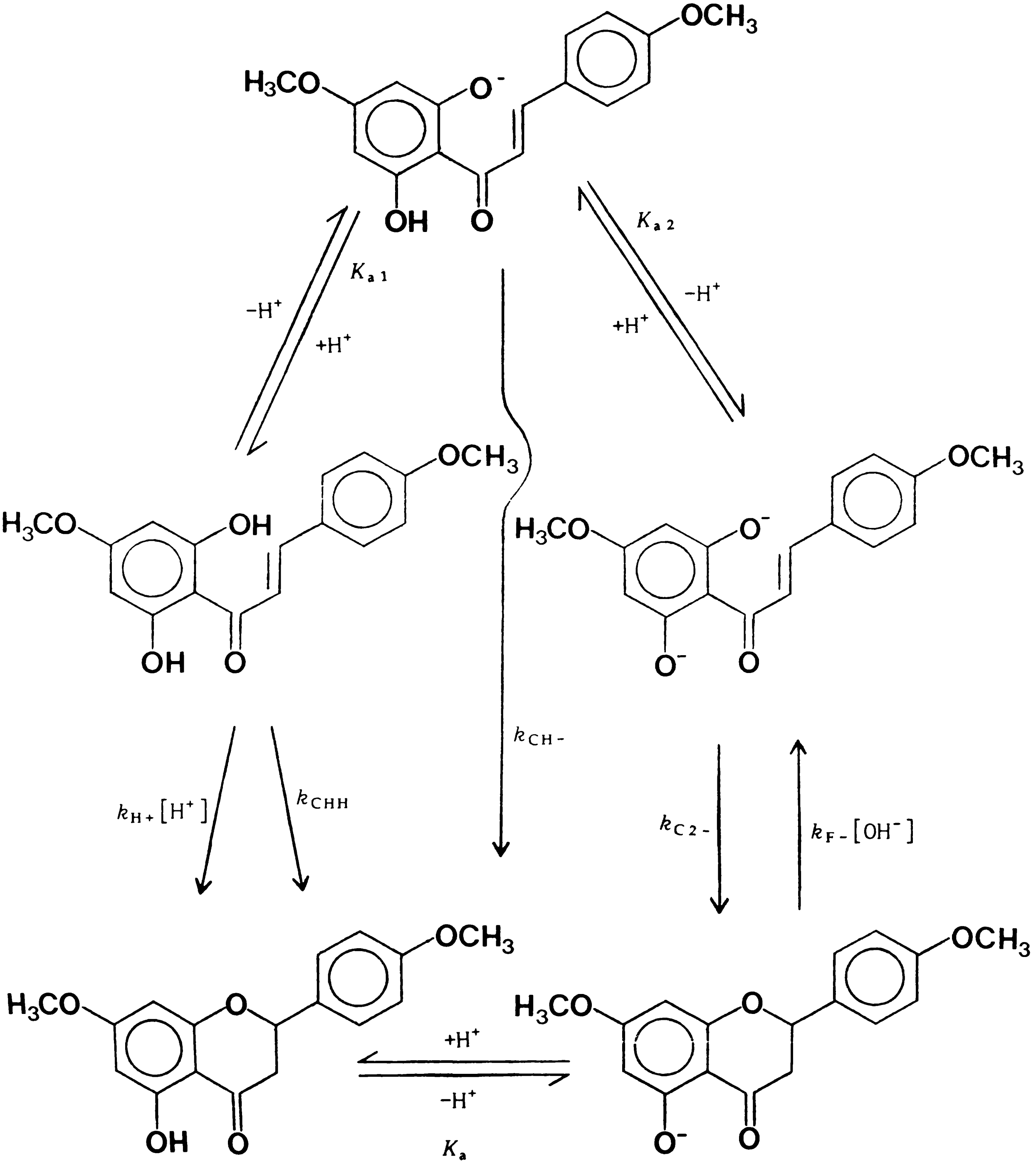


FIGURE 2-2b Calculated pH-Rate Profile for the Equilibration of *32* and *33* (——), along with the Individual Contributions due to $k_{H^+}f_{CHH}[H^+]$ (----), $k_{CHH}f_{CHH}$ (— — —), $k_{CH^-}f_{CH^-}$ (— — — —), $k_{C^{2-}}f_{C^{2-}}$ (– – –) and $k_{F^-}[OH^-]$ (— ——— —)



SCHEME 2-1 The Probable Source of the Individual Rate Coefficients Contributing to k_{obs}, for the Reaction *32* $\leftrightharpoons$ *33* (from Equation 2-19)

agreement provides strong support for the validity of the treatment used to analyse the results, and the derivation of the rate expression (equation 2-19).

The estimated accuracy of the rate constants and pK_a values obtained by the above procedure vary considerably, depending on the form of the pH-rate profile in the pH region from which the particular constant is determined. Thus, the values of rate constants obtained from observed plateaux in the pH-rate profile, such as k_{CHH} (± 5%) and k_{CH^-} (± 3%) (for most of the chalcones in this study) are more accurate than the other constants. The value of k_{H^+} can be considered accurate to ± 5-10%, since k_{obs} in the low pH region is defined by the sum of k_{CHH} and $k_{H^+}[H^+]$ (where $[H^+]$ is known accurately). The other rate constants, k_{C2^-} and k_{F^-}, are probably accurate to ± 10-15%. The reason for the lower accuracy of these values is that in the pH region where these constants have a measurable effect on the pH-rate profile (pH 11-13.9), k_{obs} is determined by three unknowns – k_{C2^-}, k_{F^-} and pK_{a2} – all of which must be evaluated from the rate data at (usually) just six pH values. For this reason, pK_{a2} is probably accurate to 0.1-0.15, while pK_{a1}, which is estimated from the pH at which k_{obs} is half the high pH plateau rate constant (*i.e.* $\frac{1}{2}k_{CH^-}$), is probably accurate to ± 0.05.

2.2.3 The Effect of D_2O on the Rate of Chalcone Mono-anion Cyclisation

The rate constant for attainment of equilibrium (k_{obs}) was measured in the high pH plateau region in deuterium oxide solution. The results given in table 2-2 show the invariance with pD of k_{obs}, consistent with its representing just cyclisation of the chalcone mono-anion.

TABLE 2-2 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-4,4'-dimethoxychalcone (*32*) - 5-Hydroxy-4',7-dimethoxyflavanone (*33*) Equilibrium in 4% Ethanolic D_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

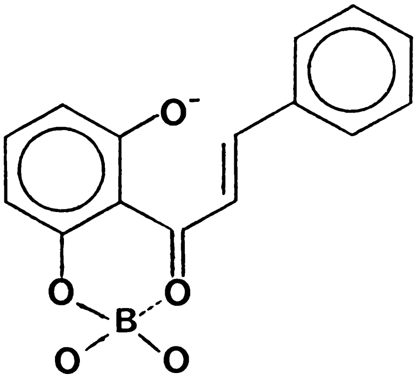
pD	BUFFER	λ/nm	k_{obs}/s^{-1}
9.91	CARBONATE	365	7.70×10^{-4}
10.47	CARBONATE	365	7.13×10^{-4}
10.47	CARBONATE	365	7.38×10^{-4}
10.47	CARBONATE	365	7.52×10^{-4}
10.93	CARBONATE	365	7.33×10^{-4}
10.93	CARBONATE	365	7.37×10^{-4}

The kinetic isotope effect, then, is:

$$\frac{k_H}{k_D} = \frac{4.30\times10^{-3}}{7.5\times10^{-4}} = 5.7$$

for the cyclisation of the chalcone mono-anion.

2.2.4 The Effect of Borate Buffer on the Rate of Attainment of Equilibrium

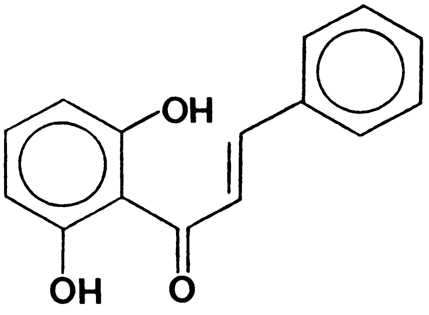


The cyclisation reaction was conducted in a borate buffer, in a preliminary experiment to ascertain whether the borate, which is capable of coordinating adjacent carbonyl and hydroxy groups (as shown above), had any major catalytic effect on the rate of cyclisation.

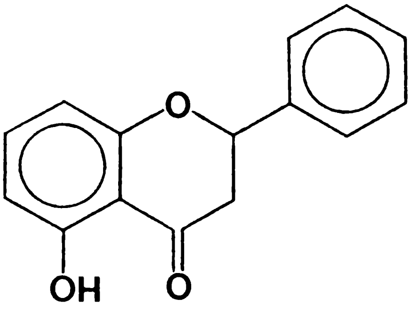
The pH of the standard sodium borate buffer (0.01 mol l^{-}) at 30°C is 9.14, which is in the region of the higher pH-rate plateau. Thus there is no need to consider the degree of ionisation of the chalcone when working in this pH region; the measured rate constant closely approximates the rate coefficient of cyclisation of the mono-anion. It should be noted, however, that the ionic strength of the borate buffer is very much lower than the standard conditions used elsewhere in these studies, so that direct comparison of the rate constants obtained by the two methods is not strictly valid, although if the rate difference is large, tentative qualitative conclusions may be drawn.

In the borate buffer, k_{obs} was found to be 3.40×10^{-3} s^{-1} at 30°C. This is about 80% of the plateau rate constant under the standard conditions: so there is no evidence of special catalysis associated with borate. The decrease in rate is not sufficiently large to enable any further conclusions to be drawn from this result. However the desirability of a more thorough investigation, involving a determination of the effect of different borate buffer concentrations at constant ionic strength on k_{obs}, is indicated by this preliminary result.

2.3 The 2',6'-Dihydroxychalcone – 5-Hydroxyflavanone Equilibrium



34



35

2.3.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of 2',6'-dihydroxychalcone (*34*) or 5-hydroxyflavanone (*35*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0-13.83. Repetitive scans at a number of pH values (figure 2-3) showed (by the tight isosbestic points obtained) that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. First-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-3.

TABLE 2-3 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxychalcone (*34*) – 5-Hydroxyflavanone (*35*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	315	$2.57(0.02)\times10^{-6}$
2.00	HCl	315	$1.65(0.07)\times10^{-6}$
3.98	ACETATE	315	$6.29(0.05)\times10^{-6}$
4.68	ACETATE	315	$2.83(0.02)\times10^{-5}$

TABLE 2-3 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
4.80	PHTHALATE	315	$3.43(0.02)\times10^{-5}$
5.43	ACETATE	315	$1.79(0.003)\times10^{-4}$
5.43	ACETATE	315	$1.80(0.02)\times10^{-4}$
5.88	PHOSPHATE	315	$3.64(0.02)\times10^{-4}$
6.48	PHOSPHATE	315	$1.50(0.03)\times10^{-3}$
6.48	PHOSPHATE	315	$1.43(0.01)\times10^{-3}$
6.49	PHOSPHATE	315	$1.50(0.01)\times10^{-3}$
6.48	PHOSPHATE	315	$1.49(0.01)\times10^{-3}$
6.82	PHOSPHATE	315	$2.91(0.02)\times10^{-3}$
6.83	PHOSPHATE	315	$2.84(0.01)\times10^{-3}$
7.35	PHOSPHATE	315	$7.52(0.03)\times10^{-3}$
7.35	PHOSPHATE	315	$7.45(0.02)\times10^{-3}$
7.76	BICINE	315	$1.11(0.007)\times10^{-2}$
7.76	BICINE	315	$1.17(0.005)\times10^{-2}$
8.65	BICINE	315	$1.83(0.01)\times10^{-2}$
8.66	BICINE	315	$1.95(0.008)\times10^{-2}$
9.34	CARBONATE	315	$2.04(0.01)\times10^{-2}$
9.34	CARBONATE	315	$2.06(0.01)\times10^{-2}$
10.34	CARBONATE	315	$2.00(0.006)\times10^{-2}$
10.34	CARBONATE	315	$2.00(0.004)\times10^{-2}$
11.31	NaOH	315	$1.59(0.006)\times10^{-2}$
11.31	NaOH	315	$1.59(0.007)\times10^{-2}$
11.83	NaOH	315	9.79×10^{-3}
11.83	NaOH	315	$1.03(0.005)\times10^{-2}$
12.31	NaOH	315	$5.44(0.01)\times10^{-3}$
12.31	NaOH	315	$5.45(0.02)\times10^{-3}$
12.83	NaOH	293	$2.74(0.02)\times10^{-3}$
12.83	NaOH	293	$2.75(0.01)\times10^{-3}$
13.31	NaOH	293	$2.60(0.01)\times10^{-3}$
13.31	NaOH	293	$2.64(0.03)\times10^{-3}$
13.83	NaOH	293	$3.97(0.02)\times10^{-3}$
13.83	NaOH	293	$3.94(0.05)\times10^{-3}$
13.83	NaOH	294	$3.92(0.02)\times10^{-3}$ †

† Determined by ring opening of the isomeric flavanone.

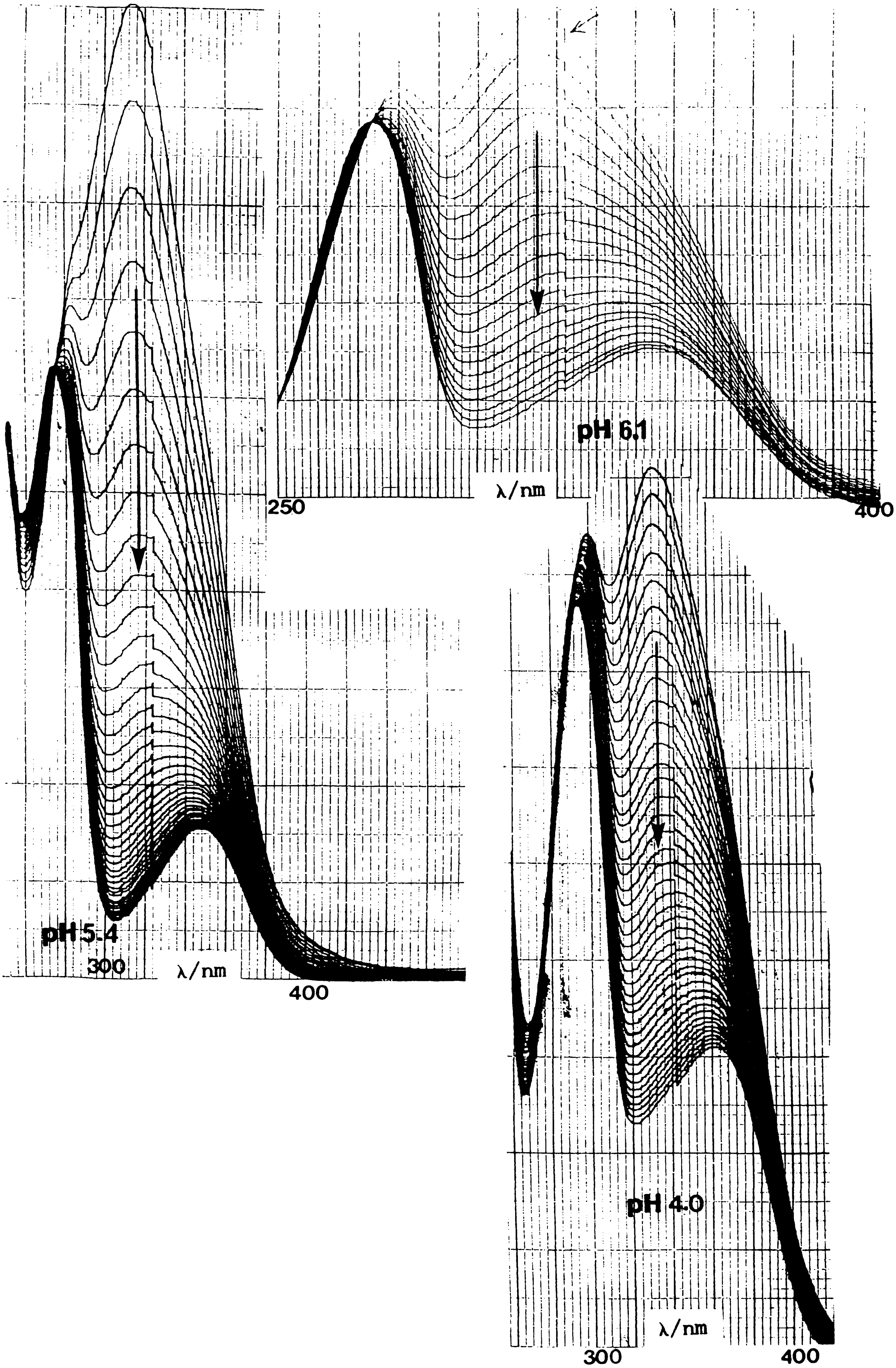


FIGURE 2-4 Repetitive Scans of the Equilibration of *34* and *35*, at various pH Values

The pH-rate data were analysed as outlined previously, to provide the pK_a values for the chalcone, and the rate coefficients for the reaction:

$$k_{H^+} = 9.6\times10^{-7}\ l\ mol^{-1}\ s^{-1}$$

$$k_{CHH} = 1.6\times10^{-6}\ s^{-1}$$

$$k_{CH^-} = 2.10\times10^{-2}\ s^{-1}$$

$$k_{C2^-} = 1.3\times10^{-3}\ s^{-1}$$

$$k_{F^-} = 2.6\times10^{-3}\ l\ mol^{-1}\ s^{-1}$$

$$pK_{a1} = 7.6$$

$$pK_{a2} = 11.7$$

In figure 2-5 the experimental values of k_{obs} are plotted against pH, while the line represents the calculated values based on the rate constants and pK_a values given above, and the usual rate expression (equation 2-19).

2.3.2 The Effect of D_2O on the Rate of Chalcone Mono-anion Cyclisation

The rate constant for the approach to equilibrium (k_{obs}) was determined in the high pH plateau region in deuterium oxide solution. The results are given in table 2-4.

TABLE 2-4 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxychalcone (***34***) – 5-Hydroxyflavanone (***35***) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
10.37	CARBONATE	315	$6.10(0.02)\times10^{-3}$
10.96	CARBONATE	315	$5.60(0.03)\times10^{-3}$

The kinetic isotope effect for the cyclisation of the mono-anion,

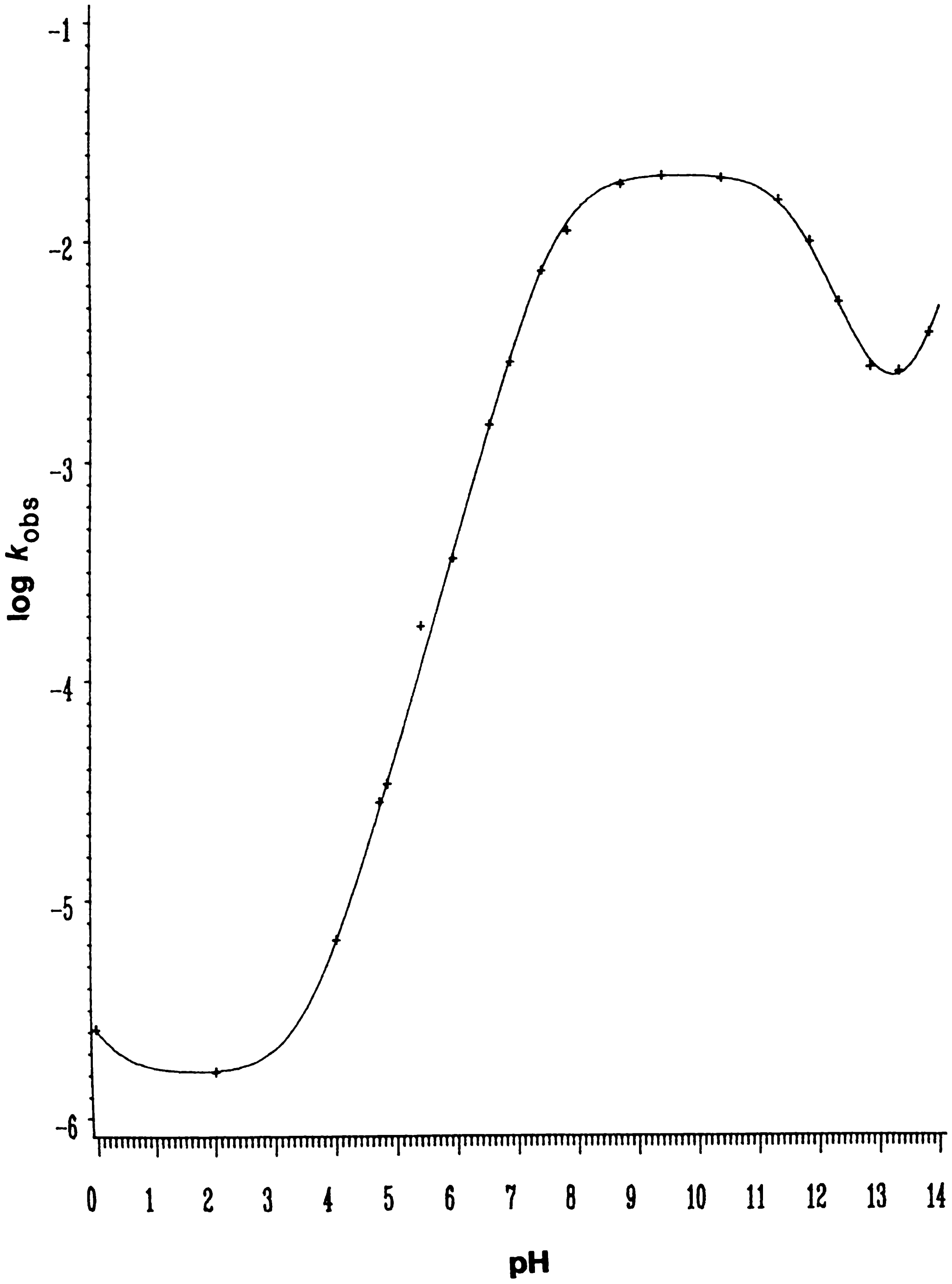


FIGURE 2-5 pH-Rate Profile for the Equilibration of *34* and *35*: Experimental Values (+); Curve (——) Calculated from Equation 2-19, and the Rate Coefficients and pK_a values Given on Page 78 then, is:

$$\frac{k_H}{k_D} = \frac{2.10\times10^{-2}}{6.1\times10^{-3}} = 3.4$$

2.3.3 The Effect of Buffer Concentration on the Rate of the Chalcone Mono-anion Cyclisation

The effect of the buffer concentration on the rate of approach to equilibrium was investigated in the high pH plateau region. For two different carbonate buffers (*ca*. pH 9.4 and 10.1), with μ kept constant at 1.0 mol l^{-1} (KCl), the rate constants for the approach to equilibrium were measured at 0.02, 0.05, 0.08 and 0.10 mol l^{-1} total carbonate concentration. The results are shown in table 2-5.

TABLE 2-5 The Effect of Buffer Concentration on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxychalcone (**34**) - 5-Hydroxyflavanone (**35**) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl).

[BUFFER] mol l^{-1}	$10^2 k_{obs}(\sigma)/s^{-1}$	
	$[HCO_3^-]:[CO_3^{2-}]$ 2:8	7:3
0.02	2.06(0.008)	2.05(0.002)
0.02	2.00(0.006)	2.16(0.01)
0.02		2.03
0.05	2.14(0.009)	2.26(0.009)
0.05	2.18(0.01)	2.25(0.008)
0.08	2.21(0.009)	2.36(0.006)
0.08	2.22(0.01)	2.42(0.01)
0.08		2.21(0.007)
0.10	2.22(0.02)	2.44(0.004)
0.10	2.22(0.009)	2.43(0.01)

The rate constant shows a weak dependence on the buffer concentration as shown in figure 2-6.

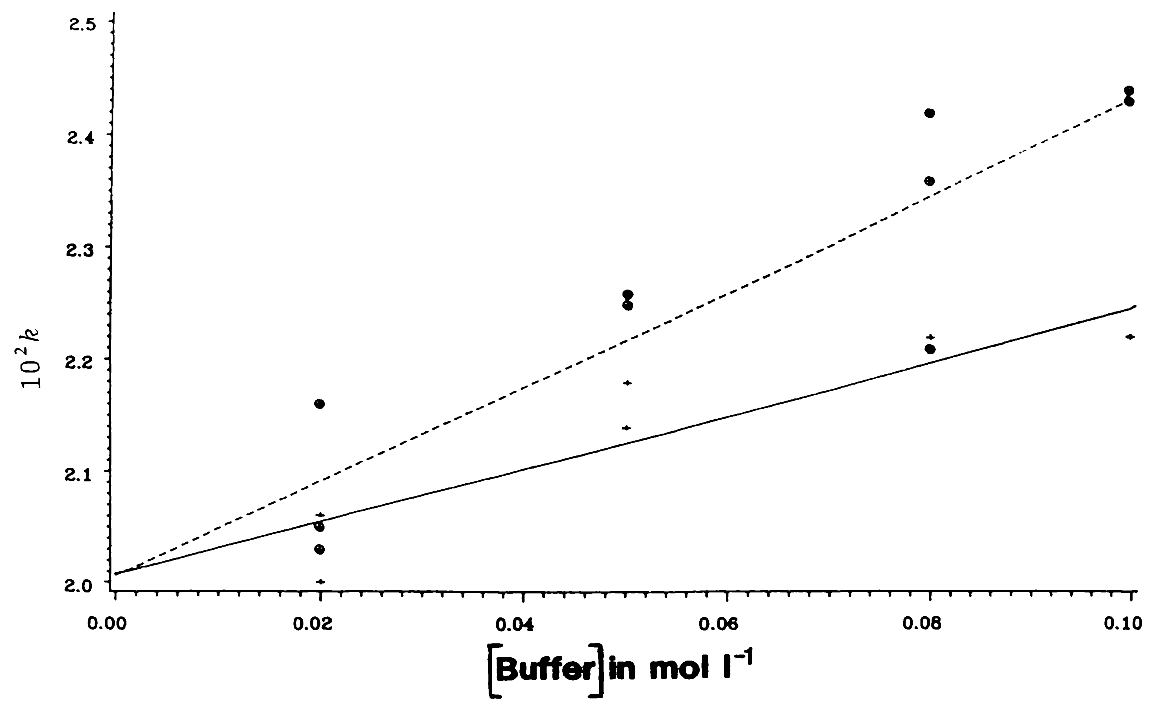


FIGURE 2-6 The Effect of Buffer Concentration on k_{obs} for the Equilibration of *34* and *35*

The slope for the 7:3 $[HCO_3^-]:[CO_3^{2-}]$ buffer appears to be **higher** than that for the 2:8 $[HCO_3^-]:[CO_3^{2-}]$ buffer. This suggests that HCO_3^- might be the major catalytic species.

2.3.*4* The Effect of Temperature on the rate of the Chalcone Mono-Anion Cyclisation

The rate constant for attainment of equilibrium was determined in the high pH plateau region at various temperatures in 3:7 $[HCO_3^-]:[CO_3^{2-}]$ buffer (see section 2.1.7). The results are reported in table 2-6.

A plot of $\ln k_{obs}$ versus T^{-1} (figure 2-7) gave a straight line, which by least-squares regression analysis had a slope of -7370 (σ = 110) K and an intercept of 20.4 (σ = 0.4). Thus, from equation 2-11 we can calculate the Arhennius activation parameters E_a = 61 3 (σ = 0.9) kJ mol^{-1} and $A = e^{20.4}$ = 7 (σ = 3)x10^8. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^\ddagger$ = 58.8 (σ = 0.9) kJ mol^{-1}; $\Delta S^\ddagger$ = -84 (σ = 3) J mol^{-1} K^{-1}; $\Delta G^\ddagger$ = 84 (σ = 2) kJ mol^{-1}.

TABLE 2-6 The Effect of Temperature (in 3:7 $[HCO_3^-]:[CO_3^{2-}]$ Buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxychalcone (***34***) – 5-Hydroxyflavanone (***35***) Equilibrium in 4% Ethanolic H_2O, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3 k_{obs}(\sigma)$ /s^{-1}
16.83	3.449	6.56(0.006)
20.60	3.404	9.75(0.008)
23.40	3.372	11.3(0.09)
26.65	3.336	15.0(0.06)
30.00	3.299	20.5(0.1)
33.70	3.259	27.5(0.09)
37.35	3.221	35.8(0.05)
40.55	3.188	45.0(0.1)
43.80	3.155	58.0(0.3)

The same procedure as above was also carried out using a deuterium oxide- (rather than a water-) based carbonate buffer (see section 2.1.3). Due to the limited amount of the deuterium-based buffer solution available, measurements at fewer temperatures were made, and hence a lower accuracy was achieved. The results are given in table 2-7.

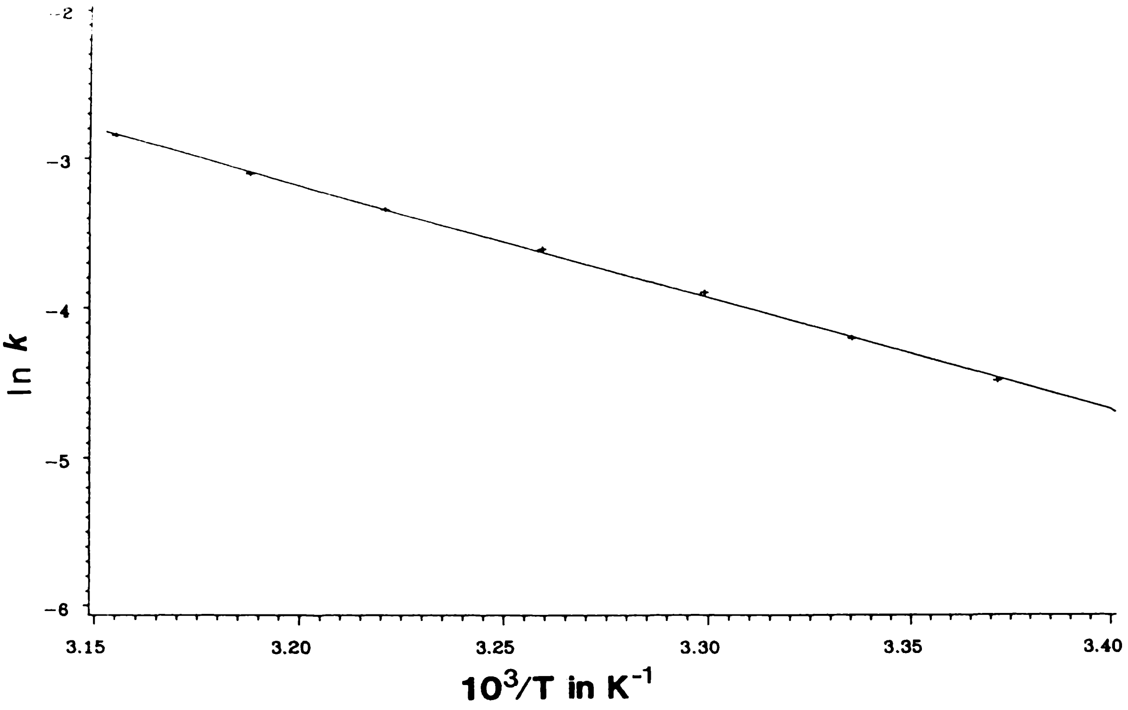


FIGURE 2-7 Plot of logk_{obs} versus T^{-1} in the High-pH Plateau Region for the Equilibration of *34* and *35*

A plot of lnk_{obs} versus T^{-1} gave a straight line (figure 2-8), which by least-squares regression analysis had a slope of -7530 (σ = 160) K and an intercept of 19.8 (σ = 0.5). Thus, from equation 2-11 we can calculate the Arrhenius activation parameters E_a = 62.6 (σ = 1.3) kJ mol^{-1} and A = $e^{19.8}$ = 4 (σ = 3)$\times 10^8$. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^{\ddagger}$ = 60 (σ = 1.3) kJ mol^{-1}; $\Delta S^{\ddagger}$ = -89 (σ = 4) J mol^{-1} K^{-1}; $\Delta G^{\ddagger}$ = 87 (σ = 2.6) kJ mol^{-1}.

2.3.5 The Rate Constant for the 2',6'-Dihydroxychalcone Mono-anion Cyclisation under the Conditions of Old[46,71]

It was desired to compare the rate constant for ring closure of the 2'-hydroxy-6'-methoxychalcone anion with that for the 2',6'-dihydroxychalcone mono-anion. The reason was that the two anions should be electronically and sterically quite similar, except for the potential for intramolecular hydrogen bonding, which is absent in the

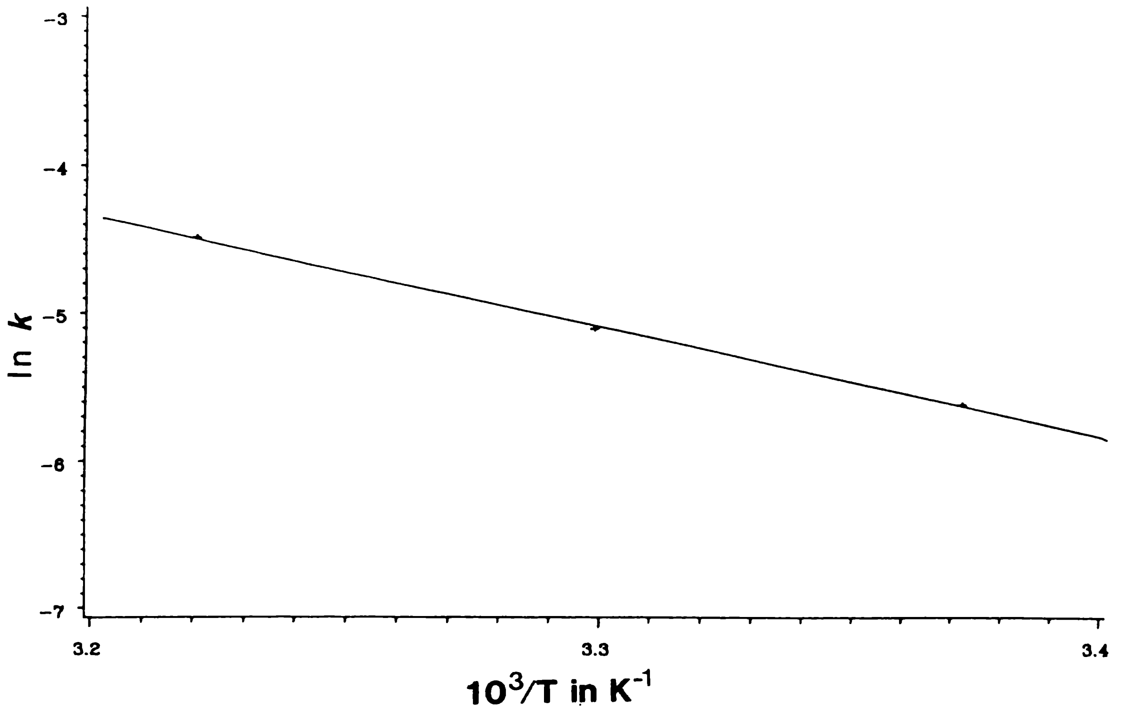


FIGURE 2-8 Plot of $\log k_{obs}$ versus T^{-1} in the High-pH Plateau Region for the Equilibration of *34* and *35* in D_2O

TABLE 2-7 The Effect of Temperature (in 1:1 $[DCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxychalcone (*34*) – 5-Hydroxyflavanone (*35*) Equilibrium in 4% Ethanolic D_2O, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3 k_{obs}(\sigma)$ /s^{-1}
16.83	3.449	1.97(0.02)
20.60	3.404	2.87(0.01)
23.40	3.372	3.66(0.02)
30.00	3.299	6.10(0.02)
37.35	3.221	11.3(0.2)

former chalcone anion. Such a comparison could provide information as to whether 2′,6′-dihydroxychalcones are inherently more reactive towards cyclisation than 2′-hydroxychalcones, or whether the instability of 2′,6′-dihydroxychalcones is perhaps due to other factors, such as a low first pK_a.

It was obviously more convenient to determine the rate of cyclisation of 2′,6′-dihydroxychalcone mono-anion (in the high pH plateau region) under the reaction conditions used by Old[46,71] than it was to repeat the analysis of Old for 2′-hydroxy-6′-methoxychalcone under the conditions of the present study; since 2′-hydroxy-6′-methoxychalcone exhibits no pH-rate plateau, estimation of the rate coefficient for the cyclisation of its anion requires detailed analysis of kinetic data collected over a wide pH range.[46,71] Therefore, k_{obs} was determined (in duplicate) in carbonate buffers where $[HCO_3^-]:[CO_3^{2-}]$ ranged from 9:1 to 1:9 (pH *ca.* 8.9-9.5). The chalcone (125 μl) was added from a stock dioxane solution to 2.50 ml of the buffer (μ = 0.50 mol l^{-1} with KCl; [buffer] = 0.02 mol l^{-1}) at 30°C. The pH of the reaction solutions was determined (at 25°C), just as a precaution to be sure that the buffers spanned the high pH plateau range. Clearly, this was the case, as the measured pH values span a 1.5 unit range, and the k_{obs} values in the 1:9 and 9:1 buffers are slightly lower than the buffers of intermediate pH, as can be seen clearly from the results in table 2-8.

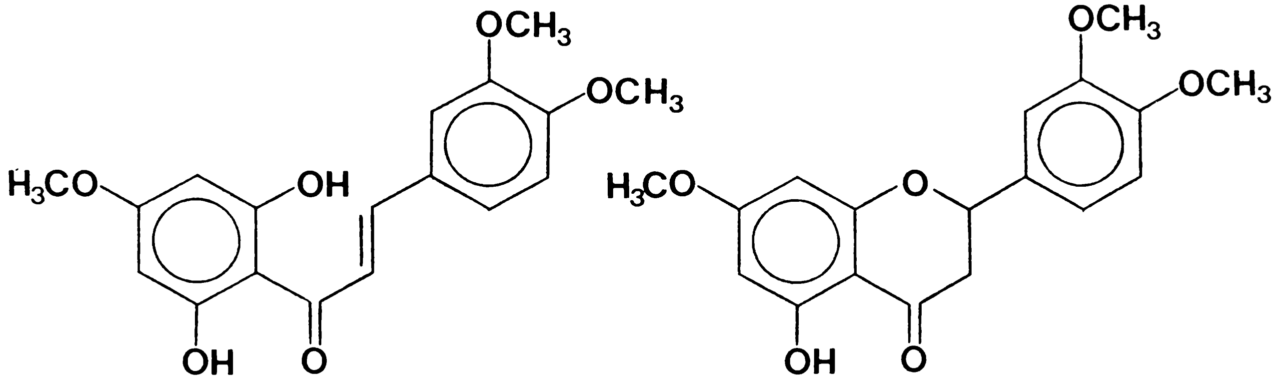
TABLE 2-8 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxychalcone – 5-Hydroxyflavanone Equilibrium in Various Carbonate Buffers in aqueous 5% Dioxane, μ = 0.50 mol l^{-1} (KCl), [Buffer] = 0.02 mol l^{-1} at 30°C.

$[HCO_3^-]:[CO_3^{2-}]$	pH(25°C)	$10^2k_{obs}(\sigma)/s^{-1}$
9:1	8.94	1.92(0.02) & 1.94(0.008)
7:3	9.46	1.98(0.005) & 2.00(0.003)
5:5	9.80	1.95(0.007) & 2.00(0.004)
3:7	10.11	1.92(0.005) & 1.94(0.009)
1:9	10.48	1.84(0.005) & 1.85(0.007)

We can estimate from the results in table 2-8 that $k_{CH^-} = 2.05\times10^{-2}$ s^{-1} under the conditions used by Old[46,71] in his study. For comparison, the value obtained in their study, for cyclisation of 2'-hydroxy-6'-methoxychalcone, was $k_{C^-} = 5.3\times10^{-4}$ s^{-1}.

It should be pointed out at this stage that the change of the reaction conditions, from those of the present study to those of Old,[46,71] resulted in only a very small change in the rate coefficient of 2',6'-dihydroxychalcone mono-anion cyclisation. It would seem unlikely, then, that the same change in reaction conditions would produce major changes in the other rate constants involved in the isomerisation reaction. Therefore it is justifiable to make tentative comparisons of other rate coefficients, even when they are measured under the (slightly) different conditions used in this, and in Old's study.

2.4 The 2′,6′-Dihydroxy-3,4,4′-trimethoxychalcone – 5-Hydroxy-3′,4′,7-trimethoxyflavanone Equilibrium



36 *37*

2.4.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of 2′,6′-dihydroxy-3,4,4′-trimethoxychalcone (*36*) or 5-hydroxy-3′,4′,7-trimethoxyflavanone (*37*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0–13.83. Repetitive scans at several pH values (figure 2-9) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. The first-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-9.

TABLE 2-9 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2′,6′-Dihydroxy-3,4,4′-trimethoxychalcone (*36*) – 5-Hydroxy-3′,4′,7-trimethoxyflavanone (*37*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	370	$1.55(0.03)\times10^{-5}$
0.00	HCl	370	$1.54(0.02)\times10^{-5}$
1.94	HCl	370	$7.87(0.07)\times10^{-6}$
1.94	HCl	370	$7.74(0.06)\times10^{-6}$

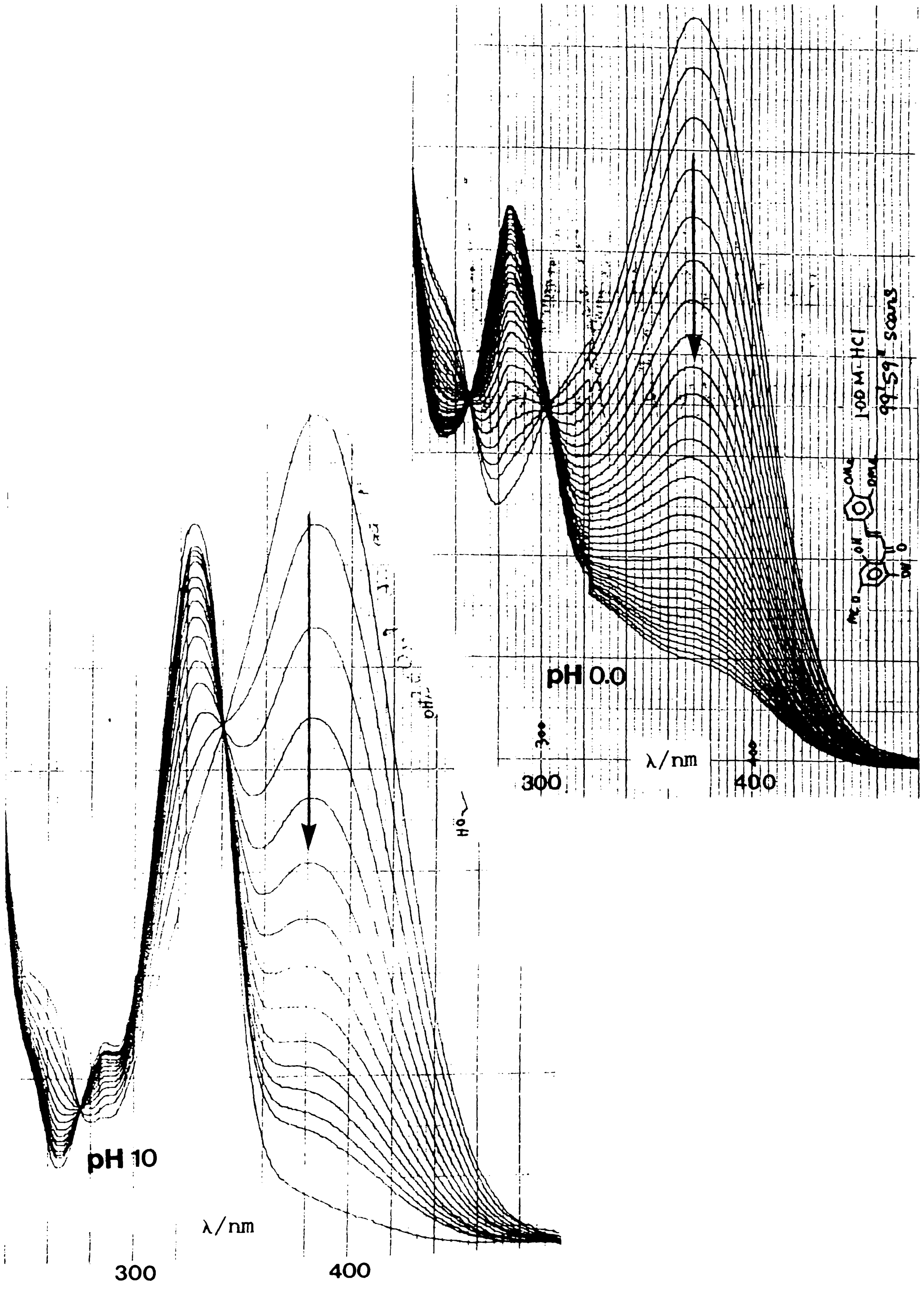


FIGURE 2-9 Repetitive Scans of the Equilibration of *36* and *37*, at various pH Values

TABLE 2-9 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
4.00	ACETATE	370	$1.53(0.01)\times10^{-5}$
4.00	ACETATE	370	$1.93(0.02)\times10^{-5}$
4.69	ACETATE	370	$4.86(0.04)\times10^{-5}$
5.46	ACETATE	370	$2.28(0.01)\times10^{-4}$
5.46	ACETATE	370	$2.20(0.01)\times10^{-4}$
5.88	PHOSPHATE	370	$4.10(0.01)\times10^{-4}$
6.47	PHOSPHATE	370	$1.35(0.008)\times10^{-3}$
6.48	PHOSPHATE	370	$1.36(0.008)\times10^{-3}$
6.81	PHOSPHATE	370	$2.25(0.03)\times10^{-3}$
6.81	PHOSPHATE	370	$2.41(0.01)\times10^{-3}$
7.31	PHOSPHATE	370	$3.81(0.01)\times10^{-3}$
7.31	PHOSPHATE	370	$3.82(0.01)\times10^{-3}$
7.76	BICINE	370	$4.15(0.02)\times10^{-3}$
7.76	BICINE	370	$4.21(0.02)\times10^{-3}$
8.68	BICINE	370	$4.36(0.02)\times10^{-3}$
8.69	BICINE	370	$4.41(0.02)\times10^{-3}$
9.32	CARBONATE	370	$4.45(0.02)\times10^{-3}$
9.33	CARBONATE	370	$4.55(0.01)\times10^{-3}$
10.37	CARBONATE	370	$4.30(0.02)\times10^{-3}$
10.37	CARBONATE	370	$4.13(0.02)\times10^{-3}$
11.31	NaOH	370	$4.05(0.01)\times10^{-3}$
11.31	NaOH	370	$4.02(0.02)\times10^{-3}$
11.83	NaOH	370	$3.45(0.02)\times10^{-3}$
11.83	NaOH	370	$3.53(0.01)\times10^{-3}$
12.31	NaOH	370	$2.82(0.008)\times10^{-3}$
12.31	NaOH	370	$2.76(0.01)\times10^{-3}$
12.83	NaOH	338	2.28×10^{-3}
12.83	NaOH	338	$2.29(0.01)\times10^{-3}$
13.31	NaOH	338	$2.21(0.02)\times10^{-3}$
13.31	NaOH	338	$2.21(0.01)\times10^{-3}$
13.83	NaOH	338	$3.43(0.03)\times10^{-3}$ †
13.83	NaOH	338	$3.44(0.02)\times10^{-3}$ †

† Determined by ring opening of the isomeric flavanone.

Computer analysis of the results, as outlined previously, gave the chalcone's pK_a values and the individual rate coefficients for the reaction:

k_{H+} = 7.8x10^{-6} l mol^{-1} s^{-1}

k_{CHH} = 7.6x10^{-6} s^{-1}

k_{CH-} = 4.40x10^{-3} s^{-1}

k_{C2-} = 1.3x10^{-3} s^{-1}

k_{F-} = 2.0x10^{-3} l mol^{-1} s^{-1}

pK_{a1} = 6.8

pK_{a2} = 12.3

In figure 2-10 the experimental points are plotted as crosses, while the line is the theoretical curve based on the rate constants and pK_a values given above, and on equation 2-19.

2.4.2 The Effect of D_2O on the Rate of the Chalcone Mono-Anion Cyclisation

The rate of approach to equilibrium was also measured in deuterium oxide in the high pH plateau region. The resulting values of k_{obs} are given in table 2-10.

TABLE 2-10 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-3,4,4'-trimethoxychalcone (*36*) - 5-Hydroxy-3',4',7-trimethoxyflavanone (*37*) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
10.39	CARBONATE	370	8.70(0.06)x10^{-4}
10.39	CARBONATE	370	8.80(0.07)x10^{-4}
10.96	CARBONATE	370	8.60(0.03)x10^{-4}
10.96	CARBONATE	370	8.39(0.06)x10^{-4}

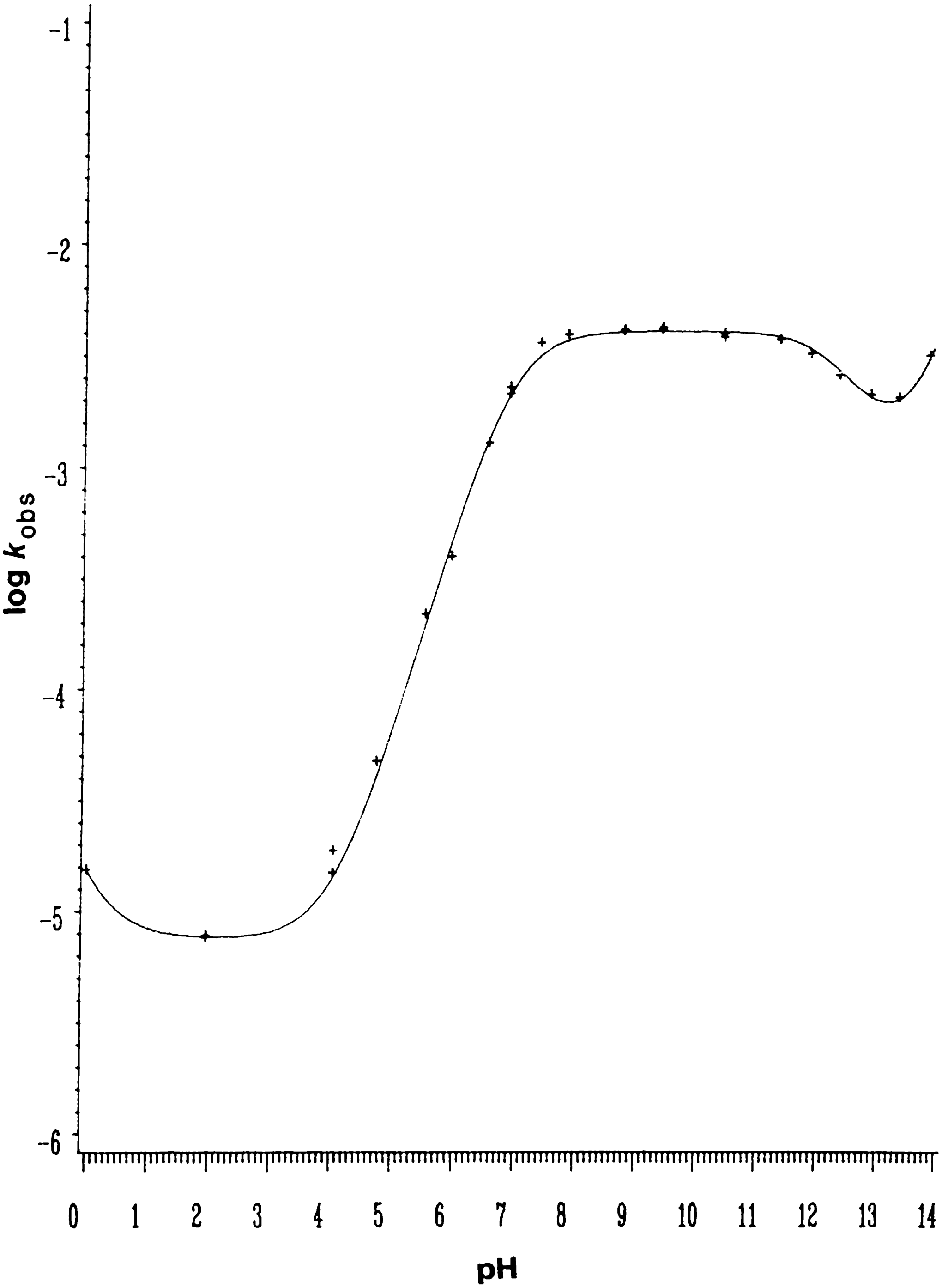


FIGURE 2-10 pH-Rate Profile for the Equilibration of *36* and *37*: Experimental values (+); Curve (——) Calculated From Equation 2-19, and the Rate and Equilibrium Constants Given in the Text (Page 90)

The kinetic isotope effect for the cyclisation of the mono-anion, then, is:

$$\frac{k_H}{k_D} = \frac{4.40\times10^{-3}}{8.7\times10^{-4}} = 5.0$$

2.4.3 The Effect of Temperature on the rate of the Chalcone Mono-Anion Cyclisation

The effect of temperature on the observed rate constant in the high pH plateau region was studied. The results are given in table 2-11.

TABLE 2-11 The Effect of Temperature (in 3:7 $[HCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-3,4,4'-trimethoxychalcone (*36*) – 5-Hydroxy-3',4',7-trimethoxyflavanone (*37*) Equilibrium in 4% Ethanolic H_2O, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3k_{obs}(\sigma)$ /s^{-1}
18.7	3.426	1.63(0.01)
18.7	3.426	1.67(0.01)†
24.3	3.362	2.69(0.01)
24.3	3.362	2.73(0.009)†
30.0	3.299	4.30(0.02)
36.85	3.226	7.89(0.02)
36.85	3.226	7.60(0.02)

† $[HCO_3^-]:[CO_3^{2-}]$ = 4:6.

A plot of $\ln k_{obs}$ versus T^{-1} (figure 2-11) gave a straight line, which by least-squares regression analysis had a slope of -7690 (σ = 95) K and an intercept of 19.9 (σ = 0.3). Thus, from equation 2-11 we can calculate the Arhennius activation parameters E_a = 63.9 (σ = 0.8) kJ mol^{-1} and $A = e^{19.9}$ = 4.5 (σ = 2)$\times10^8$. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^{\ddagger} = 61.4$ ($\sigma = 0.8$) kJ mol^{-1}; $\Delta S^{\ddagger} = -88$ ($\sigma = 3$) J mol^{-1} K^{-1}; $\Delta G^{\ddagger} = 88$ ($\sigma = 1.6$) kJ mol^{-1}.

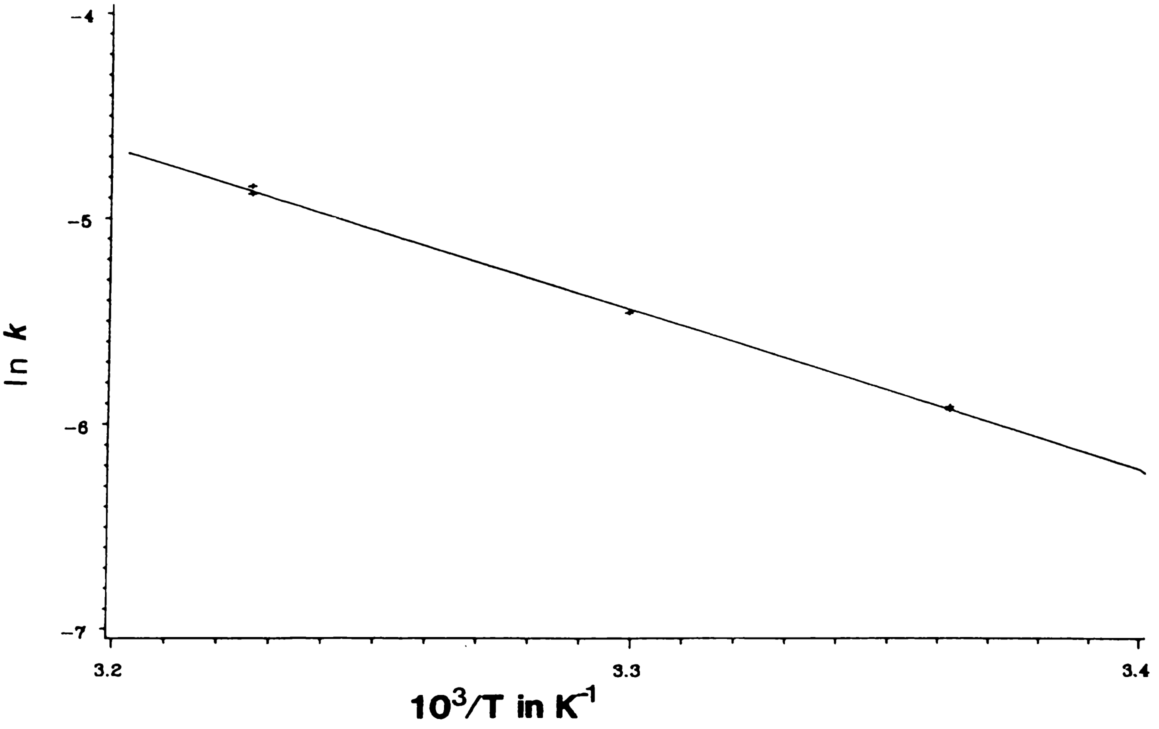
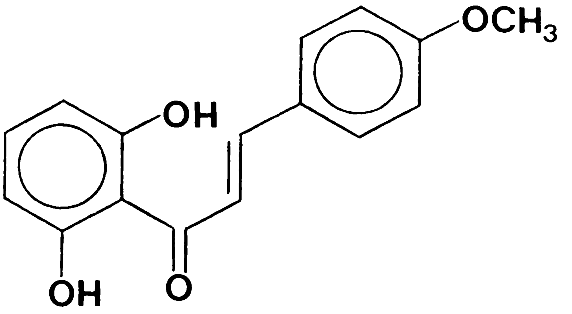


FIGURE 2-11 Plot of $\ln k_{obs}$ (in the High-pH Plateau Region) Versus T^{-1} for the Equilibration of *36* and *37*

2.5 The 2',6'-Dihydroxy-4-methoxychalcone - 5-Hydroxy-4'-methoxyflavanone Equilibrium



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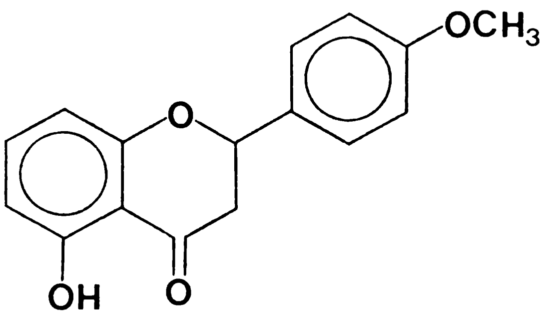

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2.5.*1* The Effect of pH on the Rate of Equilibration

The rate of conversion of 2',6'-dihydroxy-4-methoxychalcone or 5-hydroxy-4'-methoxyflavanone to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0-13.83. Repetitive scans at several pH values (figure 2-12) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. First-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-12.

TABLE 2-12 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-4-methoxychalcone (*38*) - 5-Hydroxy-4'-methoxyflavanone (*39*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	346	$8.87(0.02)\times10^{-6}$
1.94	HCl	365	$5.03(0.03)\times10^{-6}$
3.07	PHTHALATE	346	$4.82(0.09)\times10^{-6}$
3.99	ACETATE	346	$1.32(0.01)\times10^{-5}$

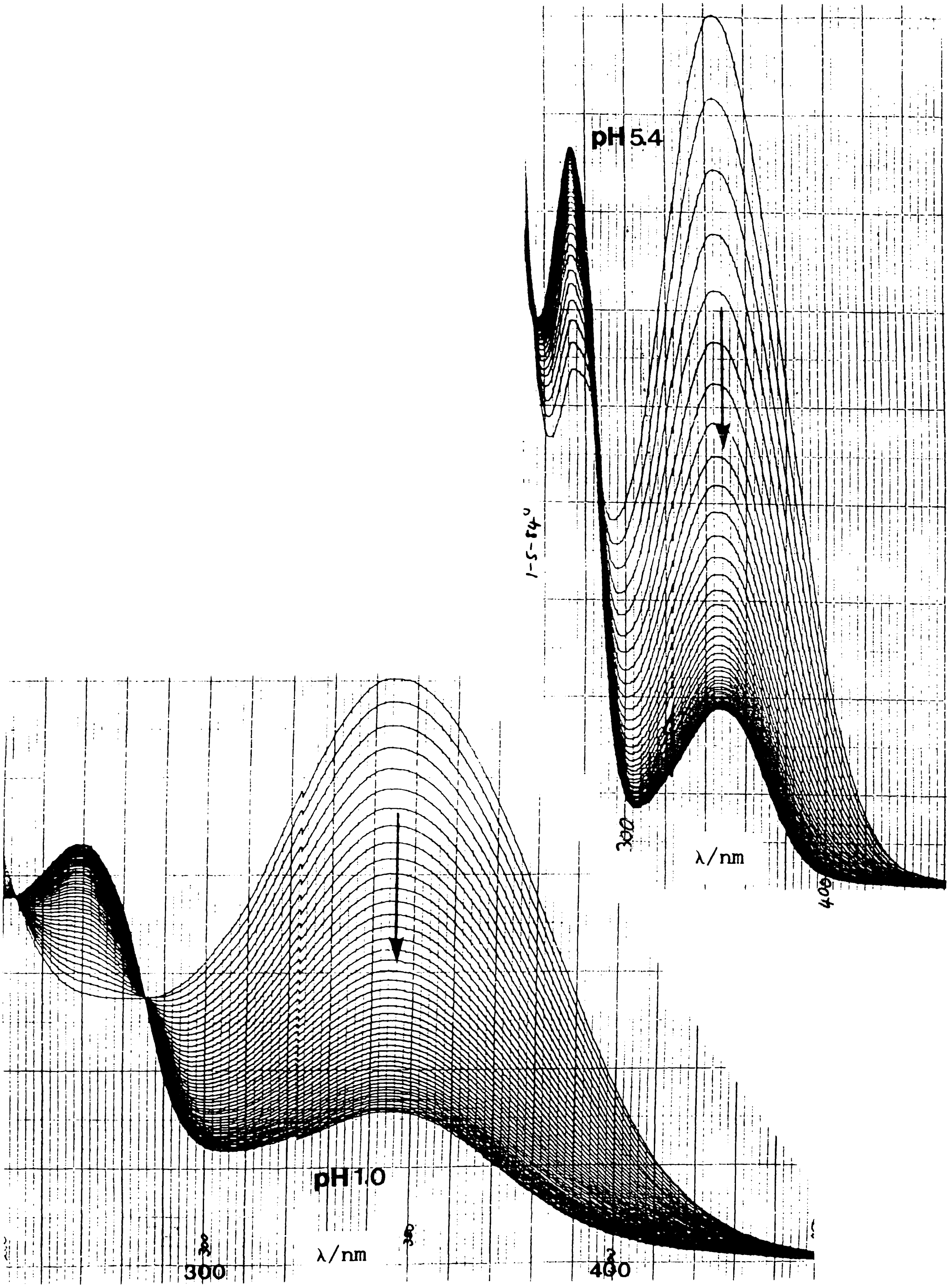


FIGURE 2-12 Repetitive Scans of the Equilibration of *38* and *39*, at Various pH Values

TABLE 2-12 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
4.69	ACETATE	346	$3.92(0.02)\times10^{-5}$
4.81	PHTHALATE	346	4.14×10^{-5}
5.44	ACETATE	346	$2.05(0.003)\times10^{-4}$
5.88	PHOSPHATE	346	$4.24(0.008)\times10^{-4}$
6.48	PHOSPHATE	346	$1.65(0.008)\times10^{-3}$
6.83	PHOSPHATE	346	$3.12(0.01)\times10^{-3}$
6.83	PHOSPHATE	346	$3.02(0.02)\times10^{-3}$
7.34	PHOSPHATE	346	$7.29(0.02)\times10^{-3}$
7.35	PHOSPHATE	346	$7.19(0.02)\times10^{-3}$
7.76	BICINE	346	$1.05(0.004)\times10^{-2}$
7.77	BICINE	346	$1.06(0.003)\times10^{-2}$
8.64	BICINE	346	$1.78(0.007)\times10^{-2}$
8.65	BICINE	346	$1.78(0.007)\times10^{-2}$
9.34	CARBONATE	346	$2.01(0.005)\times10^{-2}$
9.34	CARBONATE	346	$2.01(0.006)\times10^{-2}$
10.36	CARBONATE	346	$1.88(0.007)\times10^{-2}$
10.37	CARBONATE	346	$1.86(0.006)\times10^{-2}$
11.31	NaOH	346	$1.48(0.003)\times10^{-2}$
11.31	NaOH	346	$1.48(0.006)\times10^{-2}$
11.83	NaOH	346	9.35×10^{-3}
11.83	NaOH	346	$9.58(0.05)\times10^{-3}$
12.31	NaOH	346	$5.00(0.03)\times10^{-3}$
12.31	NaOH	346	$5.12(0.01)\times10^{-3}$
12.83	NaOH	322	$2.90(0.03)\times10^{-3}$
12.83	NaOH	322	$3.04(0.02)\times10^{-3}$
13.31	NaOH	322	$3.15(0.01)\times10^{-3}$
13.31	NaOH	322	$3.14(0.03)\times10^{-3}$
13.83	NaOH	322	5.44×10^{-3} †
13.83	NaOH	322	$5.44(0.01)\times10^{-3}$ †

† Determined by ring opening of the isomeric flavanone.

Computer analysis of the results, as outlined previously, gave the chalcone's pK_a values and the individual rate coefficients for the

reaction:

k_{H+} = 3.93×10^{-6} l mol^{-1} s^{-1}

k_{CHH} = 4.94×10^{-6} s^{-1}

k_{CH-} = 2.05×10^{-2} s^{-1}

k_{C2-} = 1.3×10^{-3} s^{-1}

k_{F-} = 4.0×10^{-3} l mol^{-1} s^{-1}

pK_{a1} = 7.6

pK_{a2} = 11.7

In figure 2-13 the experimental points are plotted as crosses, while the line is the theoretical curve, based on the rate constants and pK_a values given above and equation 2-19.

2.5.2 The Effect of D_2O on the Rate of the Chalcone Mono-anion Cyclisation

The rate constant for the approach to equilibrium was also measured in deuterium oxide in the high pH plateau region. The resulting values of k_{obs} are given in table 2-13.

TABLE 2-13 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-4-methoxychalcone (*38*) – 5-Hydroxy-4'-methoxyflavanone (*39*) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)$/s^{-1}
10.38	CARBONATE	346	3.64(0.02)$\times10^{-3}$
10.96	CARBONATE	346	3.40(0.01)$\times10^{-3}$

The approximate kinetic isotope effect for the cyclisation of the mono-anion, then, is:

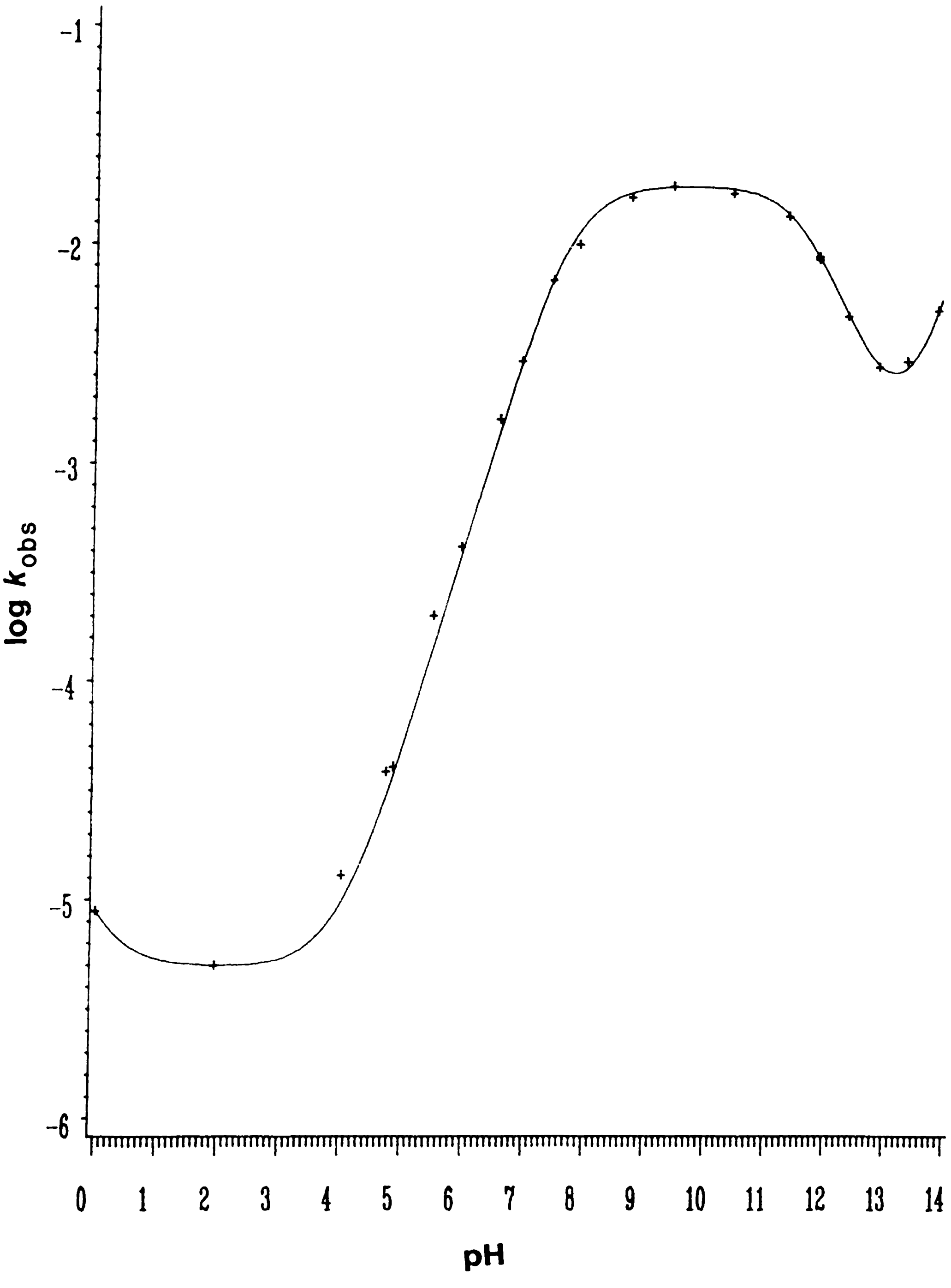


FIGURE 2-13 pH-Rate Profile for the Equilibration of *38* and *39*: Experimental Values (+) from Table 2-12; Curve (——) Calculated from Equation 2-19 and the Rate Coefficients and pK_a Values Given in the Text (Page 97)

$$\frac{k_H}{k_D} = \frac{2.05\times10^{-2}}{3.6\times10^{-3}} = 5.7$$

2.5.3 The Effect of Temperature on the rate of the Chalcone Mono-Anion Cyclisation

The effect of temperature on the observed rate constant in the high pH plateau region was also investigated. The results are given in table 2-14.

TABLE 2-14 The Effect of Temperature (in 3:7 $[HCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-4-methoxychalcone – 5-Hydroxy-4'-methoxyflavanone Equilibrium in 4% Ethanolic H_2O, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3 k_{obs}(\sigma)$ /s^{-1}
16.83	3.449	6.55(0.005)
20.60	3.404	9.71(0.005)
23.40	3.372	11.3(0.1)
26.65	3.336	14.8(0.09)
30.00	3.299	20.1(0.1)
33.70	3.259	26.2(0.08)
37.35	3.221	33.9(0.03)
40.55	3.188	43.0(0.1)
43.80	3.155	51.9(0.2)

A plot of $\ln k_{obs}$ versus T^{-1} (figure 2-14) gave a straight line, which by least-squares regression analysis had a slope of -7050 (σ = 110) K and an intercept of 19.3 (σ = 0.35). Thus, from equation 2-11 we can calculate the Arhennius activation parameters E_a = 58.6 (σ = 0.9) kJ mol^{-1} and $A = e^{19.3} = 2.4$ (σ = 1)$\times10^8$. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^{\ddagger} = 56.1$ ($\sigma = 0.9$) kJ mol^{-1}; $\Delta S^{\ddagger} = -93$ ($\sigma = 3$) J mol^{-1} K^{-1}; $\Delta G^{\ddagger} = 84$ ($\sigma = 2$) kJ $^{-1-1}$

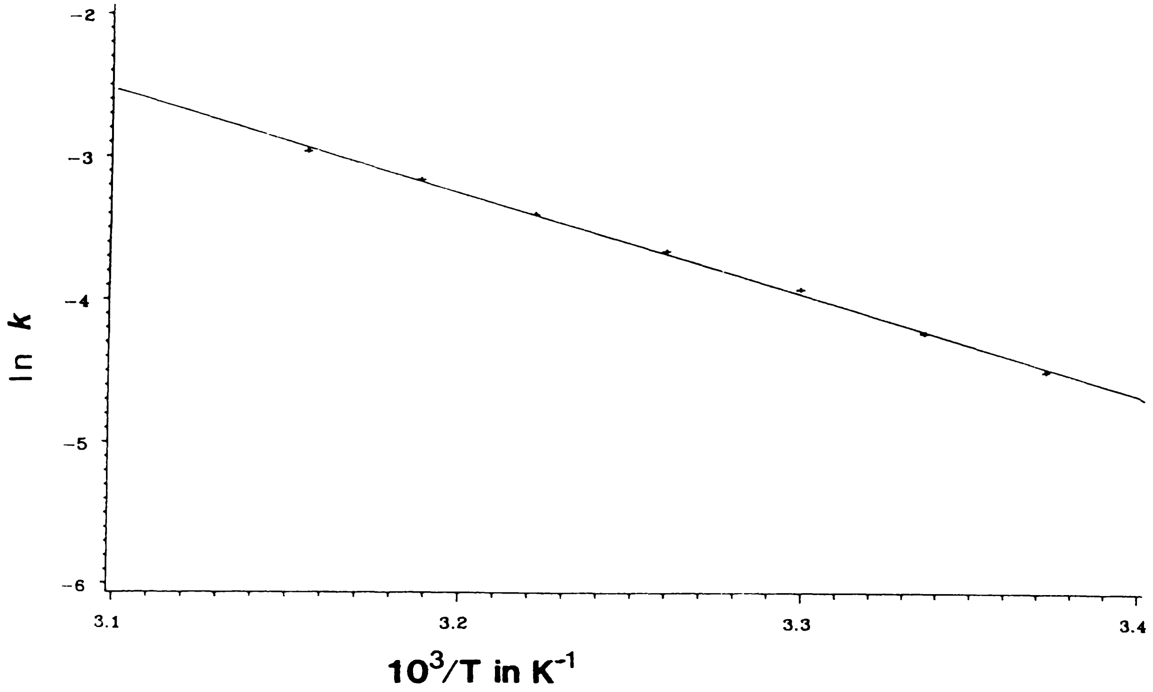


Figure 2-14 Plot of lnk_{obs} Versus T^{-1} for the Equilibration of *38* and *39*

The same procedure as above was carried out using a deuterium oxide- (rather than a water-) based carbonate buffer (see section 2.1.*3*). Due to the limited amount of the deuterium-based buffer solution available, measurements at fewer temperatures were made, and hence a lower accuracy was achieved. The results are given in table 2-15.

A plot of lnk_{obs} versus T^{-1} (figure 2-15) gave a straight line, which by least-squares regression analysis had a slope of -7830 ($\sigma = 190$) K and an intercept of 20.2 ($\sigma = 0.6$). Thus, from equation 2-11 we can calculate the Arhennius activation parameters $E_a = 65.1$ ($\sigma = 1.6$) kJ mol^{-1} and $A = e^{20.2} = 6$ ($\sigma = 5$)$\times 10^8$. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^{\ddagger} = 62.6$ ($\sigma = 1.6$) kJ mol^{-1}; $\Delta S^{\ddagger} = -85$ ($\sigma = 5$) J mol^{-1} K^{-1}; $\Delta G^{\ddagger} = 88.5$ ($\sigma = 3$) kJ mol^{-1}.

TABLE 2-15 The Effect of Temperature (in 1:1 $[DCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-4-methoxychalcone – 5-Hydroxy-4'-methoxyflavanone Equilibrium in 4% Ethanolic D_2O, $\mu = 1.0$ mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3 k_{obs}(\sigma)$ /s^{-1}
16.83	3.449	1.09(0.01)
20.60	3.404	1.66(0.02)
23.40	3.372	2.00(0.01)
30.00	3.299	3.64(0.02)
37.35	3.221	6.65(0.007)

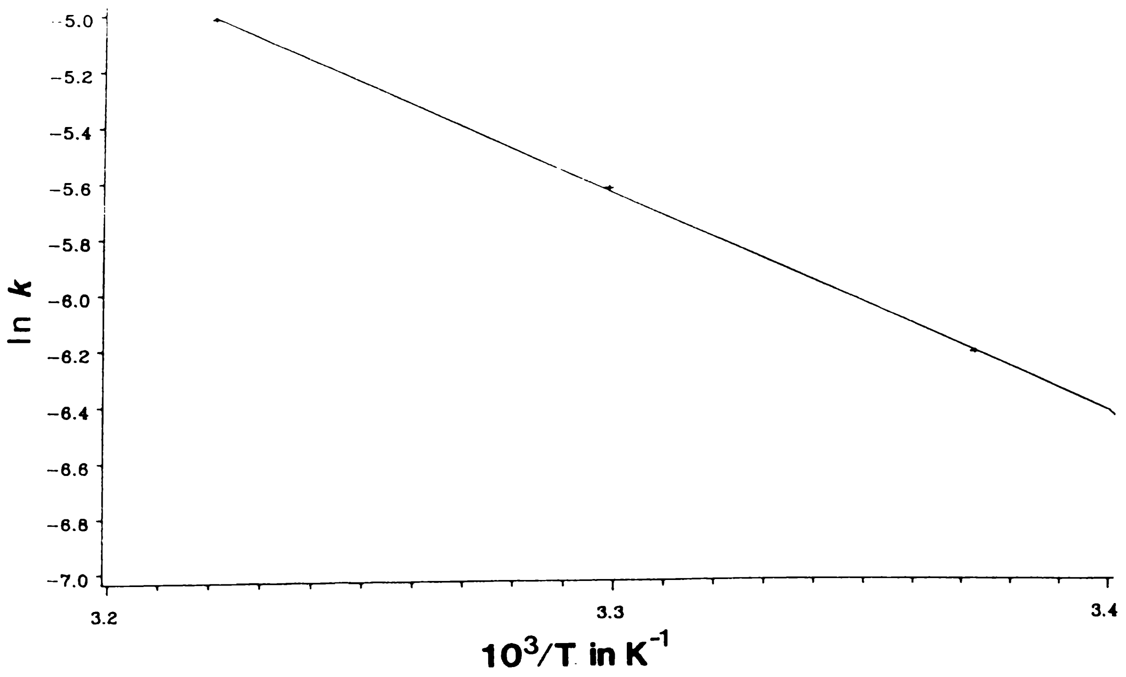
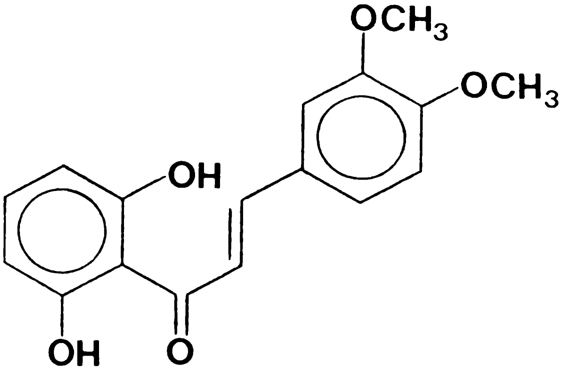


Figure 2-15 Plot of $\ln k_{obs}$ Versus T^{-1} for the Equilibration of *38* and *39* in D_2O

2.6 The 2',6'-Dihydroxy-3,4-dimethoxychalcone – 5-Hydroxy-3',4'-dimethoxyflavanone Equilibrium



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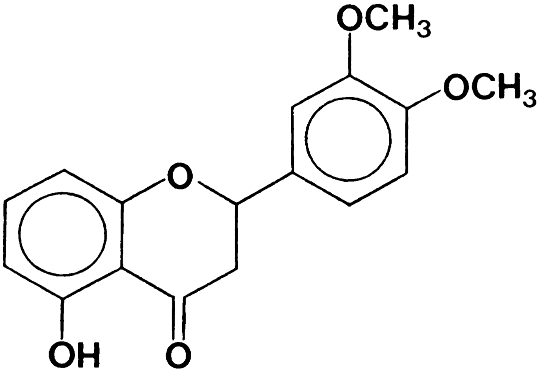

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2.6.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of 2',6'-dihydroxy-3,4-dimethoxychalcone (*40*) or 5-hydroxy-3',4'-dimethoxyflavanone (*41*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0-13.83. Repetitive scans at several pH values (figure 2-16) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. The first-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-16.

TABLE 2-16 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-3,4-dimethoxychalcone (*40*) – 5-Hydroxy-3',4'-dimethoxyflavanone (*41*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	365	$8.2(0.1)\times10^{-6}$
1.94	HCl	365	$3.95(0.01)\times10^{-6}$
3.99	ACETATE	355	$2.06(0.02)\times10^{-5}$

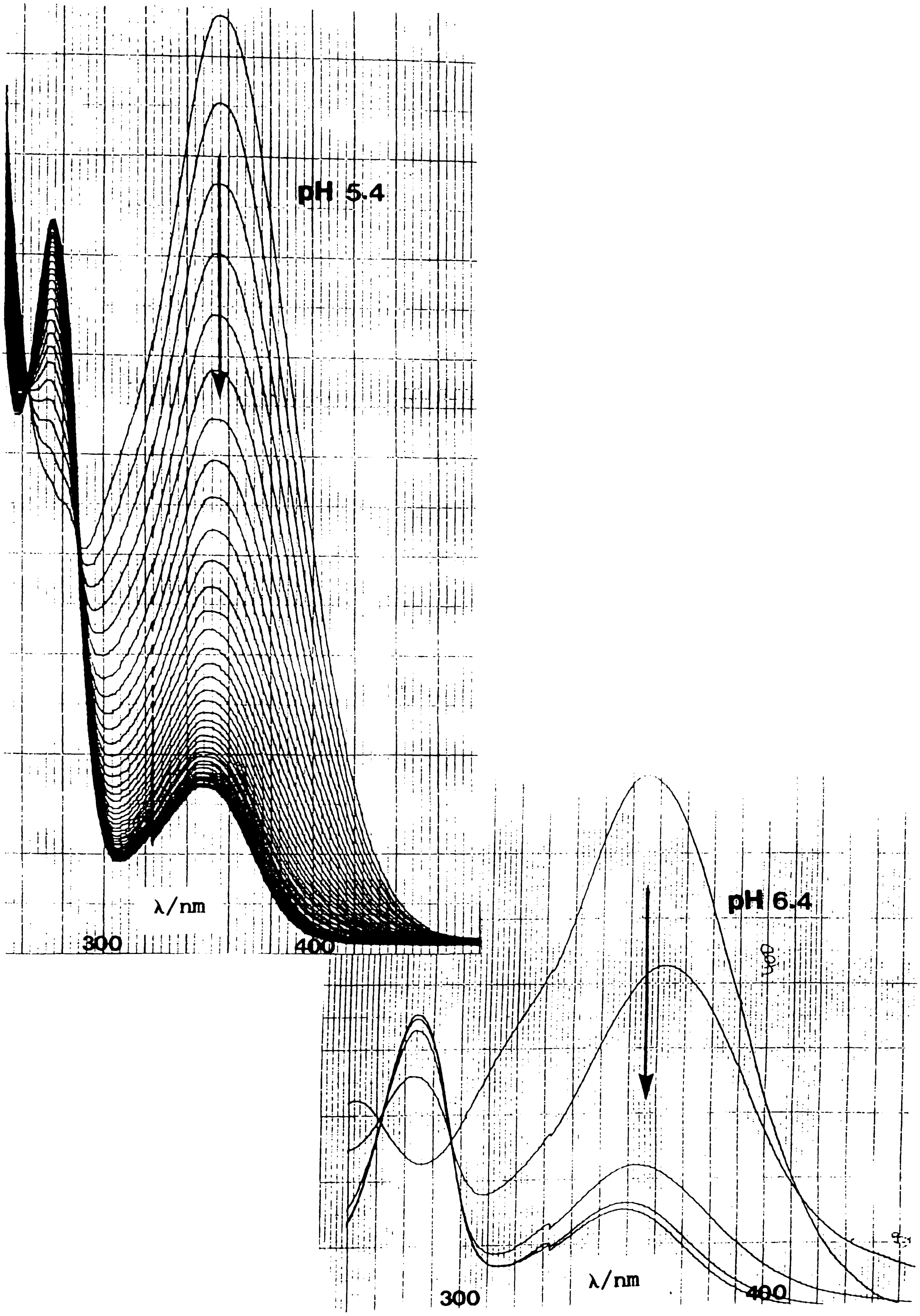


FIGURE 2-16 Repetitive Scans of the Isomerisation of *40* and *41*, at Various pH Values

TABLE 2-16 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
4.69	ACETATE	355	$3.95(0.02)\times10^{-5}$
4.81	PHTHALATE	355	$4.00(0.02)\times10^{-5}$
5.45	ACETATE	355	$1.96(0.005)\times10^{-4}$
5.88	PHOSPHATE	355	$4.03(0.008)\times10^{-4}$
6.49	PHOSPHATE	355	$1.58(0.008)\times10^{-3}$
6.49	PHOSPHATE	355	$1.57(0.01)\times10^{-3}$
6.84	PHOSPHATE	355	$3.01(0.01)\times10^{-3}$
6.84	PHOSPHATE	355	$2.98(0.009)\times10^{-3}$
7.34	PHOSPHATE	355	$7.29(0.02)\times10^{-3}$
7.34	PHOSPHATE	355	$7.41(0.02)\times10^{-3}$
7.76	BICINE	355	$1.11(0.005)\times10^{-2}$
7.77	BICINE	355	$1.12(0.006)\times10^{-2}$
8.66	BICINE	355	$1.82(0.01)\times10^{-2}$
8.65	BICINE	355	$1.80(0.008)\times10^{-2}$
9.34	CARBONATE	355	$1.99(0.01)\times10^{-2}$
9.34	CARBONATE	355	$2.00(0.01)\times10^{-2}$
10.40	CARBONATE	355	$1.87(0.007)\times10^{-2}$
10.39	CARBONATE	355	$1.92(0.02)\times10^{-2}$
11.31	NaOH	355	$1.51(0.005)\times10^{-2}$
11.31	NaOH	355	$1.51(0.008)\times10^{-2}$
11.83	NaOH	355	$9.74(0.07)\times10^{-3}$
11.83	NaOH	355	$9.67(0.05)\times10^{-3}$
12.31	NaOH	355	$4.90(0.02)\times10^{-3}$
12.31	NaOH	355	$4.93(0.02)\times10^{-3}$
12.83	NaOH	333	$2.96(0.009)\times10^{-3}$
12.83	NaOH	333	$2.97(0.007)\times10^{-3}$
13.31	NaOH	333	$2.77(0.02)\times10^{-3}$
13.31	NaOH	333	$2.70(0.02)\times10^{-3}$
13.83	NaOH	333	$5.23(0.03)\times10^{-3}$ [†]
13.83	NaOH	333	$5.31(0.02)\times10^{-3}$ [†]

[†] Determined by ring opening of the isomeric flavanone.

Computer analysis of the results, as outlined previously, gave the chalcone's pK_a values and the individual rate coefficients for the

reaction:

k_{H+} = 4.3×10^{-6} l mol^{-1} s^{-1}

k_{CHH} = 3.9×10^{-6} s^{-1}

k_{CH-} = 2.04×10^{-2} s^{-1}

k_{C2-} = 1.1×10^{-3} s^{-1}

k_{F-} = 4.1×10^{-3} l mol^{-1} s^{-1}

pK_{a1} = 7.6

pK_{a2} = 11.7

In figure 2-17 the experimental points are plotted as crosses, while the line is the theoretical curve (equation 2-19) based on the rate constants and pK_a values given above.

2.6.2 The Effect of D_2O on the Rate of the Chalcone Mono-anion Cyclisation

The rate of approach to equilibrium was also measured in deuterium oxide in the high pH plateau region. The resulting values of k_{obs} are given in table 2-17.

TABLE 2-17 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2′,6′-Dihydroxy-3,4-dimethoxychalcone (*40*) – 5-Hydroxy-3′,4′-dimethoxyflavanone (*41*) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
10.38	CARBONATE	355	$4.20(0.01)\times10^{-3}$
10.96	CARBONATE	355	$3.95(0.02)\times10^{-3}$

The approximate kinetic isotope effect for the cyclisation of the mono-anion, then, is:

$$\frac{k_H}{k_D} = \frac{2.04\times10^{-2}}{4.2\times10^{-3}} = 4.9$$

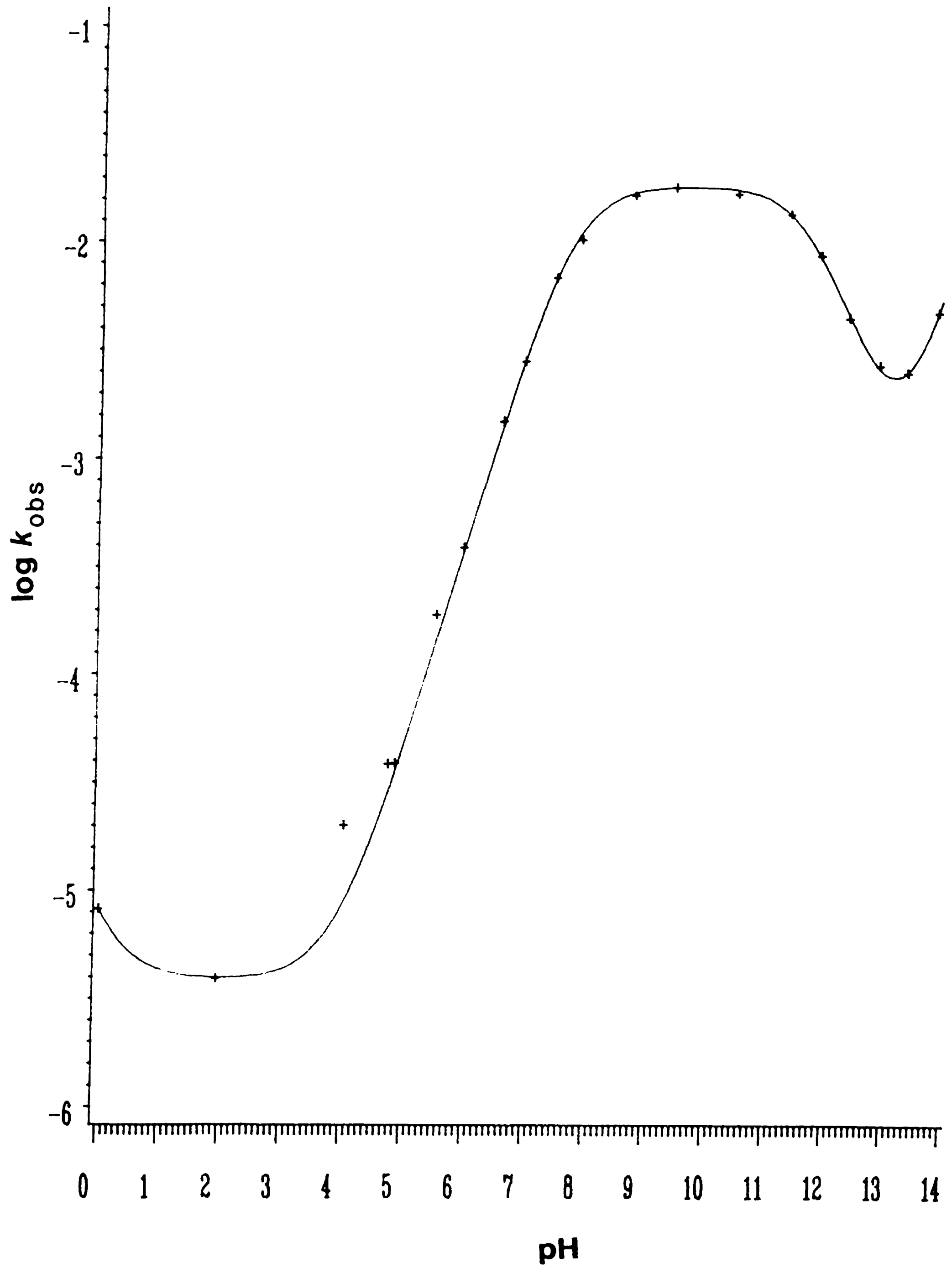
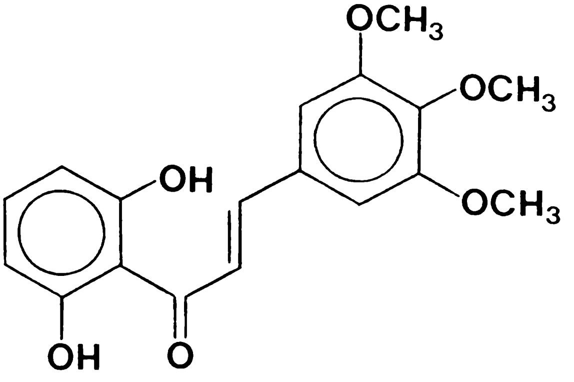


FIGURE 2-17 pH-Rate Profile for the Isomerisation of *40* and *41*: Experimental Values (+) from Table 2-16; Curve (——) Calculated from the Rate Coefficients and pK_a Values Given in the Text (Page 105), and Equation 2-19

2.7 The 2',6'-Dihydroxy-3,4,5-trimethoxychalcone - 5-Hydroxy-3',4',5'-trimethoxyflavanone Equilibrium



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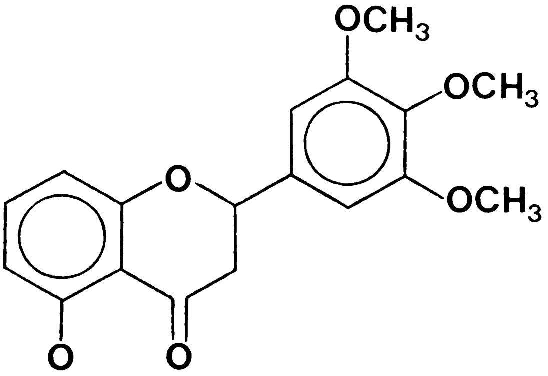

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2.7.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of 2',6'-dihydroxy-3,4,5-trimethoxychalcone (*42*) or 5-hydroxy-3',4',5'-trimethoxyflavanone (*43*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0-13.83. Repetitive scans at several pH values (figure 2-18) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. The first-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-18.

TABLE 2-18 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-3,4,5-trimethoxychalcone (*42*) - 5-Hydroxy-3',4',5'-trimethoxyflavanone (*43*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	337	$2.96(0.02)\times10^{-6}$
2.00	HCl	337	$1.65(0.04)\times10^{-6}$
4.00	ACETATE	337	$8.9(0.1)\times10^{-6}$

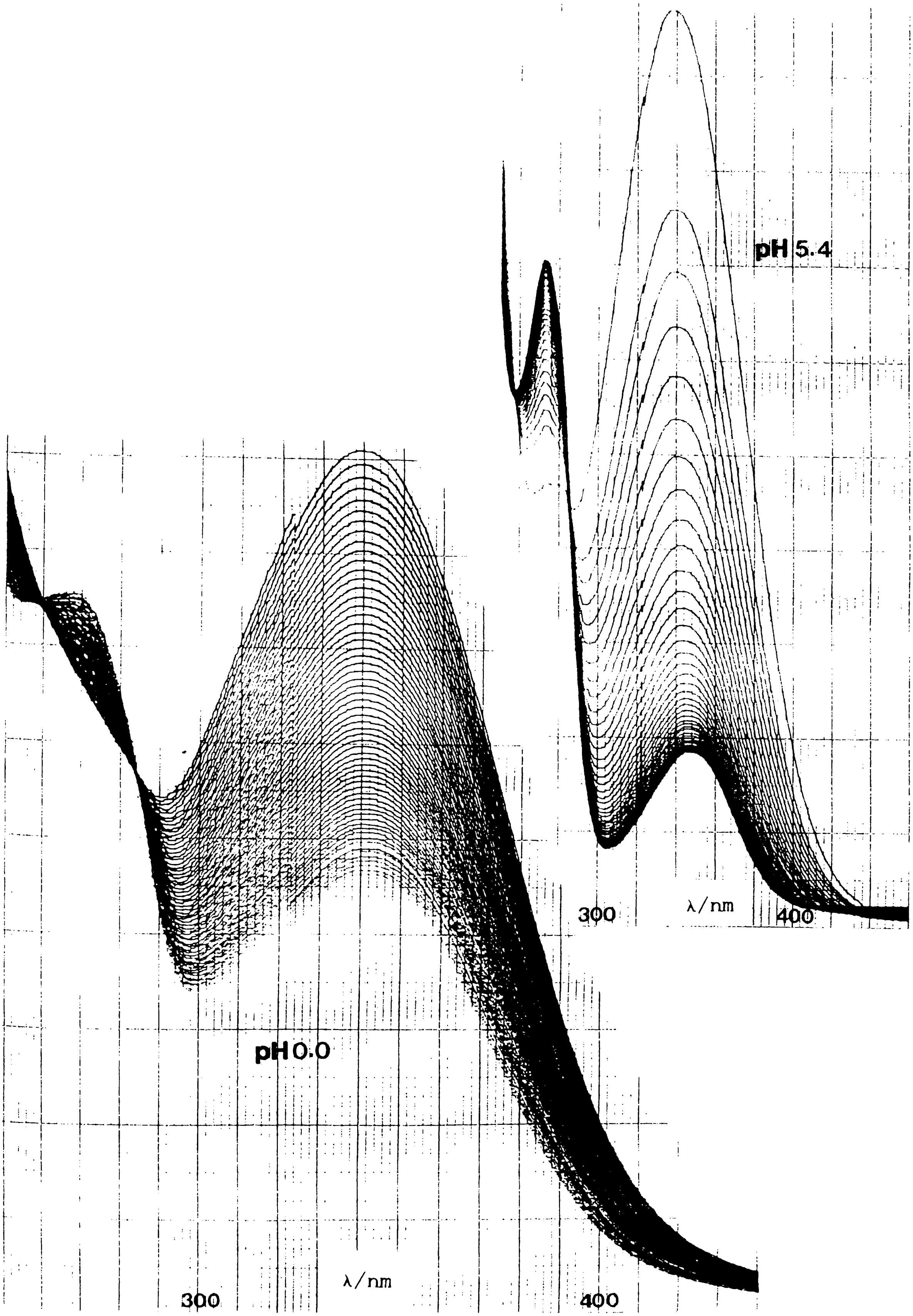


FIGURE 2-18 Repetitive Scans of the Isomerisation of *42* and *43*, at various pH Values

TABLE 2-18 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
4.00	ACETATE	337	$8.5(0.2)\times10^{-6}$
4.69	ACETATE	337	$3.32(0.02)\times10^{-5}$
5.45	ACETATE	337	$1.97(0.007)\times10^{-4}$
5.88	PHOSPHATE	337	$3.83(0.008)\times10^{-4}$
6.48	PHOSPHATE	337	$1.51(0.006)\times10^{-3}$
6.48	PHOSPHATE	337	$1.51(0.008)\times10^{-3}$
6.82	PHOSPHATE	337	$2.97(0.009)\times10^{-3}$
6.82	PHOSPHATE	337	$2.96(0.01)\times10^{-3}$
7.31	PHOSPHATE	337	$6.78(0.04)\times10^{-3}$
7.31	PHOSPHATE	337	$7.65(0.03)\times10^{-3}$
7.76	BICINE	337	$1.25(0.004)\times10^{-2}$
7.76	BICINE	337	$1.24(0.006)\times10^{-2}$
8.66	BICINE	337	$1.92(0.008)\times10^{-2}$
8.67	BICINE	337	$1.90(0.009)\times10^{-2}$
9.33	CARBONATE	337	$2.10(0.006)\times10^{-2}$
9.34	CARBONATE	337	2.09×10^{-2}
10.38	CARBONATE	337	$1.95(0.003)\times10^{-2}$
10.38	CARBONATE	337	$1.92(0.004)\times10^{-2}$
11.31	NaOH	337	$1.55(0.004)\times10^{-2}$
11.31	NaOH	337	$1.54(0.005)\times10^{-2}$
11.83	NaOH	337	$9.72(0.05)\times10^{-3}$
11.83	NaOH	337	$9.72(0.03)\times10^{-3}$
12.31	NaOH	337	$5.06(0.02)\times10^{-3}$
12.31	NaOH	337	$5.09(0.01)\times10^{-3}$
12.83	NaOH	314	$2.81(0.02)\times10^{-3}$
12.83	NaOH	314	$2.80(0.01)\times10^{-3}$
13.31	NaOH	314	$2.29(0.02)\times10^{-3}$
13.31	NaOH	314	$2.25(0.008)\times10^{-3}$
13.83	NaOH	314	$3.82(0.02)\times10^{-3}$ †
13.83	NaOH	314	$3.82(0.02)\times10^{-3}$ †

† Determined by ring opening of the isomeric flavanone.

Computer analysis of the results, as outlined previously, gave the chalcone's pK_a values and the individual rate coefficients for the

reaction:

$k_{H+} = 1.4\times10^{-6}\ l\ mol^{-1}\ s^{-1}$

$k_{CHH} = 1.6\times10^{-6}\ s^{-1}$

$k_{CH-} = 2.10\times10^{-2}\ s^{-1}$

$k_{C2-} = 1.2\times10^{-3}\ s^{-1}$

$k_{F-} = 2.5\times10^{-3}\ l\ mol^{-1}\ s^{-1}$

$pK_{a1} = 7.6$

$pK_{a2} = 11.7$

In figure 2-19 the experimental points are plotted as crosses, while the line is the theoretical curve based on the rate constants and pK_a values given above.

2.7.2 The Effect of D_2O on the Rate of the Chalcone Mono-anion Cyclisation

The rate of approach to equilibrium was also measured in deuterium oxide in the high pH plateau region. The resulting values of k_{obs} are given in table 2-19.

TABLE 2-19 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-3,4,5-trimethoxychalcone (**42**) – 5-Hydroxy-3',4',5'-trimethoxyflavanone (**43**) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
10.36	CARBONATE	337	$7.04(0.02)\times10^{-3}$
10.93	CARBONATE	337	$6.52(0.03)\times10^{-3}$

The approximate kinetic isotope effect for the cyclisation of the mono-anion, then, is:

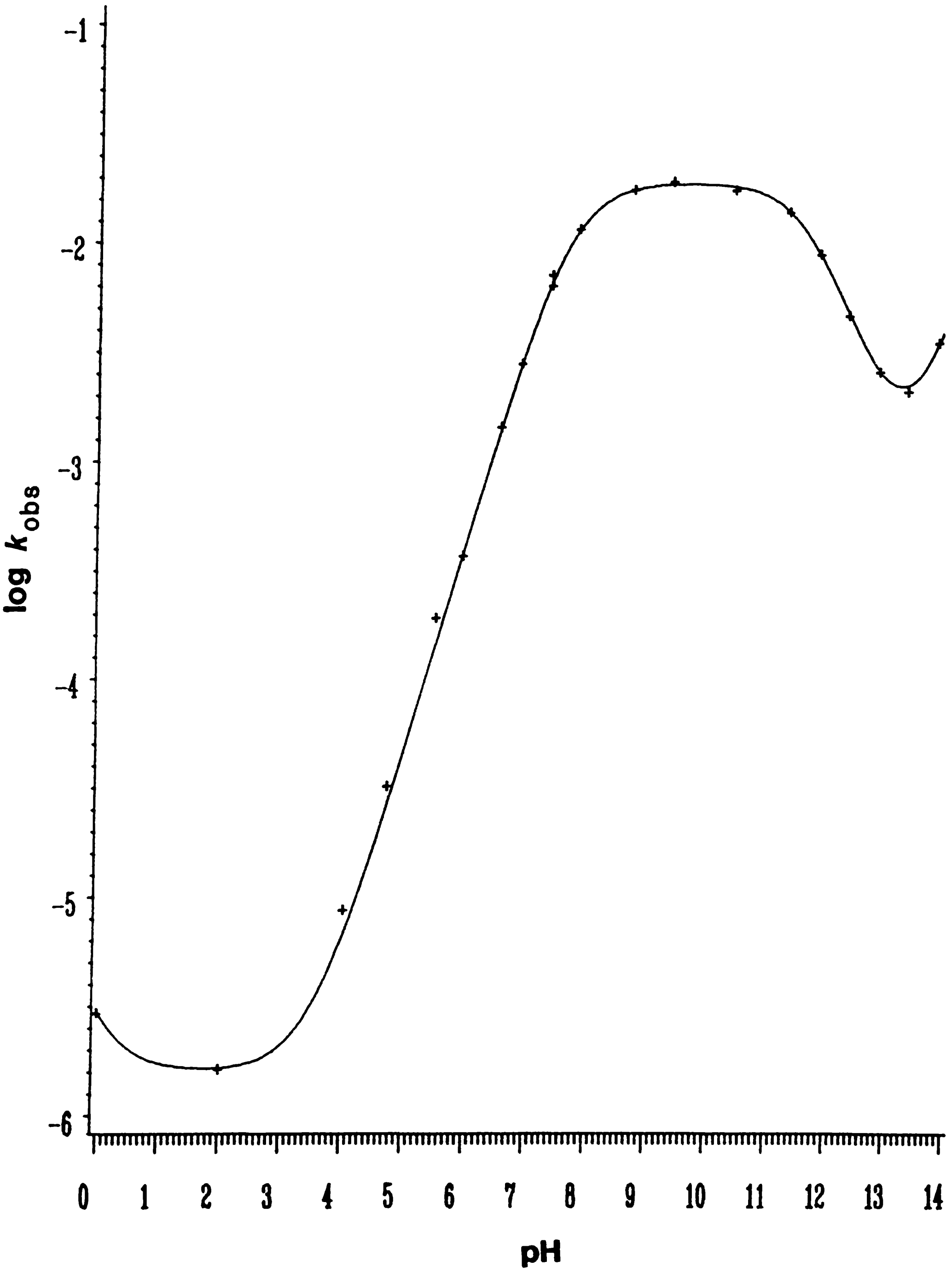


FIGURE 2-19 pH-Rate Profile for the Equilibration of *42* and *43*: Experimental Values (+) from Table 2-18; Curve (——) Calculated from the Rate Coefficients and pK_a Values Given in the Text (Page 111), and Equation 2-19

$$\frac{k_H}{k_D} = \frac{2.10\times10^{-2}}{7.0\times10^{-3}} = 3.0$$

2.7.3 The Effect of Temperature on the rate of the Chalcone Mono-Anion Cyclisation

The effect of temperature on the observed rate constant in the high pH plateau region was also investigated. The results are given in table 2-20.

TABLE 2-20 The Effect of Temperature (in 3:7 $[HCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-3,4,5-trimethoxychalcone (*42*) – 5-Hydroxy-3',4',5'-trimethoxyflavanone (*43*) Equilibrium in 4% Ethanolic H_2O, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3k_{obs}(\sigma)$ /s^{-1}
12.8	3.497	5.01(0.06)
12.8	3.497	4.94(0.04)†
17.65	3.439	7.56(0.06)
17.65	3.439	7.06(0.04)
24.3	3.362	12.3(0.05)
24.3	3.362	13.1(0.05)†
30.0	3.299	19.5(0.03)
30.0	3.299	19.2(0.04)
36.85	3.226	33.6(0.2)
36.85	3.226	33.4(0.1)

† $[HCO_3^-]:[CO_3^{2-}]$ = 4:6

A plot of $\ln k_{obs}$ versus T^{-1} (figure 2-20) gave a straight line, which by least-squares regression analysis had a slope of -7020 (σ = 80) K and an intercept of 19.2 (σ = 0.3). Thus, from equation 2-11 we can calculate the Arhennius activation parameters E_a = 58.4 (σ = 0.7) kJ mol^{-1} and A = $e^{19.2}$ = 2.2 (σ = 0.7)$\times10^8$. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^{\ddagger} = 55.9$ ($\sigma = 0.7$) kJ mol^{-1}; $\Delta S^{\ddagger} = -93.5$ ($\sigma = 2$) J mol^{-1} K^{-1}; $\Delta G^{\ddagger} = 84$ ($\sigma = 1.4$) kJ mol^{-1}.

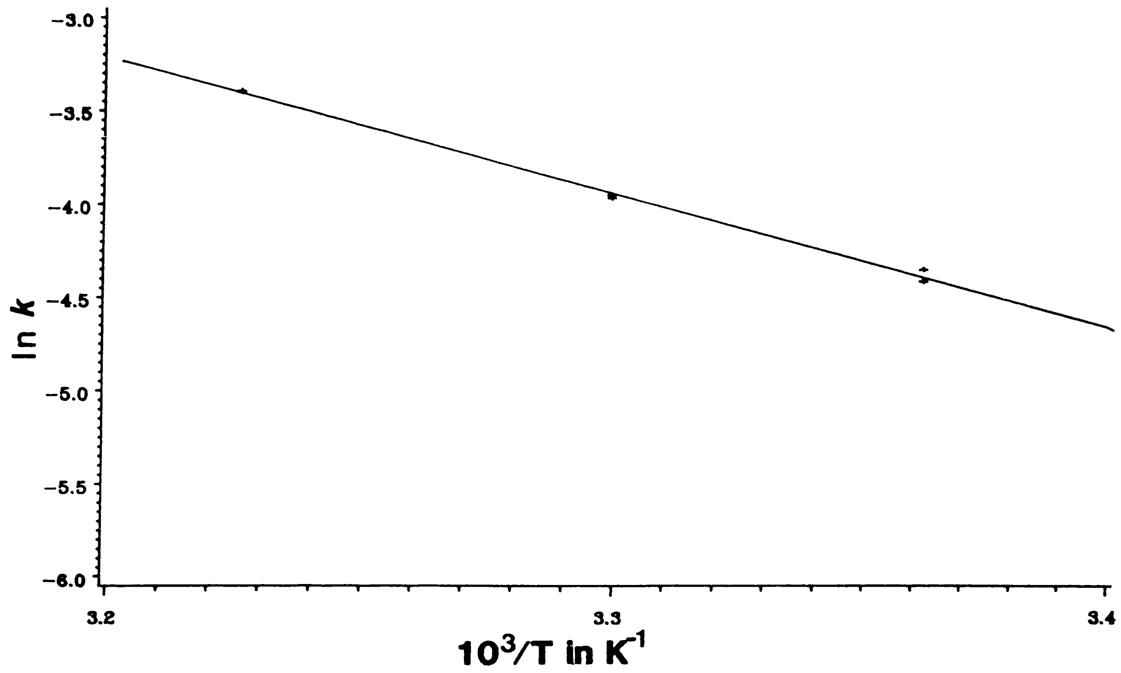
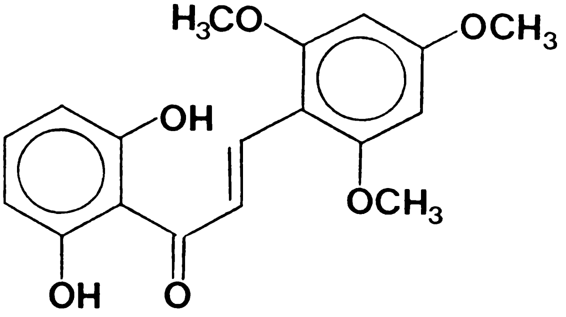


FIGURE 2-20 Plot of lnk_{obs} Versus T^{-1} for the Equilibration of *42* and *43*

2.8 The 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone – 5-Hydroxy-2′,4′,6′-trimethoxyflavanone Equilibrium



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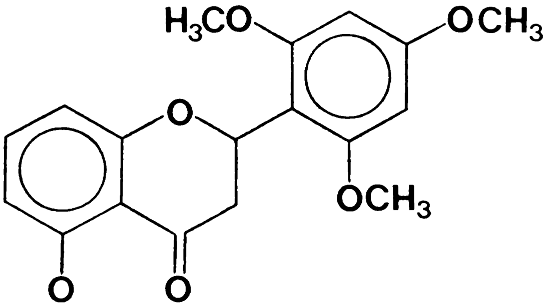

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2.8.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of 2′,6′-dihydroxy-2,4,6-trimethoxychalcone (*44*) or 5-hydroxy-2′,4′,6′-trimethoxyflavanone (*45*) to its equilibrium mixture was measured at 30°C in 4% aqueous ethanol solution, over the pH range 0.0-13.83. Repetitive scans at several pH values (figure 2-21) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. The first-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-21.

TABLE 2-21 The Effect of pH on the First-Order Rate Constant for the Attainment of the 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone (*44*) – 5-Hydroxy-2′,4′,6′-trimethoxyflavanone (*45*) Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	376	$1.55(0.009)\times10^{-3}$
0.00	HCl	376	$1.58(0.005)\times10^{-3}$
1.00	HCl	376	$1.46(0.008)\times10^{-4}$

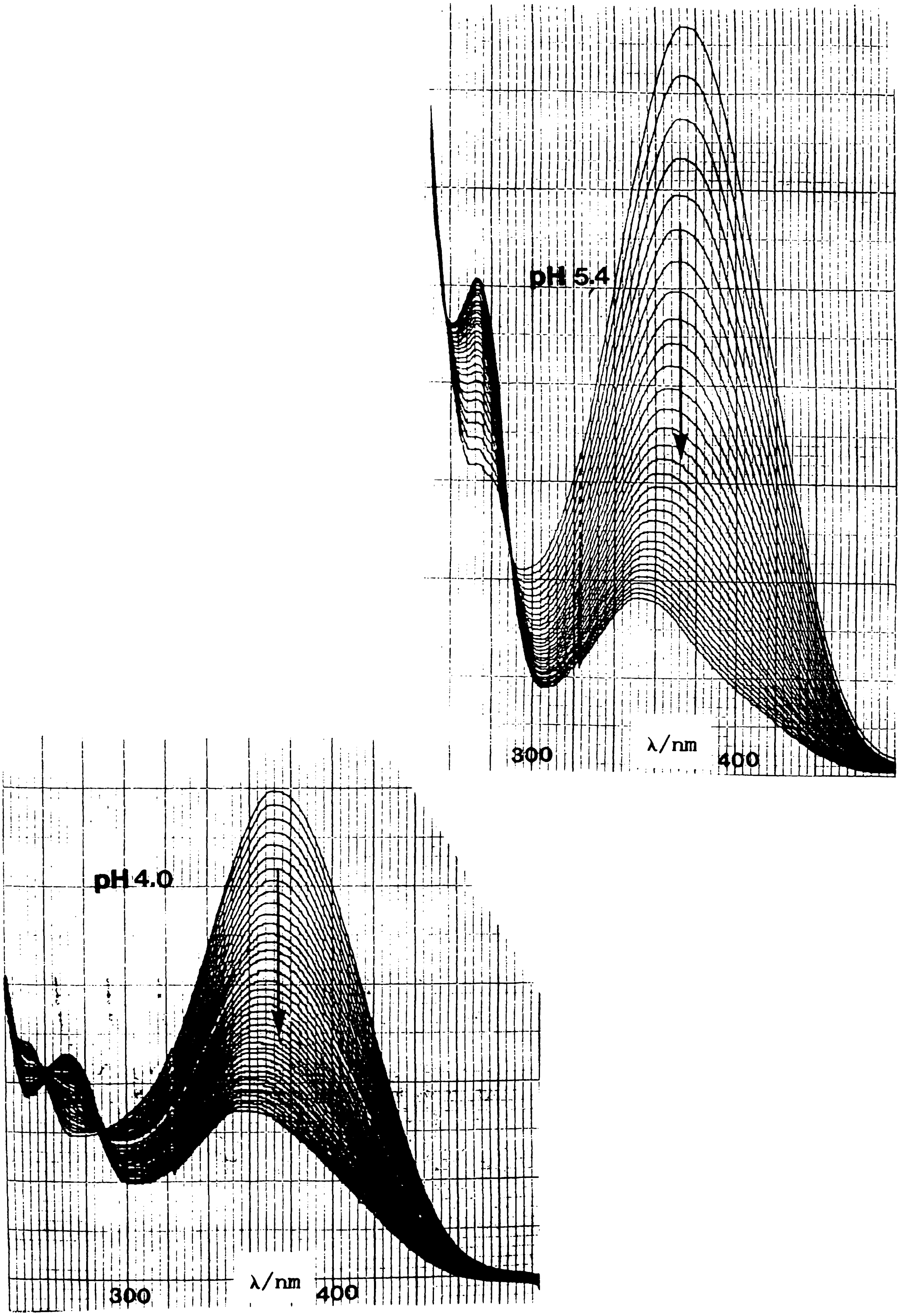


FIGURE 2-21 Repetitive Scans of the Isomerisation of *44* and *45*, at Various pH Values

TABLE 2-21 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
2.00	HCl	376	$1.66(0.009)\times10^{-5}$
3.07	PHTHALATE	376	$6.50(0.08)\times10^{-6}$
3.98	ACETATE	376	$5.53(0.05)\times10^{-6}$
4.69	ACETATE	376	$8.60(0.03)\times10^{-6}$
5.45	ACETATE	376	$1.89(0.01)\times10^{-5}$
5.88	PHOSPHATE	376	$3.54(0.03)\times10^{-5}$
6.48	PHOSPHATE	376	$1.05(0.006)\times10^{-4}$
6.48	PHOSPHATE	376	$9.83(0.006)\times10^{-5}$
6.79	PHOSPHATE	376	$1.72(0.01)\times10^{-4}$
6.79	PHOSPHATE	376	$1.62(0.01)\times10^{-4}$
7.34	PHOSPHATE	376	$3.95(0.03)\times10^{-4}$
7.34	PHOSPHATE	376	$3.98(0.08)\times10^{-4}$
7.77	BICINE	376	$4.80(0.05)\times10^{-4}$
7.77	BICINE	376	$4.90(0.08)\times10^{-4}$
8.65	BICINE	376	$7.70(0.1)\times10^{-4}$
8.65	BICINE	376	$7.90(0.1)\times10^{-4}$
9.35	CARBONATE	376	$9.33(0.03)\times10^{-4}$
9.35	CARBONATE	376	$9.10(0.06)\times10^{-4}$
10.38	CARBONATE	376	$1.01(0.007)\times10^{-3}$
10.38	CARBONATE	376	$1.01(0.004)\times10^{-3}$
11.31	NaOH	376	$9.10(0.03)\times10^{-4}$
11.31	NaOH	376	$9.10(0.04)\times10^{-4}$
11.31	NaOH	376	$9.12(0.04)\times10^{-4}$
11.83	NaOH	376	$8.08(0.07)\times10^{-4}$
11.83	NaOH	376	$8.16(0.07)\times10^{-4}$
12.31	NaOH	376	$6.31(0.02)\times10^{-4}$
12.31	NaOH	376	$6.24(0.04)\times10^{-4}$
12.83	NaOH	339	$5.81(0.02)\times10^{-4}$
12.83	NaOH	339	$5.84(0.02)\times10^{-4}$
13.31	NaOH	339	$8.28(0.07)\times10^{-4}$
13.31	NaOH	339	$8.56(0.08)\times10^{-4}$
13.83	NaOH	339	$1.84(0.009)\times10^{-3}$ †
13.83	NaOH	339	$1.83(0.008)\times10^{-3}$ †

† Determined by ring opening of the isomeric flavanone.

Computer analysis of the results, as outlined previously, gave the chalcone's pK_a values and the individual rate coefficients for the reaction:

k_{H+} = 1.50×10^{-3} l mol^{-1} s^{-1}

k_{CHH} = 6.5×10^{-6} s^{-1}

k_{CH-} = 9.8×10^{-4} s^{-1}

k_{C2-} = 3.5×10^{-4} s^{-1}

k_{F-} = 1.5×10^{-3} l mol^{-1} s^{-1}

pK_{a1} = 7.4

pK_{a2} = 12.1

In figure 2-22 the experimental points are plotted as crosses, while the line is the theoretical curve (equation 2-19) based on the rate constants and pK_a values given above.

2.8.2 The Effect of D_2O on the Rate of the Chalcone Mono-anion Cyclisation

The rate of approach to equilibrium was also measured in deuterium oxide in the high pH plateau region. The resulting values of k_{obs} are

TABLE 2-22 The Effect of pD on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-2,4,6-trimethoxychalcone (*44*) – 5-Hydroxy-2',4',6'-trimethoxyflavanone (*45*) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
10.37	CARBONATE	376	$1.35(0.01)\times10^{-4}$
10.97	CARBONATE	376	$1.40(0.04)\times10^{-4}$
10.98	CARBONATE	376	$1.32(0.01)\times10^{-4}$
10.93	CARBONATE	376	$1.34(0.01)\times10^{-4}$

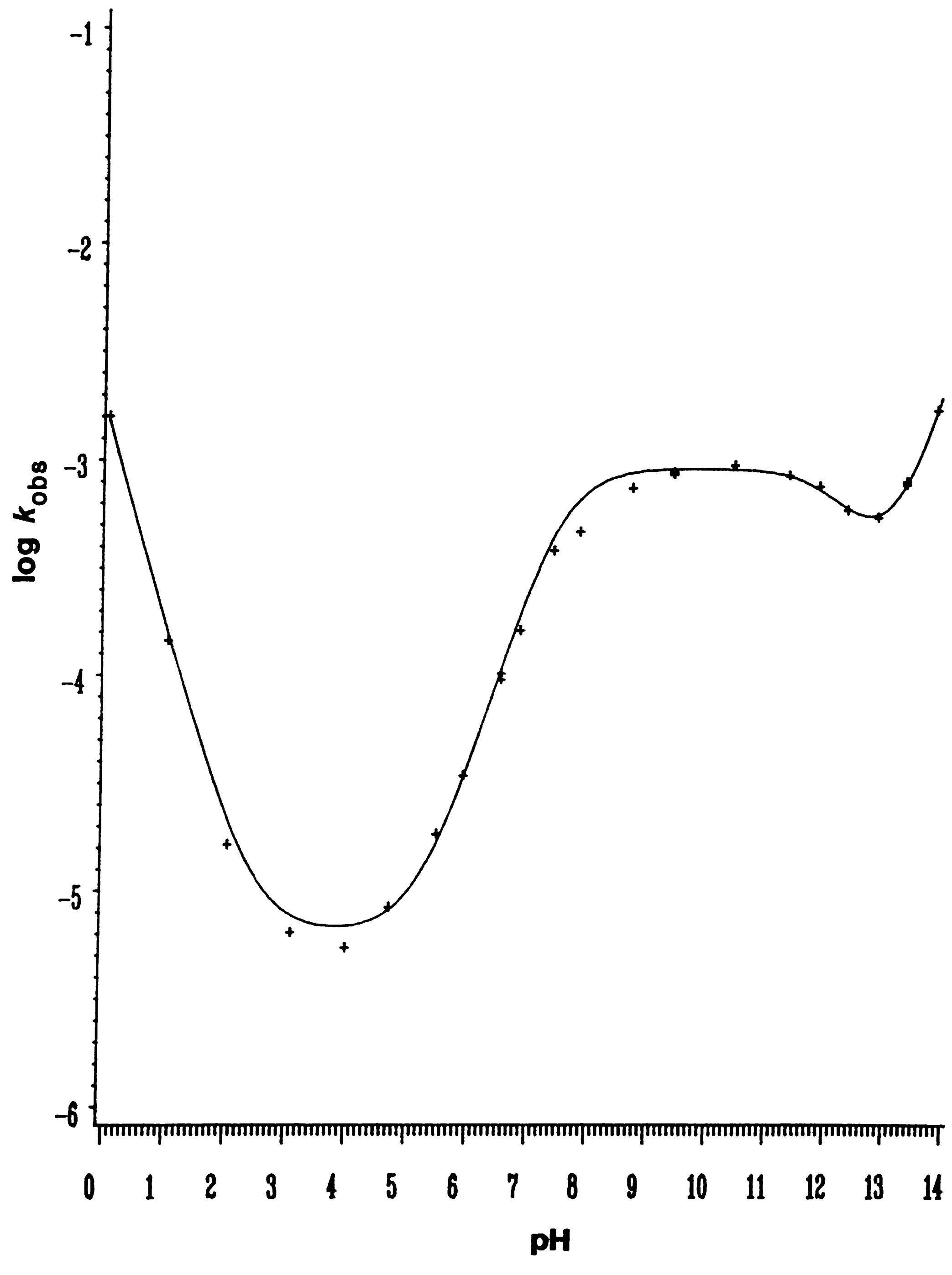


FIGURE 2-22 pH-Rate Profile for the Isomerisation of *44* and *45*: Experimental Values (+) from Table 2-21; Curve (——) Calculated from the Rate Coefficients and pK_a Values Given in the Text (Page 118), and Equation 2-19

given in table 2-22.

The kinetic isotope effect for the cyclisation of the mono-anion, then, is:

$$\frac{k_H}{k_D} = \frac{9.8\times10^{-4}}{1.3\times10^{-4}} = 7.5$$

2.8.3 The Effect of Buffer Concentration on the Rate of the Chalcone Mono-anion Cyclisation

The effect of the buffer concentration on the rate of approach to equilibrium was investigated in the high pH plateau region. For two different carbonate buffers (*ca.* pH 9.4 and 10.1), with μ kept constant at 1.0 mol l^{-1} (KCl), the rate constants for the approach to equilibrium were measured at 0.02, 0.05, 0.08 and 0.10 mol l^{-1} total carbonate concentration. The results are shown in table 2-23.

TABLE 2-23 The Effect of Buffer Concentration on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-2,4,6-trimethoxychalcone – 5-Hydroxy-2',4',6'-trimethoxyflavanone Equilibrium in 4% Ethanolic H_2O at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

[BUFFER] /mol l^{-1}	$10^3 k_{obs}(\sigma)/s^{-1}$	
	$[HCO_3^-]:[CO_3^{2-}]$ 2:8	7:3
0.02	1.03(0.008)	1.03(0.01)
0.05	1.15(0.009)	1.30(0.01)
0.08	1.22(0.007)	1.52(0.02)
0.10	1.29(0.007)	1.61(0.03)

The rate shows a weak dependence on the buffer concentration, as shown in figure 2-23.

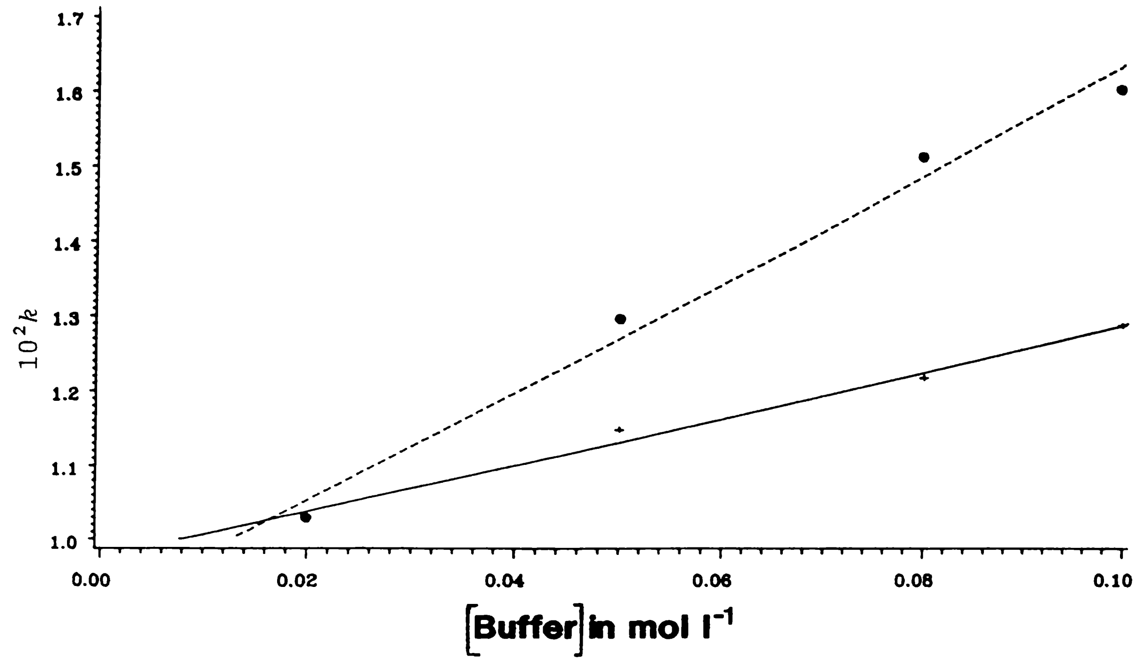


FIGURE 2-23 The Effect of Buffer Concentration on k_{obs} for the Equilibration of *44* and *45*

The slope for the 7:3 $[HCO_3^-]:[CO_3^{2-}]$ buffer appears to be higher than that for the 2:8 $[HCO_3^-]:[CO_3^{2-}]$ buffer. This suggests that HCO_3^- might be the major catalytic species.

2.8.*4* The Effect of Temperature on the rate of the Chalcone Mono-Anion Cyclisation

The effect of temperature on the observed rate constant in the high pH plateau region was also investigated. The results are given in table 2-24.

A plot of $\ln k_{obs}$ versus T^{-1} (figure 2-24) gave a straight line, which by least-squares regression analysis had a slope of -8760 (σ = 60) K and an intercept of 22.0 (σ = 0.2). Thus, from equation 2-11 we can calculate the Arhennius activation parameters E_a = 72.9

TABLE 2-24 The Effect of Temperature (in 1:9 $[HCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 2',6'-Dihydroxy-2,4,6-trimethoxychalcone (*44*) – 5-Hydroxy-2',4',6'-trimethoxyflavanone (*45*) Equilibrium in 4% Ethanolic H_2O, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^4 k_{obs}(\sigma)$ /s^{-1}
17.9	3.436	3.10(0.02)
17.9	3.436	2.89(0.04)
23.8	3.368	5.47(0.05)
23.8	3.368	5.53(0.04)
30.0	3.299	10.1(0.07)
30.0	3.299	10.1(0.04)
38.78	3.206	22.6(0.2)
38.78	3.206	22.4(0.1)
39.8	3.195	25.6(0.1)
39.8	3.195	24.6(0.1)[†]
48.90	3.105	53.9(0.2)

[†] $[HCO_3^-]:[CO_3^{2-}]$ = 4:6

(σ = 0.5) kJ mol^{-1} and A = $e^{22.0}$ = 36 (σ = 8)x10^8. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^\ddagger$ = 70.4 (σ = 0.5) kJ mol^{-1}; $\Delta S^\ddagger$ = -70.4 (σ = 1.7) J mol^{-1} K^{-1}; $\Delta G^\ddagger$ = 91.7 (σ = 1) kJ mol^{-1}.

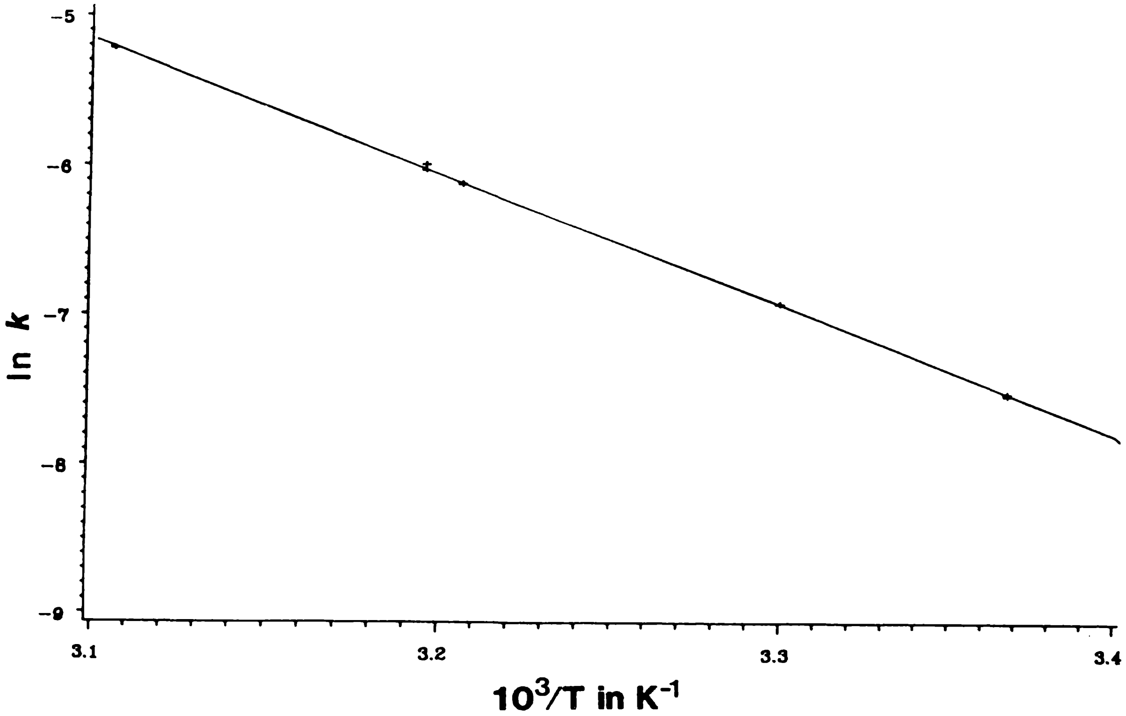
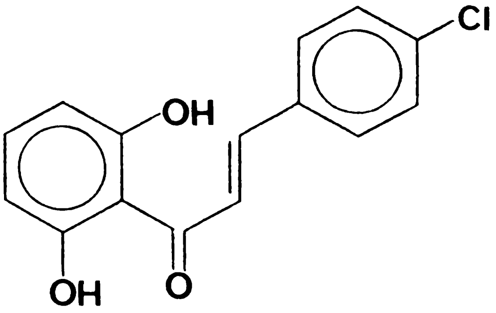


FIGURE 2-24 Plot of $\ln k_{obs}$ Versus T^{-1} for the Equilibration of *44* and *45*

2.9 The 4-Chloro-2',6'-dihydroxychalcone – 4'-Chloro-5-hydroxyflavanone Equilibrium



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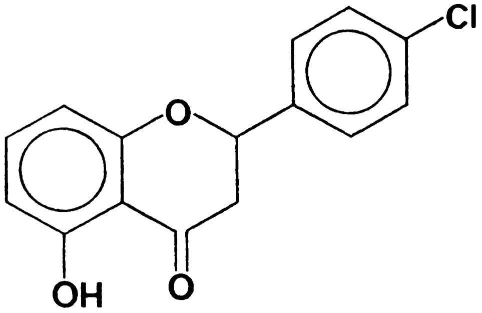

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2.9.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of 4-chloro-2',6'-dihydroxychalcone (*46*) or 4'-chloro-5-hydroxyflavanone (*47*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 2.99-13.83. Repetitive scans at several pH values (figure 2-25) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. However at pH 0-2.99, repetitive scans showed that the reaction was not proceeding cleanly to equilibrium. The problem appeared to be due to the low solubility of the flavanone in the reaction solution, and could not practically be overcome by use of 4.0 cm silica cells or by lowering the chalcone concentration being used. Therefore, no rate data were obtained at pH 0.0-2.99 for this chalcone. First-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-25.

Computer analysis of the results, as outlined previously, gave the chalcone's pK_a values and the individual rate constants for the

TABLE 2-25 The Effect of pH on the First-Order Rate Constant for the Attainment of the 4-Chloro-2',6'-dihydroxychalcone (*46*) - 4'-Chloro-5-hydroxyflavanone (*47*) Equilibrium Aqueous in 4% Ethanol at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
2.99	HCl	321	$9.21(0.2)\times10^{-6}$
3.99	ACETATE	326	$1.76(0.05)\times10^{-5}$
4.72	ACETATE	326	$3.74(0.04)\times10^{-5}$
5.46	ACETATE	321	$1.62(0.01)\times10^{-4}$
5.88	PHOSPHATE	321	$3.63(0.01)\times10^{-4}$
6.48	PHOSPHATE	321	$1.73(0.03)\times10^{-3}$
6.48	PHOSPHATE	321	$1.72(0.01)\times10^{-3}$
6.78	PHOSPHATE	321	$2.59(0.02)\times10^{-3}$
6.82	PHOSPHATE	321	$2.83(0.05)\times10^{-3}$
6.82	PHOSPHATE	321	$2.81(0.04)\times10^{-3}$
7.22	PHOSPHATE	321	$5.94(0.03)\times10^{-3}$
7.23	PHOSPHATE	321	$5.94(0.04)\times10^{-3}$
7.34	PHOSPHATE	321	$6.87(0.03)\times10^{-3}$
7.34	PHOSPHATE	321	$6.93(0.04)\times10^{-3}$
7.76	BICINE	321	$1.11(0.009)\times10^{-2}$
7.77	BICINE	321	$1.09(0.01)\times10^{-2}$
8.64	BICINE	321	$1.96(0.005)\times10^{-2}$
8.65	BICINE	321	$1.97(0.01)\times10^{-2}$
9.33	CARBONATE	321	$2.08(0.02)\times10^{-2}$
9.33	CARBONATE	321	$2.08(0.01)\times10^{-2}$
10.40	CARBONATE	321	$1.99(0.02)\times10^{-2}$
10.40	CARBONATE	321	$1.94(0.01)\times10^{-2}$
10.40	CARBONATE	321	$1.88(0.02)\times10^{-2}$
10.40	CARBONATE	325	$1.96(0.006)\times10^{-2}$
10.42	CARBONATE	321	$1.81(0.01)\times10^{-2}$
10.42	CARBONATE	321	$1.85(0.02)\times10^{-2}$
11.31	NaOH	321	$1.57(0.006)\times10^{-2}$
11.31	NaOH	321	$1.54(0.009)\times10^{-2}$
11.31	NaOH	321	$1.42(0.004)\times10^{-2}$
11.83	NaOH	321	$1.05(0.005)\times10^{-2}$
11.83	NaOH	321	$1.00(0.005)\times10^{-2}$

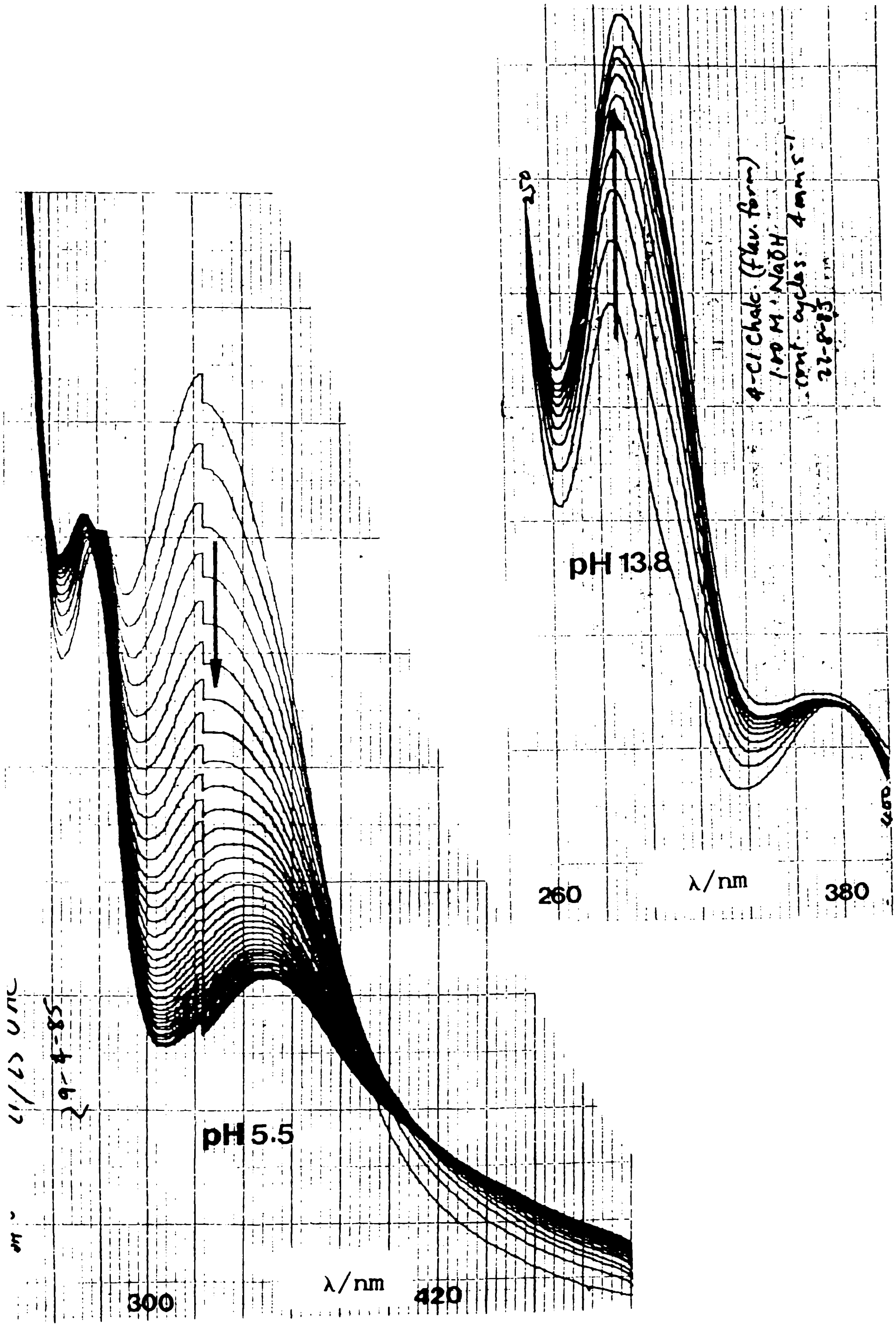


FIGURE 2-25 Repetitive Scans of the Isomerisation of *46* and *47*, at Various pH Values

TABLE 2-25 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
12.31	NaOH	321	$6.29(0.03)\times10^{-3}$
12.31	NaOH	321	$6.27(0.03)\times10^{-3}$
12.83	NaOH	294	$3.56(0.02)\times10^{-3}$
12.83	NaOH	294	$3.51(0.02)\times10^{-3}$
13.31	NaOH	294	$3.05(0.03)\times10^{-3}$
13.31	NaOH	294	$3.15(0.02)\times10^{-3}$
13.83	NaOH	293	$4.42(0.06)\times10^{-3}$ †
13.83	NaOH	293	$4.62(0.06)\times10^{-3}$ †

†Determined by ring opening of the isomeric flavanone.

reaction given below. Unfortunately, due to to the problems encountered at low pH, k_{H^+} was undefined, and the value of k_{CHH} was subject to considerable uncertainty.

$k_{CHH} = 9\times10^{-6}\ s^{-1}\ (\pm\ 100\%)$

$k_{CH^-} = 2.10\times10^{-2}\ s^{-1}$

$k_{C^{2-}} = 2.0\times10^{-3}\ s^{-1}$

$k_{F^-} = 2.4\times10^{-3}\ l\ mol^{-1}\ s^{-1}$

$pK_{a1} = 7.6$

$pK_{a2} = 11.7$

In figure 2-26 the experimental points are plotted as crosses, while the line is the theoretical curve (equation 2-19) based on the rate constants and pK_a values given above.

2.9.2 The Effect of D_2O on the Rate of the Chalcone Mono-anion Cyclisation

The rate of approach to equilibrium was also measured in deuterium oxide in the high pH plateau region. The resulting values of k_{obs} are given in table 2-26.

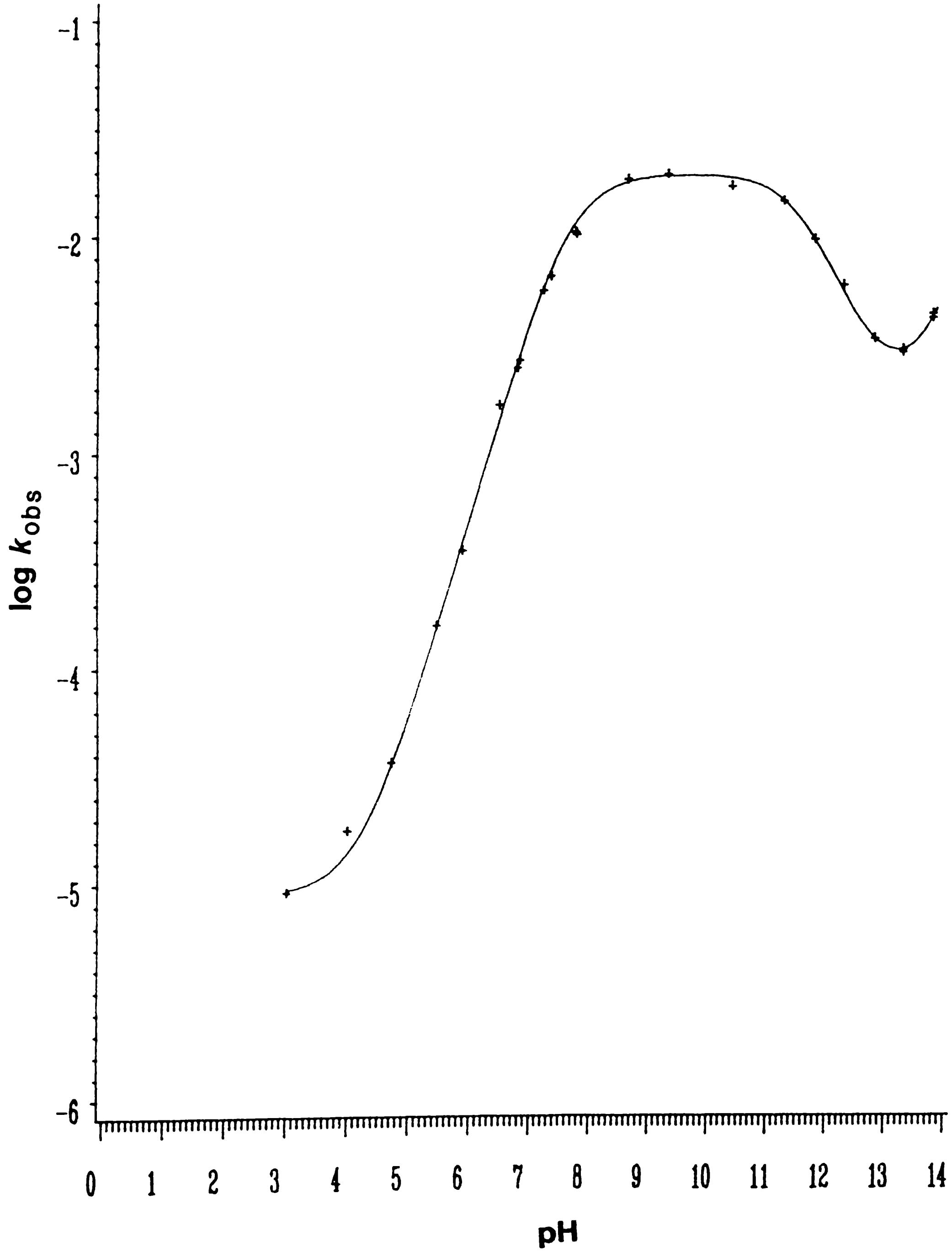


FIGURE 2-26 pH-Rate Profile for the Isomerisation of *46* and *47*: Experimental Values (+) from Table 2-25; Curve (——) Calculated from the Rate Coefficients and pK_a Values Given in the Text (Page 127), and Equation 2-19

TABLE 2-26 The Effect of pD on the First-Order Rate Constant for the Attainment of the 4-Chloro-2',6'-dihydroxychalcone (*46*) – 4'-Chloro-5-hydroxy-4'-chloroflavanone (*47*) Equilibrium in 4% Ethanolic Deuterium Oxide at 30°C, $\mu = 1.0$ mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pD	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
10.37	CARBONATE	321	$7.13(0.07)\times10^{-3}$
10.96	CARBONATE	321	$6.32(0.05)\times10^{-3}$

The approximate kinetic isotope effect for the cyclisation of the mono-anion, then, is:

$$\frac{k_H}{k_D} = \frac{2.10\times10^{-2}}{7.2\times10^{-3}} = 2.9$$

2.9.3 The Effect of Temperature on the rate of the Chalcone Mono-Anion Cyclisation

The effect of temperature on the observed rate constant in the high pH plateau region was also investigated. The results are given in table 2-27. A plot of $\ln k_{obs}$ versus T^{-1} (figure 2-27) gave a straight line, which by least-squares regression analysis had a slope of -7140 (σ = 140) K and an intercept of 19.6 (σ = 0.5). Thus, from equation 2-11 we can calculate the Arhennius activation parameters E_a = 59.4 (σ = 1) kJ mol^{-1} and $A = e^{19.6}$ = 3.2 (σ = 2)$\times10^{8}$. From these were determined the enthalpy, entropy and Gibbs free energy of activation for the reaction using equations 2-13, 2-15 and 2-17: $\Delta H^{\ddagger}$ = 56.9 (σ = 1) kJ mol^{-1}; $\Delta S^{\ddagger}$ = -91 (σ = 4) J mol^{-1} K^{-1}; $\Delta G^{\ddagger}$ = 84.3 (σ = 2.3) kJ mol^{-1}.

TABLE 2-27 The Effect of Temperature (in 7:3 $[HCO_3^-]:[CO_3^{2-}]$ buffer) on the First-Order Rate Constant for the Attainment of the 4-Chloro-2',6'-dihydroxychalcone (*46*) – 4'-Chloro-5-hydroxyflavanone (*47*) Equilibrium in 4% Ethanolic H_2O, $\mu = 1.0$ mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

TEMPERATURE/°C	$10^3/T$ IN K^{-1}	$10^3k_{obs}(\sigma)$ /s^{-1}
17.9	3.436	6.88(0.04)
17.9	3.436	7.08(0.05)
18.7	3.426	7.86(0.04)
23.8	3.368	11.3(0.07)
23.8	3.368	11.2(0.09)
24.3	3.362	12.0(0.03)
24.3	3.362	11.8(0.08)
30.0	3.299	18.1(0.1)
30.0	3.299	18.5(0.2)
36.85	3.226	30.7(0.2)
36.85	3.226	31.7(0.1)

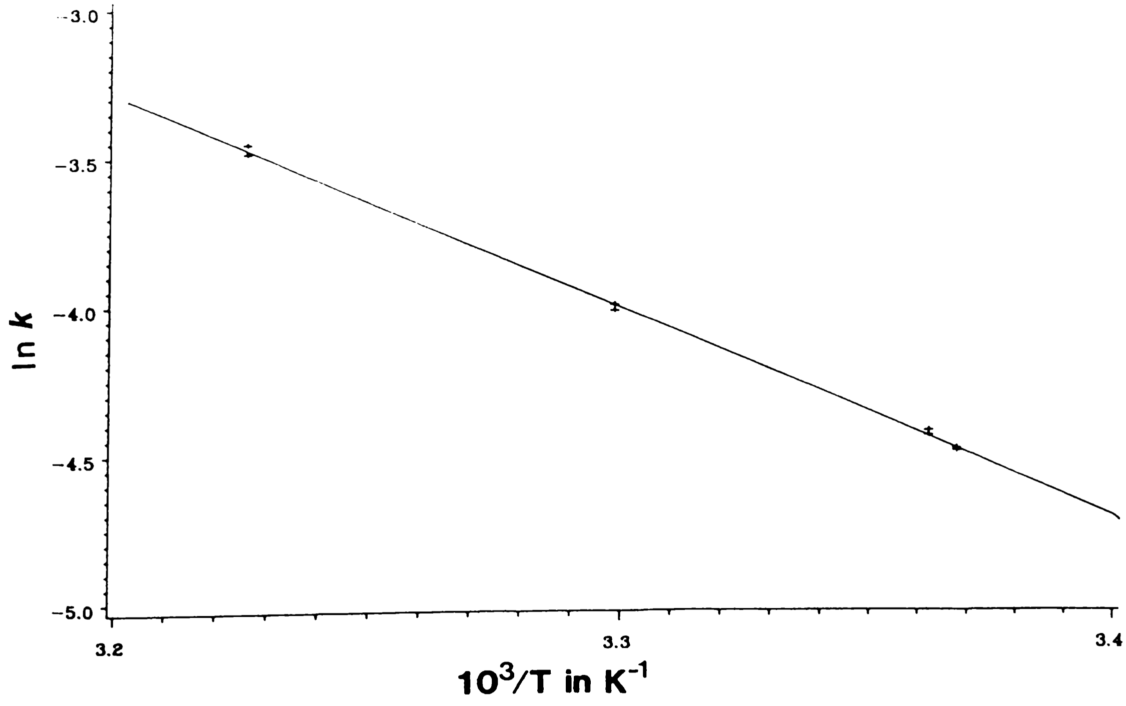
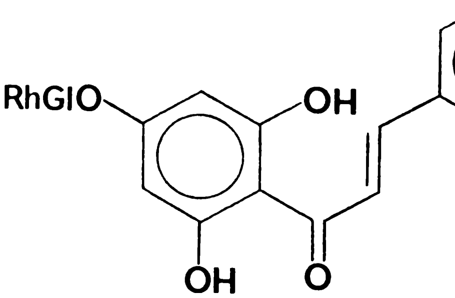
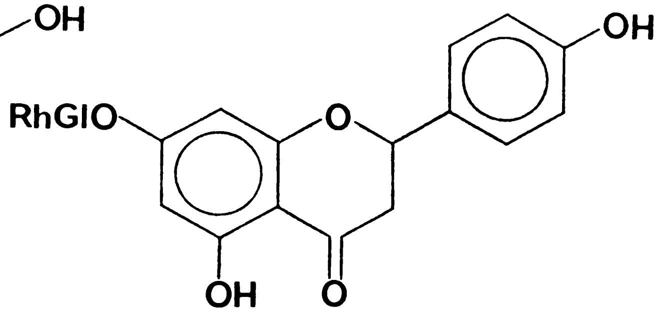


FIGURE 2-27 Plot of lnk_{obs} Versus T^{-1} for the Equilibration of *46* and *47*

2.10 The Naringin-chalcone - Naringin Equilibrium



48



18

2.10.1 The Effect of pH on the Rate of Equilibration

The rate of conversion of naringin-chalcone (2',4,6'-trihydroxy-4'-rhamnoglucosyloxychalcone) (*48*) or naringin (4',5-dihydroxy-7-rhamnoglucosyloxyflavanone) (*18*) to its equilibrium mixture was measured at 30°C in 4% ethanolic H_2O solution, over the pH range 0.0-13.83. Repetitive scans at a number of pH values (figure 2-28) showed that the reaction proceeded cleanly to equilibrium under the conditions used, and that the major product at equilibrium was the flavanone, except at very high pH. First-order rate constants were determined for the approach to equilibrium, and the results are reported in table 2-28.

TABLE 2-28 The Effect of pH on the First-Order Rate Constant for the Attainment of the Naringin-chalcone - Naringin Equilibrium in Aqueous 4% Ethanol at 30°C, μ = 1.0 mol l^{-1} (KCl), [Buffer] = 0.01 mol l^{-1}.

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
0.00	HCl	365	$2.16(0.005)\times10^{-5}$
0.00	HCl	365	2.15×10^{-5}
1.00	HCl	365	$1.41(0.002)\times10^{-5}$

†Determined by ring opening of the isomeric flavanone.

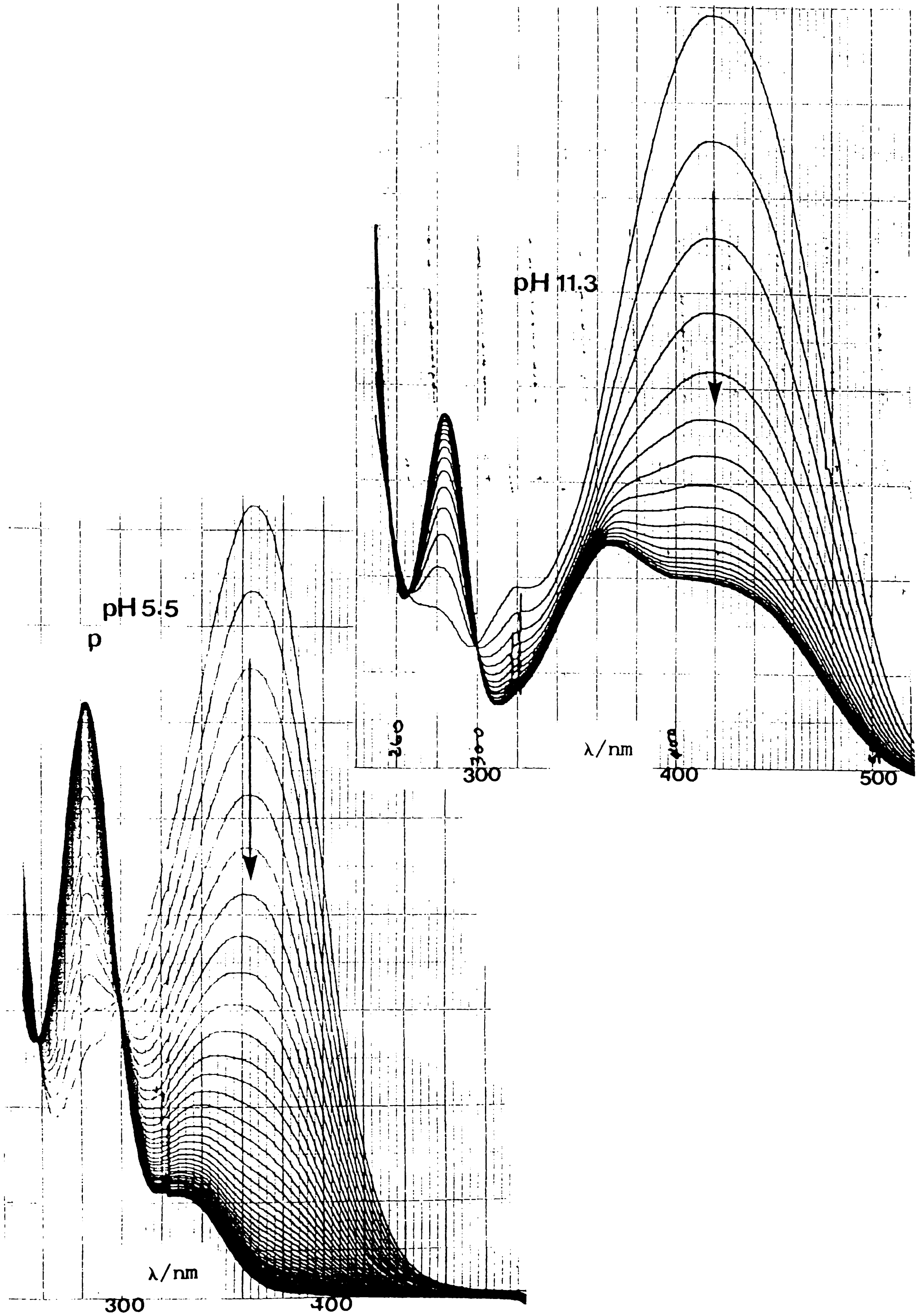


FIGURE 2-28 Repetitive Scans of the Isomerisation of *48* and *18*, at Various pH Values

TABLE 2-28 Continued

pH	BUFFER	λ/nm	$k_{obs}(\sigma)/s^{-1}$
2.00	HCl	365	$1.40(0.02)\times10^{-5}$
3.99	ACETATE	365	$2.90(0.05)\times10^{-5}$
3.99	ACETATE	285	$3.21(0.04)\times10^{-5}$
3.99	ACETATE	365	$3.43(0.05)\times10^{-5}$
4.71	ACETATE	365	$7.60(0.03)\times10^{-5}$
5.46	ACETATE	365	$3.92(0.008)\times10^{-4}$
5.88	PHOSPHATE	365	$6.87(0.02)\times10^{-4}$
6.48	PHOSPHATE	365	$2.23(0.01)\times10^{-3}$
6.48	PHOSPHATE	365	$2.30(0.006)\times10^{-3}$
6.80	PHOSPHATE	365	$3.53(0.02)\times10^{-3}$
6.80	PHOSPHATE	365	$3.52(0.02)\times10^{-3}$
7.28	PHOSPHATE	365	$5.02(0.02)\times10^{-3}$
7.28	PHOSPHATE	365	$4.99(0.02)\times10^{-3}$
7.76	BICINE	365	$4.49(0.02)\times10^{-3}$
7.76	BICINE	365	$4.53(0.02)\times10^{-3}$
8.70	BICINE	365	$3.15(0.01)\times10^{-3}$
8.70	BICINE	365	$3.13(0.008)\times10^{-3}$
9.33	CARBONATE	425	$1.70(0.005)\times10^{-3}$
9.31	CARBONATE	425	$1.71(0.005)\times10^{-3}$
10.35	CARBONATE	425	$8.88(0.01)\times10^{-4}$
10.36	CARBONATE	425	$8.83(0.009)\times10^{-4}$
11.31	NaOH	425	$6.84(0.02)\times10^{-4}$
11.31	NaOH	425	$6.67(0.01)\times10^{-4}$
11.83	NaOH	400	$6.10(0.01)\times10^{-4}$
11.83	NaOH	400	$6.13(0.03)\times10^{-4}$
12.31	NaOH	380	$5.44(0.02)\times10^{-4}$
12.31	NaOH	380	$5.48(0.03)\times10^{-4}$
12.31	NaOH	380	$5.54(0.009)\times10^{-4}$ †
12.31	NaOH	380	$5.59(0.02)\times10^{-4}$ †
12.83	NaOH	375	$6.28(0.01)\times10^{-4}$ †
12.83	NaOH	375	$6.28(0.01)\times10^{-4}$ †
13.31	NaOH	375	$1.16(0.02)\times10^{-3}$ †
13.31	NaOH	375	1.13×10^{-3} †
13.83	NaOH	375	$2.92(0.02)\times10^{-3}$ †
13.83	NaOH	375	$2.91(0.02)\times10^{-3}$ †

2.10.2 Analysis of the pH-Rate Data

The pH-rate profile for this compound is quite different from those of the previous eight 2′,6′-dihydroxychalcones. The reverse reaction becomes significant at a much lower pH than usual; ring opening of naringin is accurately measurable at pH 12.3 compared to *ca.* 13.8 for most of the other chalcones in this study. The usual high pH plateau (pH 9-11) is absent; in fact the rate drops sharply at about pH 7-8, presumably because a second rate-reducing ionisation intervenes at a pH lower than that necessary for mono-anion formation to be complete. From inspection of the structures of naringin (*18*) and its isomeric chalcone (*48*) it is clear that the extra (when compared to the other chalcones and flavanones studied here) phenolic group in each would be expected to affect the pH-rate profile; indeed, the numerous changes in the wavelength most suitable for following the course of the reaction attests to the presence of a larger than usual number of ionised species across the pH range studied. In order to analyse the pH-rate profile, it was necessary to estimate the approximate pK_a values of the phenolic groups, and to correlate these with changes in the pH-rate profile.

The "extra" phenolic group is in the 4-position in the chalcone, and is some distance from the 2′- and 6′-phenolic groups which are directly involved in the ring closure. Even though the 4-OH is conjugated to the A-ring, we have seen for the other chalcones that when the B-ring contains methoxy groups (not in the 2- and 6-positions), there is no measurable effect on the pK_a values of the A-ring hydroxyls. It was therefore expected that the 4-OH group would have little effect on the pK_a values of the A-ring, and that, conversely, the pK_a of the 4-OH was expected to be about that of an isolated phenolic group, modified by conjugation with the α,β-unsaturated ketone moiety. Thus it was expected that $pK_a(4\text{-OH}) \approx 9.6$ (from ref. 131).

The pK_{a1} of the 2′,6′-dihydroxy system of the A-ring was thought likely to be *ca.* 6.9 (as usual), so that the first ionisation of the chalcone will occur at one of the A-ring hydroxyls. The second ionisation (pK_{a2}) will be at the 4-hydroxyl, since the pK_a for ionisation of the second A-ring hydroxyl is normally > 12, and is hardly likely to be lowered by the presence of the (albeit distant) 4-hydroxyl or its oxyanion; and while conjugation of the 4-hydroxyl with a mono-anionic A-ring might be expected to raise its pK_a, it was still expected that $pK_{a2} = pK_a(4\text{-OH}) = 8\text{-}10$. Ionisation of the second A-ring hydroxyl may be made more unfavourable than usual due to conjugation with the 4-oxyanion, and it was expected that $pK_{a3} \geqslant 12.3$.

Combining this analysis with the form of the pH-rate profile suggests that we have: a typically unreactive neutral chalcone; a highly reactive chalcone mono-anion (as usual); a much less reactive di-anion, with $pK_{a2} - pK_{a1} \approx 1\text{-}2$; and an even less reactive tri-anion, with $pK_{a3} > 12$.

In the case of the flavanone form, clearly the 4′-hydroxyl group will ionise first. The pK_a for the 4′-hydroxyl group should be about that expected for an isolated phenolic group (*ca.* 9-10, from[131]), since the conjugation present in the chalcone (to the α,β-unsaturated ketone and the A-ring) is not possible in the flavanone. The 5-hydroxyl group, which is capable of intramolecular hydrogen bonding, ought to have $pK_a(5\text{-OH}) \approx 10$, as discussed previously for the other flavanones, since the presence of the rather remote and unconjugated 4′-oxyanion within the molecule would be expected to have only a small effect on the ionisation of the 5-hydroxyl.

In the pH region where naringin mono-anion is likely to be the major flavanone species present (*ca.* pH 8-10), the eight other flavanones in this study would exist largely in the un-ionised form. In the case of all eight of these flavanones, the concentration of hydroxide ion was too low at such pH values to effect a significant level of ring opening. For naringin (***18***) then, it is hardly likely that the mono-anion form present at such pH values will be significantly more reactive than the eight un-ionised flavanones investigated previously. Therefore ring opening of the naringin 4′-mono-anion was not expected to contribute detectably to the rate of approach to equilibrium.

In fact, the reverse reaction does not begin to measurably affect the pH-rate profile until about pH 12.3, where ionisation of the 5-hydroxyl should be nearly complete. Thus, the major contribution to the pH-rate profile from the reverse reaction was expected to be due to ring opening of the naringin di-anion.

The above analyses lead to the conclusion that the equation defining the observed rate constant for the approach to equilibrium should be of the form:

$$k_{obs} = k_{H+}f_{CHHH}[H^+] + k_{CHHH}f_{CHHH} + k_{CHH-}f_{CHH-} + k_{CH2-}f_{CH2-} + k_{C3-}f_{C3-} + k_{F2-}f_{F2-}[OH^-] \quad ......2\text{-}20$$

with the symbols having their usual meaning, but with the subscripts: CHHH representing the neutral chalcone; CHH- representing the chalcone mono-anion; CH2- representing the chalcone di-anion; C3- representing the chalcone tri-anion; and F2- the flavanone di-anion.

The value of f_{F2-} was assumed to be 1.0 in the usual manner, since only in the pH region where $f_{F2-} \approx 1$ was the hydroxide concentration sufficiently high for the reverse reaction to contribute significantly to the observed rate constant for the isomerisation (as judged from the repetitive scans at various pH values, and from the fit of the experimental data to the theoretical profile based on equation 2-20).

Analysis of the pH-rate data was complicated by the computer programme being designed for analysis of chalcones containing only two ionisable phenolic groups. To overcome this problem it was necessary to perform the analysis in two parts.

Because $pK_{a1} \approx 7$ and $pK_{a3} \approx 12$, they are sufficiently different that they can be treated to a good approximation as independent. So the first part of the analysis was performed by ignoring the chalcone tri-anion and reverse reactions altogether, and obtaining approximate parameters for k_{H+}, k_{CHHH}, k_{CHH-}, k_{CH2-}, pK_{a1} and pK_{a2} to obtain the best possible fit with the pH-rate profile at pH < 11.

In the second part of the analysis, the acid-catalysed and neutral chalcone cyclisations and pK_{a1} were ignored. Thus, below *ca.* pH 7, the chalcone was assumed to be predominantly in the mono-anion form. The approximate parameters obtained from the first part of the analysis were then used to analyse the pH-rate data above *ca.* pH 8 to give values for k_{C3-}, k_{F2-} and pK_{a3}, and to modify the previous estimates for k_{CHH-}, k_{CH2-} and pK_{a2}. These values were then re-used in the first analysis, and the cycle of the two types of analysis continued until a satisfactory agreement between the calculated and the observed rates was obtained.

The first analysis gave good agreement with k_{obs} until *ca.* pH 10, after which a steadily increasing deviation was observed. On the other hand, the second analysis gave a good agreement only above *ca.* pH 9. Between pH 9 and pH 10 the two analyses gave very similar calculated values for k_{obs}. A full calculated pH-rate profile was obtained by using the first analysis for pH 0-9.0, and the second analysis for pH 10.0-14.0. For pH 9.1-9.9, calculated values of k_{obs} were obtained using both analyses and a weighting scheme, such that at pH 9.1 the first analysis value was given 9/10 weight while at pH 9.9 it was given 1/10 weighting. The line in figure 2-29 results from this method of analysis (based on equation 2-20) and the rate coefficients and pK_a values given below, while the crosses represent the experimental values of k_{obs}.

TABLE 2-29 Method Used in the Analysis of the Naringin-chalcone (*48*) pH-Rate Data

Symbol in Computer Programme	Equivalent Symbol from Equation 2-20 Used in the	
	First Analysis	second Analysis
k_{H+}	k_{H+}	0.00
k_{CHH}	k_{CHHH}	k_{CHH-}
k_{CH-}	k_{CHH-}	k_{CH2-}
k_{C2-}	k_{CH2-}	k_{C3-}
k_{F-}	0.00	k_{F2-}
pK_{a1}	pK_{a1}	pK_{a2}
pK_{a2}	pK_{a2}	pK_{a3}

k_{H+} = 7.5×10^{-6} l mol^{-1} s^{-1}

k_{CHHH} = 1.4×10^{-5} s^{-1}

k_{CHH-} = 7.5×10^{-3} s^{-1}

k_{CH2-} = 8.0×10^{-4} s^{-1}

k_{C3-} = 3.0×10^{-4} s^{-1}

k_{F2-} = 2.6×10^{-3} l mol^{-1} s^{-1}

pK_{a1} = 6.9

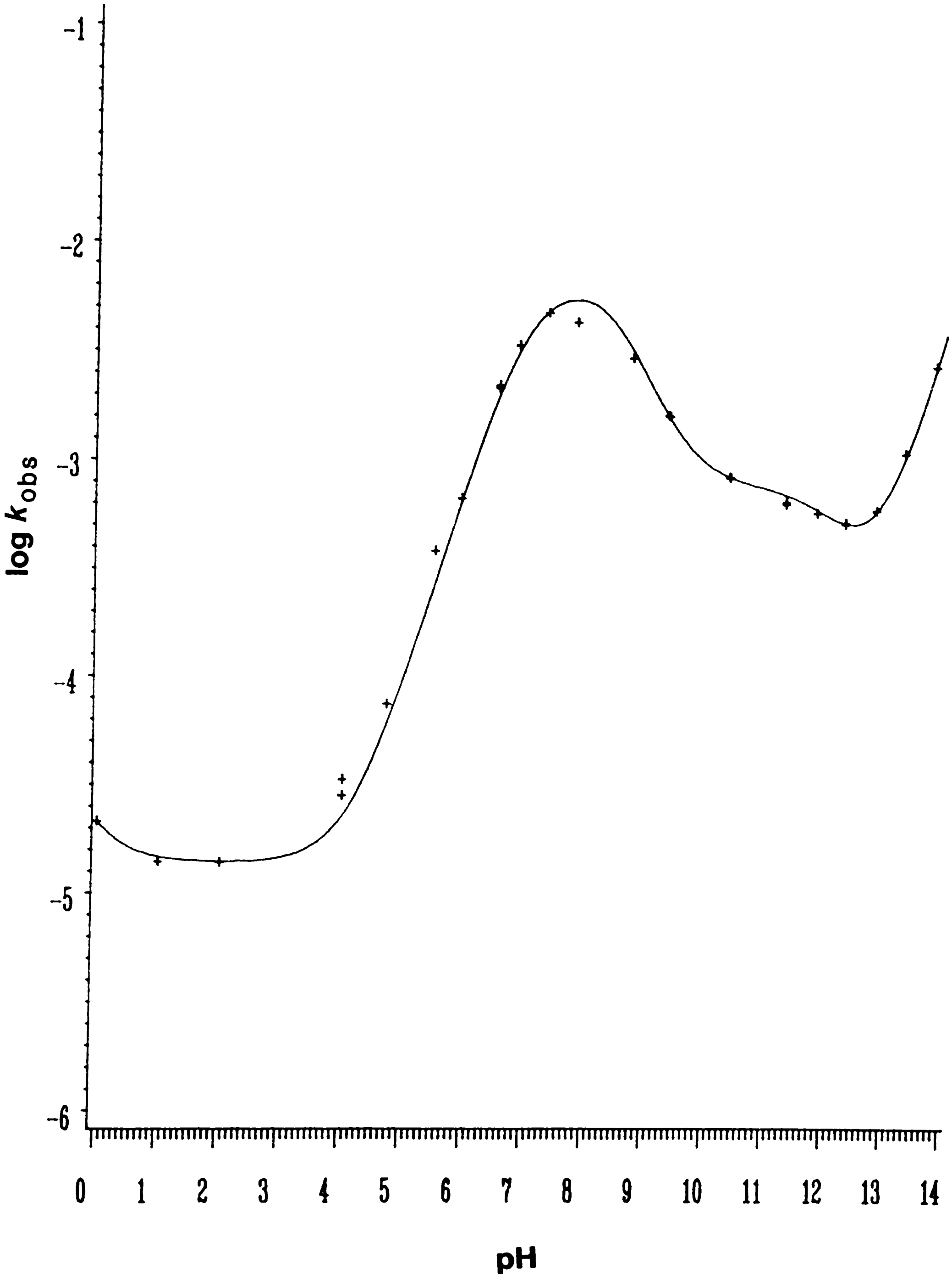


FIGURE 2-29 pH-Rate Profile for the Equilibration of *48* and *18*: Experimental Values (+) from Table 2-28; Curve (——) Calculated from the Rate Coefficients and pK_a Values Given in the Text (Pages 138 and 140), and Equation 2-20

$pK_{a2} = 8.5$

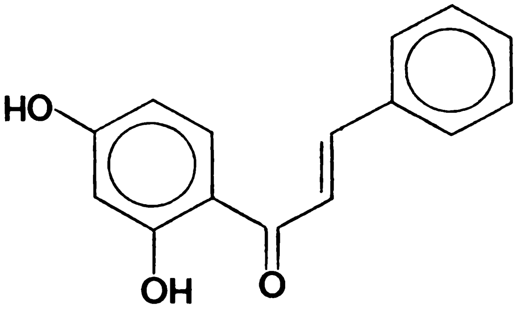
$pK_{a3} = 12.0$

It should be pointed out at this stage that the value for k_{H^+} may well contain some contribution from cyclisation of species generated by partial or total deglycosylation of naringin-chalcone under the acidic conditions present. This possibility, which is unlikely to be readily detectable by u.v. spectroscopy, was not investigated in this study.

The non-plateau rate coefficients for the cyclisation of ***48*** (*i.e.* all but k_{CHHH}) are probably only accurate to ± 15%, due to the problems inherent in estimating rate coefficients from non-linear pH-rate data (see section 2.2) for discussion. The value of k_{F2-} is probably accurate to ± 5-10%, since it is the dominant term in the pH-rate profile at pH > 12.5.

Because of the absence of a plateau in the pH-rate profile representing ring closure of the chalcone mono- or di-anion, it was not convenient to measure the kinetic isotope effect, or the effect of temperature on the rate coefficients for the cyclisation of these species.

2.11 Rate of Attainment of the 2',4'-Dihydroxychalcone - 7-Hydroxyflavanone Equilibrium: An Analysis of Earlier Results



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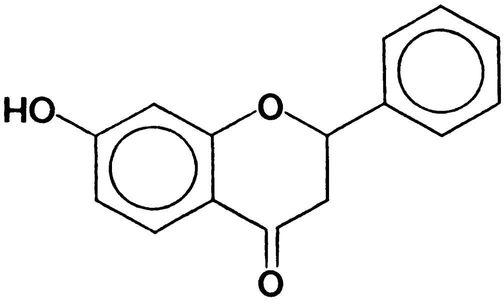

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2.11.*1* The Effect of pH on the Rate of Equilibration

The pH-rate profile presented by Old^{46,71} for the rate of attainment of the 2',4'-dihydroxychalcone (*17*) – 7-hydroxyflavanone (*16*) equilibrium was not analysed to give rate coefficients and pK_a values at the time. The reason for this was that it was considered that the rate of the forward reaction could be determined by up to three rate coefficients and two pK_a values, and the rate of the reverse reaction by up to two rate coefficients and one pK_a; thus up to eight unknown values might need to be determined in an analysis of the pH-rate profile. The authors did point out, however, that the rate coefficient for the reverse reaction involving the flavanone anion must be at least twenty times smaller than that for its methyl ether analogue, 7-methoxyflavanone.

The analysis of the rate data of Old^{46,71} in table 2-30 was undertaken with the aid of the computer programme developed for the analysis of the pH-rate data of 2',6'-dihydroxychalcones. The task turned out, in fact, not to be a difficult one, as a number of simplifying assumptions can be made: (i) in the pH region of the data

(9-13) all the flavanone that is present will be in the anionic form, since the pK_a of the flavanone is expected[131] to be *ca.* 7.4. Even if

TABLE 2-30 Effect of pH on the Observed Rate Constant for the Attainment of the 2',4'-Dihydroxychalcone (*17*) – 7-Hydroxyflavanone (*16*) Equilibrium at 30°C and μ=0.5 mol l^{-1}, [Buffer] = 0.02 mol l^{-1}, in H_2O Containing 5% Dioxane, as Determined by Old[46,71]

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}{}^{a}/s^{-1}$
8.90	0.25	0.25 ± 0.01
9.38	0.33	0.33 ± 0.01
10.40	2.35	2.35 ± 0.07
10.81	4.22 4.37	4.30 ± 0.13
11.07	7.15 7.23	7.19 ± 0.22
11.40	12.37 12.48	12.4 ± 0.4
12.70	42.5 43.0	42.8 ± 1.3
13.83[b]	167	167 ± 5

[a] Mean values taking 3% experimental error in k_{obs} into account.
[b] 1.00 mol l^{-1} NaOH *i.e.* $\mu \neq 0.5$ mol l^{-1}.

this assumption were not entirely accurate (*e.g.* at the lower pH values), experience with the 5-hydroxyflavanones suggests that ionisation of a neutral flavanone's phenolic group occurs much more readily than that of the much less acidic 3-proton, and that as a result ionisation occurs in preference to ring opening of the neutral flavanone; (ii) there is unlikely to be any significant contribution, in the pH region studied, from the neutral chalcone – the reason being that pK_{a1} of the chalcone is expected[130] to be *ca*. 7.4, so that the fraction of neutral chalcone in the pH region of interest is likely to be very small. Furthermore, the rate coefficient for the cyclisation of the neutral chalcone is certain to be considerably smaller than that for the cyclisation of the di-anion (which involves nucleophilic attack by phenoxide oxygen rather than by phenolic oxygen). These two assumptions reduce the problem to one of determining pK_{a2} of the chalcone, the rate coefficients for ring closure of the chalcone mono- and di-anions, and the second order rate coefficient for ring opening of the flavanone anion. The analysis is made even easier when it is realised that the rate coefficient for cyclisation of the chalcone mono-anion (2′-OH-4′-O$^-$; attack by 2′-OH) should be considerably smaller than that for the chalcone di-anion (2′,4′-di-O$^-$; attack by 2′-O$^-$).

Recently, Forlong *et al*.[72,73] have reported kinetic studies of the cyclisation of several chalcones to their flavanones, including the cyclisation of 2′,4′-dihydroxychalcone to 7-hydroxyflavanone at pH 9-13. They also determined the rate coefficient for the chalcone di-anion cyclisation at various temperatures. From a (linear) graph of their rate constants versus the inverse of the absolute temperature, it was possible to estimate the rate coefficient for cyclisation of the chalcone di-anion to be *ca*. 3.4×10^{-3} s^{-1} at 30°C. Although their studies had not been carried out at constant ionic strength, it was considered unlikely that the rate coefficient under Old's reaction conditions (μ = 0.5 mol l^{-1}) would be very different from this value. The reason was that, in the present study, it was demonstrated that the rate coefficient for the cyclisation of 2′,6′-dihydroxychalcone mono-anion was the same under the reaction conditions used by Old[46,71] as it was under the the reaction conditions used by conditions of the present study (μ = 1.0 mol l^{-1}) at 30°C, suggesting that the reaction is not very sensitive to ionic strength effects.

Old[71] also measured the rate constant for attainment of the equilibrium in 1.00 mol l^{-1} sodium hydroxide under the usual reaction conditions (except of course that the ionic strength was greater than 0.5 mol l^{-1}), and found it to be 1.67×10^{-2} s^{-1}. It was considered likely, in view of the apparent low sensitivity of the cyclisation to ionic strength effects, that this value ought not to be very different from the rate coefficient predicted by extrapolation from the pH-rate data at μ = 0.5 mol l^{-1}. This was indeed subsequently found to be the case, as is shown by the good agreement between the observed and calculated rate constants (k_{obs}) for the reaction in the high pH region (see figure 2-30).

2.11.2 Analysis of the pH-Rate Data

Initially it was assumed that k_{H+} = 0, k_{CH2} = 0, k_{CH-} = 0, k_{C2-} = 3.40×10^{-3} s^{-1} (estimated from the data of Furlong *et al.*,[72] as discussed above), k_{F-} = 1.4×10^{-2} l mol^{-1} s^{-1} (estimated from k_{obs} in 1.00 mol l^{-1} NaOH, from Old[71]), pK_{a1} = 7, and pK_{a2} = 11.6 (estimated from the data of Furlong *et al.*[72]); where the symbols have their usual meaning (see section 2.2.2), and the calculations are based on equation 2-19. Trial and error showed that the best fit to the data at pH > 10 was obtained with pK_{a2} = 11.65, k_{C2-} = 3.40×10^{-3} s^{-1} and k_{F-} = 1.40×10^{-2} l mol^{-1} s^{-1}. Then the fit of the curve to the data at pH 9-10 was improved by adjusting k_{CH-}, by trial and error. A reasonable fit was obtained with k_{CH-} = 1.8×10^{-5} s^{-1}, although the estimated error for this value was 0.3×10^{-5} s^{-1}. An accurate value for this rate coefficients can only be obtained by obtaining rate data at lower pH values (where the mono-anion is the dominant form in solution), and by determining pK_{a1}. The results of the above analysis of the results of Old[46,71] for the 2',4'-dihydroxychalcone (*17*) – 7-hydroxyflavanone (*16*) equilibrium are collated below.

$$pK_{a2} = 11.65\ (\pm 0.05)$$

$$k_{CH^-} = 1.8(\pm 0.3)\times 10^{-5}\ s^{-1}$$

$$k_{C2^-} = 3.40(\pm 0.05)\times 10^{-3}\ s^{-1}$$

$$k_{F^-} = 1.40(\pm 0.05)\times 10^{-2}\ l\ mol^{-1}\ s^{-1}$$

In figure 2-30, the experimentally determined values of k_{obs} are plotted as crosses, while the line (from equation 2-19) is based on the above rate coefficients and pK_{a2}, and on pK_{a1} = 7.

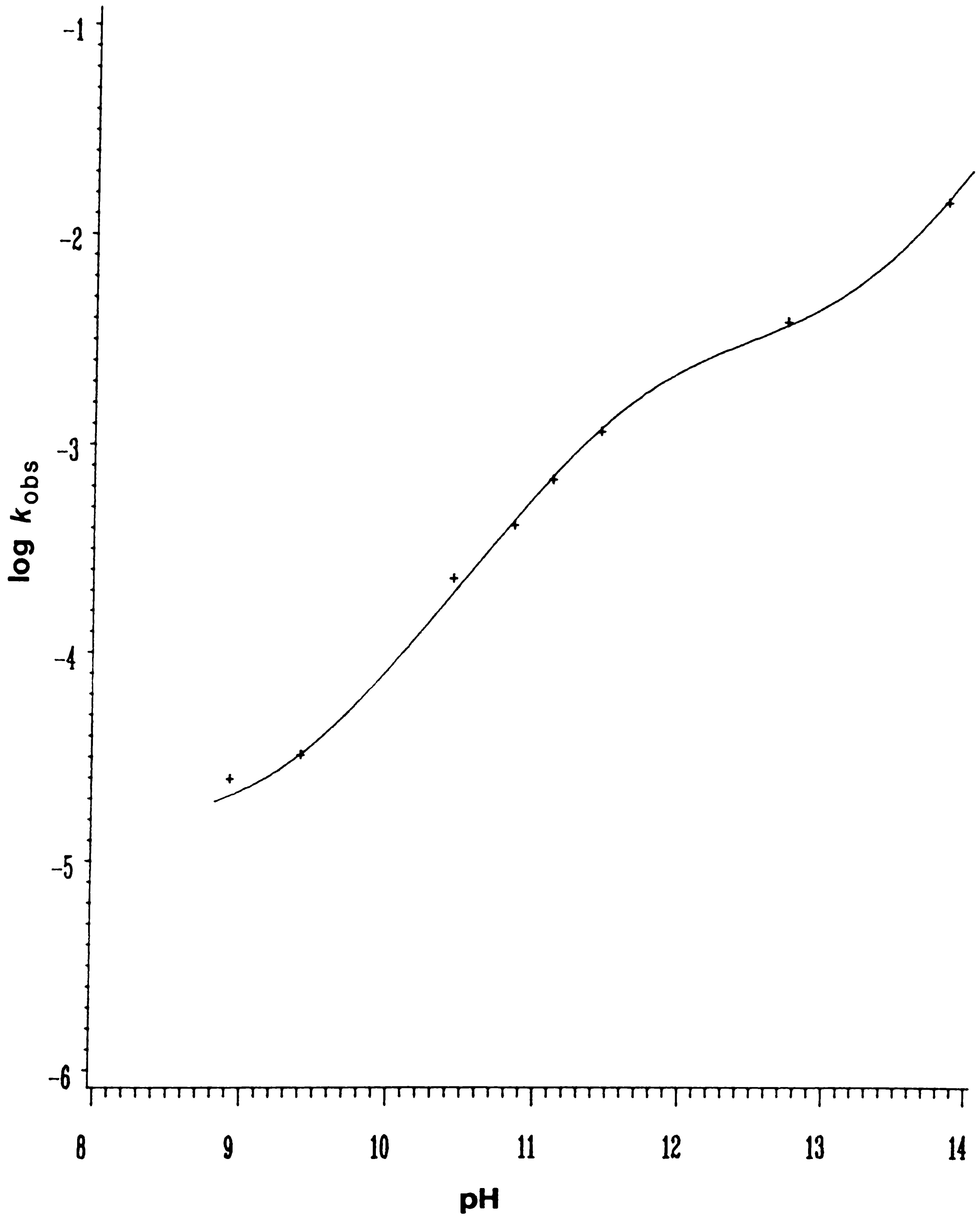


FIGURE 2-30 pH-Rate Profile for the Isomerisation of *16* and *17*: Experimental Values (+) from Old[46,71]; Curve (——) Calculated from the Rate Coefficients and pK_a Values Given in the Text (Page 145), and Equation 2-19

CHAPTER 3

3.1 Product Isotope Effect

3.1.*1* Introduction

A kinetic isotope effect (k.i.e.) was observed in the cyclisation of most of the 2′,6′-dihydroxychalcone mono-anions investigated in this study (see chapter 2). The magnitude of the isotope effect was such ($2.9 \leqslant k_H/k_D \leqslant 7.5$) that it was considered almost certain that the phenomenon was due, at least largely, to a primary isotope effect; thus a proton (or deuteron) is transferred in the transition state of the rate-limiting step. The question remains, however, as to whether the proton that is transferred in the rate-limiting step is the one which ends up at the 3-position of the flavanone product. The answer to this question can provide important information about the mechanism of the cyclisation (see section 5.3.*4*).

One method which can be used to help answer this question is to perform the cyclisation in mixed H_2O—D_2O solutions. Cyclisation with incorporation of hydrogen then competes with the cyclisation incorporating deuterium into the flavanone product. If no H/D exchange occurs between the solvent and the product, and if the cyclisation step is essentially irreversible, then the relative rates of hydrogen and deuterium incorporation into the product can be determined from an analysis of the H:D ratio in the product (provided the ratio of H_2O:D_2O in the reaction medium is known). The relative rates of incorporation of H and D into the product can be referred to as the product isotope effect (p.i.e.). If the p.i.e. is very different from the k.i.e., then either the proton that appears in the product is not the one that is being transferred in the rate-limiting step (which must precede the proton transfer to the 3-position); or if proton transfer to the 3-position is rate-limiting, then the k.i.e. must be changed away from the p.i.e. due the superimposition of an equilibrium isotope effect on an earlier step. The converse, however, does not hold: if the p.i.e. is approximately the same as the k.i.e., then we cannot be sure that the proton in the product is the same one that is transferred in the rate-limiting step, as the p.i.e. and the k.i.e. may just happen to coincide.

This method has been used by Evans and Kirby,[124] who used mass spectrometry to determine the level of deuterium incorporation in their products. It was hoped that the same method would be suitable for the investigation of the cyclisation of 2′,6′-dihydroxychalcone mono-anions.

3.1.2 Experimental Method

The cyclisation reactions needed to be carried out in the high-pH plateau region of the pH-rate profile, *ca.* pH 10, to ensure that the cyclisation proceeded mainly *via* the chalcone mono-anion. A dilute carbonate buffer was made up by weighing out appropriate amounts of potassium carbonate, bicarbonate and chloride into a 10 ml volumetric flask, so that when the volume was made up, $[HCO_3^-]/[CO_3^{2-}] \approx 1$, $[HCO_3^-] + [CO_3^{2-}] = 0.01$ mol l^{-1}, and $\mu = 1.0$ mol l^{-1} (with KCl). The volume was made up with carefully weighed amounts of H_2O (double-distilled from glass) and D_2O (purity > 99.7 atom % D), so that the mole ratio of D to H in the solution was *ca.* 3:2. Because the weights of all reagents present were accurately recorded, it was subsequently possible to accurately calculate the mole ratio of D:H, including a small correction for H added as HCO_3^-.

To perform the cyclisation, 3 mg of the chalcone was added to a mixture containing 4.0 ml of the above buffer, 4.0 ml hexane and 0.5 ml dioxane, and the mixture was shaken vigorously until it was colourless (4 hours for 2',6'-dihydroxy-4-methoxychalcone, and overnight for 2',6'-dihydroxy-4,4'-dimethoxychalcone). For each chalcone, exactly the same procedure was carried out using instead its isomeric flavanone, as a standard, and in order to determine whether or not the H/D in the solvent was exchanging with the cyclised product under the reaction conditions used. The organic phase was then separated and added to chloroform (10 ml), which was then washed twice with a large excess of water (to convert any phenolic O*D* to O*H*), before drying over magnesium sulphate and removal of the solvent *in vacuo*. The pairs of samples, along with a sample of the isomeric flavanones prepared as described in chapter 7, were sent to the D.S.I.R. (Mt. Albert) for analysis on a Kratos MS30 mass spectrometer. Each sample was analysed by averaging the relative intensities of the peaks in the molecular ion region for 50 scans. The results of this procedure are given in table 2-1.

3.1.3 The Results

From the results in table 3-1 we can see that the flavanone standard gave peak intensities that are not significantly different from those obtained from the all-H flavanone. This shows that negligible proton exchange took place at the 3-position under the reaction conditions. This leads to the conclusion that H/D exchange

TABLE 3-1 Relative Intensities of Molecular Ion Peaks (Average of 50 Scans)

Compound	Sample	D2O/H2O	Relative Intensity (σ)		
			$M^{+\cdot}-1$	$M^{+\cdot}$	$M^{+\cdot}+1$
5-hydroxy-4'-methoxyflavanone	all-H*	-	73.01(1.88)	100.0	17.37(0.48)
	standard†	1.32	70.44(1.23)	100.0	17.70(0.40)
	cyclised†	1.32	64.30(1.34)	100.0	30.38(0.62)
5-hydroxy-4,4'-dimethoxyflavanone	all-H*	-	62.26(-)	100.0	17.37(-)
	standard†	1.52	60.88(1.06)	100.0	18.92(0.50)
	cyclised†	1.52	50.27(1.10)	100.0	47.12(1.24)

*Prepared by the procedure given in chapter 7.
†Prepared as outlined earlier (section 3.1.2)

between the flavanone (once it has been formed from the chalcone), and the solvent, does not take place to any significant extent. Presumably this is because the flavanone, once formed, partitions mainly into the organic phase, and is no longer available to the aqueous solvent for exchange reactions. Thus it would appear that the cyclisation reaction, as a result of the experimental conditions used here, is essentially irreversible, and therefore that the H:D ratio in the product is kinetically controlled.

To determine the level of deuterium incorporation, ideally we would obtain the all-H and both (3-D_{eq} and 3-D_{ax}) mono-deuterated (at the 3- position) flavanone mass spectra. The intensities of the ions in table 3-1 should then be analysable in terms of the sum of the contributions from the all-H and both mono-3-D spectra, weighted to take account of their relative abundances in the mixture being analysed. Unfortunately the mass spectra of the pure mono-deuterated flavanones were not readily obtainable. However it was possible to analyse the spectra by assuming that the spectra of the pure mono-D flavanones were identical to that of the all-H spectrum in the molecular ion region, except that they are 1 mass unit heavier (since one H has been replaced by D, per molecule). Such an approximation should hold fairly well for the $M^{+\cdot}$ and $M^{+\cdot}+1$ peaks, but this might not be so for the $M^{+\cdot}-1$ peak; the mono-D flavanone $M^{+\cdot}$ ion may lose either H or D (*i.e.* 1 or 2 mass units), and this loss may also be subject to an isotope effect which cannot be determined from the all-H sample. In any case only a proportionately rather small change in the $M^{+\cdot}-1$ peak intensity (in comparison to that of the $M^{+\cdot}$ peak) is observed between the all-H and cyclised forms of the flavanone (in table 3-1). Any analysis of the level of deuterium incorporation into the cyclised product based on changes in the $M^{+\cdot}-1$ peak intensity will therefore be subject to some uncertainty, and of limited accuracy.

3.1.*4* Analysis of the Results

(i) 2′,6′-dihydroxy-4-methoxychalcone

Let f_D be the fraction of the cyclised product containing one deuteron, and similarly f_H is the fraction containing no deuterium, then the ratio of the $M^{+\cdot}+1$ to $M^{+\cdot}$ intensities in the cyclised product is:

$$\frac{M^{+\cdot}+1}{M^{+\cdot}} = \frac{30.38}{100.0} = \frac{(M_H^{+\cdot}+1)f_H + (M_D^{+\cdot})f_D}{(M_H^{+\cdot})f_H + (M_D^{+\cdot}-1)f_D}$$

where $M_H^{+\cdot}$ is the intensity of the $M^{+\cdot}$ peak of the standard sample, and $M_D^{+\cdot}$ is the intensity of the molecular ion peak for the mono-D flavanone (calculated as in section 3.1.*3*). Thus:

$$0.3038 = \frac{17.70f_H + 100.0f_D}{100.0f_H + 70.44f_D}$$

$$30.38f_H + 21.40f_D = 17.70f_H + 100.0f_D$$

$$12.68f_H = 78.60f_D$$

$$\frac{f_H}{f_D} = 6.20 \ (\sigma = 0.5)$$

Because increasing the value of [D]/[H] in the cyclisation medium will proportionately decrease f_H/f_D, we need to correct for the relative concentrations of D_2O and H_2O used in the original experiment. Thus:

$$\frac{[\text{di-H flavanone}]}{[\text{mono-D flavanone}]} = \frac{f_H}{f_D} \times \frac{[D_2O]}{[H_2O]} = 6.20 \times 1.32 = 8.18 \ (\sigma = 0.7)$$

An analogous analysis using the values of $M^{+\cdot}-1/M^{+\cdot}$ for the product gave a p.i.e. of 9.4, while using $M^{+\cdot}+1/M^{+\cdot}-1$ gave a p.i.e. of 8.4. The value for the p.i.e. obtained by the first analysis (p.i.e. = 8.2) is probably the most accurate for the reasons outlined at the end of section 3.1.*3*.

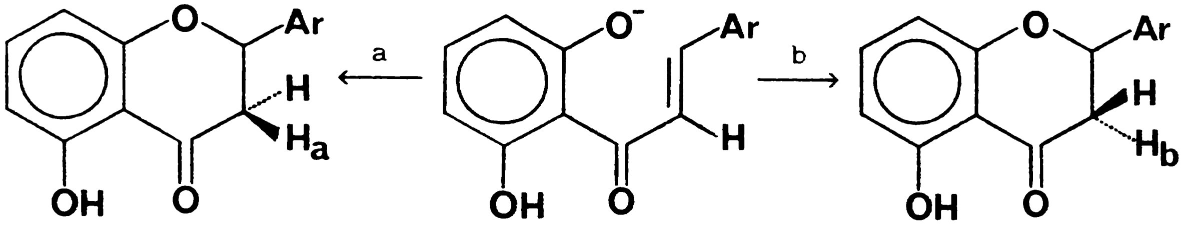
(ii) 2',6'-dihydroxy-4,4'-dimethoxychalcone

An identical analysis to that above was carried out for the data from 5-hydroxy-4',7-dimethoxyflavanone (table 3-1). Using $M^{+\cdot}+1/M^{+\cdot}$ gave a p.i.e. of 3.84 ($\sigma = 0.3$), while using $M^{+\cdot}-1/M^{+\cdot}$ and $M^{+\cdot}-1/M^{+\cdot}+1$ gave p.i.e.s of 4.15 and 3.92 respectively.

3.2 Stereochemistry of Cyclisation

3.2.*1* Introduction

The cyclisation of 2',6'-dihydroxychalcone mono-anions involves, overall, addition of a phenoxide ion and of H^+ to the chalcone double bond. The flavanones possess an asymmetrically substituted carbon atom (at C-2 in the flavanone nomenclature), and so have the potential for optical activity. Of course the chalcones must necessarily cyclise to a racaemic mixture of the flavanone under the conditions used in this study, as neither the chalcones themselves, nor the buffer solutions, contain a chiral centre capable of inducing a preferred asymmetric conformation. There are two possible stereochemically distinct orientations for the attachment of the added proton at the 3-position. The added proton can end up in the equatorial position (H_a; scheme 3-1) by addition of the proton to the face of the double bond opposite that where the phenoxide ion attacks. Alternatively, the added proton can appear in the axial position (H_b; scheme 3-1) by adding to the same face of the double bond as does the phenoxide ion.



SCHEME 3-1 Possible Orientations for Attachment of the Proton at C-3 During 2',6'-Dihydroxychalcone Mono-Anion Cyclisation

If the added proton appears preferentially in either the axial or the equatorial positions of the flavanone, then we may be able to draw conclusions about the mechanism of the cyclisation, or the nature of the transition state. The only practical way to distinguish between a proton in the 3-position of a flavanone derived from the α-position of its chalcone, and one derived from the solvent, is by isotopic labelling of either the solvent, or of the (α-position of the) chalcone.

Hahlbrock *et al.*,[50] as mentioned in chapter 1, have investigated the stereochemistry of cyclisation of 2',4,4'-trihydroxychalcone, supposedly under the influence of enzymic (chalcone - flavanone isomerase) catalysis. They conducted the cyclisation of the normal all-protio chalcone in deuterium oxide, and also the cyclisation of the α-D chalcone in water. Both methods gave essentially the same ratios for the addition of solvent H/D to the two possible sites (equatorial and axial) at C-3 in the flavanone. The ratios were determined from the ratio of the integrals of the 3-H_{ax} and 3-H_{eq} signals in the ^1H n.m.r. spectra of the cyclised products.

It was hoped that a similar method could be applied to the study of the stereochemistry of the uncatalysed cyclisation of 2',6'-dihydroxychalcone mono-anions.

3.2.2 2',6'-Dihydroxy-4-methoxychalcone

To a carbonate buffer (*ca.* 0.05 mol l^{-1} $KHCO_3$, 0.05 mol l^{-1} K_2CO_3) in D_2O (*ca.* 50 ml) was added 50 mg 2',6'-dihydroxy-4-methoxychalcone in dioxane (*ca.* 5 ml), and the mixture was shaken overnight. The resultant colourless solution was then added to aqueous carbonate buffer (*ca.* 50 ml; pH 9.5 prepared as described in chapter 2), and the product was extracted into chloroform (*ca.* 25 ml). The chloroform solution was washed twice with a large excess of water, then dried over magnesium carbonate and the solvent removed *in vacuo*.

The resultant colourless solid was examined by ^1H n.m.r. at 90 MHz (JEOL FX90Q FT instrument) [in $(CD_3)_2CO$]. The spectrum obtained (figure 3-1) had insufficient resolution for an analysis of the region of interest. It was therefore necessary to obtain the spectrum at higher field. 200 MHz ^1H n.m.r. spectra were duly obtained (on a Bruker AC200 FT instrument, at the Forestry Research Institute, Rotorua) in both $(CD_3)_2CO$ and C_6D_6, and the integrals of the various peaks were determined. The spectrum in $(CD_3)_2CO$ contained a spurious peak in the region of interest, possibly due to traces of water in the solvent. With C_6D_6 as the solvent (figure 3-2) no interfering peaks were present, and to aid in analysis of this spectrum, the spectrum of the corresponding all-H flavanone was obtained for comparison (figure 3-3).

The assignments in the all-H spectrum at 200 MHz were straight forward and unambiguous, and are shown in figure 3-3. The 2-H signal was a doublet of doublets, due to couplings to 3-H_{ax} and 3-H_{eq}. Similarly, both the 3-H_{ax} and 3-H_{eq} signals were also doublets of doublets. Together these three signals form an ABX system. A spin simulation experiment was performed using the chemical shift and coupling constant parameters obtained at 200 MHz, to give the expected spectra at 90 and at 60 MHz (figure 3-4). The increasing overlap of the 3-H_{ax} and 3-H_{eq} signals that occurs with decreasing field strength makes it clear why the original spectrum, at 90 MHz (figure 3-1), provided insufficient resolution for the desired stereochemical analysis. An unusual feature of the spectra (figures 3-2 and 3-3) was the set of satellite peaks around the 2′,6′-H and 3′,5′-H peaks. These were not the result of any impurities, but rather, were due to second-order effects in the A_2X_2 system of the flavanone B-ring. Confirmation of this was obtained by the use of a computer-assisted spin simulation experiment, where the coupling constants were determined by trial and error (to give sensible values). The resultant simulated spectrum is shown in figure 3-5 alongside the original spectrum obtained in C_6D_6.

The analysis of the all-H spectrum of the flavanone made analysis of the mono-deuterated flavanone ^1H n.m.r. spectrum simple. The D-H coupling constant is much smaller (*ca.* 6 fold) than the equivalent H-H coupling constant, and signals coupled to deuterium are also subject to considerable line broadening. If the deuteron appears in the equatorial 3-position, then no 3-H_{eq} signal will be present, and the 3-H_{ax} signal (2.51 ppm in figure 3-2) will then be split by coupling with the 2-H ($J \approx 13$ Hz) and broadened by coupling with the 3-D; the 2-H (4.67 ppm in figure 3-2) signal will appear as a doublet, $J \approx 13$ Hz (due to coupling with the 3-H_{ax}), with little line broadening - since the 2-H couples only weakly with the 3-H_{eq}. Alternatively, the deuteron may appear in the axial 3-position: in this case no 3-H_{ax} signal will be observed, and the 3-H_{eq} signal (2.27 ppm in figure 3-2) will be split into a doublet ($J \approx 3$ Hz) by coupling with the 2-H, and broadened by coupling with the 3-D_{ax}; the 2-H signal (4.67 ppm in figure 3-2) should appear as a doublet, $J \approx 3$ Hz (due to coupling with the 3-H_{eq}), with considerable line broadening due to the coupling with the 3-D_{ax}.

A small amount of undeuterated flavanone was also present, as can be seen from the sharp, low intensity pair of doublets of doublets present in figure 3-2, in the usual positions of the 3-H signals for the all-H flavanone. These signals are a good deal sharper than those of the mono-deuterated compound because of the absence of the broadening effect of coupling to deuterium. The source of this all-H flavanone is the cyclisation of the chalcone *via* the ^{1}H contaminants in the reaction medium. The cyclisation *via* these ^{1}H contaminants will be several times more rapid than cyclisation involving deuterium, since both product and kinetic isotope effects have been observed on the cyclisation of this chalcone.

It was considered highly likely that some H/D exchange would take place between the 3-position of the flavanone, and the solvent (D_2O), either *via* the reverse reaction or (more probably) *via* base-catalysed enolisation. It was hoped that the rate of exchange would be sufficiently low under the mildly basic conditions to be negligible. In addition, it was observed that some of the flavanone precipitated during the course of the cyclisation reaction, and the flavanone in this solid phase is unlikely to participate much in the deuterium exchange reactions. If the integral of the 2-H (which is probably not subject to significant isotopic exchange under the reaction conditions) is assigned a value of 1.00, then the sum of the integrals for the 3-H_{eq} and 3-H_{ax} signals is 0.87. Presumably, then, 13% of the flavanone present is in the 3-D_2 form. Thus a small, but significant, fraction of the flavanone formed is subject to subsequent exchange with the solvent.

The ratio of the of the integrals for the 3-H_{ax}:3-H_{eq} signals in figure 3-2 is 52:48, suggesting that 52% of the deuterium adds to the axial position (path b) and 48% to the equatorial position (path a). This makes the probably valid assumption that the exchange reaction (mentioned above) affects both isomers to an equal extent, but given the small but significant amount of exchange that takes place, we can only say with any certainty that the ratio of axial to equatorial addition is in the region of 1:1.

3.2.3 2',6'-Dihydroxychalcone

In order to avoid the uncertainties that occurred as a result of the presence of isotopic exchange between the product and the solvent, a modified procedure was adopted. Essentially the same method as was used for the p.i.e. experiments - except that D_2O, rather than a mixture of H_2O and D_2O, was used as the solvent for the cyclisation step - since the results of the p.i.e. experiment had shown that under these conditions significant H/D exchange between the flavanones and the solvent did not occur.

Thus 30 mg of 2',6'-dihydroxychalcone was added to a D_2O–carbonate buffer (as described in 3.2.2) (4.0 ml), and hexane (4.0 ml) and dioxane (0.5 ml) were added. The mixture was then shaken overnight, and the resultant colourless product extracted with chloroform (20 ml). The organic phase was then washed twice with a large excess of water, dried over magnesium sulphate, and the solvent removed *in vacuo* to afford a colourless solid [*m/z* 242 (18%), 241 ($M^{+\cdot}$, 100) 240 (70), 164 (53), 137 (25), 136 (74), 108 (27)].

The colourless solid was then examined by ^1H n.m.r. At 90 MHz the axial and equatorial 3-protons were again insufficiently separated for analysis. However at 200 MHz (figure 3-6), excellent separation and resolution were obtained, and the form of the spectrum for the 2-H and 3-H signals was essentially the same as for the mono-D 5-hydroxy-4',7-dimethoxyflavanone spectrum of section 3.2.2. The integrals for the 3-H_{ax}, 3-H_{eq} and 2-H signals were accurately determined. When the integral for the 2-H signal was assigned a value of 1.000, the integral for 3-H_{ax} was 0.445, and that for 3-H_{eq} was 0.550. The total intensity of the 3-protons was therefore 99.5% of the intensity for the 2-proton, indicating that exchange between the product, and the deuterium of the solvent, had not taken place. Therefore the ratio of the integrals of the axial and equatorial 3-protons is representative of the ratio of the stereochemical preference of the cyclisation reaction. Thus 45% of the added deuterium appears in the axial position (route b), and 55% appears in the equatorial position (route a).

We see, then, that for both of these chalcones, there is only a small preference for attack *via* the slightly less sterically hindered route a. This will be discussed in terms of possible mechanisms for 2',6'-dihydroxychalcone mono-anion cyclisation mechanisms in chapter 5.

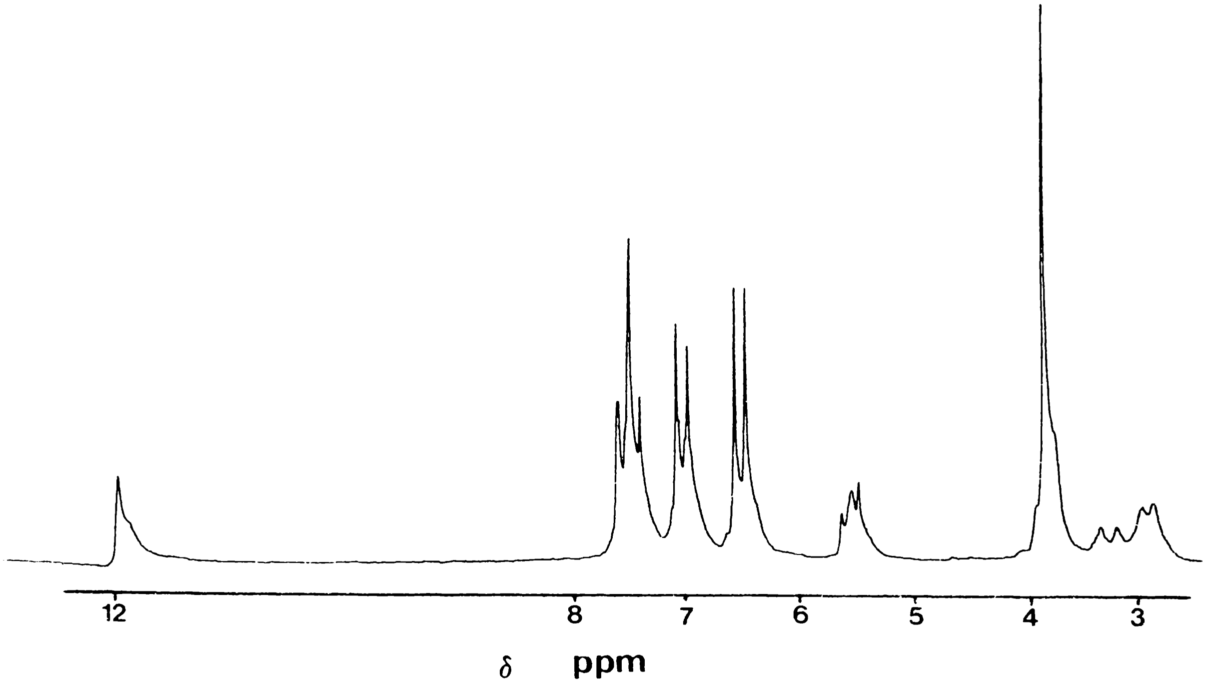


FIGURE 3-1 90 MHz ^{1}H n.m.r. Spectrum of 3-deuterio-5-Hydroxy-4'-methoxyflavanone in $(CD_3)_2CO$

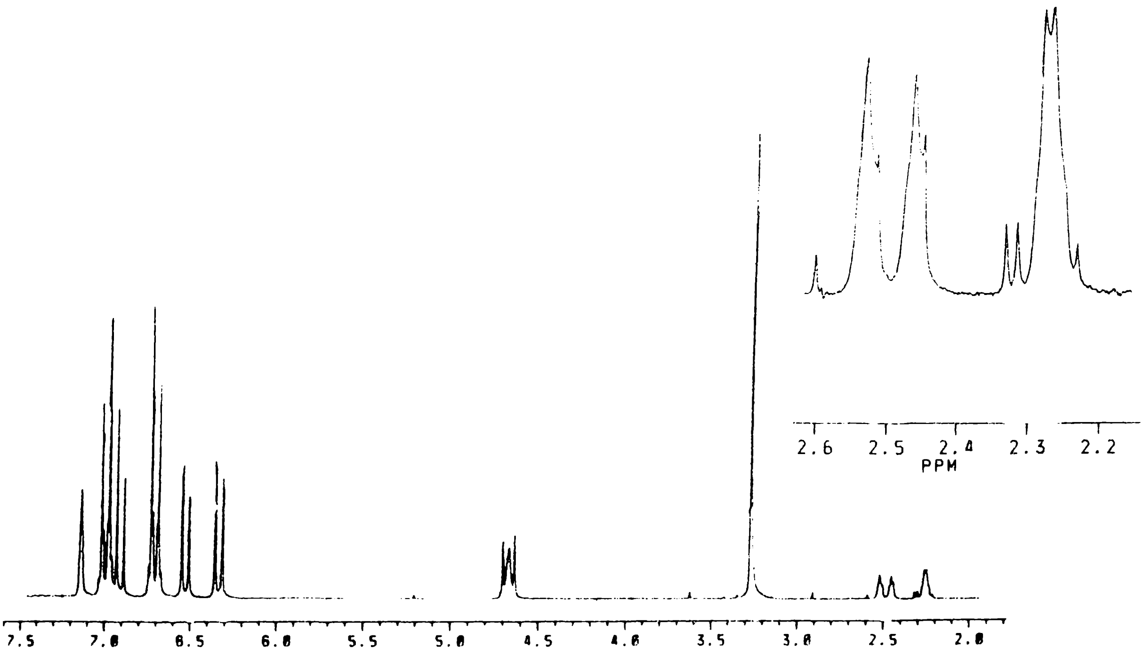


FIGURE 3-2 200 MHz ^{1}H n.m.r. Spectrum of 3-deuterio-5-Hydroxy-4'-methoxyflavanone in C_6D_6

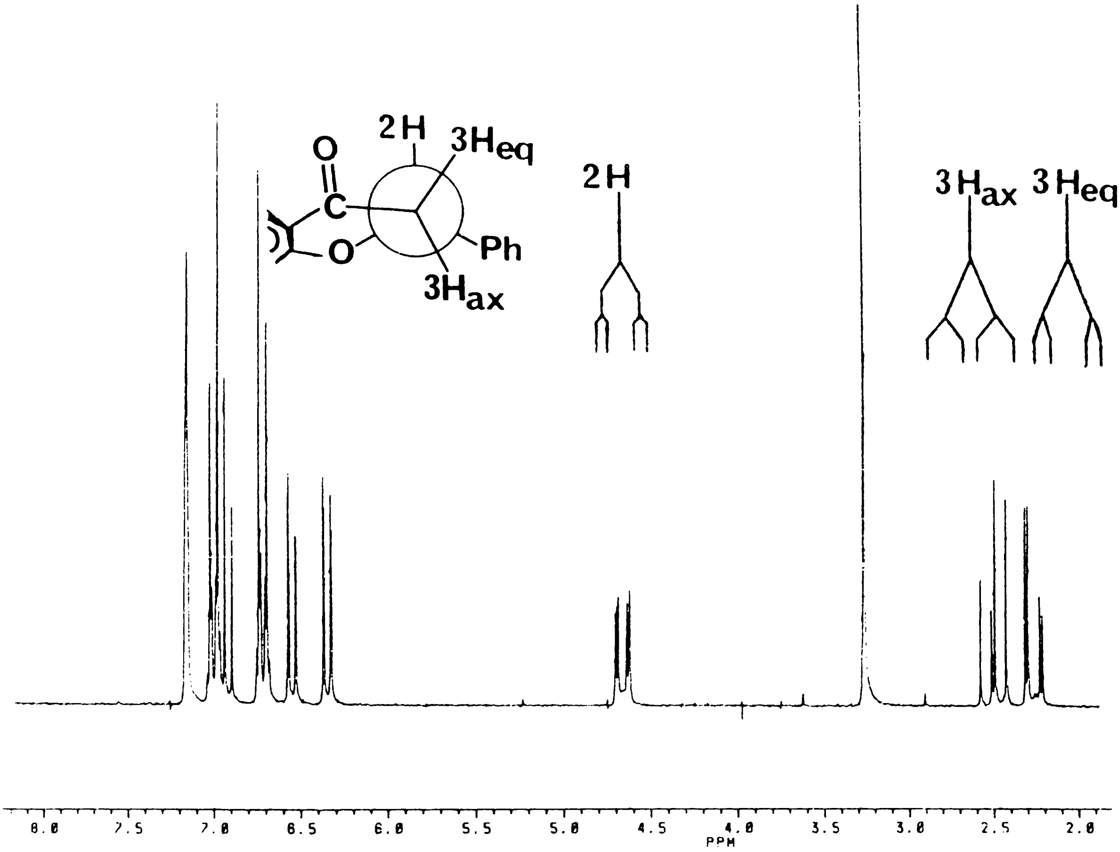


FIGURE 3-3 200 MHz ^{1}H n.m.r. Spectrum of 5-Hydroxy-4'-methoxyflavanone in C_6D_6

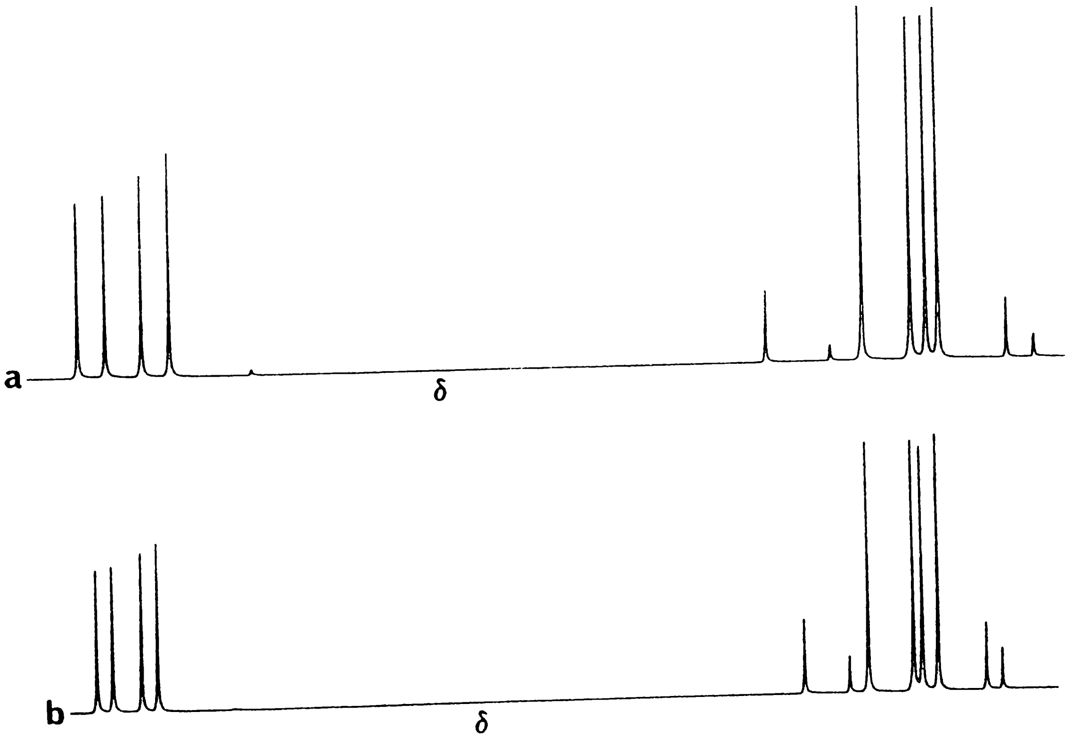


FIGURE 3-4 Computer-Assisted Spin Simulation of the ABX Region of 5-Hydroxy-4'-methoxyflavanone in C_6D_6: (*a*) at 60 MHz; (*b*) at 90 MHz

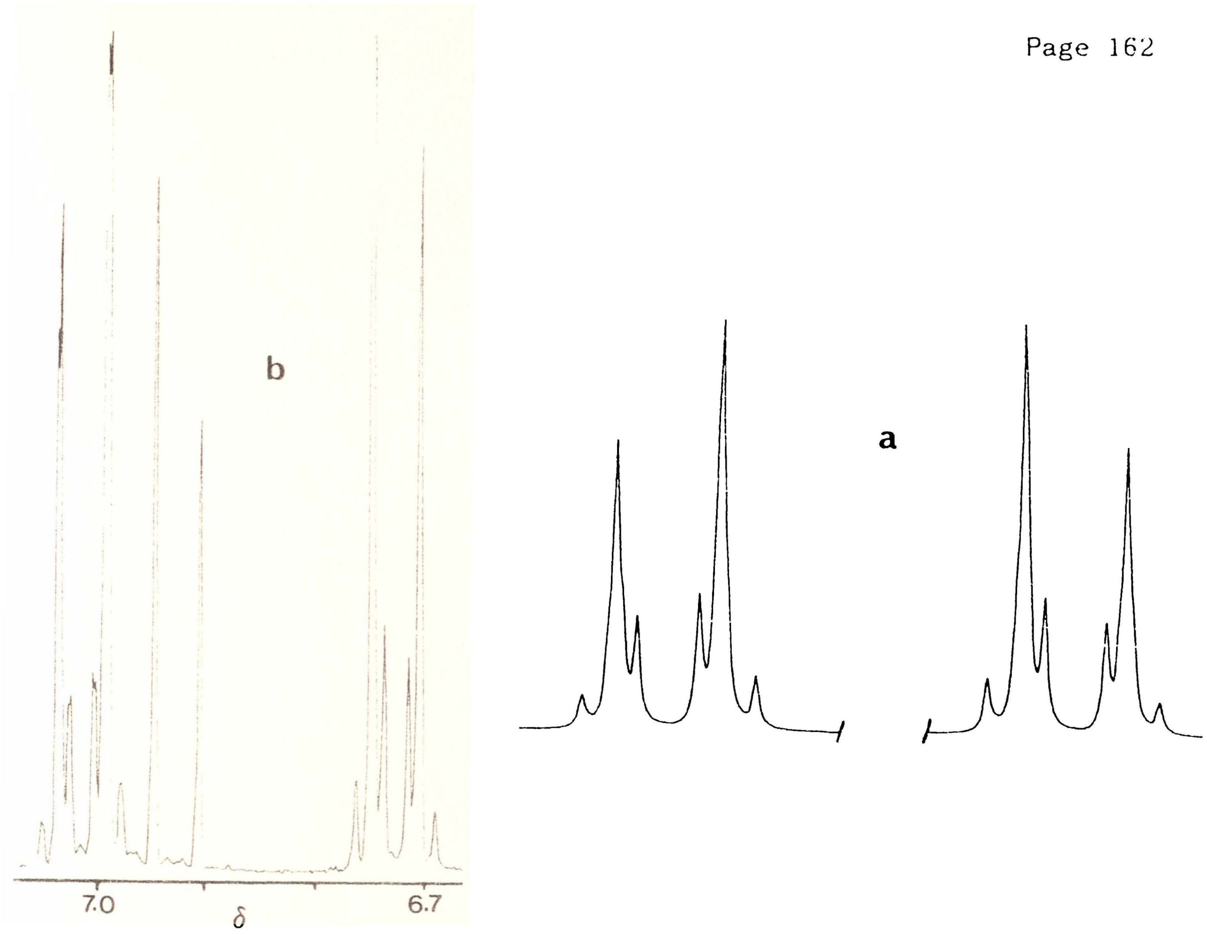


FIGURE 3-5 Computer-Assisted Spin Simulation of the A_2X_2 Pattern of 5-Hydroxy-4′-methoxyflavanone at 200 MHz (*a*); and the Actual Spectrum of the Same Region (*b*)

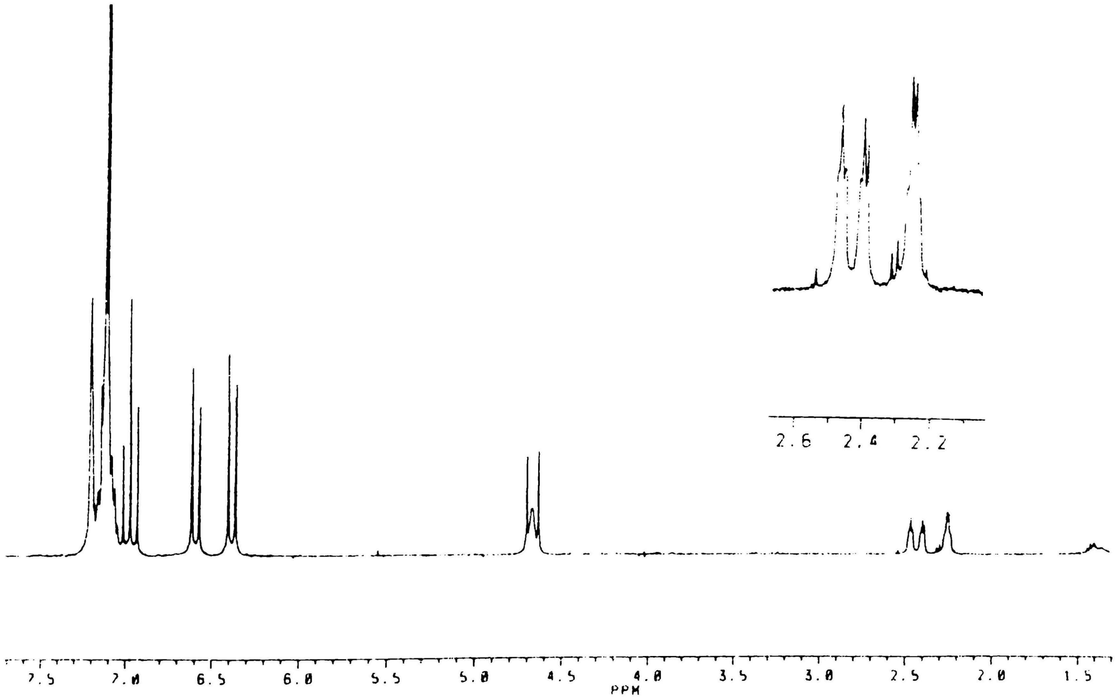


FIGURE 3-6 200 MHz ^{1}H n.m.r. Spectrum of 3-deuterio-5-Hydroxyflavanone in C_6D_6

CHAPTER 4

Crystallography

4.1 Introduction

Although a number of chalcones[132-138] have had their crystal structures determined by X-ray diffraction, crystal structure determinations for 2'-hydroxychalcones are rare,[90,139-142] while none has been performed on a 2',6'-dihydroxychalcone in spite of the importance of this class of natural products. The structures found for the 2'-hydroxychalcones mentioned above are very similar to that predicted[143] for 2'-hydroxychalcone itself from molecular orbital calculations.

It was decided that it was desirable to perform a crystal structure determination on a 2',6'-dihydroxychalcone, since it was considered possible that structural features of the molecule could be responsible, at least in part, for the enhanced reactivity of the 2',6'-dihydroxy- over the 2'-hydroxy-chalcones, towards cyclisation.

In addition, it was hoped to determine the crystal structure of the 2',6'-dihydroxychalcone's isomeric 5-hydroxyflavanone. The results of the flavanone crystal structure would be of interest because it has been suggested in the past (*e.g.* ref. 60) that the difficulties encountered in the isolation of 2',6'-dihydroxychalcones (as compared to simple 2'-hydroxychalcones) is due to the enhanced stability of the (cyclised) 5-hydroxyflavanones (as compared to simple flavanones), rather than to any fundamental difference in reactivity between the two classes of chalcone. A crystal structure determination of the isomeric 5-hydroxyflavanone, then, could possibly show structural features indicating higher stability to ring opening than for simple flavanones. There is, however, no indication of any major difference between the structures of the three reported[144-146] 5-hydroxyflavanone crystal structures, and that reported for 5,7,4'-trimethoxyflavanone,[147] apart from the absence of an intramolecular hydrogen bond in the latter.

It was decided to attempt to determine the crystal structures of 2',6'-dihydroxy-4,4'-dimethoxychalcone (*32*) and its isomer, 5-hydroxy-4',7-dimethoxyflavanone (*33*), since in addition to being readily available, both compounds are also well characterised natural products. Crystals of the chalcone were easily obtained as described in chapter 7, but problems were experienced in growing suitable crystals of the flavanone. The flavanone crystals grew as long, thin, tapering needles under all recrystallisation conditions tried, and eventually the largest of these needles were used in the X-ray diffraction experiments. The space group and unit cell dimensions were successfully determined for the crystals of both compounds, and then samples were sent to the University of Canterbury for data collection.

Good data were obtained for the flavanone (*33*), but in the case of the chalcone (*32*), no data could be obtained due to twinning of the chalcone crystals, despite the excellent appearance of the orange prismatic crystals. Because diffraction-quality crystals of 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***), in the form of deep-red prisms, were available from the procedure described in chapter 7, determination of the crystal structure of this compound was attempted. Again the space group was determined and the unit cell dimensions estimated at Waikato, then crystals were forwarded to the University of Canterbury, where data collection was successfully performed.

4.2.*1* Data Collection and Structural Refinement for 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone (***44***)

Suitable crystals were obtained from the procedure described in chapter 7.

Crystal Data.— $C_{18}H_{18}O_6$, M = 330.3. Monoclinic, a = 8.864(8), b = 14.32(1), c = 13.12(2) Å, β = 106.34(8)°, V = 1590 Å^3 (from 16 automatically centred reflections, λ = 0.7107 Å), space group $P2_1/c$ (No. 14), Z = 4, D_c = 1.37 g cm^{-3}, F(000) = 696.00. Deep-red prisms. μ(Mo-K_α) = 0.63 cm^{-1}.

Data Collection and Processing.— Nicolet XRD P3 four-circle diffractometer at -100°C, with graphite-monochromated Mo-K_α radiation (0.7107 Å); 2934 reflections were measured, $3.0° \leqslant \theta \leqslant 55.0°$, 2830 unique, giving 2052 with $I > 3\sigma(I)$. Data were corrected for Lorentz and polarisation effects, but not for absorption.

Structure Analysis and Refinement.— Solved by direct methods. Refined by full-matrix least squares, with methoxy and hydroxy carbon and oxygen atoms anisotropic, and all other non-hydrogen atoms isotropic. Hydrogen atoms were included in calculated positions with fixed temperature factors, except for the hydroxy hydrogen atoms, which were located in a penultimate difference map and were refined with fixed temperature factors. Refinement converged with R = 0.0598 and R_w = 0.0690, where $w = 4.5808[\sigma^2(F_o) + 0.001F_o{}^2]^{-1}$. No parameter shifted by more than 0.2σ in the final refinement cycle, and a final difference map gave no features greater than 0.65 e Å^{-3}. All calculations were performed with SHELX-76.[148]

4.2.2 Discussion

The crystal structure of 2',6'-dihydroxy-2,4,6-trimethoxychalcone (*44*) has many features in common with those determined for other 2'-hydroxychalcones. Note that the labelling for the crystal structure is different to that used elsewhere in this thesis: to avoid confusion in the text, "atom(number)" refers to the appropriate atom under the crystal structure labelling scheme (figure 4-1); while "number-atom" refers to the appropriate atom using the standard labelling (scheme 1-1).

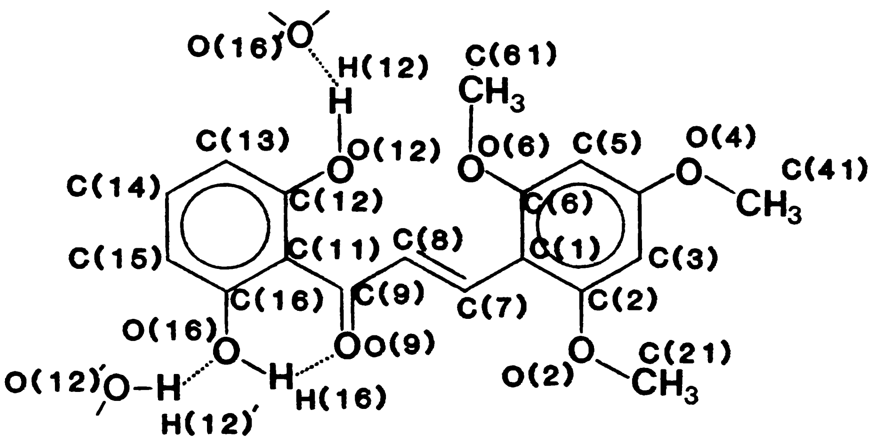


FIGURE 4-1 Labelling Scheme for 2',6'-Dihydroxy-2,4,6-trimethoxychalcone (*44*)

The molecule is very nearly planar, a feature it shares with all the 2'-hydroxychalcones whose structures have been determined;[90,139,140,142] the maximum deviation of any non-hydrogen atom from the mean plane defined by the heavy atoms of the molecule is *ca.* 0.3 Å. The dihedral angle between the two aromatic rings is 5.5°, while the angle between the A-ring and the plane of the three carbon bridge is 9.9°, and that between the B-ring and the C_3 bridge is 6.1°. The atoms O(9), O(12), O(16) and H(16) all lie very close to the plane of the A-ring; presumably, in the case of O(9) and H(16), this is due to the influence of the intramolecular hydrogen bond. In contrast to

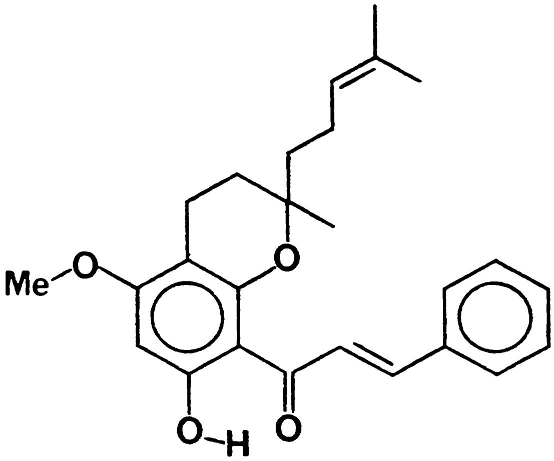
H(16), H(12) lies 0.2 Å out of the plane of the A-ring, an effect attributable to its participation in intermolecular hydrogen bonding to the O(16)′ of a neighbouring molecule. As for the B-ring, this too is quite planar, with all the methoxy oxygens lying within 0.1 Å, and all the methoxy carbons within 0.25 Å, of the plane of the B-ring. Planarity is not such a common feature among chalcones which lack a 2′-hydroxy group; of the chalcones lacking a 2′-hydroxy group studied to date, only 4′-methoxychalcone[134] and both[135,137] forms of chalcone itself, are approximately planar. On the other hand, 4,4′-dimethyl-,[132] 4′-bromo-,[133] 4-bromo-2′-nitro-[136] and[136] 3-chloro-2′-nitro-chalcones are distinctly non-planar. It seems, therefore, that the intramolecular hydrogen bonding present in 2′-hydroxy-substituted chalcones favours molecular planarity, which is otherwise only weakly favoured for other chalcone substitution patterns.

The present chalcone (***44***) possesses the *cisoid* conformation in the enone bridge, as do almost all of the chalcones whose structures have been reported. Only 3-chloro-2′-nitrochalcone[136] has been found to exist in the *transoid* conformation in the solid state. However it must be borne in mind that the conformation of the molecule in the solid state may not necessarily be the only (or even the preferred) conformation in solution.

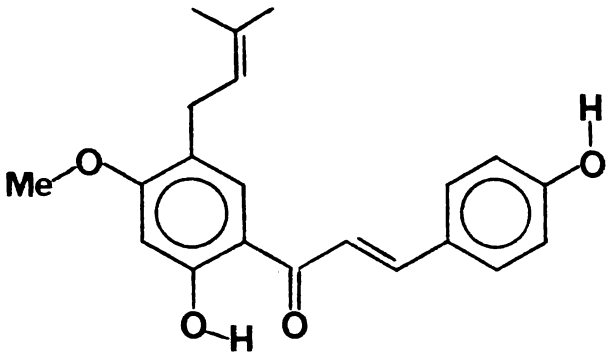
The molecule possesses a large (123.8°) angle at the carbonyl carbon, [C(8)-C(9)-C(11)], a feature it shares[90] with boesenbergin A (***49***), and which has previously been attributed[90] to the effect of hydrogen bonding. However the equivalent bond angles in two other[139,140] 2′-hydroxychalcones, which contain intramolecular hydrogen bonds but lack 6′-substituents, are normal (*ca.* 120°), suggesting instead that steric repulsion between the 6′-O and the α-H may be responsible. In accord with this is the C(9)-C(11)-C(12) angle of 125.0°, and the observed close approach of O(12) to H(8), these atoms being only 2.06 Å apart in the present chalcone. However, the corresponding angle in bavachalcone[142] (*50*), a compound in which no bulky substituents are present in the 6'- position, is also 123.8°, so that steric crowding is probably not solely responsible for the large angle in the present chalcone. In fact a 4° opening of a bond angle is not a very high energy process, so that packing or electronic effects could easily account for this phenomenon. Even so, the O(6)-H(8), O(9)-H(7) and O(2)-H(7) distances in the present chalcone are all *ca.* 2.2 Å [the sum of the Van der Waal's radii (H and O) is[127c] 2.6 Å], so that the central area of the chalcone is clearly sterically crowded (see figure 4-4).

The angles at the α- [C(7)-C(8)-C(9)] and β- [C(1)-C(7)-C(8)] carbons are respectively smaller and larger than normal, presumably to better accommodate the steric bulk of the 2- and 6-methoxy groups. Another feature of the structure is that the four aromatic C-C bonds adjacent to the junction of the two aromatic rings to the propenone unit [C(11)-C(12), C(11)-C(16), C(1)-C(2) and C(1)-C(6)] are significantly longer (average: 1.418(3) Å) than the other aromatic C-C bonds [average: 1.382(3) Å]. This may be due to delocalisation of electron density from the adjacent O atoms onto the conjugated propenone system (this feature is also present[90] in the A-ring of boesenbergin A, where the C-1' to C-2' and C-2' to C-6' bond lengths are *ca.* 1.43 Å). This feature is also found, to a lesser extent, in the A-rings of the 2',4'-di-O- substituted chalcones whose structures[139,140,142] have been determined. It has been found that a similar shortening of the C-1' to C-2', C-1' to C-6', C-3' to C-4' and C-4' to C-5' bonds occurs in substituted acetophenones (*51*); when

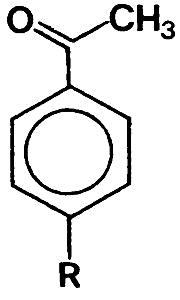
R = NO_2 the effect[149] was said to be due to the effect of electron-withdrawing substituents at C1′ and C4′; and where R = NH_2 the effect[150] was attributed to the interaction of a strong π-donor and a π-acceptor. In comparison, for acetophenone (***51***; R = H) itself,[151] all the aromatic bond lengths are the same within experimental error, while for 4′-hydroxyacetophenone[152] (***51***; R = OH) no pattern in the aromatic C—C bond lengths was apparent.



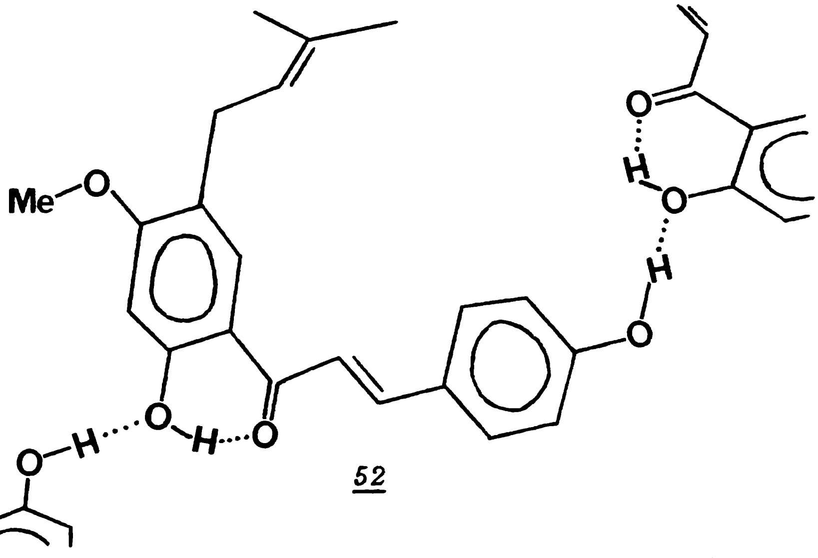
Boesenbergin A (***49***)



Bavachalcone (***50***)



51



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Possibly related to these variations in aromatic bond lengths is the small endocyclic bond angle at the junction of the aromatic rings with the propenone unit of the chalcone. Also, the endocyclic bond angles at the carbon atoms *ortho*-, *meta*- and *para*- to the junction are respectively larger, slightly smaller and larger, than 120° for both the A- and B-rings of 2',6'-dihydroxy-2,4,6-trimethoxychalcone. This feature is also present in the A-rings of all the 2'-hydroxychalcones whose structures have been determined[90,139-142] (all of which are 2',4'-di- or 2',4',6'-tri-*O*-substituted). A similar, although not completely analogous pattern of bond angles was again present[149,150] in the substituted acetophenones mentioned above, but was completely absent in acetophenone[151] itself.

The chalcone obviously possesses a strong intramolecular hydrogen bond (as most 2'-hydroxychalcones probably do). The length of the C=O bonds in all the 2'-hydroxychalcones whose structures have been determined are somewhat longer than those that have been found for simple chalcones lacking the 2'-hydroxy group (1.26-1.27 Å compared to *ca.* 1.23 Å), presumably as a result of the intramolecular hydrogen bond. All the bond lengths and angles involved in the intramolecular hydrogen bond are essentially the same as the corresponding bond lengths and angles in the 2'-hydroxychalcones (where the position of the intramolecularly bonded hydrogen atom[90,142] has been located).

In addition to the intramolecular hydrogen bond, the present chalcone contains an intermolecular hydrogen bond. As expected, the intermolecular bond is weaker than the intramolecular bond, as is shown by the relative lengths of these two bonds. Bavachalcone[142] (*50*) is the only other chalcone containing an intermolecular hydrogen bond where the hydrogen atom involved has been located. The intermolecular hydrogen bond of bavachalcone has almost exactly the same O-H length (1.68 Å) and O-H-O angle (166°) as that present in 2',6'-dihydroxy-2,4,6-trimethoxychalcone (tables 4-1 and 4-2). In the case of bavachalcone, the intermolecularly bonded hydrogen atom bonds to the oxygen atom of the intramolecularly bonded phenolic group, as shown in structure **52**, so that the phenolic oxygen is approximately

Table 4-1 Bond Lengths (in Å) for 2',6'-dihydroxy-2,4,6-trimethoxychalcone (*44*)

C(1) ---C(2)	1.417(4)	C(11) ---C(12)	1.418(4)
C(1) ---C(6)	1.416(4)	C(11) ---C(16)	1.419(4)
C(1) ---C(7)	1.437(4)	C(12) ---C(13)	1.383(4)
C(2) ---C(3)	1.380(4)	C(12) ---O(12)	1.351(4)
C(2) ---O(2)	1.363(3)	C(13) ---C(14)	1.380(4)
C(3) ---C(4)	1.387(4)	C(14) ---C(15)	1.373(5)
C(4) ---C(5)	1.391(4)	C(15) ---C(16)	1.383(4)
C(4) ---O(4)	1.351(4)	C(16) ---O(16)	1.357(4)
C(5) ---C(6)	1.376(4)	C(21) ---O(2)	1.429(4)
C(6) ---O(6)	1.356(3)	C(41) ---O(4)	1.433(4)
C(7) ---C(8)	1.342(4)	C(61) ---O(6)	1.428(4)
C(8) ---C(9)	1.445(4)	O(9) ---H(16)	1.48(4)
C(9) ---C(11)	1.469(4)	O(12) ---H(12)	0.97(4)
C(9) ---O(9)	1.271(3)	O(16) ---H(16)	0.96(4)
		O(16)'---H(12)	1.70

TABLE 4-2 Bond Angles (in Degrees) for 2',6'-dihydroxy-2,4,6-trimethoxychalcone (*44*)

C(2) -C(1) -C(6)	116.2(3)	C(9) -C(11) -C(12)	125.0(3)
C(2) -C(1) -C(7)	117.8(3)	C(9) -C(11) -C(16)	118.6(3)
C(6) -C(1) -C(7)	126.0(3)	C(12) -C(11) -C(16)	116.4(3)
C(1) -C(2) -C(3)	122.6(3)	C(11) -C(12) -C(13)	121.1(3)
C(1) -C(2) -O(2)	115.0(3)	C(11) -C(12) -O(12)	118.9(3)
C(3) -C(2) -O(2)	122.4(3)	C(13) -C(12) -O(12)	120.0(3)
C(2) -C(3) -C(4)	118.4(3)	C(12) -C(13) -C(14)	119.9(3)

TABLE 4-2 Continued

C(3)	-C(4)	-C(5)	121.7(3)	C(13)	-C(14)	-C(15)	121.3(3)
C(3)	-C(4)	-O(4)	123.6(3)	C(14)	-C(15)	-C(16)	119.2(3)
C(5)	-C(4)	-O(4)	114.6(3)	C(11)	-C(16)	-C(15)	121.9(3)
C(4)	-C(5)	-C(6)	119.0(3)	C(11)	-C(16)	-O(16)	120.3(3)
C(1)	-C(6)	-C(5)	122.1(3)	C(15)	-C(16)	-O(16)	117.8(3)
C(1)	-C(6)	-O(6)	115.6(2)	C(2)	-O(2)	-C(21)	118.5(3)
C(5)	-C(6)	-O(6)	122.3(3)	C(4)	-O(4)	-C(41)	118.0(2)
C(1)	-C(7)	-C(8)	130.4(3)	C(6)	-O(6)	-C(61)	118.3(2)
C(7)	-C(8)	-C(9)	118.9(3)	C(9)	-O(9)	-H(16)	102(1)
C(8)	-C(9)	-C(11)	123.8(3)	C(12)	-O(12)	-H(12)	111(2)
C(8)	-C(9)	-O(9)	119.0(3)	C(16)	-O(16)	-H(16)	101(2)
C(11)	-C(9)	-O(9)	117.2(3)	O(9)	-H(16)	-O(16)	159(4)
				O(12)	-H(12)	-O(16)'	166

trigonally coordinated. The situation is analogous to the situation found in the present chalcone, where the H(12) of one molecule is bonded intermolecularly to the O(16) (which is itself involved in an intramolecular hydrogen bond) of a neighbouring molecule.

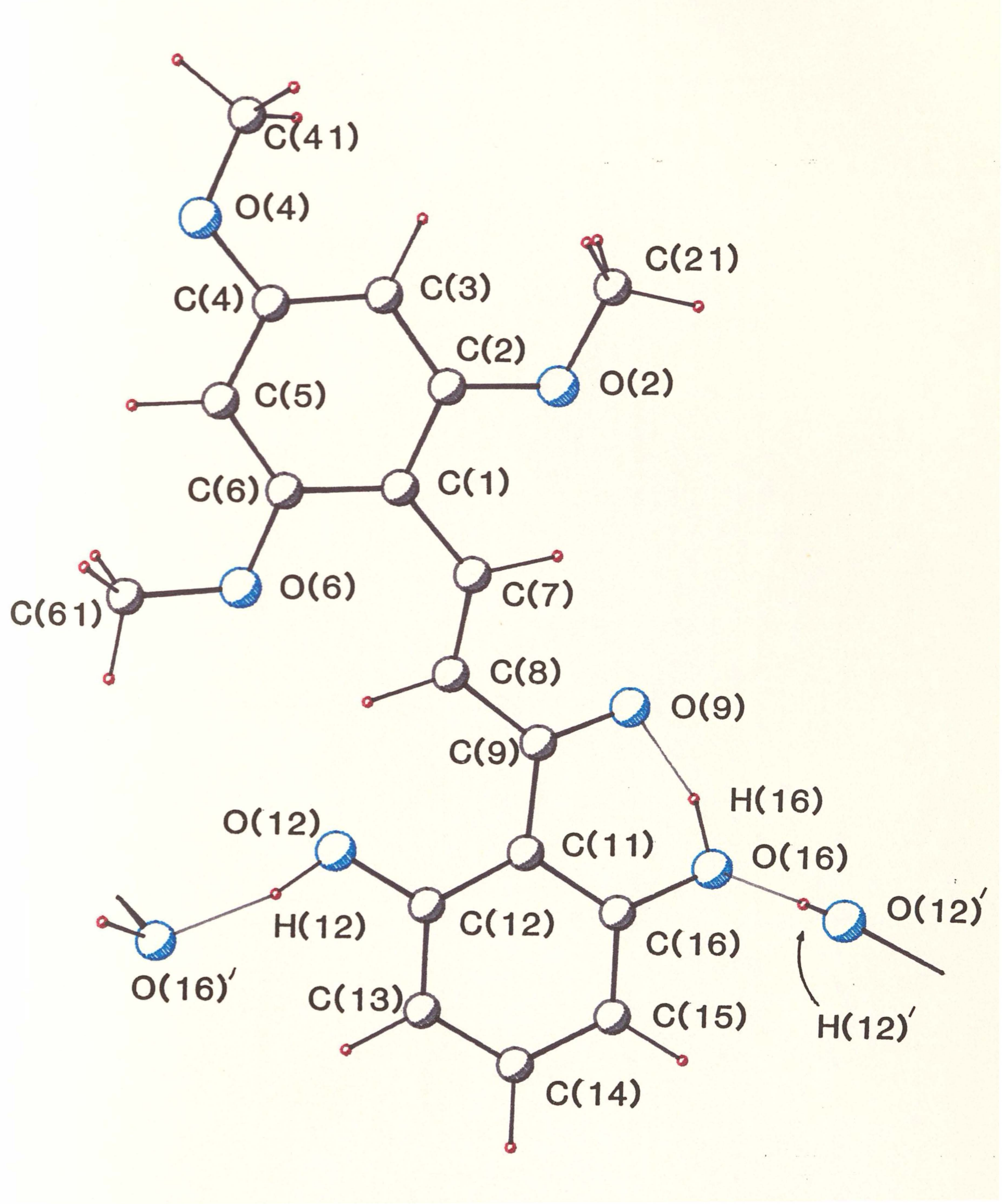


FIGURE 4-2 View of 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone (*44*) Perpendicular to the Molecular Plane

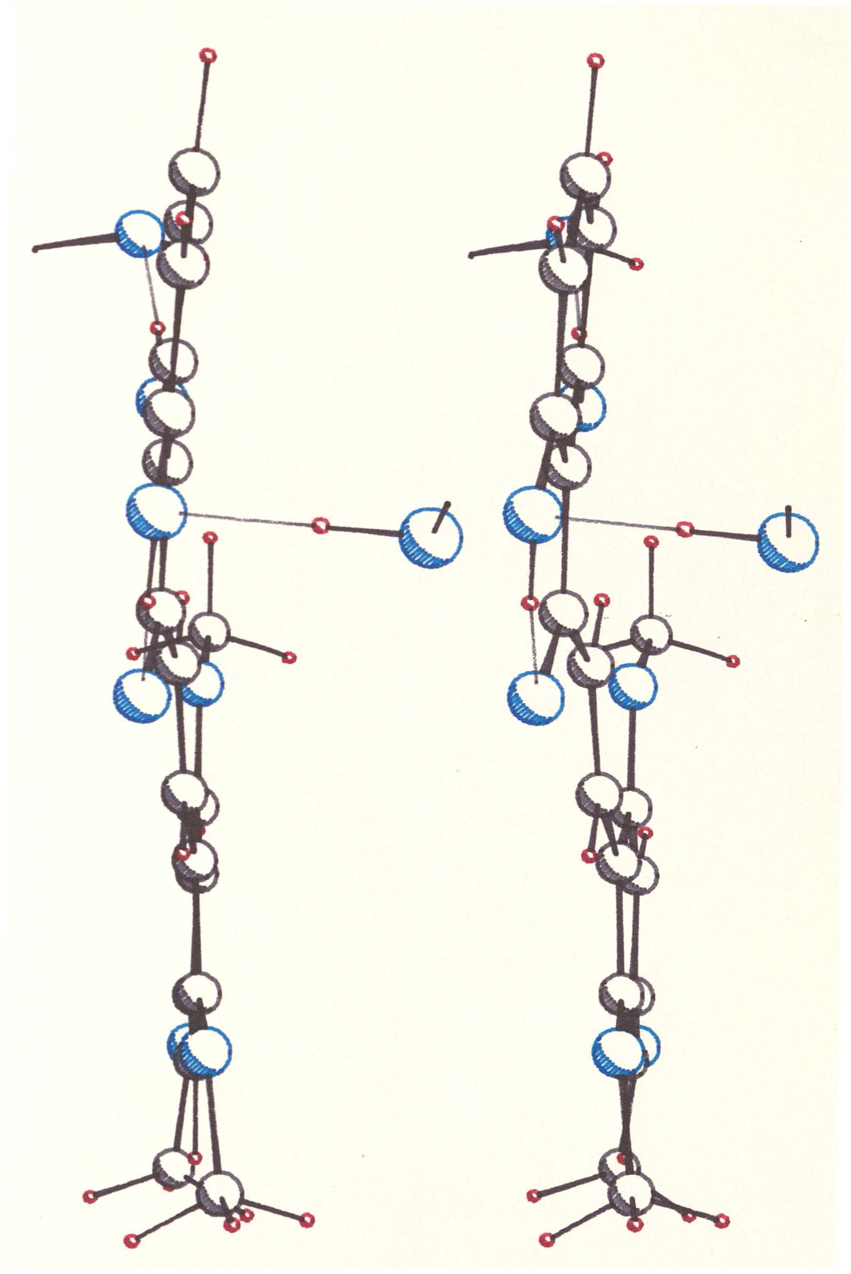


FIGURE 4-3 Stereo View of 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone (*44*) Seen from *ca*. 10 Degrees out of the Molecular Plane

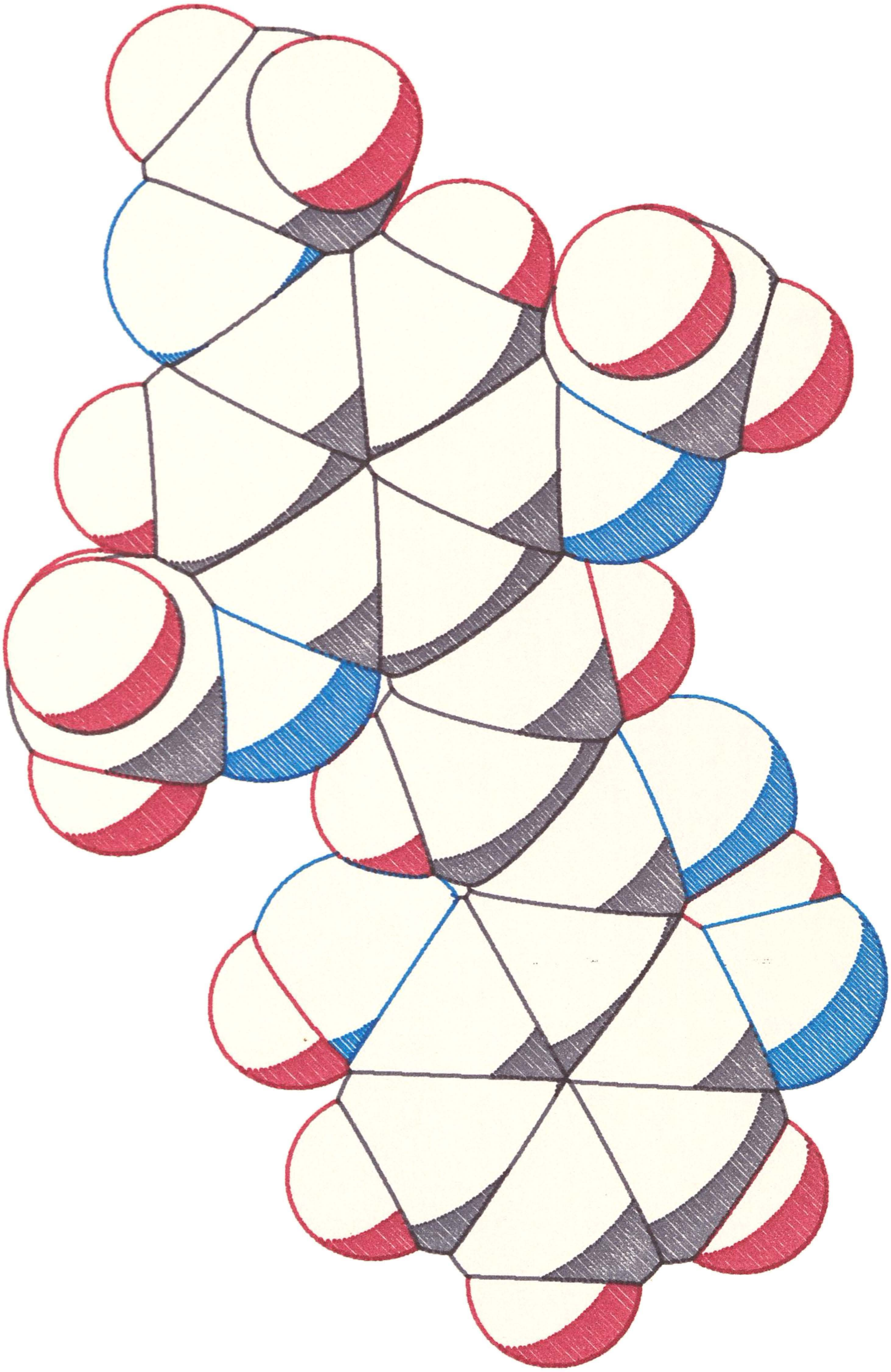


FIGURE 4-4 Space-Filling View of 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone (*44*) Perpendicular to the Molecular Plane: r_O = 1.30 Å; r_C = 1.45 Å; r_H = 1.00 Å

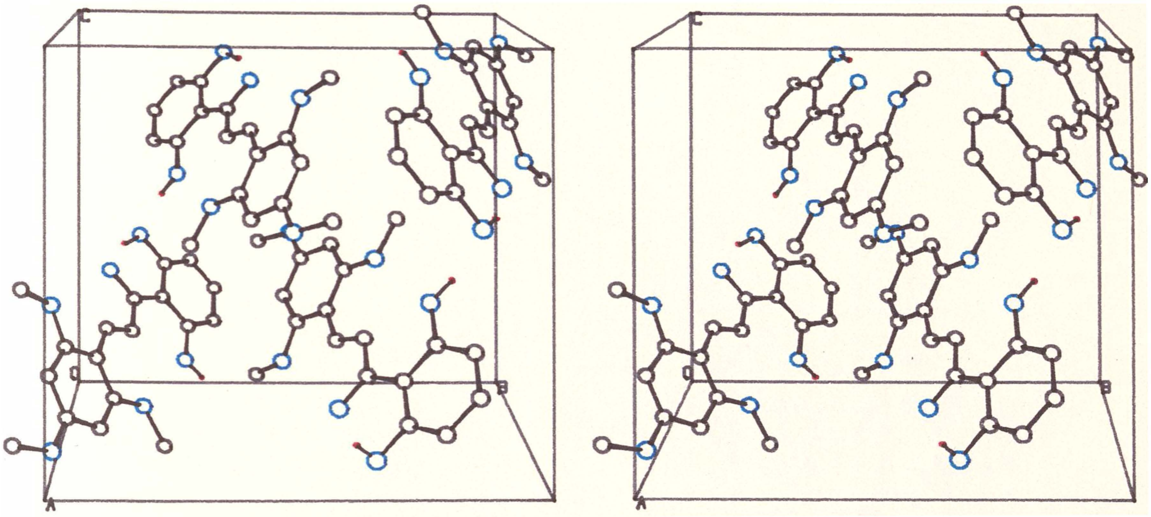


FIGURE 4-5 Stereo View of the Unit Cell of 2′,6′-Dihydroxy-2,4,6-trimethoxychalcone (***44***)

4.3.*1* Data Collection and Structural Refinement for 5-Hydroxy-4′,7-dimethoxyflavanone (***33***)

Suitable crystals were obtained from the procedure described in chapter 7.

Crystal Data.— $C_{17}H_{16}O_5$, M = 300.3. Monoclinic, a = 17.38(2), b = 5.321(4), c = 30.74(3) Å, β = 98.27(7)°, V = 2814 $Å^3$ (from 20 automatically centred reflections, λ = 0.7107 Å), space group *C*2/*c* (No. 15), Z = 8, D_c = 1.42 g cm^{-3}, F(000) = 1264.00. Colourless needles. $\mu(\text{Mo-}K_\alpha)$ = 0.6 cm^{-1}.

Data Collection and Processing.— Nicolet XRD P3 four-circle diffractometer at -100°C, with graphite-monochromated Mo-K_α radiation (0.7107 Å); 2044 reflections were measured, $3.0° \leqslant \theta \leqslant 45°$, 1755 unique, giving 871 with $I > 3\sigma(I)$. Data were corrected for Lorentz and polarisation effects, but not for absorption.

Structure Analysis and Refinement.— Solved by direct methods. Refined by full-matrix least squares, with all atoms except carbons C(4) to C(6) and C(8) to C(10) anisotropic. Hydrogen atoms were included in calculated positions with fixed temperature factors, except for the hydroxy hydrogen atom, which was located in a penultimate difference map and was refined with a fixed temperature factor. The 2-C atom proved to be very anisotropic when refined as a single atom, and it was evident that disorder, involving equal occupation of lattice sites by both enantiomers [which differ in configuration at 2-C] was responsible. Thus, 2-C was finally allowed to refine as two half-weighted atoms, to give two separate positions for 2-C, each corresponding to one enantiomer of the compound. As a result of this, the bond lengths and angles involving 2-C are of low precision. Refinement converged with $R = 0.0674$ and $R_w = 0.0701$, where $w = [\sigma^2(F_o) + 0.0008F_o{}^2]^{-1}$. No parameter shifted by more than 0.1σ in the final refinement cycle, and a final difference map gave no features greater than 0.25 e Å^{-3}. All calculations were performed with SHELX-76.[146]

4.3.2 Discussion

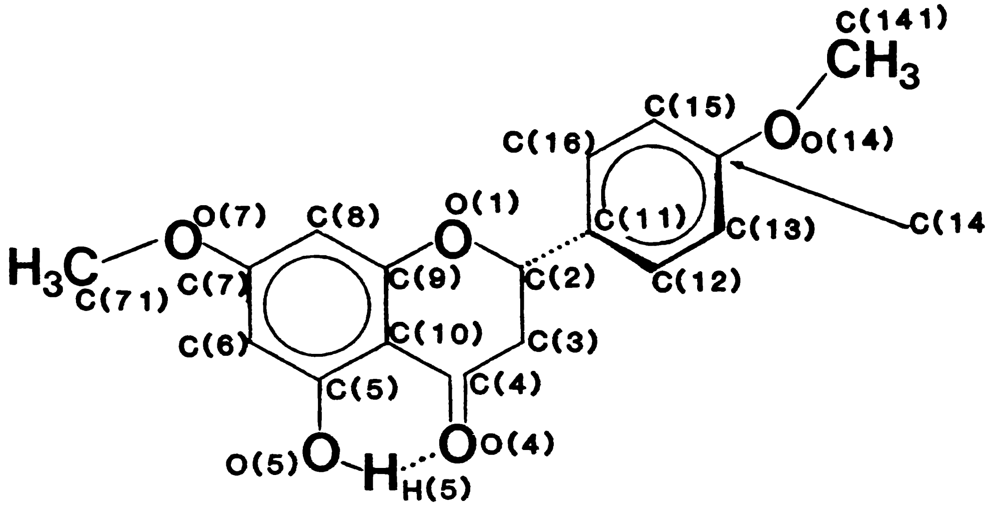


FIGURE 4-6 Crystal Structure Labelling Scheme for 5-Hydroxy-4',7-dimethoxyflavanone (*33*)

Analysis of the structure of 5-hydroxy-4',7-dimethoxyflavanone (*33*) was complicated by the presence of both 2*R*- and 2*S*-isomers, with molecules having either configuration being distributed at random throughout the crystal lattice. The B-ring of the molecule is twisted the same way (and by approximately the same amount) with respect to the rest of the molecule, regardless of which configuration is present at 2-C, presumably as a result of crystal packing effects. The 2-C and B-ring atoms were therefore found at positions averaged for the two isomers, although 2-C was subsequently resolved into two half-weighted atoms [C(2) and C(2)']. Bond lengths and angles involving the B-ring atoms, and especially C(2) and C(2)', are of lower precision than than those in the rest of the molecule.

In other respects, 5-hydroxy-4',7-dimethoxyflavanone is very similar to other flavanones whose structures have been determined[144-147,153] previously. As in the chalcone above, the flavanone contains an intramolecular hydrogen bond. This flavanone is the first 5-hydroxyflavanone in which the position of the 5-hydroxyl hydrogen has been determined, and as expected, its position was consistent with a strong hydrogen bond to the neighbouring carbonyl group. The length of the carbonyl C=O bond is slightly long compared to its analogue[147] which contains a 5-methoxy group (rather than a 5-hydroxy group), 1.252(8) Å compared to 1.224(8) Å, presumably as a result of the hydrogen bond. However the difference between the two bond lengths is not large when compared to their estimated standard deviations, and may not be real. This view is supported by the fact that the C=O bond lengths for all the other flavanones reported in the literature[144-147,153] are close to 1.22 Å, regardless of whether a 5-hydroxy group is present in the molecule or not.

In a similar manner to the chalcone above (***44***), the endocyclic aromatic bond angle at C(10) is rather small, while those *ortho-*, *meta-* and *para-* to it are respectively larger, smaller and larger than 120°. Also the C(5)-C(10) and C(9)-C(10) bonds are marginally longer than the C(6)-C(7) and C(7)-C(8) bonds, which in turn are considerably longer than the C(5)-C(6) and C(8)-C(9) bonds, again an effect attributable to delocalisation of electron density from the O-atoms onto the aromatic ring and the conjugated carbonyl group, in a situation closely paralleling that discussed for the chalcone above (4.2.*2*).

The calculated distances (in Å) out of the plane defined by the carbon atoms of the A-ring are -0.056 for O(1), 0.303 for C(2), -0.604 for C(2)', -0.070 for C(3), -0.012 for C(4) and -0.005 for O(4). Thus, the chromanone ring of this flavanone (***33***) appears to take up the sofa conformation, with O(1), C(3) and C(4) being coplanar with the A-ring. This is exactly the situation that occurs in the 5-methoxy analogue[147] of this flavanone, 4',5,7,-trimethoxyflavanone. Furthermore, the dihedral angle between the two aromatic rings in the case of the present flavanone is 70.8°, which is exactly that reported[147] for 4',5,7-trimethoxyflavanone. All the other flavanones whose crystal structures have been reported[144-146,153] possess similar molecular conformations to that reported here for 5-hydroxy-4',7-dimethoxyflavanone (**33**). It is therefore clear that there exists no major structural difference between 5-hydroxy- and simple flavanones, apart from the presence of an intramolecular hydrogen bond in the former.

TABLE 4-3 Bond Lengths (Å) for 5-hydroxy-4',7-dimethoxyflavanone (**33**)

O(1)	---C(2)	1.38(2)	C(6)	---C(7)	1.391(9)
O(1)	---C(2)'	1.39(1)	C(7)	---O(7)	1.365(8)
O(1)	---C(9)	1.362(7)	C(7)	---C(8)	1.390(9)
C(3)	---C(4)	1.474(9)	O(7)	---C(71)	1.413(8)
C(3)	---C(2)	1.48(2)	C(8)	---C(9)	1.370(9)
C(3)	---C(2)'	1.49(2)	C(9)	---C(10)	1.400(9)
C(4)	---O(4)	1.252(8)	C(11)	---C(12)	1.37(1)
C(4)	---C(10)	1.447(9)	C(11)	---C(16)	1.39(1)
C(2)	---C(11)	1.54(2)	C(12)	---C(13)	1.38(1)
C(2)'	---C(11)	1.56(2)	C(13)	---C(14)	1.38(1)
C(5)	---O(5)	1.361(8)	C(14)	---O(14)	1.363(7)
C(5)	---C(6)	1.372(9)	C(14)	---C(15)	1.37(1)
C(5)	---C(10)	1.405(9)	O(14)	---C(141)	1.429(9)
O(5)	---H(5)	1.09(7)	C(15)	---C(16)	1.42(1)
O(4)	---H(5)	1.61			

TABLE 4-4 Bond Angles (degrees) for 5-hydroxy-4',7-dimethoxyflavanone (*33*)

C(2)	-O(1)	-C(9)	119.2(8)	O(7)	-C(7)	-C(8)	114.6(6)
C(2)'	-O(1)	-C(9)	115.0(7)	C(7)	-O(7)	-C(71)	118.4(6)
C(4)	-C(3)	-C(2)	114.5(8)	C(7)	-C(8)	-C(9)	118.3(7)
C(3)	-C(4)	-O(4)	120.1(7)	O(1)	-C(9)	-C(8)	116.9(7)
C(3)	-C(4)	-C(10)	117.6(7)	O(1)	-C(9)	-C(10)	121.2(7)
O(4)	-C(4)	-C(10)	122.2(7)	C(8)	-C(9)	-C(10)	121.9(7)
O(1)	-C(2)	-C(3)	117(1)	C(4)	-C(10)	-C(5)	122.4(7)
O(1)	-C(2)'	-C(3)	116(1)	C(4)	-C(10)	-C(9)	120.2(7)
O(1)	-C(2)	-C(11)	104(1)	C(5)	-C(10)	-C(9)	117.4(7)
O(1)	-C(2)'	-C(11)	103.9(9)	C(2)	-C(11)	-C(12)	106(1)
C(3)	-C(2)	-C(11)	111(1)	C(2)	-C(11)	-C(16)	135(1)
C(3)	-C(2)'	-C(11)	110(1)	C(12)	-C(11)	-C(16)	117.5(9)
O(5)	-C(5)	-C(6)	118.3(7)	C(11)	-C(12)	-C(13)	121(1)
O(5)	-C(5)	-C(10)	119.3(7)	C(12)	-C(13)	-C(14)	120.1(8)
C(6)	-C(5)	-C(10)	122.4(7)	C(13)	-C(14)	-O(14)	114.9(7)
C(5)	-O(5)	-H(5)	105(3)	C(13)	-C(14)	-C(15)	121.7(7)
C(5)	-C(6)	-C(7)	117.5(7)	O(14)	-C(14)	-C(15)	123.4(8)
C(6)	-C(7)	-O(7)	122.9(6)	C(14)	-O(14)	-C(141)	117.0(6)
C(6)	-C(7)	-C(8)	122.5(7)	C(14)	-C(15)	-C(16)	116.2(9)
O(4)	-H(5)	-O(5)	147	C(11)	-C(16)	-C(15)	123.2(9)

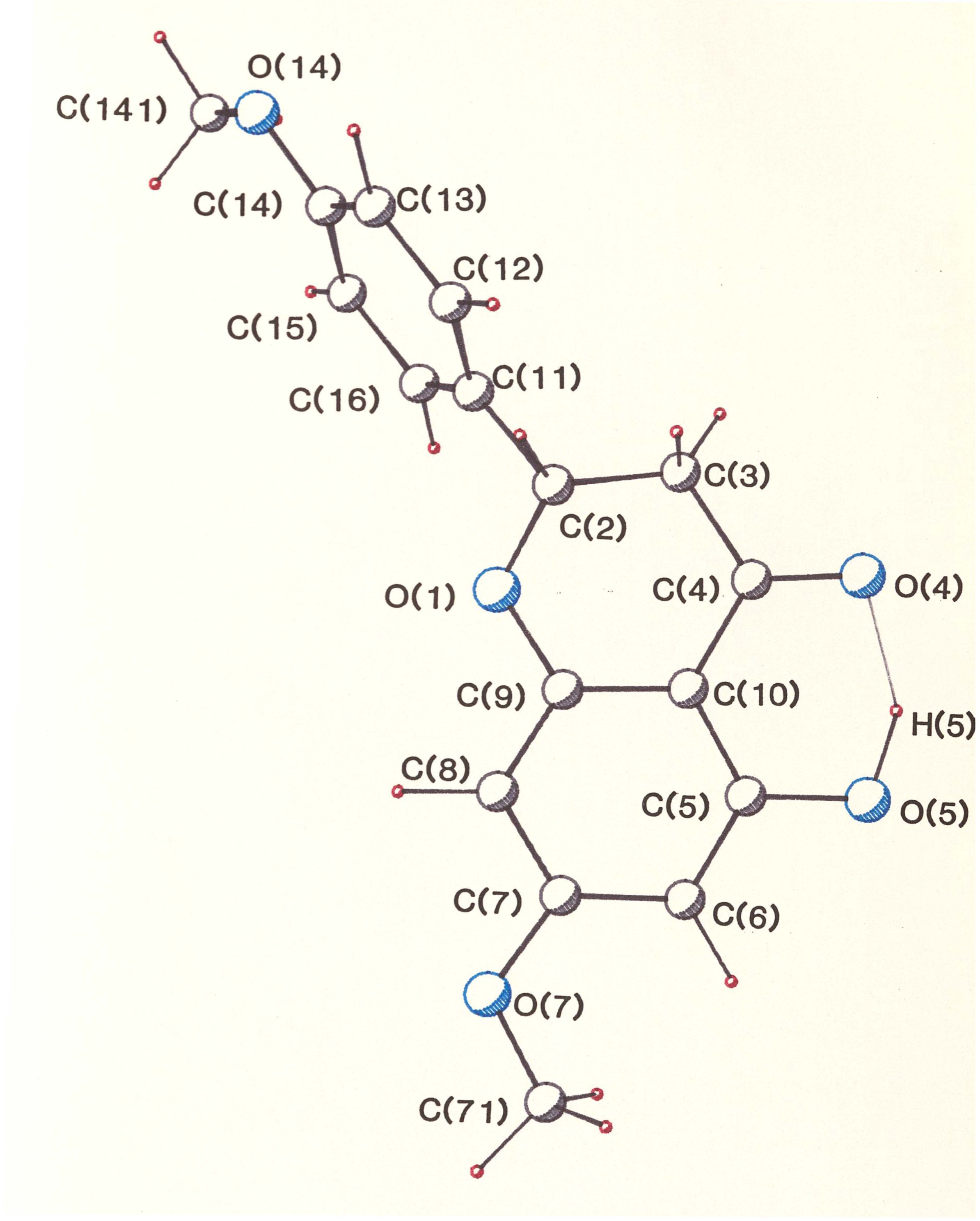


FIGURE 4-7 View of 5-Hydroxy-4′,7-dimethoxyflavanone (***33***) Perpendicular to the Plane of the A-Ring [C(2)′ Not Included for Clarity]

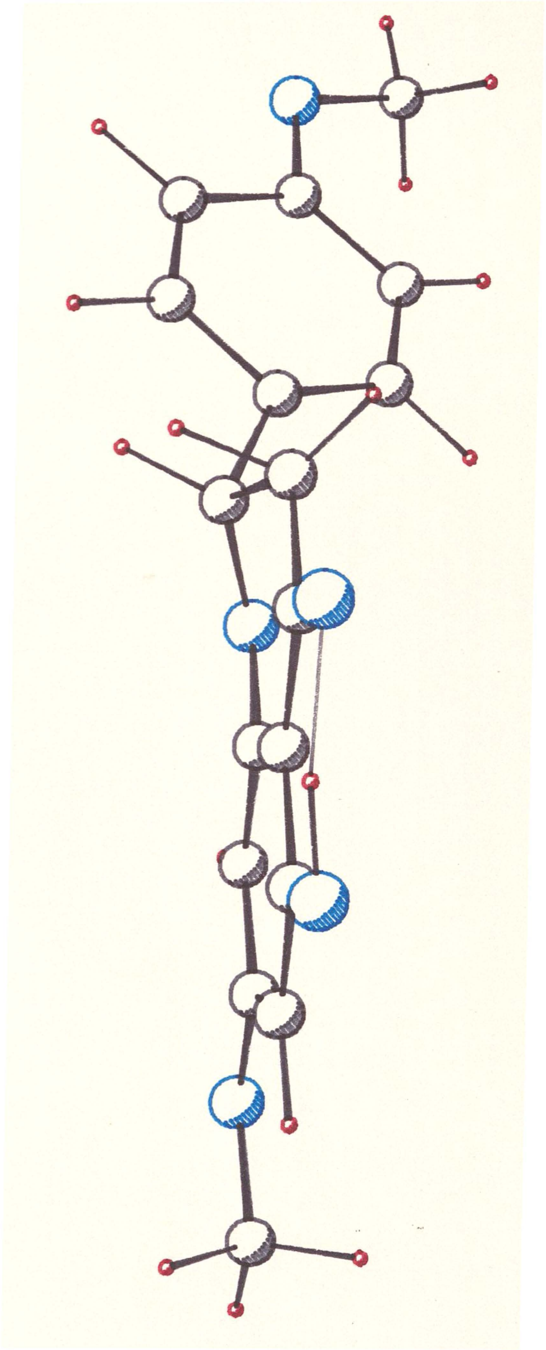


FIGURE 4-8 View of 5-Hydroxy-4',7-dimethoxyflavanone (***33***) from 10 Degrees out of the Plane of the A-Ring [C(2)' Not Included for Clarity]. The B-Ring is Aligned with Neither C(2), nor with C(2)', for the Reasons Outlined in Section 4.3

4.4 Summary

As has been pointed out by other workers,[71,73] 2'-hydroxychalcones cannot cyclise while in their preferred (*trans*-s-*cis*) conformation, since only when the 2'- oxygen atom can approach the β-carbon (*i.e.* in the less favoured *trans*-s-*trans* conformation) can cyclisation take place. Thus, if 2',6'-dihydroxy- substitution were to increase the stability of the *trans*-s-*trans* over the *trans*-s-*cis* configuration, this would provide an explanation for the enhanced susceptibility towards cyclisation of 2',6'-dihydroxychalcones.

The structure of 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***) differs in only minor details from previously examined 2'-hydroxychalcones, suggesting that the higher (than for simple 2'-hydroxychalcones) reactivity towards cyclisation of 2',6'-dihydroxychalcones is not attributable to any change in conformational preference (at least in so much as the solid state conformation is likely to reflect the preferred solution conformation).

Similarly, 5-hydroxy-4',7-dimethoxyflavanone (***33***) is essentially structurally the same as previously[144-147,153] analysed flavanone structures, and suggests no explanation for why 5-hydroxyflavanones are so much more stable than other flavanones with respect to their isomeric chalcones.

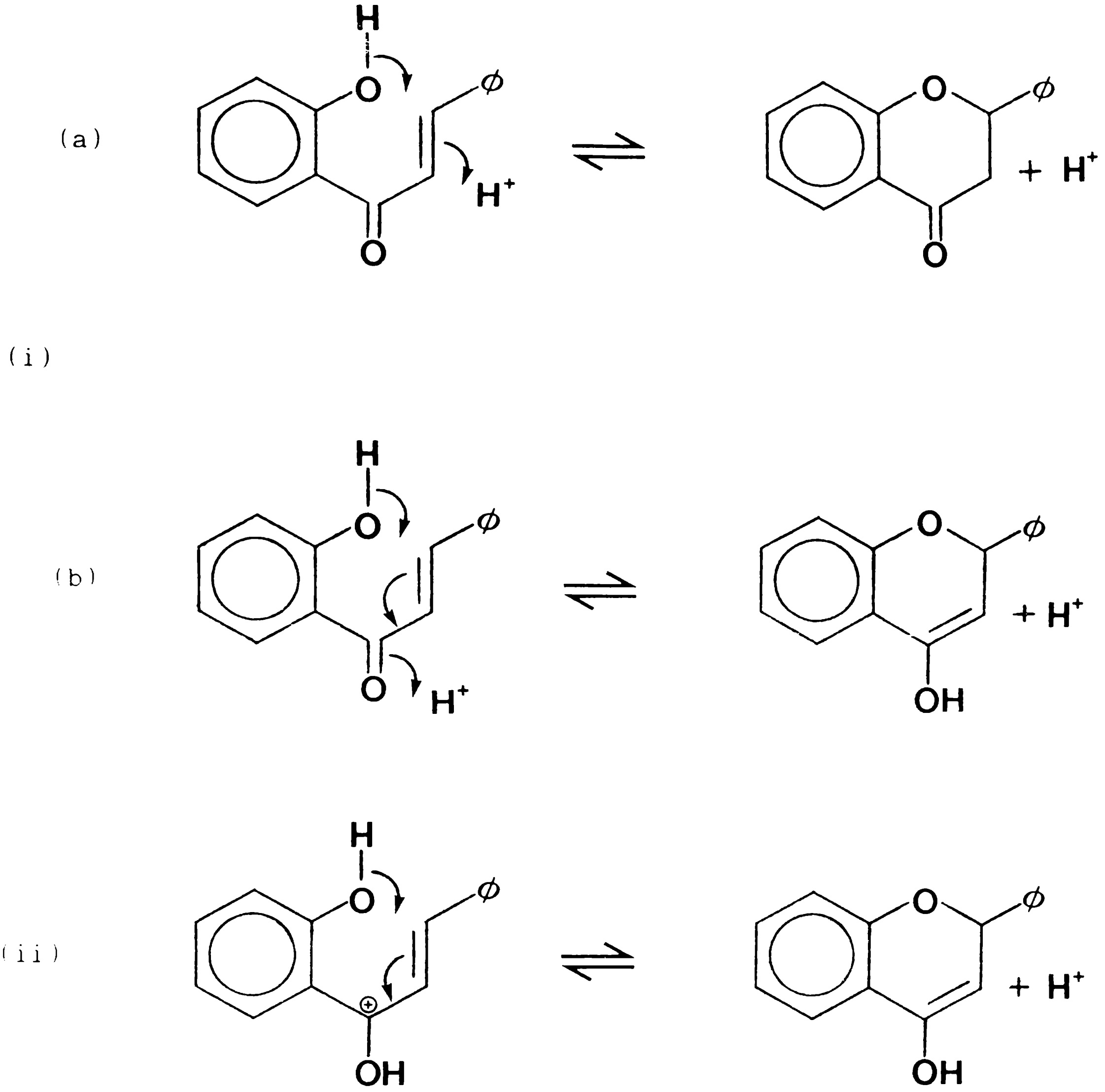
CHAPTER 5

Mechanism of the Chalcone – Flavanone Isomerisation

5.1 The Acid-Catalysed Cyclisation

The kinetic form of the rate equation for the chalcone – flavanone equilibrium at pH < 2 is $k_{obs} = k_{H^+}f_{CHH}[H^+] + k_{CHH}f_{CHH}$, where the symbols have the usual meanings (given in chapter 2). The term involving $[H^+]$ is consistent with the presence of a proton, as well as the un-ionised chalcone, in the rate-determining step. Repetitive scans of the isomerisation reaction at low pH showed clearly that the flavanone isomer is the predominant form present at equilibrium — so the increase in k_{obs} at low pH is due primarily to an increase in the rate coefficient for the chalcone cyclisation, and not to an increase in the rate coefficient for ring opening of the flavanone form. Two types of reaction mechanism (scheme 5-1) can be conceived which are consistent with the kinetic results mentioned above: (i) a concerted bimolecular cyclisation to yield either the flavanone, or its enol (which presumably would rapidly ketonise); or (ii) a pre-equilibrium protonation of the chalcone[74] followed by the cyclisation step.

Chalcones have been shown to be very weak bases. The pK_a of the protonated form of chalcone is reported[154] to be -5.73 in water, while a series of 4-substituted chalcones[155] gave $-3 \geqslant pK_a \geqslant -6$, with $\rho = -2.8$ (or $\rho^+ = -1.56$) in acetic acid – H_2SO_4. It has been demonstrated by ^{13}C n.m.r. spectroscopy that chalcones,[156] as well as other closely related α,β-unsaturated carbonyl compounds,[157] become protonated on the carbonyl oxygen. Thus, in strong acid chalcones

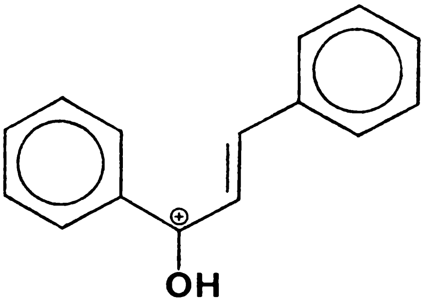


SCHEME 5-1 Possible Mechanisms for the Acid-Catalysed Cyclisation of 2'-Hydroxychalcones *via*: (i) Acid Catalysis of Cyclisation to give; (a) the Flavanone [Concerted (*i.e.* E2-type) Addition]; (b) the Flavanone Enol [Step-Wise (*i.e.* E1cB-type) Addition]. (ii) Cyclisation *via* the Protonated Chalcone

exist in form ***53***, with delocalisation of some of the charge onto the β-carbon *via* the mesomeric form, ***54***.

One notable feature of the acid-catalysed cyclisation reaction is that it is accelerated by electron-donating substituents in the

4-position. The rate coefficient for 2′,6′-dihydroxy-4-methoxychalcone (*38*) is four times that for 2′,6′-dihydroxychalcone (*34*) itself. Unfortunately, these two were the only 3- or 4-monosubstituted chalcones for which k_{H^+} was determined in the present study, so that the most we can say at this stage about the effect of B-ring substituents on the reaction is that ρ may be about -2. In contrast, 4′-substitution appears to have little effect on k_{H^+}, if the values of k_{H^+} for 2′,6′-dihydroxy-4-methoxychalcone (*38*) and 2′,6′-dihydroxy-4,4′-dimethoxychalcone (*32*) are representative. This is a little surprising if the ionisation mechanism of scheme 5-1 (ii) is operating, since the 4′-OMe group would be expected to assist formation of the conjugate acid (*53*), as well as enhancing the cyclisation step. However, the phenolic oxygen atoms in *53* ought also to be less nucleophilic, and it may be that with the 2′- and 6′-OH groups already promoting the protonation, the presence of an additional (4′-OMe) group donating electron density to the carbonyl group does not have much additional effect. Electron donation from the 4-OMe group, on the other hand, competes with electron donation from the 2′- and 6′-OH groups, the lone pair electrons of which should consequently be more available to participate in the cyclisation step. Note, however, that in contrast



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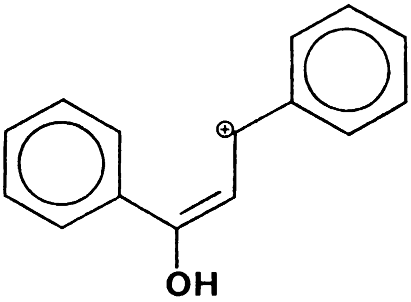

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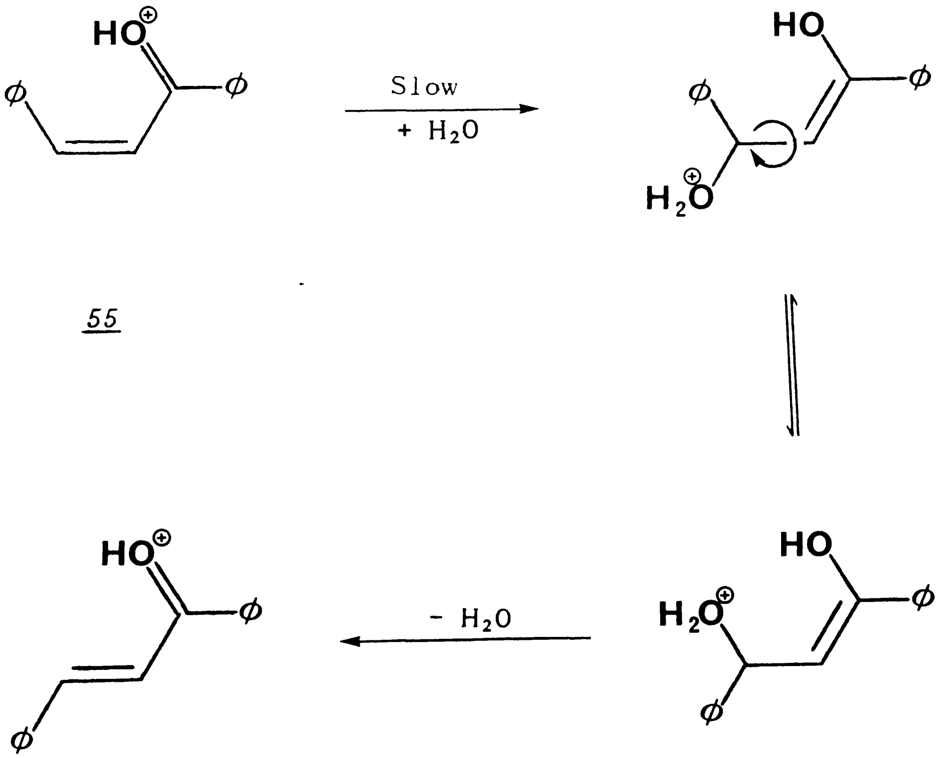
to the previous example, k_{H^+} for 2',6'-dihydroxy-3,4,4'-trimethoxychalcone (***36***) is almost double that for 2',6'-dihydroxy-3,4-dimethoxychalcone (***40***); so the effect of 4'-methoxy substitution on k_{H^+} is not a simple one.

A closely related reaction, the acid-catalysed isomerisation of *cis*-chalcones[158-162] to their *trans* isomers, has been the subject of considerable study. Two mechanisms have been observed to operate in the reaction, both involving the protonated form of the chalcones. The first, and most important, involves[158,160] the slow nucleophilic addition of H_2O to the protonated chalcone, a mechanism analogous to



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SCHEME 5-2 Proposed[158,160] Mechanism for the Water-Mediated Acid-Catalysed *cis* to *trans* Isomerisation of Chalcones the intramolecular addition of phenolic OH that would be expected to occur in the acid-catalysed cyclisation of substituted 2'-hydroxychalcones *via* mechanism (ii) of scheme 5-1.

The other mechanism[160-162] merely involved the slow isomerisation of cation ***55*** to cation ***56***, and occurred mainly where steric hindrance, or the low activity of H_2O reduced the rate of addition of water to ***55***. This mechanism was extremely sensitive to B-ring substituent effects, with $\rho = -7$.

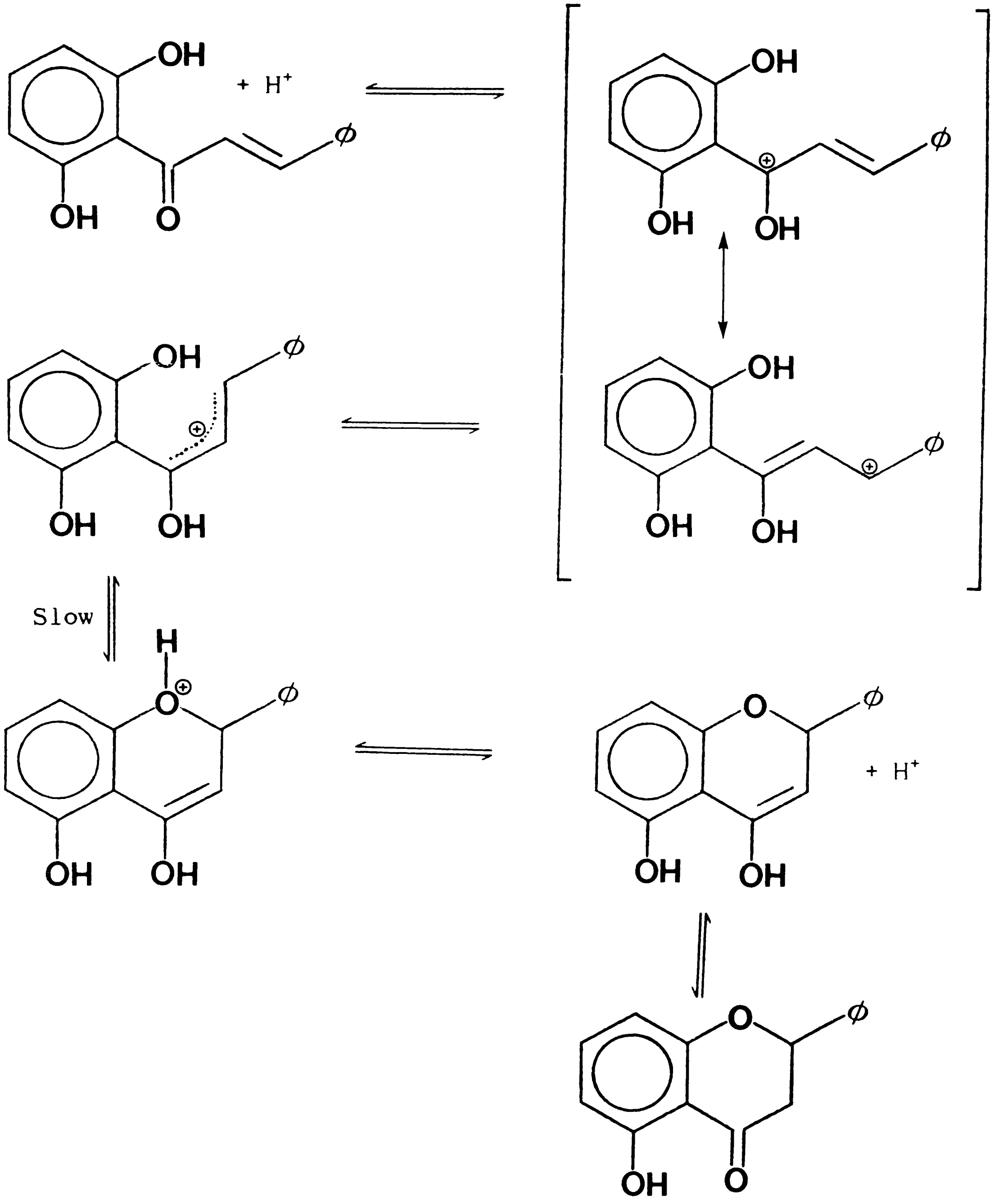
Perhaps the most surprising aspect of the chalcone cyclisation reaction was the extreme susceptibility of 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***) to acid-catalysed cyclisation. The rate coefficient, k_{H^+}, for this chalcone was 380 times that for 2',6'-dihydroxy-4-methoxychalcone (***38***), and 1000 times that for 2',6'-dihydroxy-3,4,5-trimethoxychalcone (***42***). This contrasts with the finding that the mono- and di-anions of this chalcone cyclise respectively about 20 and 4 times *slower* than those of the other B-ring substituted 2',6'-dihydroxychalcones investigated in the present study. However, Noyce and Jorgenson[161] found that *cis*-2,4,6-trimethoxychalcone was subject to unusually rapid acid catalysed isomerisation to its *trans* isomer: the rate coefficient for the reaction (which they assumed to involve the carbocation mechanism) was 3×10^3 times that for the carbocation-type isomerisation of *cis*-4-methoxychalcone, and 3×10^8 times that for *cis*-chalcone. The authors considered that steric effects were not responsible, and that the rate enhancement was an electronic effect. The authors did not, however, provide any evidence as to whether the isomerisation of this chalcone took place *via* the water-assisted or *via* the simple carbocation mechanism, so that this aspect remains open to question.

The origin of the substituent effects on the acid-catalysed cyclisation of 2′,6′-dihydroxychalcones is uncertain. If the pre-equilibrium ionisation mechanism [scheme 5-1 (ii)] applies, then the measured value of k_{H^+} will depend upon both the intrinsic rate constant for cyclisation of the ionised chalcone, and upon the pK_a of the protonated chalcone. Assuming, as seems likely, that the subsequent (possibly also acid-catalysed) ketonisation step is not rate-limiting, then changes in substitution, temperature, solvent and isotopic composition will affect k_{H^+} by affecting both the pK_a and the rate constant for ring closure of the chalcone cation. These two parameters will be affected to different extents, and it will not be possible to fully understand the reaction unless these effects can be separated kinetically. Possibly this could be done by studying the pH-rate profile of the reaction in the region pK_a + 2 > pH > pK_a - 2. Additional information could be obtained by a study of the reverse reaction, which is theoretically possible using a method that will be discussed later in this chapter (section 5.3.6).

If concerted addition of H^+ occurs, then analysis of the factors affecting k_{H^+} is simplified (assuming ketonisation to be rapid, where this occurs), since only the rate constant for cyclisation can be affected. The protonated form of the chalcone would be expected to have a different rate constant for acid-catalysed cyclisation from that of the neutral chalcone, however, so that a pK_a effect should also be observed as the pH is lowered.

The origin of the large effect of 2,4,6-trimethoxy substitution in the B-ring is, therefore, unclear. It is a little hard to explain this effect *via* concerted addition, since steric effects should hinder the approach of phenolic oxygen to the β-carbon and lower the proportion of the chalcone in the reactive s-*trans* conformation; also, electron donating substituents ought to oppose the activating (*i.e.*

electron-withdrawing) effect of the carbonyl group on the double bond. On the other hand, it is easy to see how a two-step protonation/cyclisation mechanism is consistent with the above



SCHEME 5-3 Proposed Mechanism for the Acid-Catalysed Cyclisation of 2',6'-Dihydroxychalcones, *via* their Protonated Forms

results: electron-donating substituents favour the protonation step; and relief of steric strain, as well as electronic effects, should favour the protonation of 2′,6′-dihydroxy-2,4,6-trimethoxychalcone. This chalcone has been shown to be rather sterically crowded in the central enone bridge region in the solid state (section 4-2), even though it is in the s-*cis* conformation. Clearly, an approach to planarity in this molecule will be highly unfavourable in solution, particularly so in the s-*trans* conformation. In the protonated form, however, there is no requirement for near-planarity, and the A- and B-rings are free to take up non-coplanar positions — a factor which may be particularly favourable for this chalcone (for steric reasons), and which may explain the rate enhancement.

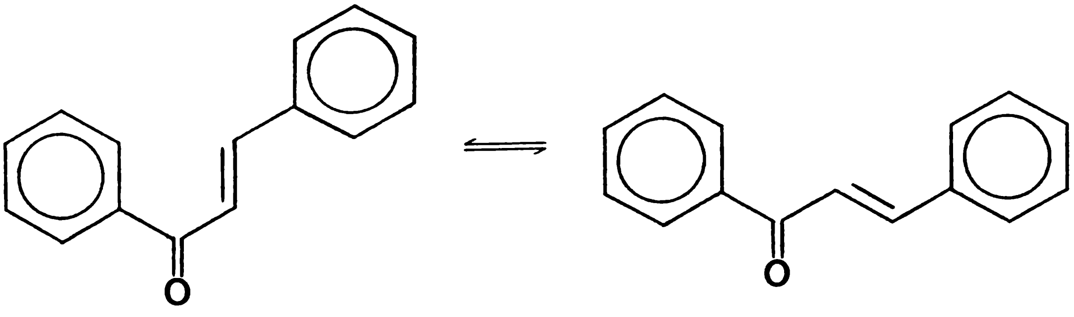
5.2 The Cyclisation of the Neutral Chalcone

The only kinetic information obtained about the neutral chalcone cyclisation in the present study is the effect of substituents on the rate coefficient for the reaction (k_{CHH}): electron-donating substituents appear to increase k_{CHH} (the value of k_{CHH} for the 4-chloro chalcone given in table 5-1 is subject to a high degree of uncertainty — see section 2.9). The addition of a 4- or 4′-methoxy group to a 2′,6′-dihydroxychalcone increases k_{CHH} *ca.* two-fold, although we can see from table 5-2 that the same does not appear to hold true for the simple 2′-hydroxychalcone system. However, the rate coefficients for the neutral 2′-hydroxychalcone cyclisations given in table 5-2 are of low accuracy, due to the method of analysing the pH-rate data,[46,71] so drawing conclusions from these results would be unwarranted. All we can safely say, in comparing the rate coefficients for the cyclisation of un-ionised 2′-hydroxy- and 2′,6′-dihydroxychalcones, is that they are of the same order of magnitude.

With this dearth of kinetic information about the reaction, we can tell little about the mechanism of the reaction. Further investigation is needed to determine if the reaction is a unimolecular cyclisation of the un-ionised chalcone, or (as seems more likely) a bimolecular water-catalysed cyclisation of the un-ionised chalcone.

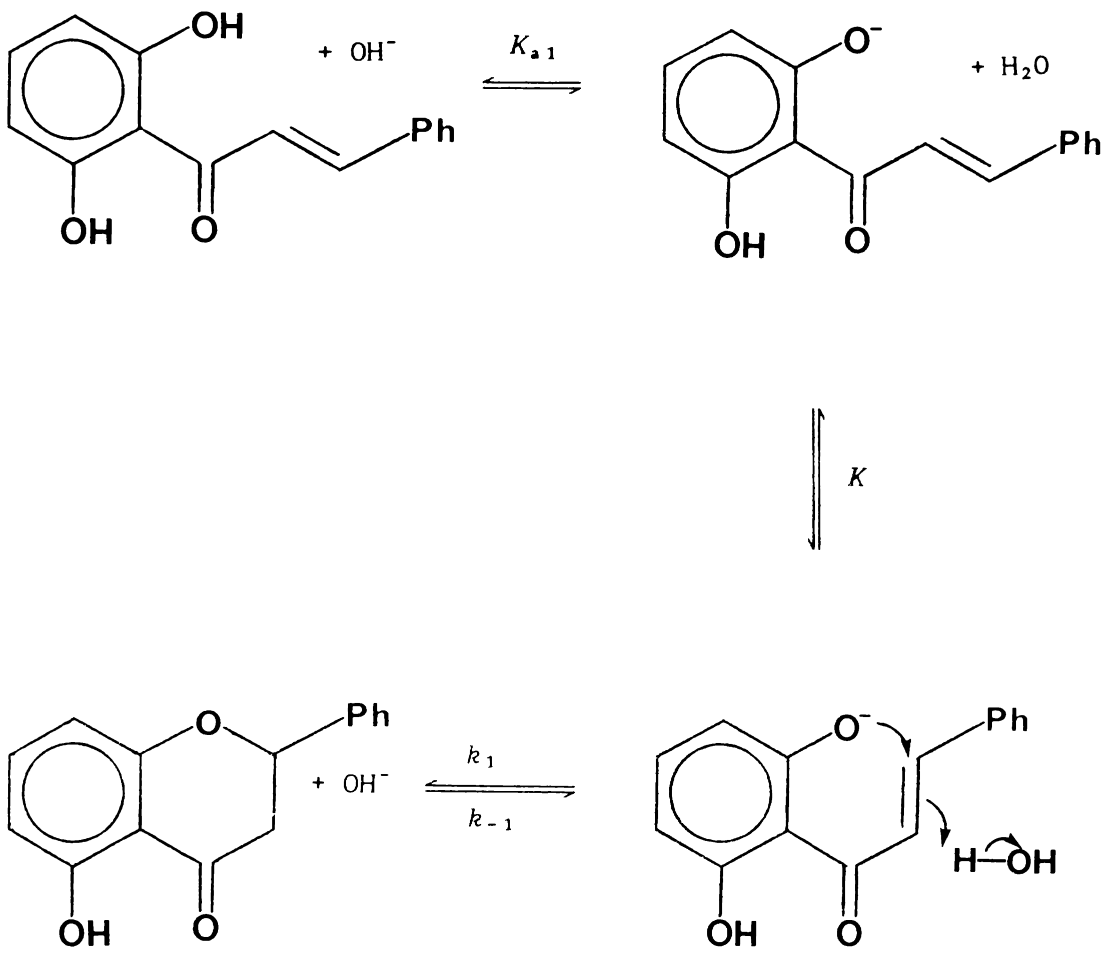
5.3.*1* Cyclisation of the Chalcone Mono-Anion

A number of mechanisms for the cyclisation of substituted 2′-hydroxychalcones in neutral to moderately basic solution have been proposed over the years, as was chronicled in chapter 1. Most of them have been based on grossly inadequate (or sometimes non-existent) kinetic data. Only since 1980 have workers begun to obtain quality kinetic data on the chalcone – flavanone isomerisation, and only two really plausible mechanisms for the base-promoted cyclisation of substituted 2′-hydroxychalcones have been proposed. These are: intramolecular attack of phenoxide oxygen on the β-carbon concerted with addition of a proton to the α-carbon^{72} (scheme 5-4) — the reverse of the well known E2 elimination mechanism; and the step-wise addition of phenoxide oxygen to the β-carbon to afford an enolate intermediate, which then ketonises to the flavanone^{46,130} (scheme 5-5)



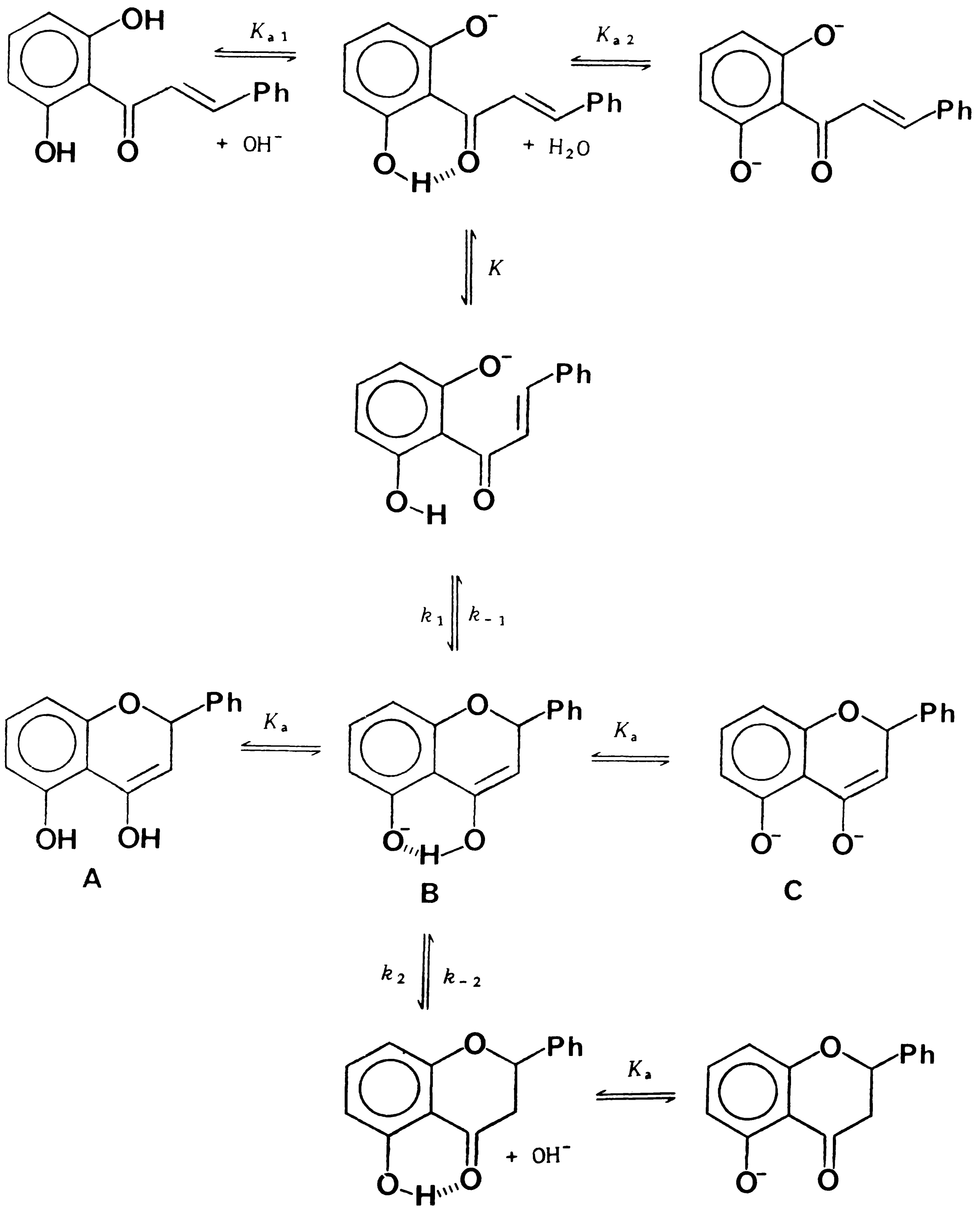
trans-s-*trans* *trans*-s-*cis*

— the reverse of the well known ElcB elimination mechanism.



SCHEME 5-4 Possible Concerted (E2) Mechanism for 2′,6′-Dihydroxychalcone Mono-Anion Isomerisation

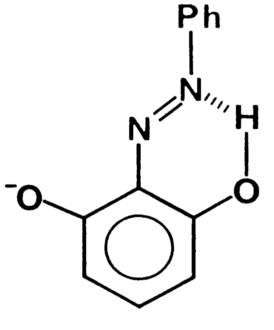
As has been pointed out before,[46,71,72] the cyclisation reaction can only take place when the chalcone is in the *trans*-s-*trans* conformation, where the phenolate oxygen is able to approach the β-carbon. The conformational isomerisation step has, of course, the



SCHEME 5-5 Possible Step-Wise (E1cB) Mechanism for Isomerisation of 2',6'-Dihydroxychalcone Mono-Anions

potential to be rate-limiting. Indeed, it has been proposed[72,73] as the slow step, and as responsible for the plateau in the pH-rate profile for cyclisation of 2',4'-dihydroxychalcone di-anion at high pH. It is unlikely, however, that this is the case, either for 2',6'-dihydroxychalcone mono-anions, or for simple 2'-hydroxychalcone anions, because all the dihydroxychalcone mono-anion cyclisations studied here were subject to considerable (*i.e.* primary) deuterium isotope effects ($k_H/k_D \geqslant 3$), as, apparently, was the cyclisation of the anion of 2'-hydroxychalcone[72] itself. It is difficult to envisage a mechanism for this s-*cis* to s-*trans* isomerisation that involves the making or breaking of bonds to hydrogen (or deuterium), and isotope effects of 3-7 hardly seem likely to be due solely to solvent isotope effects.

The ionisation step, which affords the chalcone anions, has been proposed[73] as rate-limiting at weakly alkaline pH (in the case of the 2',4'-dihydroxychalcone di-anion cyclisation). This is unlikely to be the case for the chalcones in the present study, or indeed for 2'-hydroxychalcones in general; generally, acid-base reactions at oxygen atoms are extremely rapid, so much so that they are usually[163] diffusion-controlled (k = 10^9-10^{10} l mol^{-1} s^{-1}). However the removal of hydrogen bonded protons from oxygen usually[163] occurs at a greatly reduced (albeit rapid) rate: the rate constant for proton removal from salicylate ion (pK_a = 12.13) by hydroxide in water was[163] 2.4x10^7 l mol^{-1} s^{-1}, *via* a two-step process. In cases where the rate constant for the two-step proton removal is smaller, concerted removal of the proton by hydroxide ion may be observed. For example,[164] the rate constant for one-step proton removal by hydroxide ion in water for a series of 1-phenylazo-naphthols was *ca.* 10^6-10^7 l mol^{-1}s^{-1} ($k_{two-step}$ *ca.* 10^4-10^5 l mol^{-1}s^{-1}). The rate constant for one-step proton removal for 2-phenylazoresorcinol (*57*) was[165] similarly 1.03×10^6 ($k_{\text{two-step}} = 0.42\times10^4$) 1 $\text{mol}^{-1}\text{s}^{-1}$, while that for 4-phenylazoresorcinol (*58*) was 0.98×10^6 ($k_{\text{two-step}} = 0.77\times10^4$) 1 $\text{mol}^{-1}\text{s}^{-1}$.



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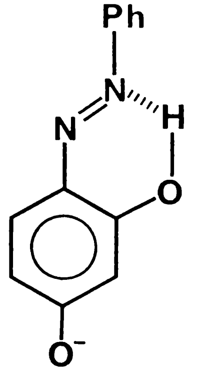

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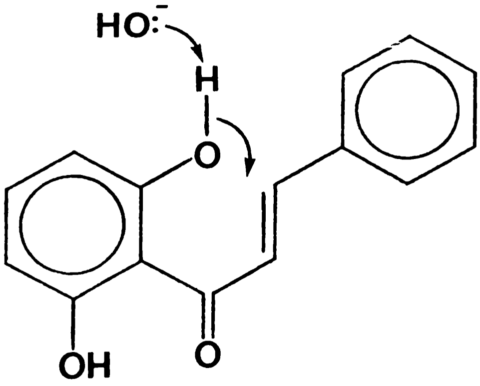
In fact the cyclisation of an ionised chalcone species need not be invoked at all, since the kinetically equivalent mechanism (scheme 5-6) involving attack of OH^- on the phenolic group, which adds to the double bond in a concerted process is also possible, although less likely. Although the ionisation step is likely to be extremely rapid, and hence not rate-limiting, the rate of the reaction will be controlled by the pH of the solution relative to pK_{a1}: k_{obs} depends upon the fraction of the chalcone present in the (reactive) ionised



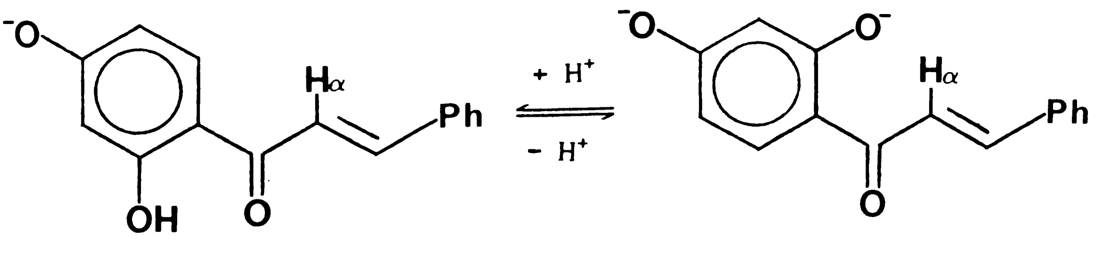
SCHEME 5-6 Possible Mechanism for Cyclisation Without Involvement of an Intermediate Chalcone Anion

form, and it is this that is responsible for the plateau in the pH-rate profile.

Thus, for the E2-type mechanism, the actual cyclisation step (k_1; scheme 5-4) must be rate-limiting, to account for the observed isotope effect. For the E1cB-type mechanism, however, the cyclisation (k_1; scheme 5-5) or the ketonisation (k_2; scheme 5-5) step is potentially rate-limiting. It has been shown[166] that isobutyraldehyde enol has a pK_a of 11.63, and that its rate coefficient for ketonisation increases with increasing pH, as the proportion of the enol in the more reactive enolate form increases, until a plateau is reached where the enol is essentially fully ionised. The form of the resultant pH-rate profile for ketonisation of this enol closely resembles that for the cyclisation of the chalcone anions. It is considered unlikely, however, that the ketonisation step is rate-limiting (and hence responsible for the form of the pH-rate profile) for the cyclisation of 2′-hydroxychalcone anions, since ketonisation of enols in basic solutions is usually rapid. For instance, the rate constant for H_2O-catalysed ketonisation of isobutyraldehyde enolate was[166] 1.18×10^{-1} l mol^{-1} s^{-1} (*i.e.* 6.5 s^{-1} in aqueous solution), which is three orders of magnitude greater than the plateau rate coefficient for cyclisation of the chalcone mono-anions. This argument by no means excludes the possibility that ketonisation is the rate-limiting step in the reaction, since the pre-equilibrium $K_{s\text{-}cis/s\text{-}trans}$ values could mean that only a small fraction of the chalcone mono-anion would be present as the enol species, so that a direct comparison of the ketonisation rate constants of these two reactions is difficult. More convincing evidence, however, comes from the usually excellent fit of the pH-rate data to the theoretical curve (equation 2-19) from pH 3 to 11. At low pH, the fraction of the cyclic product present in form A (see scheme 5-5) would increase at the expense of forms B and C, and since the un-ionised form A is likely to be much less reactive towards ketonisation than the ionised forms (B and C) (isobutyraldehyde enol was *ca*. 4×10^8 times less reactive towards ketonisation than was the corresponding enolate), a rate depression would be anticipated. Prediction of the pK_a of form A is difficult, but it is hardly likely to be so low as to lie below the pH range (3-9) over which the ionisation-associated increase in k_{obs} is observed. The observed fit of the experimental data therefore suggests that the $A \leftrightharpoons B$ equilibrium does not affect k_{obs}, and therefore implies that the ketonisation step is not rate-limiting. Thus, if the cyclisation of the chalcone mono-anions takes place *via* an E1cB-type mechanism, then it is probable that it is the cyclisation step (k_1; scheme 5-5) that is rate-limiting.

5.3.2 The Effect of Substituents on the Chalcone Mono-Anion Cyclisation

A glance at tables 5-1 and 5-2 shows that the rate coefficients for the cyclisation of 2',6'-dihydroxychalcone mono-anions are generally higher than those for comparable 2'-hydroxychalcone anions. For instance, 2',6'-dihydroxychalcone mono-anion cyclises with a rate coefficient 40 times that for the 2'-hydroxy-6'-methoxychalcone anion, and 8 times that for the electronically similar 2'-hydroxy-4'-methoxychalcone anion. The comparison with 2'-hydroxy-6'-methoxychalcone anion is not, unfortunately, a good one, since steric factors (interaction of the carbonyl group with the 6'-methoxy group) could conceivably contribute to a reduced rate coefficient for its cyclisation. The apparently higher rate coefficient for the cyclisation of the 2',6'-dihydroxychalcone mono-anion occurs despite the lower nucleophilic power of its phenolate oxygen, as compared to that of the 2'-hydroxy-6'-methoxy- and 2'-hydroxy-4'-methoxy-chalcone anions, which is reflected in their[46,71] respective pK_a values (which are 7.60, 8.95 and 9.55). Presumably, some kind of special effect due to the presence of the 6'-hydroxyl group, is responsible for this apparent rate enhancement. One possibility is that the hydrogen bond (which may be present between the 6'-hydroxy group and the carbonyl oxygen) increases the activation of the double bond towards nucleophilic attack, by withdrawing electron density from the carbonyl group. It was noted in chapter 4 that the C=O bond length for chalcones containing an intramolecular hydrogen bond was a little longer (*i.e.* the C=O bond order is lower) than for those chalcones lacking such a bond. If the cyclisation mechanism is of the E1cB-type, then the possibility exists for the 6'-hydroxy group to provide intramolecular general acid catalysis of the rate-limiting cyclisation step. This possibility has been discussed in a previous publication,[130] and will be discussed at length later in this chapter. It is also possible that the intramolecular hydrogen bond tends to hold the reacting 2'-O⁻ group in a position that is spatially more favourable for cyclisation than would normally be achieved by random



59

60

rotation of the A-ring around the C-1′—C=0 bond. However, even an ordinary 2′-hydroxychalcone anion ought to loosely take up a similar (to *60*) conformation, due to repulsion between the 2′-O^- group and the carbonyl oxygen when the two are brought into close proximity.

Forlong *et al.*[72] claim to have found evidence that the conformation of the ionised A-ring plays an important role in the cyclisation of 2′-hydroxychalcone mono-anions in solution. They state that upon ionisation, the chalcone more or less locks into the conformation shown in *60*. This claim is based upon ^{1}H n.m.r. observations of the α- and β-protons: in *59* they usually appear as a multiplet, partially obscured by the aryl protons; while in *60*, two distinct doublets (J = 15 Hz) are observed. The α-proton, it was said, resonated at lower field than the β-proton, due to the through-space interaction of 2′-O^- with α-H, as shown in *60*. This interpretation is at odds with the same authors′ data for 2′-hydroxy-4-nitrochalcone and its anion, where α-H and β-H appeared as 15 Hz doublets in both forms *59* and *60*, and in both cases the resonance at lower field was assigned to β-H. The ^{1}H n.m.r. studies of 2′-hydroxy-6′-methoxychalcones reported herein (chapter 7) suggested a more likely alternative explanation for this phenomenon: in $CDCl_3$, the spectra of the 2′-hydroxy-6′-methoxychalcones gave the α- and β-protons as a single slightly broadened peak, or as a single barely resolved doublet (actually the most intense peaks of a pair of doublets of a second-order AB system); the spectra of the same compounds in $(CD_3)_2CO$, in most cases, showed the α- and β-protons as a clearly recognizable, albeit second-order, pair of doublets. What the change in the solvent did was to increase the the separation of $\delta_{\alpha\text{-H}}$ and $\delta_{\beta\text{-H}}$ by *ca.* 10 Hz (*ca.* 0.1 ppm on a 90 MHz ^{1}H n.m.r. spectrum), with the result that the α-H/β-H system changed from a highly second-order AB system into one in which α-H and β-H, although still second-order, constituted an easily recognizable AB system. Clearly then, if merely changing the solvent from $CDCl_3$ to $(CD_2)_2CO$ increased the separation of $\delta_{\alpha-H}$ from $\delta_{\beta-H}$ sufficiently to produce an easily recognizable AB pair of doublets, then the ionisation of the 2'-hydroxy group might have a similar effect.

The introduction of a 4'-methoxy group into a 2'-hydroxychalcone system results in a decrease in the rate coefficient for cyclisation of the chalcone anion: in going from 2',6'-dihydroxy-4-methoxychalcone to 2',6'-dihydroxy-4,4'-dimethoxychalcone, the value of k_{CH^-} drops 4.8 fold, while in the simpler system, 2'-hydroxychalcone cyclises with k_{C^-} 2.8 times that for 2'-hydroxy-4'-methoxychalcone. The introduction of a 4'-methoxy group ought to have the effect of increasing the nucleophilic power of the chalcone (mono-) anion (this is reflected in the pK_a values of the above-mentioned 2',6'-dihydroxychalcones — respectively 11.7 and 12.3 — but not in the pK_a values of the 2'-hydroxychalcones, which were both 9.55), and so might be expected to increase the rate of cyclisation. Previous work, cited in chapter 1, has demonstrated the importance of the nucleophilic strength of the attacking nucleophile in intramolecular additions to activated double bonds. In the chalcone system, however, an additional effect is present that is not possible in intramolecular additions: any effect that increases the nucleophilic power of the 2'-O^- group will also decrease the ability of the carbonyl group to activate the double bond (by its electron withdrawing effect) towards nucleophilic addition. It would appear, then, that this second effect is stronger than the first. The situation is, however, far from clear-cut, and other factors would appear to be involved in the effect — since, for example, the 2',4'-dihydroxychalcone di-anion cyclises

1.25 times *faster* than does the 2′-hydroxy-4′-methoxychalcone anion, which is the reverse of what might be expected from the above analysis. Also, the importance of substituent effects on the equilibrium constant $K_{s\text{-}cis/s\text{-}trans}$ (which will in turn affect the overall rate constant for cyclisation) should not be forgotten, although the direction and magnitude of such effects would be difficult to estimate.

One of the most striking features of the 2′,6′-dihydroxychalcone mono-anion cyclisation is the extremely low sensitivity of the reaction to substitution in the B-ring: 2′,6′-dihydroxychalcones with B-rings substituted 4-H, 4-OCH_3, 4-Cl, 3,4-diOCH_3 and 3,4,5-triOCH_3 gave an average value for k_{CH^-} of 2.08×10^{-2} s^{-1}, with σ_{n-1} = 0.03×10^{-2} s^{-1} (*i.e.* a standard deviation of only 1.5% !). This amounts to a complete lack of B-ring substituent effects, which is not easily accounted for by either the E2- or E1cB-type mechanisms (whether or not cyclisation is rate-limiting). In contrast we note that Kamlet and Glover[115] observed a substantial substituent effect (ρ = 0.8) on the nucleophilic addition to β-nitrostyrenes substituted on the phenyl group, a reaction which was considered to take place *via* an E1cB-type mechanism, and Toma[111] found the addition of ethyl α-cyanobutyrate to chalcones to be subject to a B-ring substituent effect of ρ_B = 1.1. Although this lack of a B-ring substituent effect would not be so surprising if ketonisation were rate-limiting, since the reactive centre in the transition state would not be conjugated to the B-ring, the B-ring substituents would be expected to affect the preceding equilibria, which in turn play their part in determining the overall rate coefficient for the reaction. In any case, in view of the earlier discussion (section 5.3.*1*), we believe that a ketonisation step is unlikely to be rate-limiting. What we can say about the reaction is that there is no great difference in electron density at the β-carbon between the ground and transition states. Possibly this is due to the effects of the attacking nucleophile on electron density at the β-carbon being cancelled out by changes in bonding in the transition state. An alternative explanation is that there is a late transition state, where development of the negative charge takes place principally on the α-carbon and/or the carbonyl oxygen.

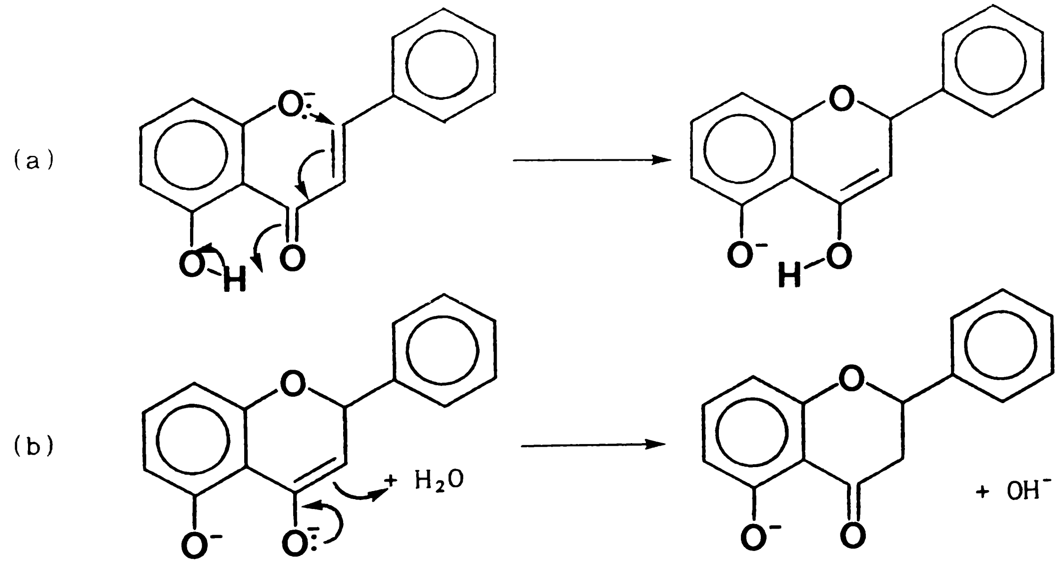
It is not possible to come to any firm conclusions about the nature of the transition state from the above results. It would be of considerable help if data from a series of B-ring substituted 2'-hydroxychalcones were available, in order to see if a similar pattern of reactivity emerged in this system. The only such results of any sort are the rather crude data of Panadam,[129] which suggest that B-ring substituent effects are quite minor ($\rho \approx 0.2$). The situation for the chalcones of the present study was made even more uncertain with the observation of a B-ring substituent effect on the chalcone mono-anion cyclisation when it was performed in deuterium oxide solution. This aspect will be discussed later, in the section (5.3.3) dealing with kinetic isotope effects on this reaction.

For two of the chalcones studied, the B-ring substituents did affect the rate of the chalcone anion cyclisation. The cyclisation of 2',6'-dihydroxy-2,4,6-trimethoxychalcone mono-anion was *ca.* 20 times slower than that of most of the other 2',6'-dihydroxychalcone mono-anions. This result was not unexpected, as even the rather poor data of Panadam[129] clearly indicated that the 2'-hydroxy-2,4,6-trimethoxychalcone anion cyclised much more slowly under weakly basic conditions than did the other 2'-hydroxychalcones in her study. Given the absence of electronic B-ring substituent effects in the other chalcones of the present study, this rate retardation may well be the result of steric effects: the crystal structure of this chalcone (section 4.2) clearly shows the central enone region of the molecule to be sterically congested. In the reactive s-*trans* conformation this steric crowding will be even more pronounced, and it is possible that either the pre-equilibrium s-*cis* to s-*trans* isomerisation equilibration favours the s-*cis* form more strongly than was the case for the other chalcones, or that the approach of the phenoxide oxygen to the β-carbon is hindered in the s-*trans* form. It may also be that this molecule prefers a non-planar conformation in solution, so as to minimise the steric interactions, and as a result is less reactive than normal. Alternatively, the combination of the powerful electronic and/or steric effects may have resulted in a change in the rate-limiting step (or even in a change in mechanism).

That the reaction is not entirely immune to purely electronic B-ring substituent effects was apparent from the analysis of the pH-rate profile of naringin chalcone. In this case, the 2'-O^- anion of naringin chalcone cyclised 9.4 times faster than did the 2',4-di-O^- di-anion. This rate retardation could be due to destabilisation of the transition state; or could alternatively be due to other effects, such as destabilisation of the s-*trans* conformation as a result of electrostatic effects.

5.3.3 Cyclisation in Deuterium Oxide

As was mentioned earlier, in all cases where cyclisation of the 2',6'-dihydroxychalcones was carried out in deuterium oxide, a substantial kinetic isotope was observed. The value of k_H/k_D varied from 2.9 to 7.5, and indicates a primary isotope effect such that the making or breaking of a bond to hydrogen (or deuterium) takes place in the transition state of the rate-determining step. A primary isotope effect of this magnitude suggests that the proton being transferred in the transition state is strongly bonded to neither the proton donor, nor the acceptor. It is obvious where the source of such an isotope effect must lie if the mechanism of the reaction is of the E2-type, since by definition the transfer of the proton is concerted with the cyclisation step in the transition state. If an ElcB-type mechanism is present, though, the situation is more complex: if the cyclisation step is rate-limiting, then the only way a primary kinetic isotope effect can be observed is if a proton is transferred in the rate-determining step — for instance if general acid catalysis takes place, as shown in scheme 5-7(a); alternatively, if the ketonisation step is rate limiting, then a primary isotope effect is to be expected, since a proton is transferred in the transition state for this reaction (scheme 5-7(b)). In the earlier discussion (section 5.3.1), however, it was noted that rate-limiting ketonisation appeared



SCHEME 5-7 Potential Sources of Isotope Effects for an ElcB-Type Mechanism: (a) Rate-Limiting Cyclisation; (b) Rate-Limiting Ketonisation

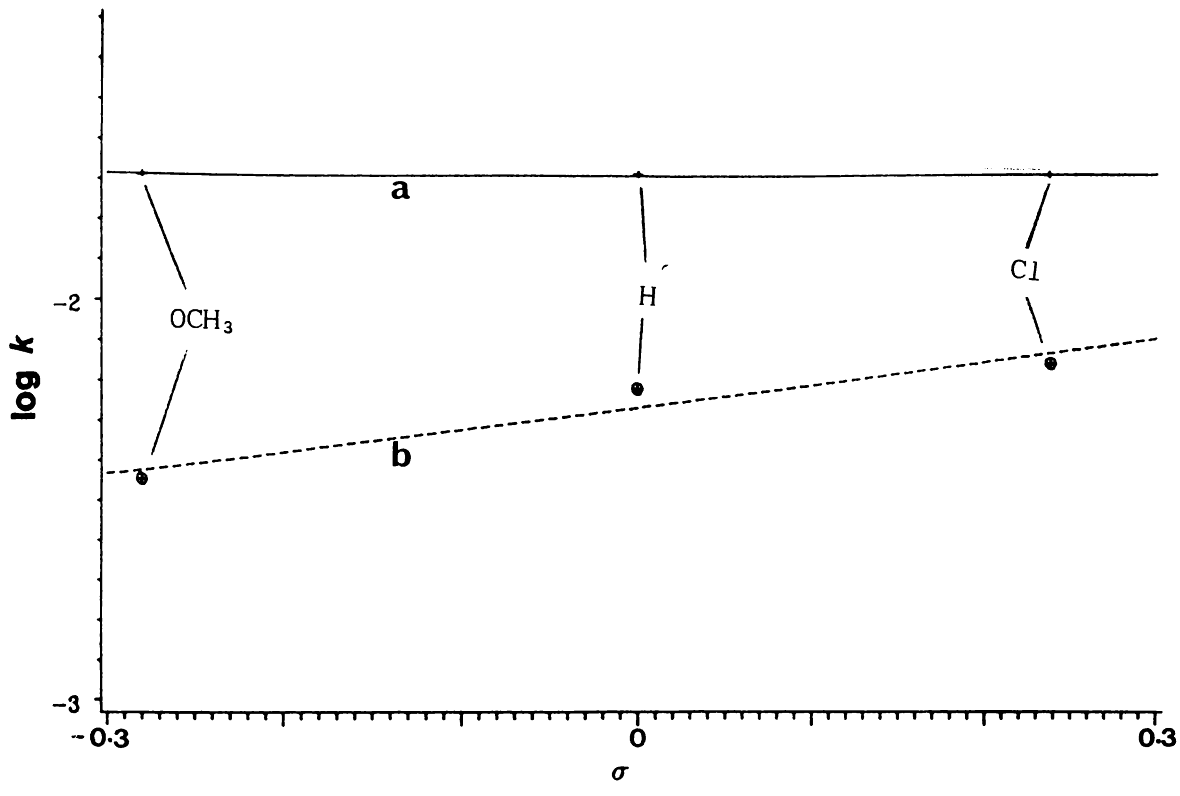


FIGURE 5-1 Logarithmic Plot of the Rate Coefficient for Cyclisation of 2',6'-Dihydroxychalcone Mono-Anions Versus their Hammett Substituent Constants:[167] (a) in H_2O; (b) in D_2O

to be inconsistent with the pH-rate profile.

Perhaps the most surprising finding about the reaction in D_2O, in view of the fact that the same reaction in H_2O was virtually independent of the B-ring substitution pattern, was that the value of the rate coefficient depends on the B-ring substitution pattern (see ~~figure 5-1).~~ Initially it was considered possible that, due to the limited number of measurements at different pDs, the value of k_{obs} obtained was not properly representative of the plateau value for the cyclisation. This uncertainty cannot, however, account for these results, since k_{obs} was determined at pDs of 10.37 and 10.96 for the 4-chloro-, 4-protio- and 4-methoxy-substituted 2',6'-dihydroxychalcones, and in each case the value of k_{obs} at the higher pD was close to 90% of that at the lower pD value. This suggests that we are looking at the high pD end of the pD-rate plateau, where the second (rate reducing) ionisation is just starting to become significant. In any event, the fact that k_{obs} at pD 10.37 for the 4-chloro compound is twice that of the 4-methoxy compound at the same pD (both would be expected to have similar pK_a values in D_2O, since their pK_a values are identical in H_2O) clearly indicates a B-ring substituent effect to be present. A Hammett plot for the reaction (figure 5-1*b*) gives $\rho \approx 0.6$ (the value is only approximate, due to the uncertainties involved in estimating such a parameter from just three data points, and the degree of correlation of these points with a straight line).

It is difficult to account for this effect: possibly performing the reaction in D_2O slows a step that is normally not rate-limiting in H_2O to an extent where another step, which *is* sensitive to B-ring substituents, becomes rate-limiting. For example, if ketonisation were rate-limiting in H_2O, performing the reaction in D_2O might slow the (normally) pre-equilibrium nucleophilic addition step to such an extent that this step became the rate-limiting one. Alternatively, the presence of a deuteron in the chalcone mono-anion (*e.g.*[130] in the intramolecularly bonded 6'- position), or a solvation effect, could alter the sensitivity of the position of the pre-equilibrium s-*cis* to s-*trans* isomerisation to B-ring substituents. In any case, the origin of the effect is uncertain and intriguing, and invites further investigation.

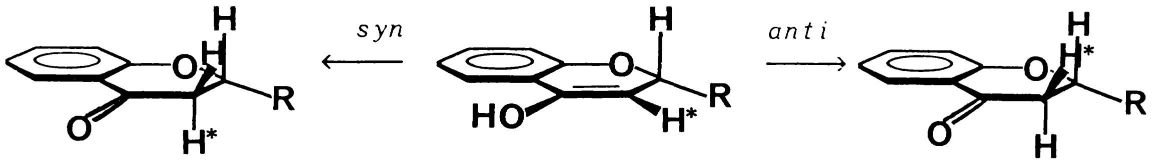
5.3.4 Product Isotope Effect

The work with p.i.e.s (in section 3.1) showed that for 2',6'-dihydroxy-4-methoxychalcone, deuterium was incorporated into the flavanone (during the chalcone mono-anion cyclisation process) 8.2 (σ = 0.7) times slower than was hydrogen, while the kinetic isotope effect for the same reaction was k_H/k_D = 5.6. On the other hand, for 2′,6′-dihydroxy-4,4′-dimethoxychalcone the p.i.e. was 3.8 (σ = 0.3) for the chalcone mono-anion cyclisation, while the k.i.e. for the same reaction was 5.7.

Because the p.i.e.s for the mono-anion cyclisation reaction are quite different from the k.i.e.s for the same reaction, we can say that the transfer of a proton to the α-C of the chalcone is not solely responsible for the k.i.e. (since if it were, the k.i.e. and the p.i.e. would be the same), and therefore a purely E2-type mechanism can be ruled out. The results do not, however, rule out an E2-type mechanism with large solvent isotope effects on its pre-equilibrium steps (*e.g.* the s-*cis* to s-*trans* equilibrium). Nor do they rule out an E2-type mechanism with some E1cB character, encompassing partial proton transfer from the 6′-OH or the solvent (to the carbonyl group), in the transition state. It could be worthwhile to investigate the manner in which the p.i.e. for this reaction varies with substitution of the chalcone, since, depending on the mechanism, the substituents may affect the p.i.e. and the k.i.e. in different ways. It should be noted that if the cyclisation of the chalcone mono-anions in deuterium oxide occurs *via* a different mechanism or rate-limiting step from that in water, then comparisons of the k.i.e. and the p.i.e. are meaningless, and in addition, the p.i.e. would probably be dependent upon $[D_2O]/[H_2O]$ (since the fraction of the product formed *via* the two different (H- and D-mediated) pathways would be dependent on the relative activities of H and D).

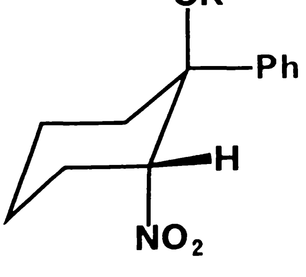
5.3.5 The Stereochemistry of the Cyclisation Reaction

The stereochemistry of the addition of deuterium to the chalcone double bond during the mono-anion cyclisation reaction was investigated (section 3.2). It was found that the addition takes place with a very low degree of specificity. This is suggestive of a reaction taking place *via* an intermediate which has a rather low steric demand for the addition of the deuteron, and is consistent with the ketonisation step of an ElcB-type cyclisation mechanism (see scheme 5-8) in which the intermediate would be expected to have some

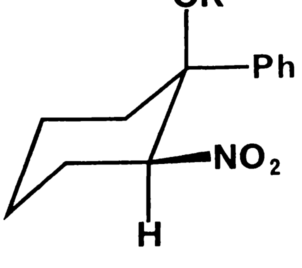


SCHEME 5-8 The Stereochemical Consequences of Ketonisation for an Enolic Intermediate in an ElcB-type Cyclisation (R = C_6H_5)

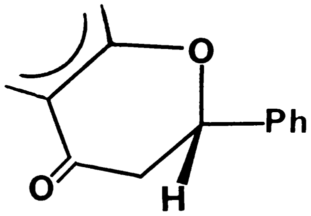
steric demand during protonation due to the presence of the adjacent B-ring. It should be noted that in both the chalcones studied, the (albeit slightly) preferred mode of addition involved proton addition to the sterically least hindered face (see figure 5-8). In no way, however, can this experiment be regarded as definitive evidence for stepwise rather than concerted addition; concerted elimination reactions occur with varying degrees of preference for *anti* over *syn* elimination, and it is quite possible for *syn* and *anti* eliminations of a concerted process to proceed at similar rates. The chalcone - flavanone isomerisation does possess factors[168] that usually favour an E1cB (over an E2) mechanism (a relatively poor leaving group, a strong activating group that causes the protons α to it to be relatively acidic, and a "relatively unstable enone form"), while possessing none of the factors commonly regarded as favouring *syn* (over *anti*) E2 elimination[169,170] (*e.g.* ion-pairing media, powerful steric effects, the presence of very strong bases). Groups that activate (*i.e.* draw electron density away from) the cleaving C-H bond are known[168,170] to decrease the usual preference for *anti* elimination in E2 reactions, as the reaction begins to approach the E1cB/E2 borderline. When powerful activating groups are present,[171] as in ***61a*** and ***61b***,[168] no stereochemical preference is observed [they both eliminate acetate or (methoxide) at almost the same rate], and there exists good evidence that the elimination takes place *via* an E1cB mechanism.[168,171] We see that the flavanone system (***61c***) is rather similar to ***61a*** and ***61b*** with respect to base-catalysed elimination, and note too that Ring *et al.*[112] have shown that the keto group strongly activates double bonds (*e.g.* more strongly than CN, SO_2R) towards nucleophilic attack, and so presumably strongly activates the reverse (elimination) reaction also.



61a



61b



61c

R = Ac, Me

5.3.6 The Reverse Reaction

Unfortunately, the reverse (ring-opening) reaction, which by the principle of microscopic reversibility must proceed *via* the same transition state as the cyclisation mechanism, was impossible to study using the techniques of the present investigation; although the reverse reaction undoubtedly occurs at pH < 10 (where the un-ionised form of the flavanone predominates), the activity of hydroxide ion is so low that ring opening is too slow (relative to cyclisation) to contribute detectably to k_{obs} ($k_{obs} = k_r + k_f$). It is possible that in the case of 2′,6′-dihydroxy-2,4,6-trimethoxychalcone, the poorer than usual fit of the pH-rate data to the curve calculated *via* equation 2-19 is due to a small contribution from ring opening of the un-ionised flavanone: the rate coefficient for the cyclisation of this chalcone mono-anion is about 20 times slower than most of the other 2′,6′-dihydroxychalcones, while the reverse of this reaction may well be relatively unaffected by the B-ring substitution pattern (compare k_{f-} for this chalcone with those for the other chalcones in table 5-1).

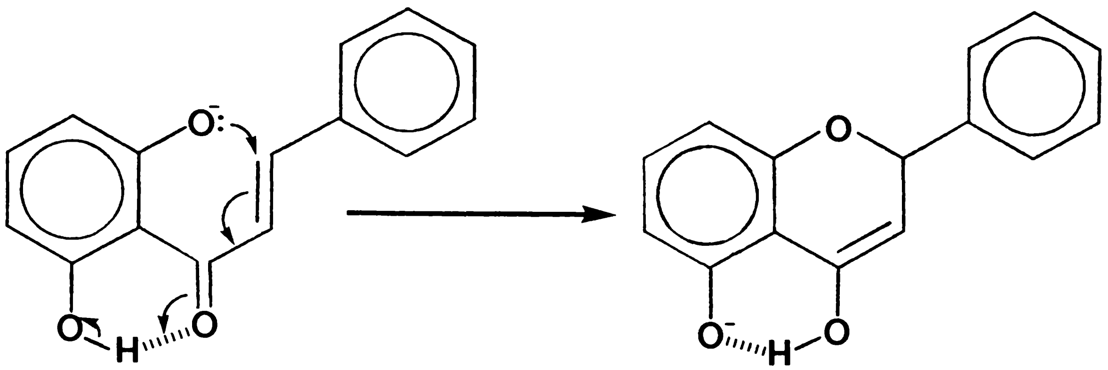
This is purely speculation, but it should be noted that it *is* theoretically possible to follow the ring-opening reaction kinetically, even at pH values where the chalcone – flavanone equilibrium lies very heavily in favour of the flavanone form. The method involves studying the rate of racaemisation of the optically active flavanones. Since the only way an optically active flavanone can undergo racaemisation is *via* ring opening (enolisation does not affect the stereochemistry at C-2), the rate of racaemisation should be equal to the rate of ring opening. The subsequent (possibly much more rapid) cyclisation reaction should not interfere with the rate measurements because it should result in racaemic flavanone, so that in a sense the ring opening observed by this method should be effectively irreversible.

Not only would such kinetic work afford information about the reverse of the cyclisation mechanism, but it could also provide mechanistic information that would otherwise be unobtainable. For instance, kinetic methods are available,[168] in the case of elimination reactions where the reverse (*i.e.* addition) step occurs at a negligible rate (which is essentially the situation in the kinetic study proposed above), to differentiate between E2 or $\mathrm{E1cB_I}$, and $\mathrm{E1cB_R}$ mechanisms. When the addition step occurs at a non-negligible rate (as was the case in the present study), then the situation becomes kinetically too complex for easy mechanistic differentiation.

This technique invites further kinetic work in this area. Some optically active flavanones are available in nature, but some enantiomeric resolutions are sure to be needed if a systematic kinetic investigation is to be carried out using this approach. The resolution of flavanone itself[172] has been reported, so major difficulties in the resolution of other flavanones and 5-hydroxyflavanones are not envisaged.

5.3.7 Discussion

The balance of the evidence presented here indicates that the cyclisation of 2',6'-dihydroxychalcone mono-anions probably takes place *via* a stepwise (*i.e.* E1cB-type) process, rather than a concerted (E2-type) one. If the reaction is an E1cB process, then this would be in accord with studies on related systems (section 1.3), where nucleophilic addition to activated double bonds is known to take place *via* an ElcB mechanism.



Scheme 5-9 Possible Intramolecular General Acid Catalysis in an ElcB-type Cyclisation

It has been proposed[130] that if an ElcB mechanism is present, then the 6'-OH group is ideally situated to provide general acid catalysis of the cyclisation step (scheme 5-9). Such an interpretation is consistent with the large k.i.e., and with the dip in the pH-rate profile that occurs when the chalcone di-anion becomes the predominant species in solution, and provides an explanation for the enhanced susceptibility of the 2',6'-dihydroxychalcones (compared to simple 2'-hydroxychalcones) towards cyclisation. The fact that the 6'-OH group may not be strongly hydrogen bonded to the carbonyl group at all, under aqueous conditions (*e.g.* ref. 173), does not detract from this hypothesis, since the hydrogen bond does not have to be present to provide the intramolecular catalysis. This explanation, however, must now be regarded with uncertainty, with the finding by Furlong and Nudelman[72] that 2'-hydroxychalcone anion also cyclises with a large k.i.e. (k_H/k_D = 5.3): so the large k.i.e. for the cyclisation of 2',6'-dihydroxychalcone mono-anions may well have another source. The fact that the di-anion is about 3 times less reactive towards cyclisation than is the mono-anion might be taken to suggest an unexpectedly high reactivity of the mono-anion, the reason being that we might expect the di-anion to be more reactive than the mono-anion, due to the stronger nucleophilic power of the anionic oxygen atoms, and to a statistical factor (2 nucleophilic O^- atoms to 1) favouring cyclisation of the di-anion over the mono-anion. This higher reactivity of the mono-anion over the di-anion, towards cyclisation, does not in fact provide firm evidence for the mono-anion being especially reactive (due, for instance, to intramolecular catalysis by the 6'-OH) because other factors (such as pre-rate-limiting-step equilibria) could be responsible (or partially responsible) for this effect. Thus, the relative positions of the s-*cis* to s-*trans* equilibria for the mono- and di-anionic species will affect their relative rate coefficients for cyclisation.

Alternatively, it could be that the presence of a 6'-O^- group adjacent to the carbonyl group (as in the di-anion) has a rate-reducing effect, rather than presence of the 6'-OH group (of the mono-anion) having a rate-enhancing effect. The di-anion cyclisation will be dealt with in more depth in the next section (5.4) of this chapter.

5.4 The Chalcone Di-Anion Cyclisation

Unlike the cyclisations of the other forms of 2',6'-dihydroxychalcones, the reverse reaction of the 2',6'-dihydroxychalcone di-anion cyclisation reaction was readily measurable (as k_{F^-}) at high pH. Since the reverse reaction necessarily proceeds by the same mechanism as the forward reaction (k_{C2^-}), there is the potential for obtaining more mechanistically useful information about the reaction than can be obtained from the study of the cyclisation reaction alone.

As was the case for the mono-anion cyclisation, the rate coefficient for the chalcone di-anion cyclisation (k_{C2-}) was almost independent of B-ring substituent effects: only the 4-chloro (1.5 times faster) and the sterically congested 2,4,6-trimethoxy (3 times slower) substituted compounds (see table 5-1) reacted at rates different from the norm (*ca.* 1.3×10^{-3} s^{-1}). Too much should not be made of the size of these differences, however, because in the pH region where k_{C2-} is important, three unknowns (k_{C2-}, k_{F-} and K_{a2}) define k_{obs}, and need to be assigned values to provide a fit to the experimental data. Consequently, these values are of lower accuracy than those which are defined by plateaux, such as k_{CH-} and k_{CH2}. The values of k_{C2-} and k_{F-} are probably accurate to ±10-15%, while the values for K_{a2} can generally be considered accurate to *ca.* ±0.1-0.15.

More surprising is the finding that the introduction of a 4′-methoxy group into the chalcones has no significant effect on k_{C2-}, which contrasts with the approximately 5-fold reduction in k_{CH-} which occurs when the same substitution change is made in the 2′,6′-dihydroxychalcone mono-anion and 2′-hydroxychalcone anion systems. Possibly this phenomenon is due to the effect of an additional methoxy group in the A-ring being swamped by the much larger electronic effects caused by the presence of the two phenoxide groups on the A-ring, in the case of the 2′,6′-dihydroxychalcone di-anions.

As was pointed out earlier, k_{C2-} is between 3 and 15 times smaller than k_{CH-} for the corresponding chalcone. This occurs in spite of a statistical factor of two in favour of di-anion cyclisation, and the increased nucleophilic power of the attacking oxygen atom in the di-anion (compared to the mono-anion), which is reflected in the respective pK_a values of the phenolate oxygen atoms of the di- (*ca.* 12) and mono-anions (*ca.* 7). As mentioned in section 5.3, this effect could be ascribed to a special role for the 6'-OH group in the mono-anion cyclisation, which is of course absent in the di-anion cyclisation. It could well be due to other factors, however: a change in the fraction of the chalcone in the reactive s-*trans* conformation between the mono- and di-anion forms; a decrease in the electron withdrawing power of the carbonyl group due to additional delocalisation of electron density from the two phenolate oxygens; or a tendency for the A-ring to lie out of the plane of the enone system (so that it is difficult for the phenolate oxygens to approach the β-carbon) due to unfavourable through-space interactions between the phenolate and carbonyl oxygens. Another possible explanation is that the cyclisation of the 2',6'-dihydroxychalcone di-anions is particularly unfavourable. For instance if, as seems likely, an E1cB-type mechanism applies, then the proximity of the 6'-O$^-$ group to the developing enolate oxygen would be expected to destabilise the transition state, resulting in a reduced rate constant for cyclisation. Some possible support comes from the observation that the rate coefficient for 2',4'-dihydroxychalcone di-anion cyclisation is greater than that for 2',6'-dihydroxychalcone di-anion, even if only by a factor of 2.6.

The second-order rate coefficient for the reverse reaction (k_{F^-}) appears to display a weak substituent effect ($\rho \approx 0.5$, figure 5-2). The magnitude of the variation in k_{F^-}, however, is not large compared to the estimated uncertainties in their values. It is noticeable that the rate coefficient for the reverse reaction is much smaller for the 5-hydroxyflavanones (table 5-1) (which occur in the anionic form in the pH region where k_{F^-} is measured) than for related non-ionisable flavanones (table 5-2). Thus, k_{F^-} for 2',6'-dihydroxychalcone is 250 times smaller than k_{OH^-} for 2'-hydroxy-6'-methoxychalcone, and 450 times smaller than that for 2'-hydroxy-4'-methoxychalcone. This effect could be due to the A-ring being a poorer leaving group for the former chalcone under the basic conditions, or more likely, to the increased difficulty of attack on the 3-H of the flavanone by hydroxide ion. In an E1cB mechanism applies, removal of a 3-H would be especially unfavourable, due to the proximity of the resultant enolate oxygen to the 5-O$^-$ group of the flavanone, and would effectively represent an increase in the pK_a for the 3-H. The effect is probably mainly due to the delocalisation of electron density from the phenolate-substituted A-ring onto the carbonyl group, however, since for ring-opening in the 2',4'-dihydroxychalcone system (where no enolate/phenoxide proximity is possible in the flavanone form – see figure 5-3), k_{OH^-} is 84 and 45 times smaller than that for the 2'-hydroxy-4-methoxy- and 2'-hydroxy-6'-methoxy-chalcone systems respectively.

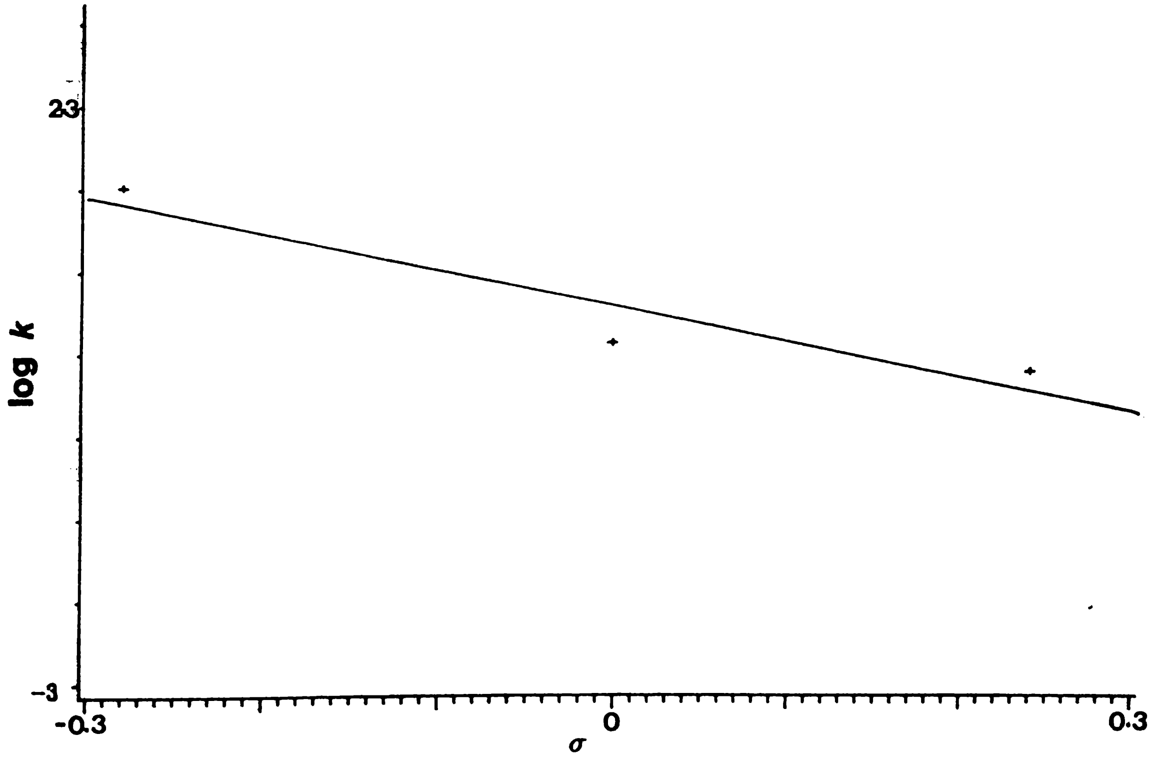


FIGURE 5-2 Plot of log k_{F^-} (from Table 5-1) versus the Hammett Substituent Constant,[167] (σ)

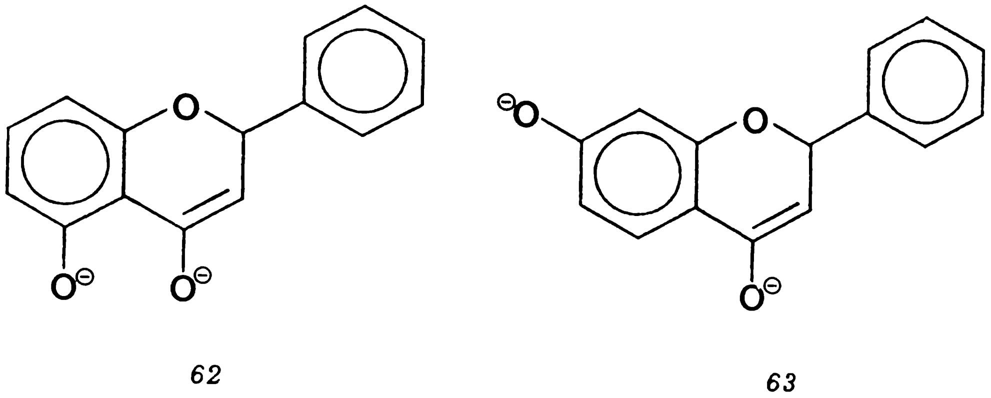
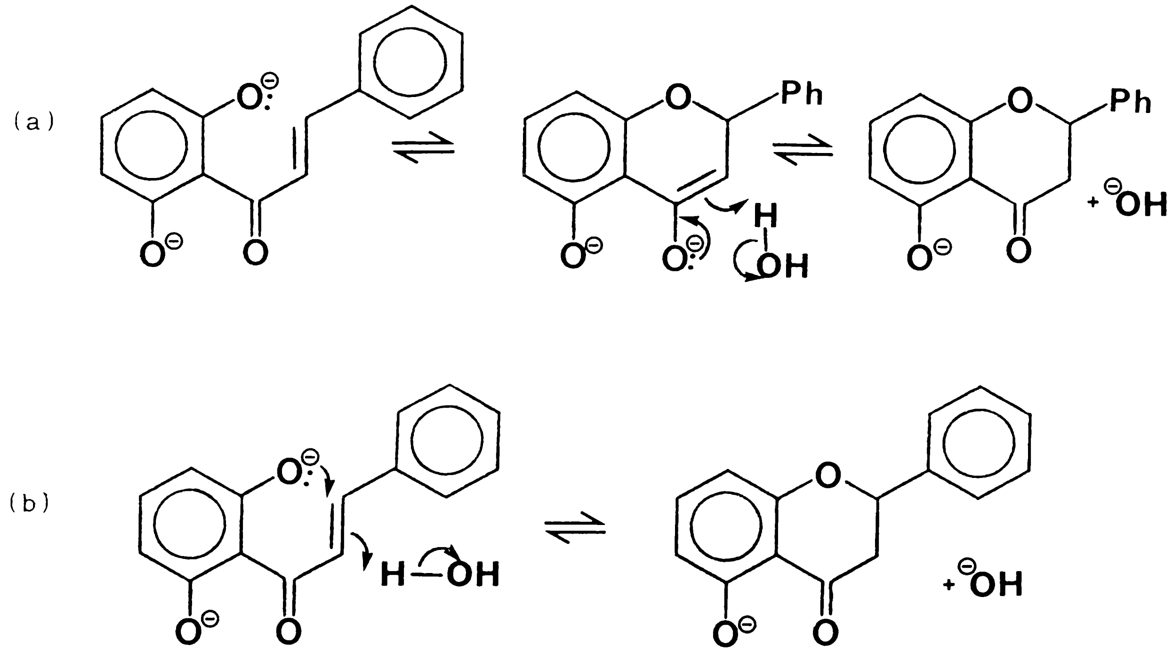


FIGURE 5-3 5-Hydroxy- (*62*) and 7-Hydroxyflavanone (*63*) Phenolate Enolates



SCHEME 5-10 The (a) E1cB and (b) E2 Mechanisms for Cyclisation/Ring-Opening of 2′,6′-Dihydroxychalcone Di-anions

Thus, as was the case for the 2′,6′-dihydroxychalcone di-anion cyclisations, there appears to be an unfavourable through-space interaction between the 5-phenoxide and carbonyl oxygens (of the flavanone) during ring opening. The effect is, however, a relatively small one

(k_{OH^-} for 2',4'-dihydroxychalcone is about 5 times k_{F^-} for 2',6'-dihydroxychalcone), and it should be noted that a similar small effect occurs in systems with non-ionising flavanones (k_{OH^-} for 2'-hydroxy-4'-methoxychalcone is 1.8 times that for 2'-hydroxy-6'-methoxychalcone) which, as was pointed out by Old,[46,71] may well be due to through-space and steric interactions.

5.5 The Chalcone – Flavanone Isomerisation in Nature

Most of the flavanones in nature contain a 5-hydroxy group (as well as a 7-*O*- substituent), for reasons that were discussed in chapter 1. These flavanones have been shown to arise from their isomeric 2',6'-dihydroxychalcones, and are often optically active.

At pH 7.0 we can calculate (from the data in table 5-1) that 2',6'-dihydroxy-4,4'-dimethoxychalcone, itself a natural product and isomeric to a naturally occurring flavanone, will isomerise with a rate constant of 2.37×10^{-3} s^{-1} at 30°C. Its half-life will thus be slightly less than 5 minutes under the conditions used in this study. If it is assumed that the rate coefficient for the reverse reaction (ring opening of the neutral flavanone) is < 1 l mol^{-1} s^{-1} (*cf.* table 5-2), then we can calculate the equilibrium constant for the formation of flavanone to be $K_{eq} > 2.4 \times 10^4$ in favour of the flavanone form, at this pH.

The above calculation raises the question of how this (and other) 2',6'-dihydroxychalcones are able to occur as natural products. Clearly, if any of the appropriate chalcone – flavanone isomerase is present in the same region as that where the chalcone synthase enzyme is generating the chalcone, then the chalcone produced will rapidly be converted into the flavanone form. Equally obviously, any 2',6'-dihydroxychalcone produced within a cell at normal physiological pH will also be converted rapidly to its flavanone isomer, even in the absence of the isomerase. In fact the synthase enzymes so far isolated are water soluble, and have pH-optima of 6.5-8.4 (see chapter 1), so presumably these chalcones are formed in approximately neutral solution *in vivo*. "Chalcone stabilising factors" have been postulated to account for the existence of 2',6'-dihydroxychalcones as isolable natural products, but it is hard to imagine what these factors might be. A more plausible explanation is that the chalcones, once formed, may be rapidly translocated into regions of the cell where they are either no longer in a hydroxylic environment, or where they are in an acidic environment from which the isomerase is excluded. The former explanation appears to be a good one, since it would also account for the fact that a large proportion of the 2',6'-dihydroxychalcones so far isolated have been located either in lipophilic plant fractions, or in lipophilic exudates. Once such chalcones are either deposited, or taken up into a non-hydroxylic environment, they are no longer highly susceptible to cyclisation: most of the chalcones in the present study were stable for > 2 years at *ca*. 4°C in the solid state, and the stability of 2',6'-dihydroxychalcone itself in dioxane solution has already been commented upon (see chapter 2).

If these chalcones isomerise so readily, why do plants require a chalcone – flavanone isomerase enzyme? Obviously, any 2',6'-dihydroxychalcones present in solution (in a cell) will be rapidly converted into the flavanone form, even without the intervention of enzymic catalysis. The most likely reason is that the plant requires some (at least) of its flavanones in one particular (2*S*) conformation. This may be because only that enantiomer of the flavanone is biologically active, or, more probably, because the enzymes for subsequent flavonoid metabolism act on only one enantiomer of the flavanone. It has been shown, at least in some plants, that the flavonoids are derived from flavanones, which in turn are derived from chalcones (see chapter 1). It is possible that in some plants, however, as pointed out by Wong and Grisebach,[174] that the flavanones are the storage forms of the chalcones, and it is the chalcones which are the direct precursors of the rest of the classes of flavonoids. If this were the case, then the isomerase enzyme would be necessary to provide sufficiently rapid conversion of the (optically active) flavanones into the chalcones.

Some workers have addressed themselves to the question of the mechanism of action of the chalcone – flavanone isomerase enzyme, without a reasonable understanding of the likely mechanisms for non enzyme-catalysed isomerisation. Two postulated mechanisms for the enzymic catalysis are given in scheme 1-4, one of which (b) seems particularly unlikely. From the foregoing mechanistic discussions in this chapter, there are several ways in which an enzyme could accelerate an E2- or an E1cB-type cyclisation without fundamentally changing the mechanism of the cyclisation. Probably the simplest and most obvious way is by removing the s-*cis* to s-*trans* equilibrium from the rate equation. An enzyme whose active site binds the chalcone in the reactive s-*trans* conformation will cause a rate enhancement. Hayes and Timmons[175] have shown that chalcone itself probably exists *ca.* 16% in the s-*trans* conformation. Such an enzyme could thus give rise to an approximately order of magnitude (or even more, if the s-*cis*:s-*trans* ratio were normally very large) increase in the rate constant for cyclisation.

The placement of suitable acidic and basic groups within the active site of the enzyme could also enhance the rate of cyclisation.

Thus, a basic group in the vicinity of the cyclising OH group (if required), to assist with proton removal, along with an acidic group adjacent to the carbonyl and/or α-carbon (dependent on whether the reaction is E2- or E1cB-type) could be responsible, at least in part, for the enzymic catalysis. It is noteworthy that in the *in vivo* formation of the chromanone LL-D253α (***24***) mentioned in chapter 1, ring closure at C-2 was stereospecific while the proton addition step (at C-3) was not. In this case at least, the enzyme does not provide the proton donation to the α-C (3-C in chromanone nomenclature): the cyclised product (presumably an enol) either ketonises with the solvent while still attached to the enzyme, or is ejected from the enzyme in the enolic form, where it subsequently ketonises. Unfortunately, it is not possible to come to any similar conclusions about the mode of action of the chalcone – flavanone isomerase, as the isolated enzymes produce flavanones with a low stereospecificity at 2-C (as well as 3-C), and no-one appears to have studied the *in vivo* isomerisation of α-^{2}H-2′-hydroxychalcones in a similar manner to the above study of LL-D253α.

5.6 Suggestions for Further Kinetic and Mechanistic Work

From the discussion in this chapter, it is clear that further work is necessary on the mechanism of the chalcone – flavanone isomerisation. Some ideas for further investigations are given below.

Studies similar to the present, and to previous[46,71,130] kinetic investigations, on a variety of 2′-hydroxy and 2′,6′-dihydroxy-chalcones are required to fully ascertain the effect of substituents on the reaction. The alternative approach to the kinetic analysis of the isomerisation outlined in section 2.1.*6* may prove useful in the case of the 2'-hydroxychalcones. Activation energy parameter data should be obtained for a series of 2'-hydroxychalcones, for comparison with the results obtained in the present study.

It would be interesting to obtain full pD-rate profiles (*i.e.* in D_2O) for chalcone – flavanone isomerisations. For instance there is little scope for a primary isotope effect on k_{C2-} at *ca.* pH 14 if the cyclisation is E1cB unless (as seems unlikely) ketonisation is rate-limiting (see scheme 5-10). Also, the origin of the unexpected substituent effect on the cyclisation of 2',6'-dihydroxychalcone mono-anions should be investigated, and more p.i.e. studies should be conducted on the chalcone – flavanone system.

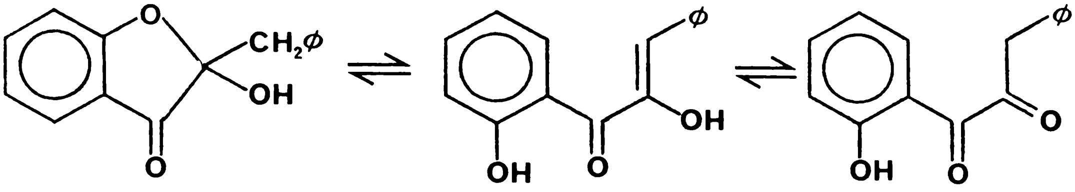
The acid-catalysed cyclisation needs further study, and it might be worthwhile to investigate both the forward and reverse reactions under highly acidic conditions ($H_0 < -3$), where the chalcone should be highly protonated.

The effect of various species in solution on the reaction should be examined. In this study it was noted that the presence of borate ions appears to slow the cyclisation of 2',6'-dihydroxychalcone mono-anions, a result that should be further investigated. Metal ions are known to be chelated by 2'-hydroxychalcones,[176,177] including Cu^{2+}, Al^{3+} and Ca^{2+}. The interaction of such ions with such chalcones is sure to affect the rate coefficients for cyclisation (and possibly also for ring opening). Study of the effects of such metal ions on the isomerisation of 2',6'-dihydroxychalcones should be particularly simple in the two plateau regions of the pH-rate profile (the 2'-hydroxychalcones may also display a plateau in their pH-rate profiles at low pH, representing cyclisation of the un-ionised chalcone).

The ring-opening reaction should be thoroughly studied over a wide pH range, using the method outlined in section 5.3 (utilising optically active flavanones) where necessary (*i.e.* where the isomerisation equilibrium lies heavily in favour of the flavanone form). Not only is this method the only easy way to obtain kinetic data about the ring-opening reaction below pH 12 (for 2',6'-dihydroxychalcones: below pH 7 for the 2'-hydroxychalcones), but it also has the potential to differentiate between the E2/E1cB$_I$, and E1cB$_R$ mechanisms of elimination (see section 5.3).

Whether the unusual effects of 2,4,6-trimethoxy substitution on the chalcone – flavanone isomerisation are due to steric, electronic, or a combination of both effects should be ascertained.

In view of the massive effects α-bromo substitution appears to have on the rate constants for the 2'-hydroxychalcone cyclisation (see chapter 1), and similarly in view of the apparently large effects of α-methoxy substitution (chapter 1), the effects of α substitution should be investigated kinetically. Such substituents seem sure to cause large changes in rate constants, not only through their effects on the susceptibility of the enone to nucleophilic attack, but also through their effects on the s-*cis* to s-*trans* equilibrium, and through their effect upon the acidity of the 3-proton of the flavanone isomer. The effect of an α-hydroxy group could prove particularly interesting, as α,2'-dihydroxychalcones have been postulated[178] as existing in base-catalysed equilibrium with their isomeric diketo and coumaran-3-one forms (but apparently not with the 3-hydroxyflavanone form, which is unstable in basic solutions) (see scheme 5-11).

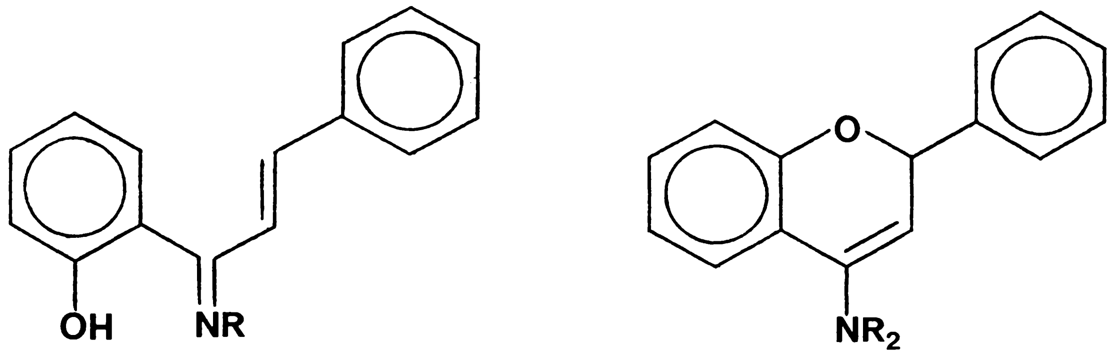


SCHEME 5-11 Proposed[178] Isomerisation of α,2'-Dihydroxychalcone

The fact that 2'-hydroxy-α-methoxychalcones isomerise to 5-membered ring compounds under acidic conditions suggests that these chalcones could yield particularly interesting kinetic data, particularly if competition between cyclisation to 5- and 6-membered ring compounds takes place. The stereochemistry of the cyclisation (which may well vary with pH) of α substituted 2'-hydroxychalcones (the 3-position in the flavanone will be chiral, and so two diastereoisomers will be possible) could provide further insights into the cyclisation mechanism.

The phenomenon of ring isomeric change (see chapter 1) in 5-hydroxyflavanones has not been the subject of any kinetic studies. Such a study could reveal the factors which control the relative stabilities of the two flavanone isomers, as well as the factors affecting the relative susceptibility of the chalcone form toward either mode of cyclisation (see figure 1-23).

Certain amine buffers have the potential to form equilibrium amounts of the imine and enamine derivatives (see scheme 5-12) of the 2'-hydroxychalcones (and their isomeric flavanones). The effects, if any, of such buffers on the chalcone - flavanone isomerisation have yet to be investigated.

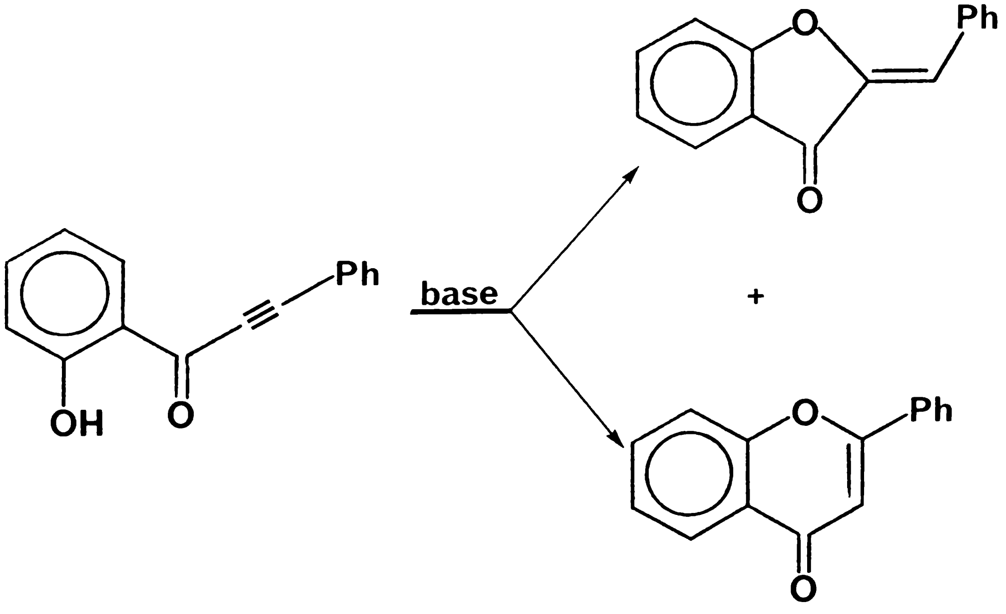


SCHEME 5-12 Possible Interactions of Amine Buffers with 2'-Hydroxychalcone and Flavanone

Clearly, in view of the apparent inadequacies of previous studies (see chapter 1), the stereochemistry of the isomerase-catalysed chalcone cyclisation needs to be reinvestigated. The study of the stereochemistry of this reaction *in vivo* could be studied by using α-^{2}H-2'-hydroxychalcones as substrates, in a similar manner to that used in the investigation of the biosynthesis of LL-D253α (see chapter 1).

The study of related isomerisations in systems similar to the chalcone should provide further information about the nature of the reaction. The simple chromanone system has not been investigated kinetically. Synthesis by condensation usually results in the chromanones directly, although occasionally the (presumably intermediate) α,β-unsaturated compounds are obtained (*e.g.* ref. 179). Similarly the isoflavanone system has not been studied, although the chalcone-type isomers to several isoflavanones have recently[180] been synthesised. Synthetic studies of the direction of cyclisation in β-2-hydroxyaroylacrylic acids have been conducted,[181] but as yet no kinetic studies appear to have taken place.

Another related reaction that may well be worthy of kinetic studies is the recently discovered[182] ring closure of *o*-hydroxyaryl phenylethynyl ketones to flavones and/or aurones (scheme 5-13). Which of the products predominates depends upon the base/solvent system used, an effect attributed to kinetic control by the authors, even though they had demonstrated some degree of reversibility was present in the reaction. This system has an advantage over the chalcone system, in that it should be kinetically simpler, since no s-*cis* to s-*trans* equilibration is possible.



SCHEME 5-13 The Cyclisation of *o*-hydroxyaryl phenylethynyl ketones[182]

Changing the nature of the nucleophilic group in the chalcone could result in interesting changes in reactivity towards cyclisation. Possible candidates for such a study include the 2'-amino- and 2'-thio-chalcones.

TABLE 5-1 Rate Coefficients and pK_a Values for Some 2',6'-Dihydroxychalcones

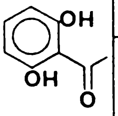
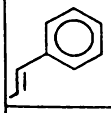
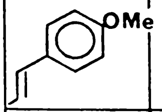
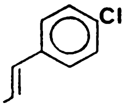
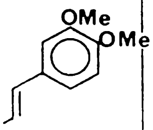
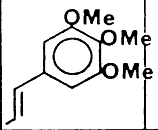
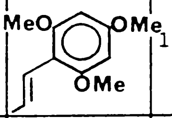
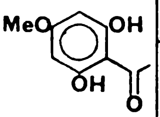
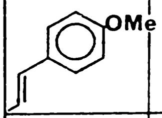
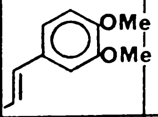
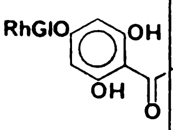
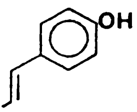
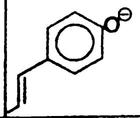
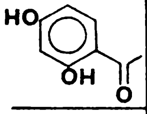
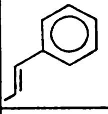
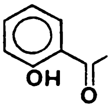
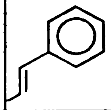
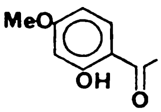
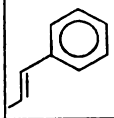
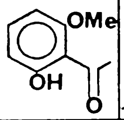
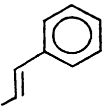
A-ring	B-ring	$10^7 k_{H^+}$	$10^6 k_{CHH}$	$10^4 k_{CH^-}$	$10^4 k_{C^{2-}}$	$10^3 k_{F^-}$	pK_{a1}	pK_{a2}	pK_{a3}
		9.6	1.6	210	13	2.6	7.6	11.7	
		39.3	4.9	205	13	4.0	7.6	11.7	
		-	9?	210	20	2.4	7.6	11.7	
		43	3.9	204	11	4.1	7.6	11.7	
		14	1.6	210	12	2.5	7.6	11.7	
		15000	6.5	9.8	3.5	1.5	7.4	12.1	
		37	9.7	42.5	13.7	1.5	6.9	12.3	
		78	7.6	44.0	13	2.0	6.8	12.3	
		75	14	75	-	-	6.9	8.5	12.0
		-	-	8.0	3.0	2.6			

TABLE 5-2 Rate Coefficients and pK_a Values for Some 2'-Hydroxychalcones

A-ring	B-ring	10^7k_{H+}	10^6k_{CHH}	10^4k_{CH-}	10^4k_{C2-}	10^3k_{F-}	pK_{a1}	pK_{a2}
		-	-	0.18	34	14	?	11.7

A-ring	B-ring	10^7k_{H+}	10^6k_{CH}	10^4k_{C-}	10^4k_{C2-}	10^3k_{OH-}	pK_{a1}	pK_{a2}
		-	10	75	-	2250	9.55	
		-	7	27	-	1170	9.55	
		-	8	5.3	-	633	8.95	

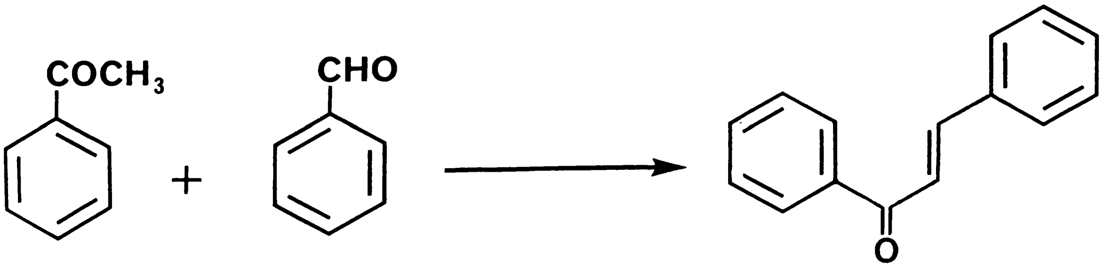
CHAPTER 6

Synthesis of Chalcones

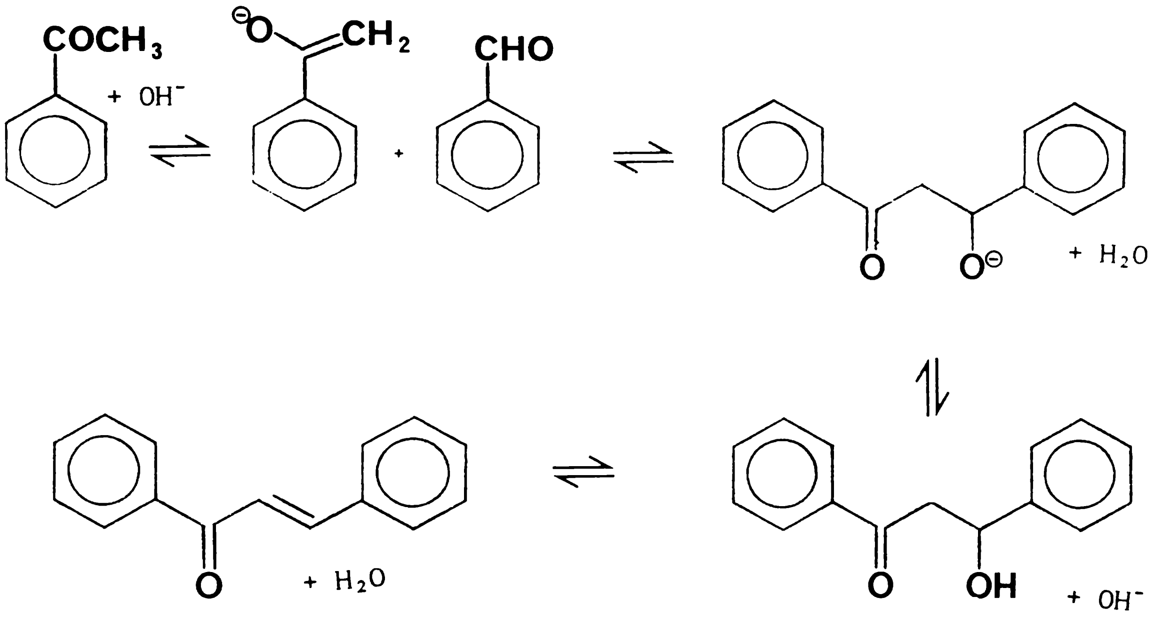
In this chapter the results of the synthetic work undertaken in the present study are discussed hand-in-hand with the literature results from related studies. Experimental detail is given in chapter 7. The implications of kinetic studies for the synthesis of both 2′-hydroxy- and 2′,6′-dihydroxychalcones, and their isomeric flavanones are considered, resulting in some improvements in current techniques, and in the development of new synthetic strategies.

6.1.*1* The Claisen-Schmidt Condensation

By far and away the most important method of synthesising chalcones is the aldol-type (Claisen-Schmidt) condensation, where, under acidic or basic conditions, an aromatic aldehyde and an acetophenone are condensed. The resulting β-hydroxy ketone[183,184] then undergoes rapid facile dehydration to yield the α,β-unsaturated ketone.

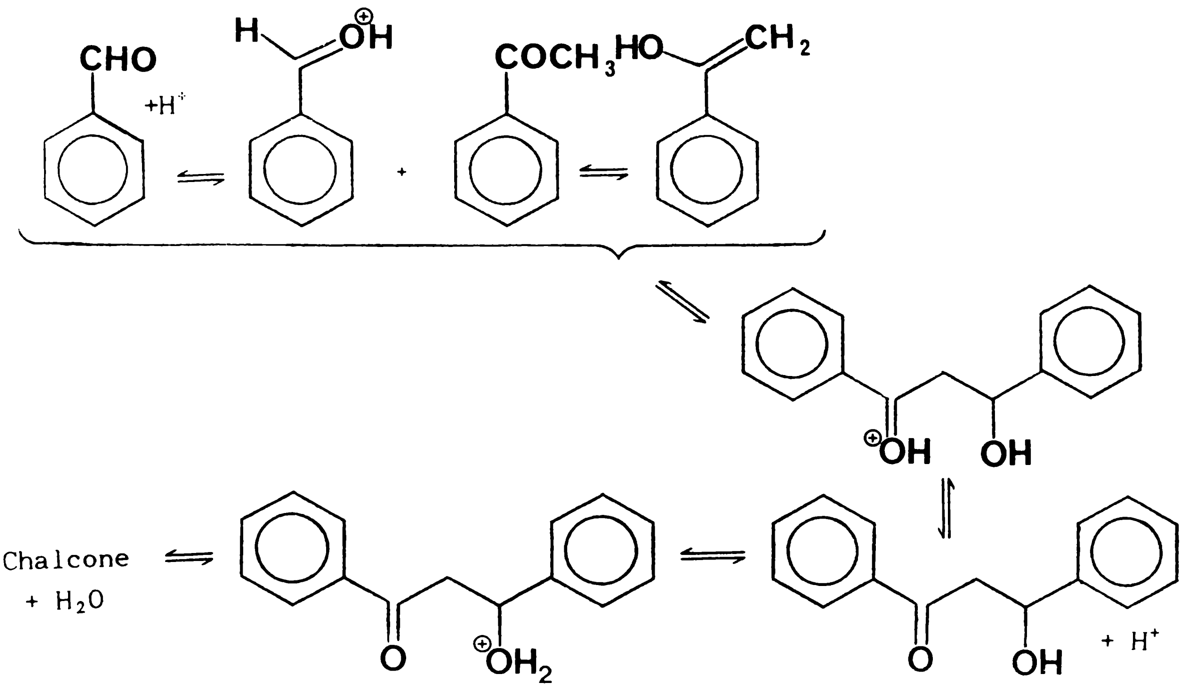


The acetophenone fragment becomes the A-ring, and the benzaldehyde fragment the B-ring. As in all methods of chalcone synthesis, the more stable *trans* isomer is produced. The kinetics of the base-catalysed condensation have been studied,[183,185-188] electron withdrawing substituents in the acetophenone enhancing (ρ = 1.6) the rate. The reaction was found[183,185-188] to be first-order in the acetophenone, the aldehyde, and in the base. In accord with this is one of the two proposed[187] mechanisms, shown in scheme 6-1.



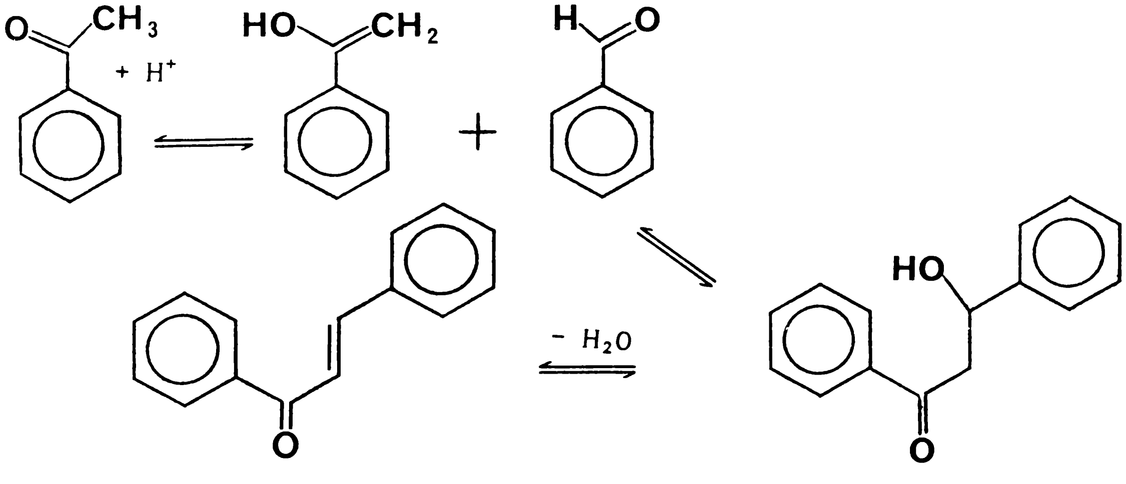
SCHEME 6-1 Proposed[187] Mechanism for the Base-Catalysed Condensation of Benzaldehydes and Acetophenones to Afford Chalcones

The acid-catalysed reaction, too, has been investigated,[184,189,190] along with a number of closely related[191-194] condensations. The reaction was favoured[190] by electron-donating substituents on the benzaldehyde (ρ = -0.25), and was[189] first-order in acetophenone, the benzaldehyde, and in H_0. The intermediacy of a ketol has been demonstrated,[184] and the following mechanism (scheme 6-2) has been put forward.[189]



SCHEME 6-2 Proposed[169] Mechanism for the Acid-Catalysed Condensation of Benzaldehydes and Acetophenones to Afford Chalcones

A more likely mechanism (see scheme 6-3) involves simply the acid-catalysed enolisation of the acetophenone. and its subsequent addition to the aldehyde.



SCHEME 6-3 Possible Mechanism for the Acid-Catalysed Condensation of Benzaldehydes and Acetophenones to Afford Chalcones

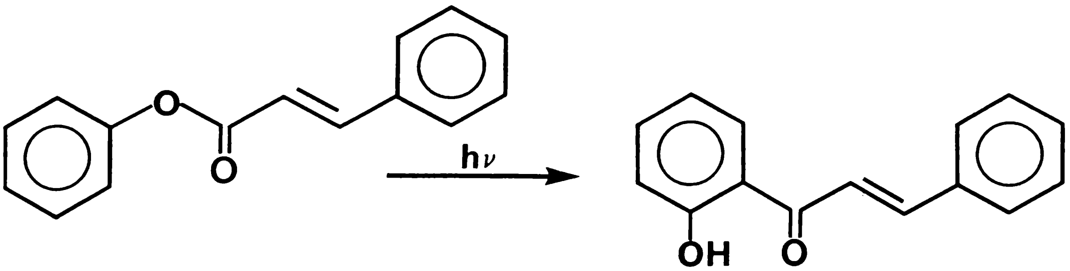
The only other synthetic methods of any importance are, by their very nature, capable of producing only 2′-hydroxychalcones with various substitution patterns.

6.1.2 Friedel-Crafts Acylation

The next most important method for synthesising chalcones is the Friedel-Crafts acylation of phenols (or their ethers) with cinnamic acids and their derivatives (usually the acid chlorides). The method seems to work reasonably well, except that in the case where the phenol is 2,4,6-trihydroxybenzene, only the 5,7-dihydroxyflavanone is isolable.[60,195,196] Presumably the chalcone that forms cyclises during the course of the reaction. This method, then, will be of no use in the synthesis of 2',6'-dihydroxychalcones.

6.1.3 Photo-Fries Rearrangement

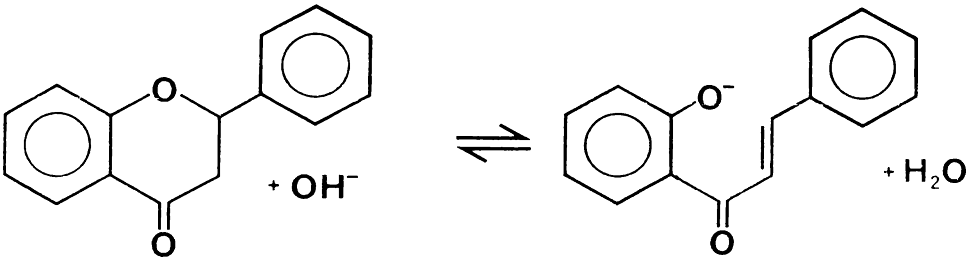
The u.v. light-catalysed rearrangement[197] of cinnamic acid esters of phenols (scheme 6-4) yields, among other products, 2'-hydroxychalcones. The method is particularly useful for 2'-hydroxy- and 2',4',6'-trihydroxy-chalcones, since the initial ester can yield only one chalcone isomer. The yields obtained in the reaction are low, however, and the desired chalcone(s) must be chromatographically separated from the unreacted ester and degradation products.



SCHEME 6-4 The Photo-Fries Rearrangement of Phenol Cinnamates to Afford 2'-Hydroxychalcones

6.1.4 Ring Opening of Flavanones

The chalcone – flavanone equilibrium discussed earlier (in chapters 1, 2 and 5) can be taken advantage of, to yield substituted 2'-hydroxychalcones. Thus, strongly basic conditions favour the formation of the chalcone isomer, although some difficulties may be experienced (*e.g.* ref. 60) when the flavanone contains a 5-hydroxy group, as was discussed in chapter 1.



SCHEME 6-5 Base-Catalysed Ring Opening of Flavanones to Afford 2'-Hydroxychalcones

6.2 Synthesis of 2'-Hydroxychalcones

For a chalcone-flavanone equilibrium to exist, the chalcone must, of course, contain a 2'-hydroxy group.

The Claisen-Schmidt condensation method is a particularly versatile one,[195] in that a great variety of substituents may be incorporated into both the acetophenone and benzaldehyde precursors. Thus, a great many 2'-hydroxychalcones have been synthesised *via* this method. According to Dhar,[195] when synthesising polyhydroxychalcones by this method it is desirable to use a "higher" concentration of alkali as the condensing agent. However, a few problems may be encountered when using the base-catalysed version of the condensation.

Firstly, under the reaction conditions required, the Cannizzaro reaction is likely to take place,[195] consuming some of the benzaldehyde. The obvious remedy to this problem is to use an excess of the benzaldehyde (if it is readily available). This will not only remove the problem of the competing (Cannizzaro) reaction producing a deficit of the benzaldehyde, but will also increase the rate of the condensation reaction according to the rate law discussed earlier (section 6.1.*1*):

$$\text{rate} = k[\text{acetophenone}][\text{benzaldehyde}][\text{base}]$$

Recommended[195] reaction times range from 12-15 hours at 50°C, to one week at room temperature.

Another problem occasionally encountered in the synthesis of 2′-hydroxychalcones is the cyclisation of the chalcones to their isomeric flavanones under the basic reaction conditions used in the Claisen-Schmidt condensation. One approach to solving this problem is to protect the hydroxy group of the acetophenone prior to the condensation, *e.g.* as the methoxymethyl ether. This type of ether is subsequently cleaved under mildly acidic conditions to yield the 2′-hydroxychalcone. Another approach, used by Old[46,71] in his synthesis of 2′-hydroxybenzochalcone, is to deliberately allow the reaction mixture to cyclise completely to the flavanone, which can then be purified. The pure flavanone can then be converted into the desired chalcone by taking advantage of the reversal of the chalcone – flavanone equilibrium at high pH. An analysis of the kinetic work of Old[46,71] (presented later in this chapter) shows that under strongly basic conditions the 2′-hydroxychalcone – flavanone equilibrium lies strongly on the side of the chalcone form (*ca.* 99% chalcone at pH 13). The same is true even in the case of Old's benzochalcone. It seems likely, therefore, that the cause of this cyclisation problem is the insolubility of the isomeric flavanone of the chalcone being synthesised, in the condensation medium; the small amount of the flavanone present at equilibrium is probably largely precipitated as it is formed, so that eventually much of the product will be present in the flavanone form in the solid phase. This is almost certainly the explanation for the problems experienced by Old[46,71] with his benzochalcone, since he also reported[46,71] solubility problems in his kinetic investigations.

Synthesis of 2'-hydroxychalcones *via* the acid-catalysed Claisen-Schmidt condensation is less common, possibly because of the great utility of the base-catalysed route, although it has been used.[195] Some flavanone will inevitably be produced with this method, since acidic conditions favour the cyclisation of 2'-hydroxychalcones to flavanones.

6.3.1 Synthesis of 2',6'-Dihydroxychalcones

Quite a number of syntheses of 2',6'-dihydroxychalcones have been reported in the literature over the years, using all the methods described above except the Friedel-Crafts acylation. The validity of many of these synthetic claims is shown to be doubtful, as a result of the spectroscopic and synthetic studies (chapter 7) and the kinetic studies (chapter 2) described in this work.

As mentioned in chapter 1, 2'-hydroxychalcones are well known as both natural and as synthetic products. However, 2',6'-dihydroxychalcones are rare as either. As discussed above, only two synthetic methods used for the production of 2'-hydroxychalcones appear to be promising as routes to 2',6'-dihydroxychalcones. These are: the ring-opening of 5-hydroxyflavanones; and the direct condensation

2',6'-dihydroxyacetophenones with benzaldehydes using acidic or basic catalysts.

6.3.2 Synthesis of 2',6'-Dihydroxychalcones by Base-Catalysed Condensation

The literature contains several reports (discussed below) of the synthesis of 2',6'-dihydroxychalcones by condensation of the appropriate acetophenone and benzaldehyde under basic conditions. In contrast to these reports, the failure of this reaction is also well documented.[71,198,199]

In my hands, base-catalysed condensation of 2',6'-di- and 2',4',6'-tri-hydroxyacetophenones with various benzaldehydes failed, in all cases, to give the desired chalcones. Rather, in most instances, a red or orange solid could be isolated from the acidified reaction mixture. These substances clearly were not the desired 2',6'-dihydroxychalcones, as they did not decolourise (*via* the facile cyclisation to flavanones, to which such chalcones are prone) in methanolic sodium acetate solutions, and they were unexpectedly soluble in low polarity solvents such as chloroform. In addition, their solubility was low in more polar solvents such as methanol. Large amounts of a red gum formed during the condensation step itself, and since the expected chalcones ought to be highly soluble in strongly basic solutions, the gum may well be a precursor of the isolable red or orange solid mentioned above. This product is therefore the result of some undesirable reaction, possibly polymerisation or oxidation.

This red or orange substance appears to have been mistaken in some instances for the desired chalcone by some workers. For instance

Seshadri and Venkateswarlu[200] have reported the preparation of 2′,6′-dihydroxy-4-methoxychalcone (*38*) and 2′,6′-dihydroxy-3,4-dimethoxychalcone (*40*) using the basic condensation method. These two "chalcones" had practically identical melting points, their ^{1}H n.m.r. spectra were not fully consistent with their claimed structures, and the authors did not report the isolation of the isomeric flavanones (as they had done with the other 2′-hydroxy-substituted chalcones whose syntheses they also reported). The syntheses of these two 2′,6′-dihydroxychalcones are reported later (in chapter 7), and from them the corresponding 5-hydroxyflavanones, too, have been isolated. Both pairs of isomers have been thoroughly characterised, but are clearly different from the products reported by Seshadri.[200] as judged by their physical and spectral characteristics. Furthermore, the kinetic studies of these compounds (reported in chapter 2) show that the isolation procedures used by Seshadri[200] {*e.g.* washing an ether solution of the "chalcone" with 5% aqueous sodium bicarbonate [the pH of a 0.1 M (*i.e.* 0.84% w/v) $NaHCO_3$ solution at 25°C is[127b] 8.4] solution} ought to have resulted either in extraction of most of the chalcone (pK_a = 7.6) present, or in the isomerisation of most of the chalcone to its isomeric 5-hydroxyflavanone.

Clearly, then, in view of the case of the two alleged chalcones discussed above, reports of the synthesis of 2′,6′-dihydroxychalcones by direct condensation under basic conditions should be regarded with suspicion. For instance, both Patil and Deshpande[201] and Hall and Glenn[202] have reported the synthesis of 2′,4,6′-trihydroxychalcone using this method. Both products gave satisfactory elemental analyses, but had different melting points. The product of Patil,[201] which was obtained in high yield, and which gave a ^{1}H n.m.r. spectrum slightly different from that expected, was reported to cyclise unexpectedly in basic hydrogen peroxide solution, to the flavanone (rather than undergoing oxidation), but in fact the product that they reported was yellow, and had the same melting point as its "chalcone" precursor. On the other hand, the product obtained by Hall[202] consisted of orange needles (obtained in low yield after chromatography) which melted at 190-192°C, then resolidified before remelting at 210-212°C. Such behaviour can occur when a chalcone which cyclises rapidly upon melting has a lower melting point than its isomeric flavanone; the chalcone melts, then cyclises to the flavanone which then solidifies, before remelting at the higher (flavanone) melting point. Such behaviour was observed in the present study for 2',4,4',6'-tetrahydroxychalcone (***85***) and for 2',6'-dihydroxy-2,4,6-trimethoxychalcone (***44***), while most of the other 2',6'-dihydroxychalcones used in this study also rapidly decolourised upon melting, a phenomenon which has been reported[61] previously.

Deshpande and Patil[203] also report the synthesis of 2',3,6'-trihydroxy-4-methoxychalcone in high yield, using essentially the same method as that in their paper[201] discussed above, although in this case the evidence for the claimed chalcone is good. Apparently, Seshadri and Trivedi[204] experienced no difficulties in the synthesis of several 3'-nitro- substituted 2',6'-dihydroxychalcones — and from them their isomeric 5-hydroxyflavanones — using the base condensation method. Possibly, the lack of difficulty experienced in the condensation was due to hydrogen bonding of the free hydroxy group to the neighbouring nitro group, or perhaps to an electronic substituent effect.

Syntheses of a number of 2',6'-dihydroxychalcones based on a phloroglucinol (1,3,5-trihydroxybenzene) A-ring have been successfully performed *via* the base-catalysed condensation method. For instance, isoxanthumol has been prepared[205] in low yield, and the compound was identical by ^{1}H n.m.r. spectroscopy with the naturally occurring[87] product. Bognar[206] *et al.* have reported the synthesis of several 4'-β-D-glucosyl ethers of 2',4,4',6'-tetrahydroxychalcone. These compounds appear to contain their isomeric flavanones as major contaminants, judging from the u.v. spectra presented by the same authors[207] in a subsequent kinetic investigation. Such contamination is hardly surprising, in view of the well documented susceptibility of 2',6'-dihydroxychalcones to cyclisation (see chapters 1 and 2). It is rather surprising that these workers[207] found their hesperetin chalcone derivative to be *ca.* 100 times less reactive towards cyclisation than the naringenin derivative at pH 7, and it may well be that this "chalcone" was really the flavanone or some other compound altogether, in view of the reported[206] difficulties experienced with its isolation and purification. The isosakuranetin chalcone[206] was at best impure, since it unaccountably formed an orange-red precipitate on standing at pH 7 (rather than precipitating just the expected colourless flavanone), and because the reported properties of this compound differ from those of the chalcone obtained by Shimokoriyama[25] from the naturally occurring flavanone.

Another example is the synthesis of 2,2',6'-trihydroxy-4'-β-D-glucosyloxychalcone, which was, however, obtained[208] in only 3% yield, after chromatographic purification.

Aurnhamer[209] *et al.* presumably prepared the 4'-(γ•γ-dimethylallyl) ether of 2',4,4',6'-tetrahydroxychalcone (which was not isolated) during their syntheses of selinone and related flavanones.

Clearly, then, base-promoted condensation cannot be regarded as a reliable method for the synthesis of substituted 2',6'-dihydroxychalcones from substituted 2',6'-dihydroxyacetophenones; and even when this method does work, the product is often obtained in rather low yields.

6.3.3 Base-Catalysed Condensation of Acetophenones and Benzaldehydes with Phenolic Groups Protected as Ethers

In order to overcome the difficulties often experienced with the condensation of 2',6'-dihydroxyacetophenones with benzaldehydes under basic conditions, some workers have made use of protecting groups, so that no free hydroxy groups are present on the reactants during the condensation step.

Using the methoxymethyl ether as a protecting group, Obara *et al.*[210] have successfully synthesised several heavily hydroxylated 2',6'-dihydroxychalcones. The major advantage of this protecting group is its facile removal under mild acidic conditions. From the pH-rate profiles in chapter 2 it can be seen that most of the 2',6'-dihydroxychalcones are most stable in the pH range 1-3, so that the rate of cyclisation of deprotected chalcones will be extremely slow under the conditions required for the removal of the methoxymethyl ether protecting group. The only drawback with this protecting group is that methoxymethyl chloride, the alkylating agent from which the protected ethers are generated, presents a considerable toxicity[211] hazard.

It can be clearly seen from the pH-rate profiles of chapter 2 that any protecting group whose removal requires strongly acidic, or neutral to basic conditions, is going to create a problem, in that under these conditions the rate of cyclisation of the chalcone (once it is deprotected) is likely to be too rapid to permit isolation of the 2',6'-dihydroxychalcone itself. It is this problem that has prevented the successful use of benzyl and methyl ethers as protecting groups for the condensation reaction — although the protected chalcones are easily generated, their deprotection results in isolation only of the flavanone isomeric to the desired chalcone.

The benzyl group has been widely used for the protection of phenolic groups. Unfortunately, removal of the benzyl group by refluxing in aqueous alcoholic acid reportedly[198,212,213] also results in the cyclisation of the chalcone to the flavanone. This was my experience also; when 2'-hydroxy-3'-benzyl-4',6'-dibenzyloxychalcone (*72*) was deprotected in this manner, the first product isolated was 5-hydroxy-6-benzyl-7-benzyloxyflavanone (*68*). It may well be that the cyclisation of the 2'-hydroxychalcone is more rapid than debenzylation under such conditions, and that subsequent removal of the benzyl group from the 5-position of the resultant flavanone follows rapidly. Such a possibility is supported by our finding that the above-mentioned 5-hydroxyflavanone undergoes cleavage of the 7-benzyloxy group only very slowly (and probably at a rate comparable to that for the 2'- and 4'-benzyl ethers of the parent chalcone), while it is well known[214] that the cleavage of ethers *peri* to a carbonyl group (as in 5-alkoxyflavanones) occurs much more readily. The other method commonly used for the removal of benzyl ether protecting groups is catalytic hydrogenation. This method is clearly unsuited where the desired product is a chalcone, since chalcones are known to undergo facile hydrogenation to dihydrochalcones under these conditions. Therefore, debenzylation by catalytic hydrogenation can only be carried out without undesirable reduction of the product, if the chalcone is first converted to its isomeric flavanone (*e.g.* ref. 215).

Similarly, the even more vigorous and acidic conditions required for the cleavage of methyl ethers has been reported[216-219] to result in only the flavanones being recovered. This method appeared to present an easy route to 5-hydroxyflavanones (*via* 2'-hydroxy-6'-methoxychalcones). In my hands, however, the refluxing of such chalcones with a mixture of hydroiodic and acetic acids generated large quantities of a gum, presumably due to some undesirable side reaction. In the case of 2'-hydroxy-4-chloro-6'-methoxychalcone (**90**), it was found that if most of the chalcone was cyclised (to the 5-methoxyflavanone) prior to the deprotection step, the desired 5-hydroxyflavanone was obtained fairly cleanly, and in fair yield (see chapter 7).

Due to the difficulties inherent in obtaining a highly toxic and possibly carcinogenic substance such as methoxymethyl chloride from abroad, and the precautions necessary in using it, an alternative protecting group with similar characteristics, but without a toxicity hazard, was sought. A promising candidate was the tetrahydropyranyl (THP) ether. These ethers are easily generated[220,221] from 2,3-dihydro-4H-pyran (henceforth referred to as dihydropyran) under mild acidic conditions, are stable to alkali, and are highly susceptible to acid-catalysed hydrolysis under very mild conditions.[221] In addition, 2,3-dihydropyran is of low toxicity.

Initially my attempts at synthesis were aimed at generating the bis-THP ether of 2',6'-dihydroxyacetophenone. After reaction of the acetophenone with a large excess of dihydropyran, the reaction mixture was diluted with ether and extracted with aqueous sodium hydroxide (2 mol l^{-1}), in order to remove the unreacted and mono-*O*-THP acetophenones. Removal of the solvent *in vacuo* afforded an oil which contained much dihydropyran, and this oil was condensed with benzaldehyde after the method of Seshadri.[200] Acidification, extraction with ether, purification by p.l.c., followed by recrystallisation afforded a small amount of red crystals. The substance decolourised when dissolved in methanolic sodium acetate, and mass spectrometry showed the substance, presumably 2',6'-dihydroxychalcone (**34**), to exhibit the expected molecular ion peak at *m/z* 240. The overall yield of the reaction was, unfortunately, very low.

It became apparent that the reason for the low yield in the above reaction was that the major product of the initial protection step was the mono-*O*-THP ether of the acetophenone, so that only a very small amount of the di-protected acetophenone was formed. Since no difficulties had been experienced with the condensation of 2'-hydroxy-6'-methoxyacetophenones with benzaldehydes to form chalcones (see chapter 7), it was envisaged that the 2'-hydroxy-6'-*O*-THP-acetophenone could be similarly condensed to form 2'-hydroxy-6'-*O*-THP-chalcones, which could subsequently be deprotected to afford the desired 2',6'-dihydroxychalcones. This method, in fact, proved to be a rapid and efficient one for the synthesis of 2',6'-dihydroxychalcones.

The mono-*O*-THP ether was isolated by adding the dihydroxyacetophenone – dihydropyran reaction mixture to ether after the protection step was complete (less than 30 minutes, as shown by t.l.c.). The ether layer was then washed with a dilute sodium carbonate solution to remove any unreacted acetophenone. Extraction with 2 mol l^{-1} sodium hydroxide, followed by careful acidification of the alkaline extracts to pH 8-10, precipitated the protected acetophenone in a fairly pure state (as judged by t.l.c.). It was found that if the mixture was over-acidified, the initial dihydroxyacetophenone was rapidly regenerated. Extraction with a non-polar organic solvent, followed by removal of the solvent *in vacuo*, usually gave the protected acetophenone as an oil, sufficiently pure to be used in the condensation step without further purification. When this oil was allowed to stand, it slowly reverted to the deprotected acetophenone. The condensation step was then carried out in the usual manner (after the method of Seshadri[200]). Usually the crude chalcone was deprotected after the condensation, and then purified by p.l.c., although in one case (*70*) the protected chalcone was isolated, purified and characterised prior to deprotection. The protected chalcone (*70*) was, at least in the crystalline form, stable at room temperature for over one year.

Essentially the same method was applied to 2',4',6'-trihydroxyacetophenone, in an endeavour to make 2',4',6'-trihydroxychalcones. Unfortunately, the required intermediate, 2'-hydroxy-4',6'-bis-*O*-THP-acetophenone could be isolated only in very low yields, and the reaction was not investigated further.[§]

6.3.*4* Acid-Catalysed Condensation of Acetophenones and Benzaldehydes

Lafon[222] claims to have synthesised several 2',4',6'-trihydroxychalcones by direct condensation of unprotected 2',4',6'-trihydroxyacetophenone with variously substituted benzaldehydes in the presence of hydrochloric and acetic acid. However, the reported properties (colour, solubility and elemental analyses) of the compounds formed are not consistent with the chalcones, and their melting points differ from those in the literature in the few cases[197,223] where the authentic chalcones are available.

Several attempts to use the method of Lafon, and minor variations on it, all failed in my hands. In no case could the desired chalcone, or even its isomeric flavanone, be isolated from the reaction mixture.

[§] The low yield of the desired bisTHP ether may be due to *C*-alkylation reactions; *C*-alkylation has been found to interfere with the protection of 1,3-dihydroxy-4-methoxybenzene with dihydropyran (C. J. Adams, unpublished work).

More promising appeared to be the method of Russel and Todd,[224] who reported the condensation of 2′,4′,6′-tribenzoyloxyacetophenone with benzaldehydes in cold ethyl acetate saturated with dry hydrogen chloride, with the resultant chalcone being isolated in high yield. This chalcone was subsequently deprotected by hydrolysis in aqueous ethanolic 3% potassium hydroxide at reflux, under nitrogen.

We used this method in several attempts to synthesise 2′,6′-di- and 2′,4′,6′-tri-hydroxychalcones from the corresponding di- and tri-benzoyloxyacetophenones. The reaction solutions slowly went yellow, but because t.l.c. indicated the presence of at least four yellow compounds, this method was not investigated further.

Swaleh *et al.*[225] have used the method of Russel and Todd[224] to form what was claimed to be 3-methoxy-2′,4,4′,6′-tetrahydroxychalcone in 96% yield, identical to that obtained by Russel and Todd. It seemed to me, however, that the product was unlikely to be the claimed chalcone, due to the rather vigorous hydrolysis and work-up procedures used; the intermediate chalcone benzoate was refluxed in 2% potassium hydroxide, acidified, taken up in ether and the ether layer *repeatedly* extracted with sodium bicarbonate "to remove the benzoic acid". For a start, it would seem unlikely that in 2% KOH the equilibrium fraction in the chalcone form would be ⩾ 96%. In addition, it would seem almost certain that repeated extraction of the ether solution of the claimed chalcone with bicarbonate would result in complete removal of the chalcone into the aqueous phase, since the results of chapter 2 suggest $pK_{a1} \leqslant 7$ for such a chalcone.

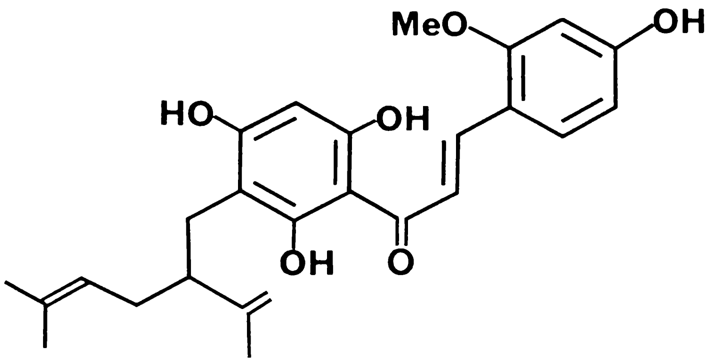
In order to see whether this was indeed the case, I conducted a test whereby 4′,5,7-trihydroxyflavanone (***4***; *ca.* 200 mg) was partitioned between equal volumes (50 ml) of ether and 1% w/w sodium bicarbonate. The above flavanone was used because it had the hydroxyl groups in the same positions as the flavanone isomeric with the "chalcone" of Swaleh *et al.*,[225] and so should have roughly the same pK_a values and water solubility. It should be noted that such a test is conservative, in that the pK_{a1} of the flavanone is likely to be somewhat higher than that of its isomeric chalcone, and that the chalcone is also likely to be more water soluble than its isomeric flavanone due to the higher degree of hydroxylation in the former. The amount of the flavanone in the ether layer was determined by separating this fraction, washing it with dilute hydrochloric acid, then removal of the solvent *in vacuo*. When dry, the sample was accurately weighed (0.1856 g) by difference. The amount in the aqueous layer was determined by acidifying the aqueous fraction with hydrochloric acid (2 mol l^{-1}), and extracting it twice with ether. The resultant ether solution was then worked up in the manner described for the ether fraction above, to afford 0.0280 g of the flavanone. These results show that 13% of the flavanone had partitioned into the bicarbonate layer. This suggests that the even more acidic chalcone would be readily removed from an ether solution by extraction with dilute bicarbonate, and that repeated extractions would completely remove all of such a chalcone, if it were present. Furthermore, the bicarbonate would also rapidly convert the extracted chalcone into the flavanone form, in view of the known susceptibility of 2',6'-dihydroxychalcones to cyclisation (chapters 1 and 2). Thus, it appears almost certain that the product was neither the claimed chalcone, nor its isomeric flavanone, and as a result the method of Russel and Todd[224] as a route to 2',6'-dihydroxychalcones must be regarded as suspect.

6.3.5 Ring-Opening of 5-Hydroxyflavanones

Advantage may be taken of the chalcone – flavanone equilibrium to generate 2',6'-dihydroxychalcones from their isomeric 5-hydroxyflavanones. Although the work of Narasimhachari[60] suggested that this method did not work for flavanones containing a 5-hydroxy group (as discussed in chapter 1), quite a number of workers have subsequently used it successfully to synthesise 2',6'-dihydroxychalcones.

The first to deliberately use this approach was Shimokoriyama,[25] who in 1957 reported the synthesis of four 4'-glycosyloxy-2',6'-dihydroxychalcones from the naturally occurring 5-hydroxy-7-glycosyloxyflavanones. The earlier (1954) reported[61] isolation of 2',4,6'-trihydroxy-4'-methoxychalcone as a natural product was probably the result of inadvertent ring opening of the naturally occurring flavanone under the strongly basic extraction conditions used. Even before these syntheses were reported, there existed strong evidence that 2',6'-dihydroxychalcones occurred in equilibrium with 5-hydroxyflavanones under both acidic and basic conditions. This evidence came from the phenomenon known as ring isomeric change, where a 6- or 8-substituted 5-hydroxyflavanone, when placed in a moderately basic or strongly acidic medium, often isomerised partially or completely, to the isomeric 8- or 6-substituted flavanone (see chapter 1). Such an isomerisation requires cleavage of the pyran ring, and implies the intermediacy of an easily cyclised chalcone-type intermediate.

Since then, base-catalysed ring opening has been used to synthesise several 2',6'-dihydroxychalcones. The natural product, pashanone (2',6'-dihydroxy-3',4'-dimethoxychalcone) has been synthesised[226] in *ca*. 50% yield from both 5-hydroxy-7,8-dimethoxy- and 5-hydroxy-6,7-di-methoxyflavanone. Kyogoku[227] has reported the synthesis of the chalcone shown below (*64*), from the naturally occurring isomeric 8-alkylflavanone.



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In 1967, Moustafa and Wong[48] reported the synthesis of 2',4,4',6'-tetrahydroxychalcone (*85*) by base-promoted ring opening of the naturally occurring flavanone (naringenin; *4*), in order to use the chalcone as a substrate in studies of the chalcone isomerase enzyme. The method has subsequently[32,53,228] been widely used in the synthesis of this and other highly hydroxylated 2',6'-dihydroxychalcones, mainly for use in enzyme studies. It seemed highly likely, in view of the work reported in chapter 2, that the isolation and purification procedures used (acidification followed by recrystallisation from aqueous alcohol) would have resulted in considerable flavanone contamination of the product. That this was indeed the case was confirmed by a published[34] u.v. spectrum of "2',4,4',6'-tetrahydroxychalcone" at pH 4, which clearly showed the flavanone (*4*) to be the major constituent of the product.

Attempts to use slight modifications of the method of Moustafa and Wong, in my hands gave a product which, although it undoubtedly contained the desired chalcone, also contained large amounts of decomposition products: t.l.c. showed numerous compounds to be present in the product of the reaction. When, however, the free (4'- and 7-)

hydroxy groups on the flavanone were protected as their THP ethers (*83*), the desired chalcone (*85*) could be obtained rapidly and conveniently in the following manner. The crude 5-hydroxy-4',7-bis-*O*-THPflavanone (*83*) was dissolved in ether, then the potassium hydroxide solution was added and the mixture shaken, and allowed to separate. The aqueous layer was then run out into an excess of dilute acid, causing rapid deprotection to occur, while the excess dihydropyran from the protection step remained behind in the ether layer. The desired chalcone (*85*) was then simply extracted from the acidified fraction, and purified.

Because the presence of the flavanone has been found to inhibit the action of the chalcone – flavanone isomerase enzyme,** the method of Moustafa and Wong[48] is clearly less than satisfactory. Obviously, it would be desirable to have a convenient, high yielding and reliable method for the synthesis of highly hydroxylated chalcones from their isomeric naturally occurring 5-hydroxyflavanones. We devised just such a method — based on the kinetic studies of Old,[46,71] and on the results reported in chapter 2 — which will be discussed in section 6.3.6. It should be pointed out at this stage, however, that even if the chalcones used in the above-mentioned enzyme studies had been pure, the usual method of administering them (as their "sodium salts") to the enzyme would have resulted in considerable non-enzymic flavanone formation, both before and after their addition to the enzyme.

The first 2',6'-dihydroxychalcone synthesised as part of this study was the well known natural product,[75,83,92] 2',6'-dihydroxy-4,4'-dimethoxychalcone (*32*), made by ring opening of the flavanone. This chalcone, which had not previously been synthesised, was identical in all respects to the natural product. Analysis of the pH-rate profile for the isomerisation of this compound gave the various rate coefficients for the cyclisation and ring-opening reactions. This, in turn, enables one to calculate the position of the chalcone – flavanone equilibrium at different pH values (above *ca.* pH 9), as well as the rate at which the equilibrium position will be approached (see section 2.1). This information allows one to choose the optimum conditions for the ring-opening (or cyclisation) reaction, as well as the work-up procedures which will result in the minimum conversion of the chalcone back into the flavanone.

Clearly, many workers have used conditions for the ring-opening reaction which were much more vigorous than necessary, no doubt resulting in an increase in undesirable side-reactions. Presumably, this is a result of the belief that 2′,6′-dihydroxychalcones are difficult to make, due to the low reactivity of their isomeric 5-hydroxyflavanones. Although it is true that 5-hydroxyflavanones are much less susceptible to ring opening than flavanones lacking an A-ring hydroxy group, figure 6-1 suggests that a good yield of 2′,6′-dihydroxychalcone should be obtainable in the presence of aqueous *ca.* 5 mol l^{-1} potassium hydroxide at 30°C: presumably the difficulties experienced with this method by some workers were the result of a poor choice of isolation and purification conditions, a factor which will be considered later. One 5-hydroxyflavanone (naringin; ***18***) was found to exist nearly entirely in the chalcone form in the presence of 1 mol l^{-1} sodium hydroxide! There is no need for the common practice of refluxing the reaction mixture: calculations show that the time taken to reach 99% of the equilibrium composition in 5 mol l^{-1} NaOH at 30°C should be of the order of 1 minute for the 2′,6′-dihydroxychalcones studied in chapter 2.

The most common error in methodology that is encountered in the literature concerning the ring-opening reaction is the "careful" acidification of the strongly basic reaction solution. It was probably this factor that was responsible for the conclusions of Narasimhachari[60] regarding the relative stabilities of 2',6'-dihydroxychalcones, and their isomeric flavanones. The pH-rate profiles of chapter 2 show that, with one exception (***44***), 2',6'-dihydroxychalcones are very stable at pH 1-3, while they are least stable at pH 6-12. A careful (*i.e.* slow) acidification of the strongly alkaline ring-opening medium will therefore result in the reactive chalcone spending a considerable length of time in a pH region in which it is highly unstable (half-life at pH 10 typically from 35 seconds to 3 minutes). The best method is to pour the basic solution into an ice-cold excess of dilute acid, with vigorous stirring. Even if the chalcone does not precipitate (thereby preventing cyclisation altogether), the rate of cyclisation in dilute acid (especially at *ca.* 0°C) should be very slow, while the product may be easily and rapidly extracted into a suitable non-hydroxylic organic solvent (in which the rate of cyclisation is negligible), from which the chalcone can readily be recovered).

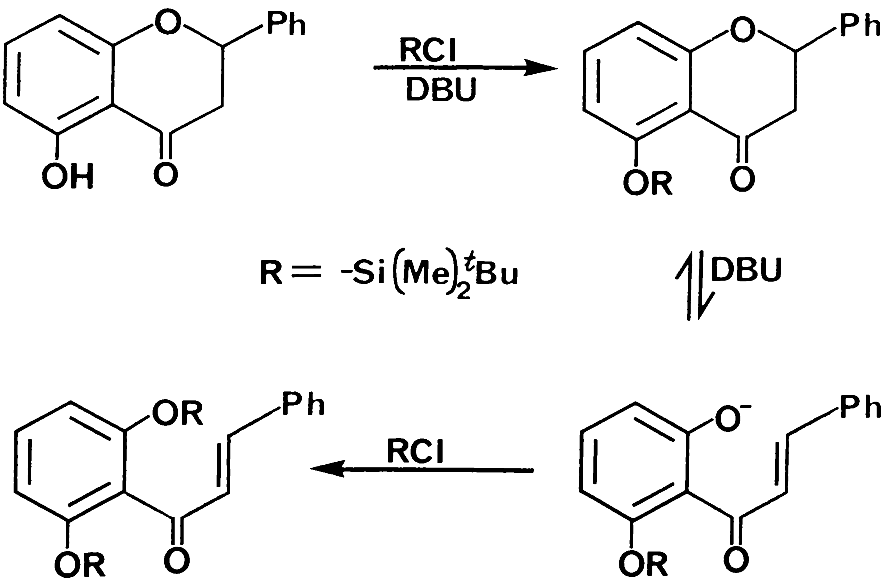
6.3.6 Base-Catalysed Irreversible Ring Opening of 5-Hydroxyflavanones

A comparison of the rate coefficients for the chalcone – flavanone equilibrium obtained in chapter 2 with those obtained by Old,[46,71] shows that 5-methoxyflavanone is nearly 1000 times more susceptible to base-catalysed ring opening than is 5-hydroxyflavanone (***35***). This suggests that if the 5-hydroxyl group of a flavanone were protected, then ring opening to the chalcone would be greatly facilitated. That this is so is supported by the fact that methylation of a 5-hydroxyflavanone with methyl iodide and potassium carbonate in acetone eventually gives the totally methylated chalcone (*e.g.* ref. 228). Similarly, even a weak base (sodium acetate) can catalyse the acetylation and ring opening of 5-hydroxyflavanones[229-231] to their fully acetylated chalcones. These methods do not, however, promise any advantages over normal synthetic methods: the problems of the methyl ether as a protecting group were discussed earlier, while basic hydrolysis of chalcone acetates can offer no advantages over the more direct base-catalysed ring opening.

In contrast, silyl ethers are ideal protecting groups for such a reaction. They are introduced under mildly basic anhydrous conditions, and can be readily removed under the weakly acidic conditions in which 2′,6′-dihydroxychalcones have been shown to be most stable. We found that when chlorotrimethylsilane was reacted with naringenin (*4*), using pyridine as the catalyst, the solution slowly went pale yellow. This suggested that if chalcone formation was taking place at all, it was occurring only slowly.

Recently, Aizpurua and Polomo[232] have introduced 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a catalyst for the insertion of even sterically bulky silyl groups onto hydroxyl groups which are of low reactivity due to such factors as hydrogen bonding or steric crowding. When I carried out the silylation of hesperetin [3′,5,7-trihydroxy-4′-methoxyflavanone; (*8*)] with chlorodimethyltertiarybutylsilane, and DBU as the catalyst, the reaction mixture became deep yellow within two days. The single yellow product was soluble in chloroform, and highly mobile on t.l.c. with chloroform as the eluent. Deprotection was carried out in aqueous alcoholic acid, and monitored by t.l.c. During the deprotection, a number of intermediate yellow products of lower mobility upon t.l.c. could be observed, and after a time, only one yellow product, immobile upon t.l.c. with chloroform, was present. Work-up then afforded the expected chalcone (*86*) in good yield.

Presumably, the method proceeds by initial rapid silylation of all the hydroxyl groups on the flavanone (including the one at the 5-position), followed by slow (DBU-catalysed) ring opening of the flavanone to give a low equilibrium concentration of the corresponding chalcone anion, which is subsequently trapped as its silyl ether, as shown in the scheme 6-5.



SCHEME 6-5 Possible Route of Ring Opening of 5-Hydroxyflavanones *via* DBU-catalysed Silylation

The utility of this reaction was subsequently extended by using trimethylsilyl ethers as the protecting groups. These ethers provide a major advantage (over most other ethers), in that they are extremely susceptible to hydrolysis in aqueous acids, thus greatly facilitating the deprotection step. In addition, the silyl halide (chlorotrimethylsilane) is both cheap and readily available. Thus, both

4-chloro-2',6'-dihydroxychalcone (*46*) and 2',4,4',6'-tetrahydroxychalcone (*85*) were conveniently synthesised from their isomeric flavanones using the DBU - chlorotrimethylsilane method.

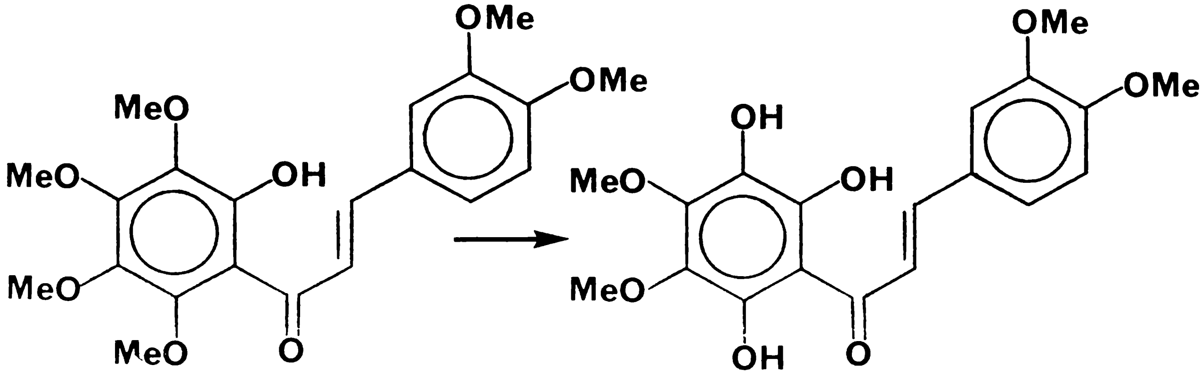
In contrast to the method of Moustafa and Wong,[48] then, this method is admirably suited to the synthesis of even highly hydroxylated chalcones, from their isomeric flavanones, and its ability to generate the pure chalcones of naturally occurring flavanones conveniently and in high yields should facilitate studies of the chalcone isomerase enzymes.

6.3.7 Miscellaneous Methods

Ramakrishnan and Kagan[197] have isomerised the mono-cinnamate esters of 1,3,5-tri- and 1,3-di-hydroxybenzenes with u.v. light, to afford several 2',4',6'-trihydroxychalcones, and 2',6'-dihydroxychalcone (*34*). The method is particularly suited to synthesis of 2',4',6'-trihydroxychalcones, since there is only one possible isomerisation product from the ester precursor. Unfortunately, the yields obtained are not high, and the mono-cinnamate esters must first be separated from the other products, after the initial ester formation. This method has also been used by Bhatia and Kagan[233] *et al.*, to synthesise 2',6'-dihydroxy-4'-methoxychalcone in low yield.

Mandloi and Sant[223] report having hydrolysed a 2'-glycosyloxy-4,4',6'-trihydroxychalcone in 7% sulphuric acid, to afford 2',4,4',6'-tetrahydroxychalcone (*85*).

Row and Sastry[234] have synthesised 2',3',6'-tri-hydroxy-3,4,4',5'-tetramethoxychalcone, by reduction of the corresponding 3',6'-quinochalcone (scheme 6-6). The quinochalcone itself was synthesised by the oxidative demethylation of 2'-hydroxy-3,3',4,4',5',6'-hexamethoxychalcone with nitric acid. Such a method is obviously of rather limited scope, synthetically, as it is only possible to generate 2',6'-dihydroxychalcones that also contain a hydroxyl group in the 3'-position.



SCHEME 6-6 Row and Sastry's[234] Route to 2',3',6'-Trihydroxy-3,4,4',5'-tetramethoxychalcone

6.4 Selective Demethylation of 5-Methoxyflavanones

Because it proved possible to generate the 2',6'-dihydroxychalcones from their corresponding flavanones, several attempts were made to synthesise 5-methoxyflavanones, and then to selectively demethylate them at the 5-position.

It has often been claimed in the literature (*e.g.* ref. 235) that aluminium chloride in benzene completely demethylates flavanones, while the same reagent in ether[236] or acetonitrile[237],[238] selectively demethylates only in the 5-position (which is *peri* to the carbonyl group). It has since been shown,[239] however, that aluminium chloride in benzene actually cleaves the flavanone structure itself, affording the demethylated acetophenone (derived from the flavanone A-ring) as the product.

In my hands, selective demethylation with aluminium chloride in ether proved to be a capricious reaction; sometimes no detectable reaction occurred at all, while in one case *ca*. 50% demethylation of 5,7-dimethoxyflavanone (*81*) was detected by ^{1}H n.m.r., giving a mixture of starting material and the desired product, 5-hydroxy-7-methoxyflavanone (*82*).

Boron trichloride is reported[240] to selectively deprotect methyl ether groups *peri* to a carbonyl group. This method, which has not previously been used with flavanones, was used on 5-methoxyflavanone (*80*). An excellent yield of the required compound, 5-hydroxyflavanone (*35*), was thus obtained. Although this particular example cannot demonstrate whether or not the method is selective when applied to flavanones, the method obviously warrants further investigation in view of its convenience and of the high yield obtained in the above case.

6.5 Conditions for the Isolation and Purification of 2',6'-Dihydroxychalcones

As a result of the kinetic and synthetic studies reported herein, it is possible to identify the optimum conditions for the isolation and purification of 2',6'-dihydroxychalcones, conditions that will result in the minimum amount of isomerisation into their 5-hydroxyflavanones. Already mentioned in connection with the base-catalysed ring-opening reaction, is the necessity to minimise the time spent by a solution of a 2',6'-dihydroxychalcone in the pH range 6-12. Thus, whenever a 2',6'-dihydroxychalcone is in a hydroxylic medium, the pH should be maintained, wherever possible, in the region 1-3.

Chromatography on silica gel was found to be a safe procedure (using non-hydroxylic solvents as eluents). During t.l.c. (on silica), 2',6'-dihydroxychalcones migrated as distinct spots: if significant isomerisation were occurring during chromatography, then a streak (due to the 5-hydroxyflavanone) emanating from the chalcone spot would have been present. It is quite likely, however, that some cyclisation of the chalcone could occur over a longer period of time, particularly if the p.l.c. plate were allowed to dry. This problem may have been responsible for the presence of *ca*. 10% naringenin (***4***) in one of my preparations of 2',4,4',6'-tetrahydroxychalcone (***85***). To be on the safe side, the chalcone should be removed from the plate immediately after the development of the chromatogram has been completed.

Recrystallisation is another area of purification in which many workers appear to have run into problems. A number of authors (*e.g.* ref. 25, 48) report having "purified" 2',6'-dihydroxychalcones by recrystallisation from water, or from aqueous alcohols. Such procedures are not to be recommended, for two reasons. Firstly, 2',6'-dihydroxychalcones can reasonably be expected to be more polar than their isomeric flavanones, and therefore ought to be more soluble than their flavanones in polar solvents. Since the flavanone is likely to be the major contaminant of a preparation of a 2',6'-dihydroxychalcone, the ideal recrystallisation medium for purification of the chalcone will be one in which the chalcone is barely soluble, while the flavanone contaminant is highly soluble. A solvent of relatively low polarity is therefore desirable.

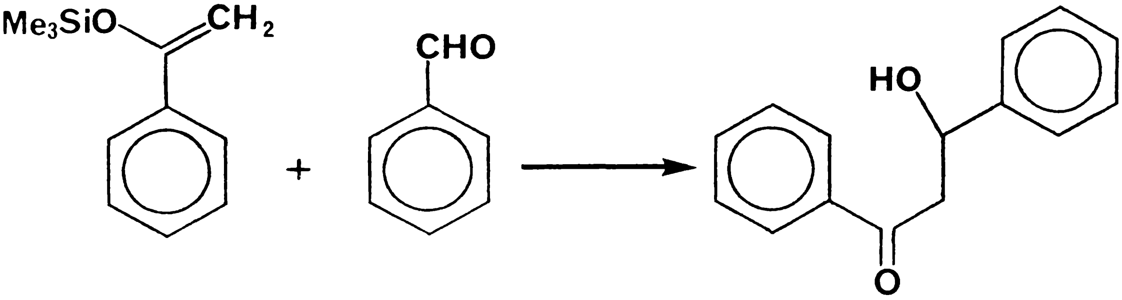
A second, and more obvious reason for not using hydroxylic solvents, such as aqueous ethanol, for recrystallisations of 2',6'-dihydroxychalcones can be seen in the pH-rate profiles in chapter 2. These chalcones all cyclised at an appreciable rate at pH 7 in aqueous solutions at 30°C. It was also noted that most of the chalcones studied here (section 2.1.5) cyclised completely upon standing in dry methanol for 1 hour. The rate constants for isomerisation of two chalcones were in fact measured in anhydrous redistilled methanol at 30°C, and their half-lives calculated: for 2',4,4',6'-tetrahydroxychalcone (*85*), 1.7 hours; and for 4-methoxy-2',3,4',6'-tetrahydroxychalcone (*86*), 6.0 hours. With the addition of water to the methanol, and with the elevated temperatures necessary for recrystallisation from such a solvent, the rate of cyclisation could easily be faster by an order of magnitude or more.

Clearly, then, the most satisfactory medium from which to perform recrystallisations of 2',6'-dihydroxychalcones is a non-hydroxylic one of relatively low polarity, which will avoid the problems discussed above. Even when a more polar solvent is required, it is usually not necessary to use alcohols, since we found solvents such as dioxane (in which we detected no measurable cyclisation - see section 2.1.5) to be excellent alternatives. It is notable also, that most of the 2',6'-dihydroxychalcones that have been isolated as natural products after recrystallisation, have been recrystallised from low polarity non-hydroxylic solvents. Should it prove necessary, for any reason, to perform the recrystallisation of a 2',6'-dihydroxychalcone from an aqueous or an alcoholic medium, the pH-rate profiles of chapter 2 clearly show that the inevitable cyclisation reaction will be minimised in the presence of *ca.* 0.01 mol l^{-1} hydrochloric acid.

6.6 Suggestions for Further Synthetic Investigations

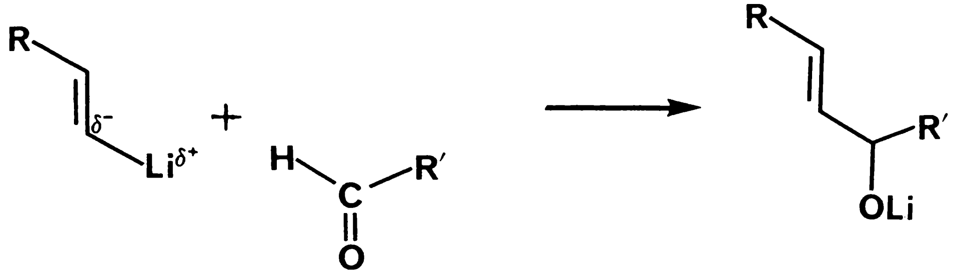
A number of promising methods for the synthesis of chalcones (and flavanones) presented themselves during the course of this investigation. Some of them have not yet been fully exploited in the flavonoid field, and warrant further investigation.

The silyl ethers of ketone enols are reported to undergo aldol condensation with benzaldehyde under mild, neutral conditions (*e.g.* ref. 241). Such a method may be capable of condensing acetophenones and benzaldehydes, which with subsequent dehydration would yield chalcones (scheme 6-7). The mild reaction conditions of the condensation reaction ought to allow the use of highly labile protecting groups (such as silyl ethers) in the generation of a wide variety of substituted chalcones.



SCHEME 6-7 Condensation of Silyl Ethers of Ketone Enols with Benzaldehyde

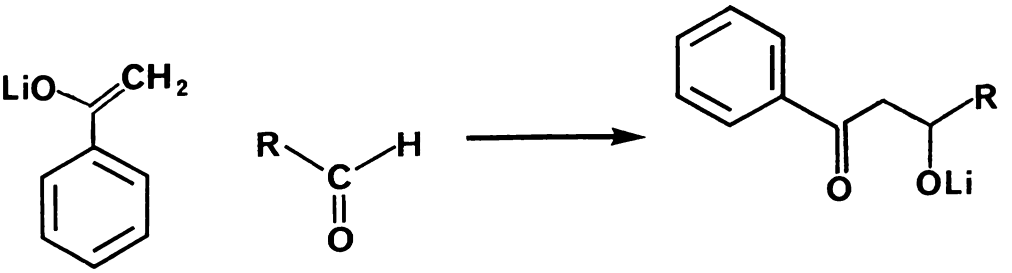
Vinyl lithium reagents add to aldehydes to give α,β-unsaturated alcohols.[242] This reaction, followed by oxidation of the hydroxy group to a keto group (scheme 6-8), could be exploited to provide a wide variety of chalcones and related compounds.



SCHEME 6-8 Addition of Vinyl Lithium to Aldehydes

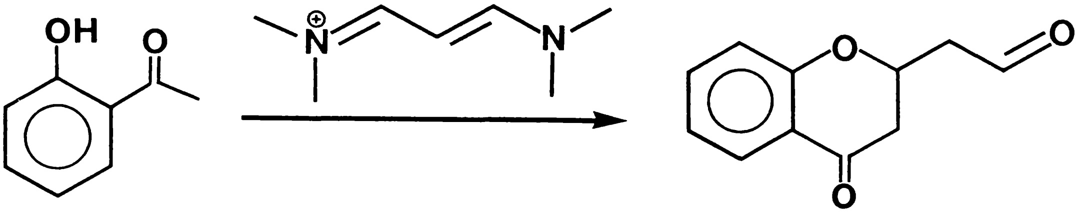
Recently,[243] the facile condensation of acetophenones and benzaldehydes to afford chalcones has been reported, using basic alumina as the catalyst. The reaction conditions were sufficiently mild that the carbomethoxy ester group was not attacked during the course of the reaction.

The lithium enolates of acetophenones have been condensed with aldehydes to afford β-keto alcohols, which were isolated. These alcohols underwent facile dehydration and ring closure (in dilute acid), giving the chromanones (scheme 6-9) directly.[244] The use of appropriate protecting groups in this reaction could well permit the synthesis of a wide variety of chalcones (and related enones), in addition to the chromanones.



SCHEME 6-9 Addition of Acetophenone Lithium Enolate to Aldehydes

The condensation of an iminium salt with the mono-methoxymethylether of 2′,6′-dihydroxyacetophenone (scheme 6-10) in pyridine, to afford a chromanone (presumably *via* the α,β-unsaturated ketone) has been reported.[245] Such a reaction may well be extendable to the synthesis of chalcones and other α,β-unsaturated ketones, as well as to chromanones.



SCHEME 6-10 The Condensation[245] of an Iminium Salt with an Acetophenone

Pinkey *et al.*[246] have recommended the addition of a quaternary ammonium salt to the base condensation medium. They claimed that this resulted in greatly increased yields of 2′-hydroxychalcones from the base-catalysed condensation of benzaldehydes and 2′-hydroxyacetophenones. The yields obtained by this method (65-95%) were not very different from those which were usually obtained in the present study, using the method of Seshadri[200] (see chapter 7). This modification of the standard method therefore appears to offer no advantages over the standard methods of chalcone synthesis.

Doshi and Ghiya[247] have used pulverised KOH in *N*,*N*-dimethylformamide (DMF) to condense acetophenones and benzaldehydes to chalcones, with good (60-85%) yields. As with the method of Pinkey,[246] however, this method appears to offer no advantages over standard methods of chalcone synthesis.

Several alternatives to the standard methods (acid- or base-promoted cyclisation of 2'-hydroxychalcones) of flavanone preparation have been reported.

Patonay *et al.*[248] have obtained a number of flavanones in adequate (40-70%) yields, by refluxing methanolic solutions of 2'-hydroxychalcones in the presence of Amberlyst A-21, a weakly basic anion exchange resin.

Kabbe[249] has condensed the enamines of aldehydes with 2'-hydroxyacetophenones in methanol in the presence of secondary amines, obtaining the chromanones in good yields. Such a method may be useful in the synthesis of a variety of flavanones (and other chromanones). If the 2'-hydroxy group were protected prior to the condensation, then it might also be possible to isolate the enones (isomeric to the chromanones) from this reaction.

A promising method for one-step synthesis of 5-hydroxyflavanones from substituted 2'-hydroxy-6'-methoxy- or 2',6'-dimethoxy-chalcones, or from 5-methoxyflavanones, has been reported.[250] Refluxing a DMF solution of the aforementioned chalcones or flavanones in the presence of equimolar quantities of powdered $NiCl_2$, Zn and KI for 2-3 hours, resulted in the 5-hydroxyflavanones, which were isolated in 70-80% yields.

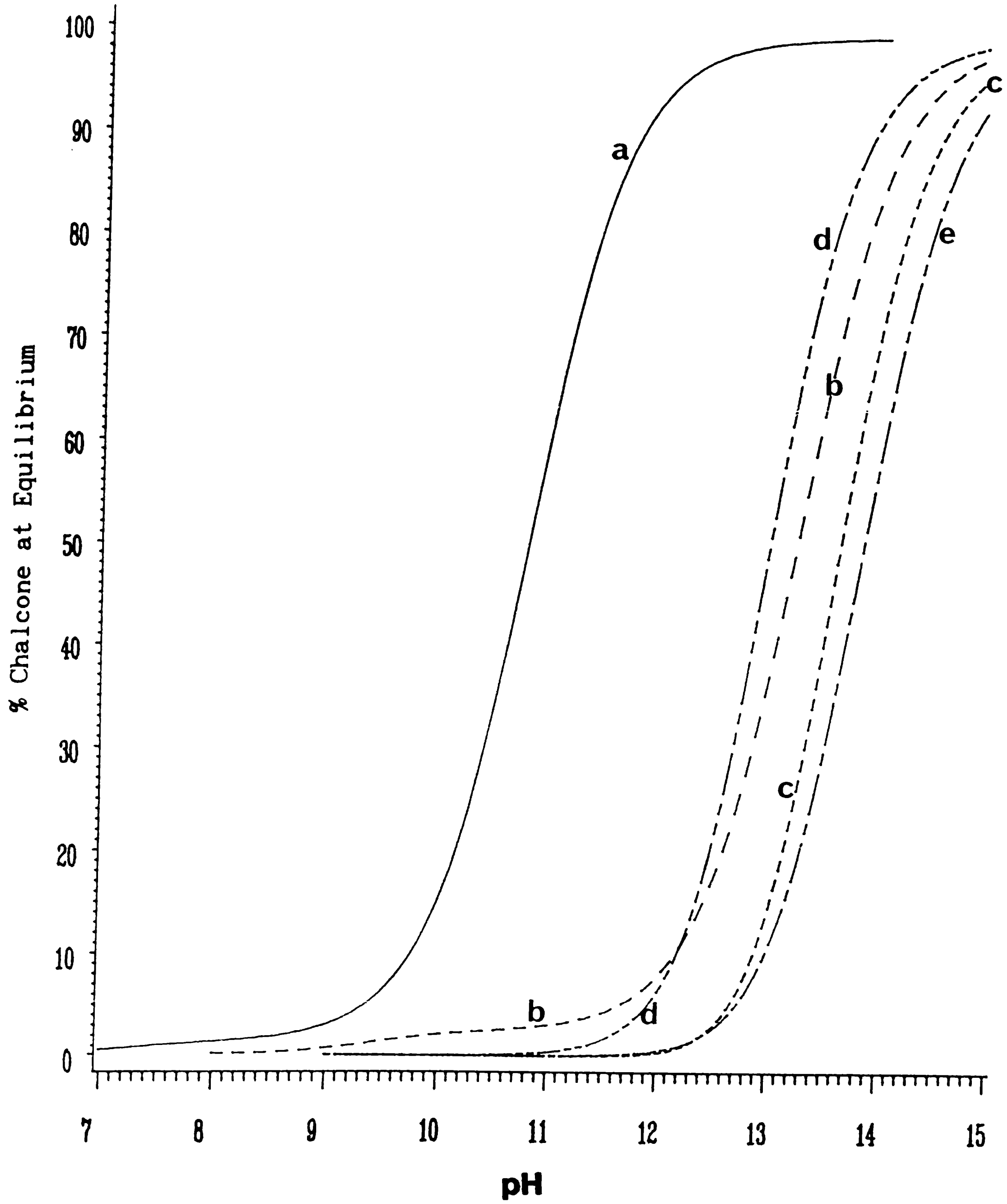


FIGURE 6-1 Fraction of Various 2'-Hydroxychalcones Present at Equilibrium as a Function of pH (Calculated from Equation 2-7, and the Rate Constants in Tables 5-1 and 5-2): (*a*) 2'-Hydroxy-6'-methoxychalcone; (*b*) 2',4'-Dihydroxychalcone (*17*); (*c*) 2',6'-Dihydroxychalcone (*34*); (*d*) Naringin-chalcone (*48*); (*e*) 2',6'-Dihydroxy-4,4'-dimethoxychalcone (*32*)

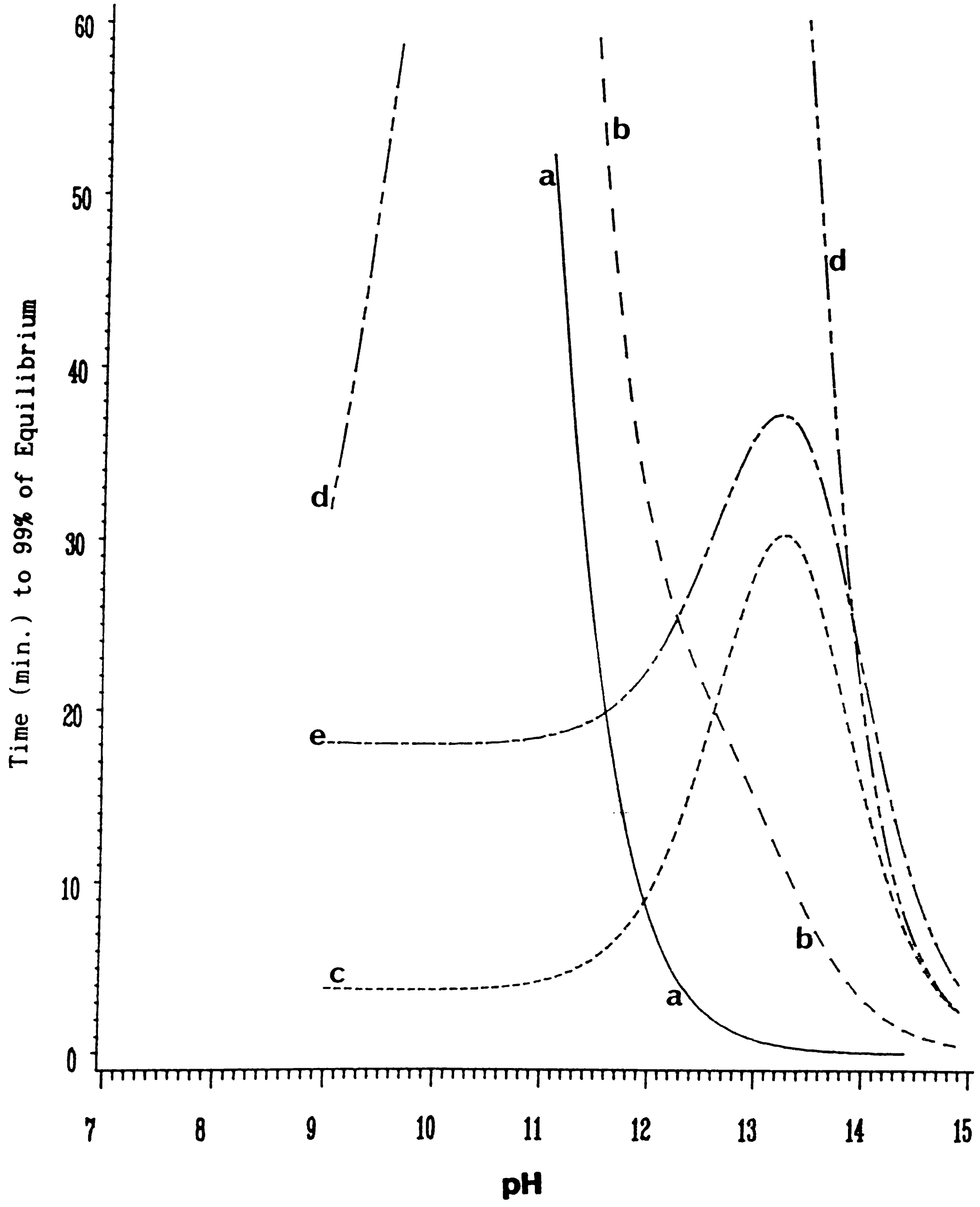


FIGURE 6-2 Time Required to Attain 99% of the Equilibrium Composition ($6.64t_{0.5}$) as a function of pH: (*a*) 2'-Hydroxy-6'-methoxychalcone; (*b*) 2',4'-Dihydroxychalcone (*17*); (*c*) 2',6'-Dihydroxychalcone (*34*); (*d*) Naringin-chalcone (*48*); (*e*) 2',6'-Dihydroxy-4,4'-dimethoxychalcone (*32*)

CHAPTER 7

EXPERIMENTAL

7.1 General Procedures

Nuclear magnetic resonance (n.m.r.) spectra.— ^{1}H and ^{13}C n.m.r. spectra were run at 89.55 and 22.49 MHz respectively, unless otherwise specified, on a JEOL FX90Q FT n.m.r. spectrometer. All chemical shifts are reported in the δ scale, in ppm from tetramethylsilane (Me_4Si). The following internal standards were used throughout (unless Me_4Si is specified as being present): for $(CD_3)_2CO$, $CD_3COCD_2\mathit{H}$ δ_H = 2.20 and $(CD_3)_2CO$ δ_C = 29.85; for $CDCl_3$, $C\mathit{H}Cl_3$ δ_H = 7.26 and $CDCl_3$ δ_C = 77.06; for $(CD_3)_2SO$, $CD_3SOCD_2\mathit{H}$ δ_H = 2.60 and $(CD_3)_2SO$ δ_C = 43.50; and for C_6D_6, $C_6D_5\mathit{H}$ δ_H = 7.40. The 200 MHz ^{1}H n.m.r. spectra were run at the Forestry Research Institute (Rotorua) on a Bruker AC200 FT n.m.r. spectrometer, and were all referenced directly to Me_4Si. Assignments of signals marked ^{a}, ^{b}, ^{c}, *etc.* are interchangeable. The assignments of the ^{13}C signals for flavanone carbons 5-9 were sometimes ambiguous, and were based on those of Markham and Chari,[251] and on comparisons with the spectra of related compounds in this study. Assignments for the ^{13}C signals for 2′-hydroxy-6′-alkoxy substituted acetophenones and chalcones were based on the work of Gommans.[252] Reported multiplicities of ^{13}C signals were determined using various spectroscopic techniques. The chemical shifts for the chalcone AB doublets (α-C, β-C) were assigned values calculated from the relationship[253] (for AB systems);

$$[(\sigma_A - \sigma_B)_{real}]^2 = [(\sigma_A - \sigma_B)_{apparent}]^2 - (J_{AB})^2$$

U.v. Spectra.— These were recorded on an Unicam SP8 500 spectrophotometer. Where absorbances were reported, solutions were made up by the addition of the appropriate solvent to a weighed amount of the compound in a 25 ml volumetric flask. For polyhydroxy chalcones and flavanones, the preferred solvent for absorbance measurements was methanol, containing 25 μl of aqueous 1 mol l^{-1} HCl, to prevent ionisation of the phenolic groups.

Mass Spectra.— Low resolution mass spectra were recorded at the Ruakura Agricultural Research Centre (RARC), on either a MAT CH5 or a Kratos MS80RFM mass spectrometer. High resolution mass spectra were recorded either at RARC on a Kratos MS80RFM mass spectrometer, or at the D.S.I.R. research centre at Mt. Albert, Auckland, on a Kratos MS30 mass spectrometer.

Elemental Analyses.— Elemental micro analyses were performed at the University of Otago, Dunedin.

Chromatography.— Preparative thin layer chromatography (p.l.c.) was carried out on 2 mm layers of silica gel (Merck 60 $PF_{254\ +\ 366}$), and thin layer chromatography (t.l.c.) on commercially prepared plates (Merck 60 F_{254}, 0.2 mm thick). Visualisation (when necessary) was carried out under ultraviolet (u.v.) light. The chromatograms were developed [in a tank saturated with the vapour of the eluent(s)], at room temperature.

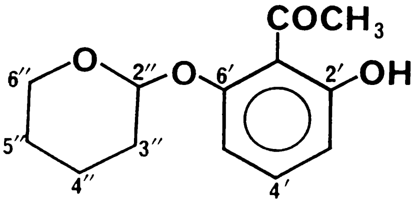
Recrystallisations.— Recrystallisations from mixed solvents were performed by dissolving the compound, with heating, in the better of the two solvents, then adding the poorer solvent under reflux, until the solution became saturated. The solution was then allowed to cool slowly to room temperature, before refrigeration of the mixture. The recrystallised solid was then recovered from the chilled solution by filtration at the pump.

Melting Points.— Melting points were determined on a Reichert Thermopan melting point apparatus, and are uncorrected. Melting points were in all cases determined at a rate of temperature change of less than 3°C min^{-1}.

Reagents.— All reagents and solvents used were either available commercially, or were prepared by procedures described in the literature. In all cases reagents and solvents displayed the appropriate boiling or melting points.

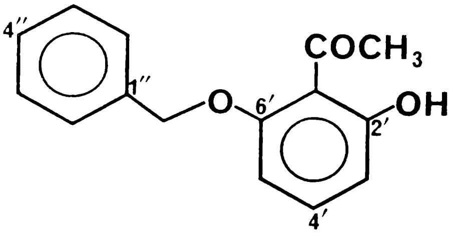
7.2 Acetophenones

2′-Hydroxy-6′-tetrahydropyranyloxyacetophenone (***65***).— To 2′,6′-dihydroxyacetophenone (0.5 g, 3.3 mmol) in dry dioxane (2 ml) was added 2,3-dihydropyran (1.5 g, 18 mmol) followed by toluene-4-sulphonic acid (5 mg) in dry dioxane (0.5 ml). The mixture, which quickly became warm, was left to stand for 3 hours. The reaction was stopped by pouring the mixture into 5% aqueous sodium bicarbonate (50 ml) and petroleum spirit (60-80°C) (50 ml) in a separating funnel and shaking, with the aqueous layer being discarded. The organic phase was then extracted with aqueous sodium hydroxide (2×10 ml; 2 mol l^{-1}), the extracts being added to 5% aqueous sodium bicarbonate (50 ml), and the mixture cautiously acidified (with HCl, 2 mol l^{-1}) until the pH was approximately 8. Extraction of the aqueous phase with petroleum spirit (60-80°C) (2×30 ml), drying over magnesium sulphate, filtration, and concentration of the organic phase *in vacuo* followed by chilling overnight (at -20°C) gave pale yellow crystals of 2′-hydroxy-6′-tetrahydropyranyloxyacetophenone (***65***) (0.46 g, 64%). It decomposed slowly on standing or more rapidly upon heating, to starting materials, and was used without further purification. The n.m.r. assignments were based on those of Gommans.[252] δ_H (90 MHz; $CDCl_3$) 1.7, 1.9, 3.7, 5.5 (br, tetrahydropyranyl protons), 2.7 (3 H, s, $COCH_3$), 6.5 (1 H, d, *J* 8 Hz, 3'-H), 6.6 (1 H, d, *J* 8 Hz, 5'-H), 7.3 (1 H, t, *J* 8 Hz, 4'-H), 13.1 (1 H, s, 2'-OH); δ_C (22 MHz; $CDCl_3$) 19.0 (t, 4''-C), 24.8 (t, 5''-C), 30.2 (t, 3''-C), 33.7 (q, 2-CH_3), 62.2 (t, 6''-C), 97.2 (d, 2''-C), 104.6 (d, 5'-C), 111.1 (d, 3'-C), 111.7 (s, 1'-C), 136.0 (d, 4'-C), 159.0 (s, 2'-C), 164.2 (s, 6'-C), 204.8 (s, C=O); *m/z* 236 ($M^{+\cdot}$, 6%), 153 (16), 152 ($M^{+\cdot}$ - 84, 100), 137 (94).



65

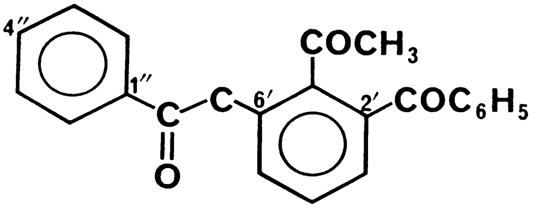
6'-Benzyloxy-2'-hydroxyacetophenone (*66*).— To potassium carbonate (1.82 g), 2',6'-dihydroxyacetophenone (*73*; 2.00 g, 13 mmol) and a few crystals of potassium iodide in refluxing dry acetone was added benzyl chloride (*ca.* 5 ml). Reflux was continued for 48 hours, then most of the solvent was removed *in vacuo* and the product taken up in chloroform. The organic fraction was then extracted with sodium hydroxide (2x50ml; 2 mol l^{-1}), and the basic extracts acidified prior to extraction with chloroform. The chloroform was then removed *in vacuo* and the residue recrystallised from methanol to afford pale yellow needles (1.73 g, 54%) of 6'-benzyloxy-2'-hydroxyacetophenone (*66*), m.p. 108°C; δ_H (90 MHz; $CDCl_3$, standard Me_4Si) 2.61 (3 H, s, $COCH_3$), 5.12 (2 H, s, OCH_2Ar), 6.45 (1 H, d, *J* 8.2 Hz, 5'-H), 6.56 (1 H, d, *J* 8.2 Hz, 3'-H), 7.32 (1 H, t, *J* 8.2 Hz, 4'-H), 7.40 (5 H, br s, 2'',3'',4'',5'',6''-H), 13.23 (1 H, s, 2'-OH); δ_C (22 MHz; $CDCl_3$, standard Me_4Si) 33.9 ($COCH_3$), 71.0 (t, OCH_2Ar), 102.2 (3'-C), 110.9 (5'-C), 111.5 (s, 1'-C), 127.9 (3''-C, 5''-C)[a], 128.4 (4''-C), 128.6 (2''-C, 6''-C)[a], 135.7 (s, 1''-C), 136.0 (4'-C), 160.6 (s, 6'-C)[b], 164.7 (s, 2'-C)[b], 205.0 (s, C=O); *m/z* 242 ($M^{+\cdot}$, 17%), 200 (14), 92 (13), 91 (100); (Found C, 74.3; H, 5.8. Calc. for $C_{15}H_{14}O_3$: C, 74.4; H, 5.8%).



66

2',6'-Dibenzoyloxyacetophenone (*67*).— To a solution of benzoic anhydride (10 g) in pyridine (10 ml) was added 2',6'-dihydroxyacetophenone (*73*; 2.00 g, 13 mmol). The mixture was allowed to stand for two days before work up (in the same manner as for the diacetoxyacetophenone above). Recrystallisation from methanol gave colourless crystals (3.84 g, 81%) of 2',6'-dibenzoyloxyacetophenone (*67*), m.p. 104°C (lit.,[258] 102-103°C); δ_H (90 MHz; solvent $CDCl_3$; standard Me_4Si) 2.47 (3 H, s, $COCH_3$), 7.1-7.6 (m), 8.0-8.2 (m); δ_C (22 MHz; solvent $CDCl_3$; standard Me_4Si) 31.1 ($COCH_3$), 120.4 (3'-C, 5'-C), 128.4 (s, 1'-C), 128.5 (s, 1''-C), 128.5 (3''-C, 5''-C)[a], 130.0 (2''-C, 6''-C)[a], 130.6 (4'-C), 133.8 (4''-C), 147.8 (s, OCOAr), 164.2 (s, 2'-C, 6'-C), 197.9 (s, C=O); *m/z* 360 ($M^{+\cdot}$, 15%), 342 (2), 239 (6), 238

(8), 106 (36), 105 (100); (Found C, 73.1; H, 4.5. Calc. for $C_{22}H_{16}O_5$: C, 73.3; H, 4.5%).



67

2′,6′-Dihydroxyacetophenone (***73***).— This compound was either prepared by the standard method,[254] or was obtained commercially (Aldrich), as a yellow powder, m.p. 156-157°C (lit.,[254,255] 154-155°C, 157°C).

2′,4′,6′-Trihydroxyacetophenone (***74***).— This substance was prepared by the standard[256] method, then recrystallised from aqueous methanol. δ_H [90 MHz: $(CD_3)_2SO$] 2.6 (3 H, s, $COCH_3$), 5.8 (2 H, s, 3′,5′-H), 12.1 (br s, ArOH); δ_C [22 MHz; $(CD_3)_2SO$] 33.3 ($COCH_3$), 95.5 (3′-C, 5′-C), 105.0 (s, 1′-C), 165.2 (s, 2′-C, 6′-C), 165.7 (s, 4′-C), 203.4 (s, C=O).

2′-Hydroxy-6′-methoxyacetophenone (***75***).— To potassium carbonate (1.82 g) and 2′,6′-dihydroxyacetophenone (2.00 g, 13 mmol) in dry acetone (*ca.* 50 ml) at reflux was added iodomethane (*ca.* 5 ml). Reflux was continued for 24 hours, after which time the solvent was removed *in vacuo* and water added (*ca.* 50 ml). The product was extracted with chloroform – petroleum spirit (1:1), and the organic layer washed with sodium hydroxide solution (4×30 ml; 2 mol l^{-1}). The basic washings were then acidified, then the product extracted in chloroform and the solvent removed *in vacuo*. Recrystallisation from hexane gave pale yellow needles (1.38 g), and a second crop was obtained from aqueous methanol (0.33 g); overall yield 78%. The appearance of the product, and its mass spectrum, was identical to that reported previously,[252,257] *m/z* 166 ($M^{+\cdot}$, 53%), 151 (100), 136 (15).

2'-Hydroxy-6'-allyloxyacetophenone.— To a solution containing allyl chloride (2.0 ml), potassium carbonate (2.0 g), and 3A molecular sieves in dry acetone was added 2',6'-dihydroxyacetophenone (**73**). The mixture was refluxed for several days before most of the solvent was removed *in vacuo*, then water was added to the residue and the product extracted into chloroform. The chloroform was then extracted with sodium hydroxide solution, which on acidification precipitated an oil. This oil was taken up in chloroform, the solvent removed *in vacuo* and the product recrystallised from hexane to a pale yellow solid whose spectral properties were consistent with the desired acetophenone, δ_C (22 MHz; $CDCl_3$) 33.9 ($COCH_3$), 69.8 (t, OCH_2), 102.2 (5'-C)^a, 110.8 (3'-C)^a, 111.5 (s, 1'-C), 118.7 (t, $CH=CH_2$), 132.4 ($CH=CH_2$), 136.0 (4'-C), 160.5 (s, 6'-C)^b, 164.7 (s, 2'-C)^b, 205.1 (s, C=O); *m/z* 192 ($M^{+\cdot}$, 100%), 177 (40), 150 (15), 149 (12), 137 (53), 135 (20), 129 (12), 108 (11), 107 (11).

2',6'-Diacetoxyacetophenone.— The parent dihydroxyacetophenone was dissolved in acetic anhydride and pyridine, and left to react for *ca*. 3 hours. The reaction solution was then added to a large excess of water and extracted with chloroform, and the chloroform layer washed thoroughly with dilute hydrochloric acid. Removal of the solvent *in vacuo* and recrystallisation from methanol gave colourless crystals with spectral properties consistent with the 2',6'-diacetoxyacetophenone, δ_C (22 MHz; $CDCl_3$) 20.7 ($OCOCH_3$), 31.0 ($COCH_3$), 120.2 (3′-C, 5′-C), 127.7 (s, 1′-C), 130.6 (4′-C), 147.6 (s, $OCOCH_3$), 168.5 (s, 2′-C, 6′-C), 198.2 (s, C=O); *m/z* 236 ($M^{+\cdot}$, 3%), 194 (33), 152 (100), 137 (96).

2′-Hydroxy-4′,6′-dimethoxyacetophenone (***76***).— To potassium carbonate (8.21 g) and iodomethane (10 ml) in dry acetone (200 ml) was added 2′,4′,6′-trihydroxyacetophenone (***74***; 5.00 g, 30 mmol). The mixture was refluxed for 24 hours, after which most of the solvent was removed *in vacuo* and the reaction solution worked up in the same manner as for 2′-hydroxy-6′-methoxyacetophenone (above), and the product recrystallised from methanol and water to give colourless crystals of 2′-hydroxy-4′,6′-dimethoxyacetophenone (***76***), m.p. 79°C (lit.,[252,259] 74-75°C, 82-83°C); δ_C (22 MHz; $CDCl_3$) 32.8 ($COCH_3$), 55.5 (OCH_3), 90.7 (3′-C)[a], 93.5 (5′-C)[a], 106.0 (s, 1′-C), 162.9 (s, 6′-C)[b], 166.1 (s, 2′-C)[b], 167.5 (s, 4′-C)[b], 203.1 (s, C=O); *m/z* 196 ($M^{+\cdot}$, 41%), 181 (100), 166 (10).

2′-Acetoxy-4′,6′-dimethoxyacetophenone.— To a solution of acetic anhydride (7 ml) in pyridine (7 ml) was added 2′-hydroxy-4′,6′-dimethoxyacetophenone (***76***; 1.24 g, 6.3 mmol) and the mixture allowed to stand for 48 hours. The reaction mixture was worked up in exactly the same manner as for 2′,6′-diacetoxyacetophenone (above), and the product recrystallised from aqueous methanol to afford colourless crystals of 2′-acetoxy-4′,6′-dimethoxyacetophenone (1.20 g, 80%), δ_H (90 MHz; $CDCl_3$) 2.15 (3 H, s, $OCOCH_3$), 2.38 (3 H, s, $COCH_3$), 3.70 (3 H, s, OCH_3), 3.74 (3 H, s, OCH_3), 6.11 (1 H, s, 5′-H)[a], 6.37 (1 H, s, 3′-H)[a]; δ_C (22 MHz; $CDCl_3$) 20.6 ($OCOCH_3$), 31.7 ($COCH_3$), 55.4 (OCH_3), 55.7 (OCH_3), 96.3 (5′-C)[a], 100.3 (3′-C)[a], 116.7 (s, 1′-C), 149.7 (s, $OCOCH_3$), 159.3 (s, 2′-C)[b], 162.2 (s, 4′-C)[b], 169.1 (s, 6'-C)^{b}, 198.9 (s, C=O); *m/z* 238 ($M^{+\cdot}$, 7%), 196 ($M^{+\cdot}$ - 42, 30), 181 (100), 178 (9).

2'-Hydroxy-3'-methyl-4',6'-dimethoxyacetophenone (*77*).— In one large scale preparation of 2'-hydroxy-4',6'-dimethoxyacetophenone (*76*), the first recrystallisation of the worked up reaction mixture from methanol and water afforded a small amount of a yellow powder, 2'-hydroxy-3'-methyl-4',6'-dimethoxyacetophenone (*77*), identical in all respects to the compound isolated by Gommans,^{252} m.p. 142-143°C (lit.,^{238,252} 141-142°C); δ_H (90 MHz; $CDCl_3$) 1.97 (3 H, s, 3'-CH_3), 2.56 (3 H, s, $COCH_3$), 3.84 (6 H, s, OCH_3), 5.88 (1 H, s, 5'-H), 13.95 (2'-OH); δ_C (22 MHz; $CDCl_3$) 7.0 (3'-CH_3), 33.0 ($COCH_3$), 55.3 (OCH_3), 85.7 (5'-C), 105.6 (s, 3'-C), 105.8 (s, 1'-C), 161.6 (s, 2'-C)^{a}, 163.5 (s, 4'-C)^{a}, 163.7 (s, 6'-C)^{a}, 203.3 (s, C=O); *m/z* 210 ($M^{+\cdot}$, 41%), 196 (17), 195 (100), 181 (19). Subsequent recrystallisations of the mother liquor afforded 2'-hydroxy-4',6'-dimethoxyacetophenone (*76*).

2'-Acetoxy-3'-methyl-4',6'-dimethoxyacetophenone.— The acetylation of 2'-hydroxy-3'-methyl-4',6'-dimethoxyacetophenone (*77*) was performed in a manner identical to that used to prepare 2'-acetoxy-4',6'-dimethoxyacetophenone (above), to afford a yellow powder with a mass-spectral fragmentation pattern consistent with the expected acetate, *m/z* 252 ($M^{+\cdot}$, 4%), 211 (21), 210 ($M^{+\cdot}$ - 42, 100), 197 (100), 192 (15), 182 (10), 181 (67), 180 (35), 176 (18), 167 (11), 152 (15), 137 (12), 109 (16).

3'-Benzyl-4',6'-dibenzyloxy-2'-hydroxyacetophenone (*78*).— To a solution of potassium carbonate (4.25 g) and benzyl bromide (10 ml) in dry acetone was added 2',4',6'-trihydroxyacetophenone (*74*; 2.60 g, 15.5 mmol). The mixture was refluxed for 24 hours, then most of the solvent was removed *in vacuo*. Water was added to the residue and the product extracted into chloroform. Removal of the chloroform *in vacuo*, followed by recrystallisation from methanol, afforded a colourless solid (1.66 g, 25%), considered to be 3′-benzyl-4′,6′-dibenzyloxy-2′-hydroxyacetophenone (***78***) from its mass spectral characteristics, m.p. 118-120°C; *m/z* 438 ($M^{+\cdot}$, 24%), 389 (17), 348 (19), 347 (56), 305 (28), 181 (15), 180 (29), 179 (11), 92 (100).

2′-Acetoxy-3′-benzyl-4′,6′-dibenzyloxyacetophenone.— Acetylation of 3′-benzyl-4′,6′-dibenzyloxy-2′-hydroxyacetophenone (***78***) was carried out by the same method as that used to prepare 2′-acetoxy-4′,6′-dimethoxyacetophenone (above), to give a colourless solid, the spectral properties of which were consistent with the expected acetate, m.p. 107-110°C; *m/z* 480 ($M^{+\cdot}$, 8%), 438 ($M^{+\cdot}$ - 42, 23), 389 (17), 348 (13), 347 (50), 305 (22), 257 (15), 181 (17), 180 (27), 92 (22), 91 (100).

2′-Hydroxy-4′,6′-bistetrahydropyranyloxyacetophenone (***79***).— This substance was prepared in exactly the same manner as 2′-hydroxy-6′-tetrahydropyranyloxyacetophenone (***65***), from 2′,4′,6′-trihydroxyacetophenone (***74***). T.l.c. of the product showed a multitude of compounds to be present, and from the mixture was isolated by p.l.c. a fraction which was considered to be the desired bisTHP ether of the acetophenone. The ^{13}C n.m.r. spectrum of this fraction was a little messy, with low ($\frac{1}{4}$ to $\frac{1}{3}$) intensity peaks present in close proximity to several of the major peaks. It is possible that these extra peaks are due to impurities, but is also quite conceivable that they are the result of different possible conformations being taken up by the two pyran rings, or of diastereoisomerism. The spectrum is given in figure 7-1.

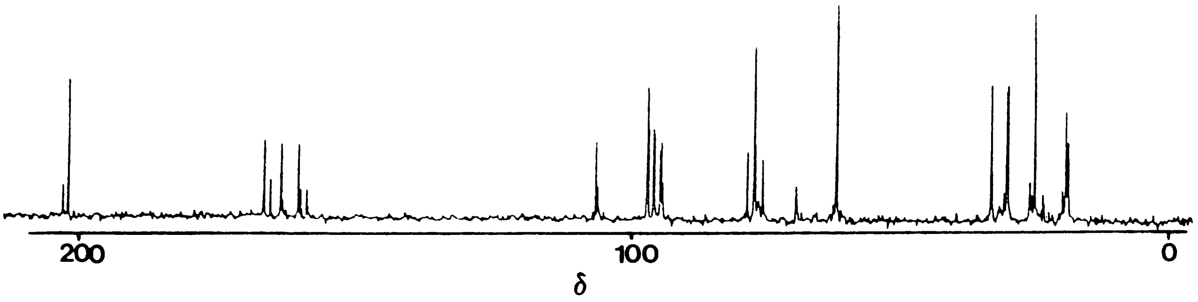


FIGURE 7-1 The ^{13}C n.m.r. Spectrum of the Fraction Containing 2′-hydroxy-4′,6′-bistetrahydropyranyloxyacetophenone (*79*)

7.3 Flavanones

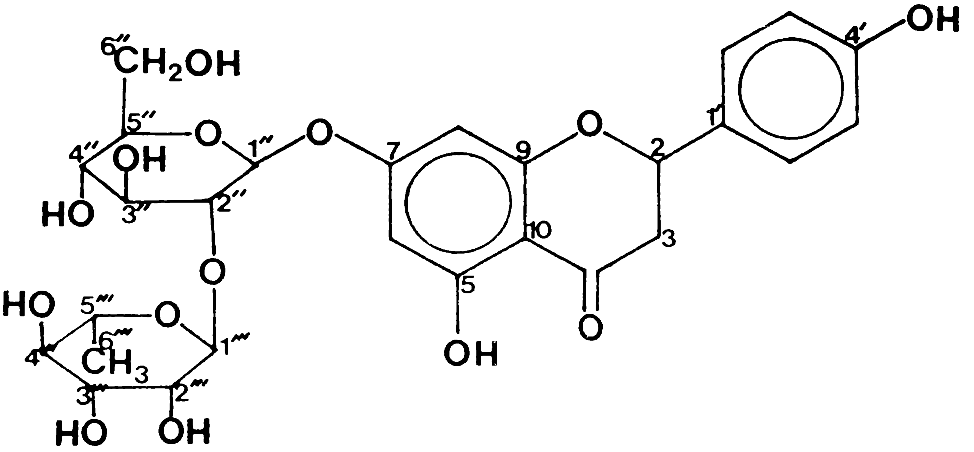
4′,5,7-Trihydroxyflavanone (naringenin) (*4*).— Commercial (±)-naringenin (Sigma) was recrystallised from methanol and water to afford cream coloured crystals of naringenin (*4*), δ_H [90 MHz; $(CD_3)_2CO$] 2.85 (1 H, dd, $J_{3\text{-}H_{ax}}$ 17, $J_{2\text{-}H}$ 3.5 Hz, 3-$H_{eq.}$), 3.33 (1 H, dd, $J_{3\text{-}H_{eq}}$ 17, $J_{2\text{-}H}$ 14 Hz, 3-$H_{ax.}$), 5.58 (1 H, dd, $J_{3\text{-}H_{ax}}$ 14, $J_{3\text{-}H_{eq}}$ 3.5 Hz, 2-H), 6.11 (2 H, s, 6-H, 8-H), 7.04 (2 H, d, J 8.5 Hz, 3′-H, 5′-H), 7.54 (2 H, d, J 8.5 Hz, 2′-H, 6′-H), 9.2 (br, 4′-OH, 7-OH), 12.38 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 43.4 (3-C), 79.9 (2-C), 95.9 (8-C)^a, 96.8 (6-C)^a, 103.2 (s, 10-C), 116.1 (3′-C, 5′-C), 129.0 (2′-C, 6′-C), 130.8 (s, 1′-C), 158.6 (s, 4′-C), 164.3 (s, 9-C)^b, 165.2 (s, 5-C)^b, 167.3 (s, 7-C)^b, 197.2 (s, C=O); *m/z* 273 (14%), 272 ($M^{+\cdot}$, 83), 271 (44), 179 (29), 166 (38), 153 (100), 152 (18), 124 (15), 121 (11), 120 (11), 119 (15), 107 (25), 91 (16).

3',5,7-Trihydroxy-4'-methoxyflavanone (hesperetin) (*8*).— (*a*) Commercial hesperetin (Sigma) was recrystallised from aqueous methanol to give straw-coloured needles of hesperetin (*8*), δ_H [90 MHz; $(CD_3)_2CO$] 2.89 (1 H, dd, $J_{3\text{-H}_{ax}}$ 17.0, $J_{2\text{-H}}$ 3.7 Hz, 3-H_{eq}), 3.32 (1 H, dd, $J_{3\text{-H}_{eq}}$ 17.0, $J_{2\text{-H}}$ 12.0 Hz, 3-H_{ax}), 4.01 (3 H, s, OCH_3), 5.58 (1 H, dd, $J_{3\text{-H}_{ax}}$ 12.0, $J_{3\text{-H}_{eq}}$ 3.7 Hz, 2-H), 6.12 (2 H, s, 6-H, 8-H), 7.12, 7.19 (3 H, 2×br s, B-ring protons), 7.87 (1 H, br s, 3'-OH), 9.75 (1 H, br s, 7-OH), 12.32 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 43.5 (3-C), 56.3 (OCH_3), 79.7 (2-C), 95.9 (8-C)^{a}, 96.8 (6-C)^{a}, 103.3 (10-C), 112.3 (5'-C)^{b}, 114.3 (2'-C)^{b}, 118.8 (6'-C), 132.8 (1'-C), 147.5 (3'-C)^{c}, 148.6 (4'-C)^{c}, 164.2 (9-C)^{d}, 165.2 (5-C)^{d}, 167.3 (7-C)^{d}, 197.0 (C=O); *m/z* 303 (12%), 302 ($M^{+\cdot}$, 70), 301 (27), 179 (28), 153 (51), 151 (10), 150 (68), 137 (100), 135 (59), 124 (20), 107 (19).

3',5,7-Trihydroxy-4'-methoxyflavanone (hesperetin) (*8*).— (*b*) Hesperetin-chalcone (*86*) was allowed to stand overnight in methanol at *ca.* 40°C, during which time the solution decolourised. The solvent was removed *in vacuo* to afford a pale yellow solid, the ^{13}C n.m.r. spectrum of which closely resembled that of hesperetin (*8*) prepared by method (*a*) (above), m.p. 227-231°C (lit.[260] for *8*, 226-228°C); δ_C [22 MHz; $(CD_3)_2CO$] 43.7, 56.5, 79.7, 96.6, 97.4, 102.6, 112.6, 114.5, 118.8, 133.3, 147.8, 164.3, 165.3, 169.9, 196.4.

4',5-Dihydroxy-7-rhamnoglucosyloxyflavanone (naringin) (*18*).— Commercial naringin (Sigma) was recrystallised from methanol and water to afford a colourless solid, identical by n.m.r. with naringin (*18*), δ_H [90 MHz; $(CD_3)_2SO$] 1.43 (3 H, d, *J* 7 Hz, 6'''-H), 3.4-4.6 (*ca.* 24 H, m, 3-H, sugar-*H*, sugar-O*H*, H_2O), 4.9-5.8 (*ca.* 5 H, br m, 2-H, sugar-*H*), 6.30 (2 H, s, 6-H, 8-H), 7.04 (2 H, d, *J* 9 Hz, 3'-H, 5'-H), 7.53 (2 H, d, *J* 9 Hz, 2'-H, 6'-H), 9.02 (1 H, br s, 4'-OH), 12.12

(1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2SO$] 18.3 (q, 6'''-C), 43.3 (t, 3-C), 62.2 (t, 6''-C), 69.1 (d, 5'''-C), 71.1, 71.7, 72.2 (d, 4''-C, 2'''-C, 3'''-C), 73.6 (d, 4'''-C), 77.5, 77.6, 78.8 (d, 2''-C, 3''-C, 5''-C), 80.0 (d, 2-C), 96.2 (d, 8-C), 97.5 (d, 6-C), 98.9 (d, 1''-C), 101.6 (d, 1'''-C), 104.5 (s, 10-C), 116.1 (d, 3'-C, 5'-C), 129.0 (d, 2'-C, 6'-C), 130.2 (s, 1'-C), 158.7 (s, 4'-C), 164.0 (s, 9-C)^a, 164.5 (s, 5-C)^a, 166.0 (s, 7-C)^a, 198.0 (s, C=O).



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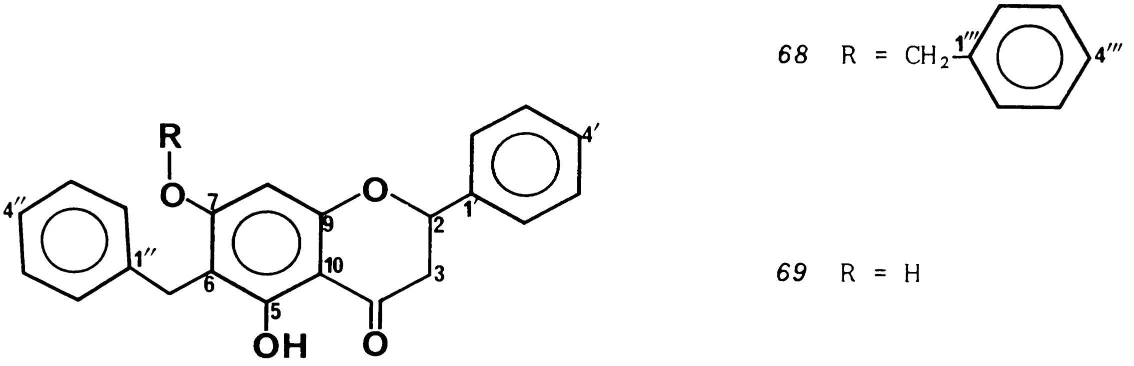
5-Hydroxy-4',7-dimethoxyflavanone (*33*).— To a suspension of naringenin (4',5,7-trimethoxyflavanone) [*4*; 5.0 g (Sigma)] in methanol (25 ml) was added a solution of KOH (6.0 g) in methanol (30 ml) over a period of 6 hours, after which the mixture was stirred for a further 3 hours. The solid that formed was collected, and extracted with chloroform and water. The chloroform layer was removed, and the solvent removed *in vacuo*. The resultant pale yellow solid was re-crystallised from methanol to afford white crystals of 5-hydroxy-4',7-dimethoxyflavanone (*33*) (3.23 g, 59%), m.p. 118°C (lit.,[261] 118°C); λ_{max} (EtOH) 325infl, 291 nm; δ_H [90 MHz; $(CD_3)_2CO$] 2.91 (1 H, dd, $J_{3\text{-}H_{ax}}$ 17, $J_{2\text{-}H}$ 4 Hz, 3-$H_{eq.}$), 3.36 (1 H, dd,

$J_{3\text{-Heq.}}$ 17, $J_{2\text{-H}}$ 13 Hz, 3-$H_{ax.}$), 3.97 (3 H, s, OCH_3), 3.99 (3 H, s, OCH_3), 5.65 (1 H, dd, $J_{3\text{-Hax.}}$ 13, $J_{3\text{-Heq.}}$ 4 Hz, 2-H), 6.19 (2 H, s, 6-H, 8-H), 7.14 (2 H, d, J 8.8 Hz, 3'-H, 5'-H), 7.64 (2 H, d, J 8.8 Hz, 2'-H, 6'-H), 12.29 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 43.5 (3-C), 55.7 (OCH_3), 56.3 (OCH_3), 79.9 (2-C), 94.6 (8-C)[a], 95.5 (6-C)[a], 103.8 (s, 10-C), 114.8 (2'-C, 6'-C), 128.9 (3'-C, 5'-C), 131.8 (1'-C), 161.0 (4'-C)[b], 164.1 (9-C)[b], 165.0 (5-C)[b], 168.9 (7-C)[b], 197.5 (C=O); *m/z* 301 (10%), 300 ($M^{+\cdot}$, 50), 299 (28), 193 (15), 167 (10), 166 (17), 138 (12), 135 (13), 134 (100), 121 (88), 119 (23), 91 (14); (Found: C, 67.7; H, 5.35. Calc. for $C_{17}H_{16}O_5$: C, 68.0; H, 5.4%).

5-Hydroxyflavanone (*35*).— (*a*) To aqueous ethanol containing a little sodium acetate was added a little 2',6'-dihydroxychalcone (*34*). The deep yellow solution rapidly decolourised, depositing colourless crystals. Recrystallisation of this solid from methanol and water gave colourless crystals of 5-hydroxyflavanone, m.p. 58-59°C (lit.,[60, 236] 152-154 C, 63-64°C); λ_{max} (EtOH) 272 nm (log ϵ 3.85) and 350 nm (log ϵ 3.44); δ_H [90 MHz; $(CD_3)_2CO$] 3.05 (1 H, dd, $J_{3\text{-Hax.}}$ 17.2, $J_{2\text{-H}}$ 3.5 Hz, 3-$H_{eq.}$), 3.46 (1 H, dd, $J_{3\text{-Heq.}}$ 17.2, $J_{2\text{-H}}$ 12.3 Hz, 3-H_{ax}), 5.79 (1 H, dd, $J_{3\text{-Hax.}}$ 12.3, $J_{3\text{-Heq.}}$ 3.5 Hz, 2-H), 6.66 (2 H, dm, J 8.5 Hz, 6-H, 8-H), 7.5-7.7 (6 H, m, 2',3',4',5',6',7-H), 11.94 (1 H, br s, 5-OH); δ_C (22 MHz; $CDCl_3$) 43.9 (t, 3-C), 79.1 (d, 2-C), 107.6 (d, 8-C)[a], 108.2 (s, 10-C), 109.6 (d, 6-C)[a], 126.2 (d, 2'-C, 6'-C)[b], 128.9 (d, 3'-C, 5'-C)[b], 128.9 (d, 4'-C), 138.4 (s, 1'-C), 138.4 (d, 7-C), 161.5 (s, 9-C)[c], 162.2 (s, 5-C)[c], 198.0 (s, C=O); *m/z* 241 (13%), 240 ($M^{+\cdot}$, 80), 239 (63), 222 (16), 163 (69), 137 (18), 136 (100), 108 (35), 104 (11), 103 (12); (Found C, 75.1; H, 5.1. Calc. for $C_{15}H_{12}O_3$: C, 75.0; H, 5.0%).

6-Benzyl-7-benzyloxy-5-hydroxyflavanone (**68**).— This flavanone was synthesised without the isolation of the intermediate 2'-hydroxychalcone (*72*). Benzaldehyde (1.04 g, 9.8 mmol) and 3'-benzyl-4',6'-dibenzyloxy-2'-hydroxyacetophenone (*78*; 0.76 g, 1.4 mmol) were condensed for 16 hours in potassium hydroxide solution in the usual way. The reaction solution was then acidified, and the crude yellow product extracted with chloroform. After removal of the solvent *in vacuo*, 4% methanolic sulphuric acid was added, and the solution was refluxed for *ca.* 48 hours. Most of the methanol was then removed *in vacuo*, and a large excess of water was added. The product was extracted in chloroform, and purified by p.l.c. to afford a colourless solid whose spectral properties were consistent with *6-benzyl-7-benzyloxy-5-hydroxyflavanone* (**68**), m.p. 131-134°C; λ_{max} (acidic methanol) 341 nm (log ϵ 3.61), 292 nm (log ϵ 4.15); δ_H [90 MHz; $(CD_3)_2CO$] 2.93 (1 H, dd, J_{3-Hax} 16, J_{2-H} 4 Hz, 3-H_{eq}), 3.42 (1 H, dd, J_{3-Heq} 16, J_{2-H} 12 Hz, 3-H_{ax}), 4.08 (2 H, s, 6-CH_2Ar), 5.37 (2 H, s, OCH_2Ar), 5.77 (1 H, dd, J_{3-Hax} 12, J_{3-Heq} 4 Hz, 2-H), 6.43 (1 H, s, 8-H), 7.3, 7.5-7.6 (15 H, m, B-ring, 2×$CH_2C_6H_5$), 12.40 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 43.7 (3-C), 71.2 (OCH_2Ar), 79.9 (2-C), 94.6 (8-C), 103.7 (s, 10-C), 109.5 (s, 6-C), 126.4, 127.1, 128.4, 128.9, 129.3 (2'-C, 6'-C, 3'-C, 5'-C, 4'-C, 2''-C, 6''-C, 3''-C, 5''-C, 4''-C, 2'''-C, 6'''-C, 3'''-C, 5'''-C, 4'''-C), 137.4 (s, 1'''-C), 140.1 (s, 1'-C)^a, 142.2 (s, 1''-C)^a, 160.2 (s, 9-C)β, 163.9 (s, 5-C)^b, 165.5 (s, 7-C)^b (A low intensity signal was present at *ca.* 195 ppm, but its chemical shift was not recorded by the technician); *m/z* 436 ($M^{+\cdot}$, 20%), 345 (23), 241 (21), 91 (100). (Found: C, 79.5; H, 5.6; $M^{+\cdot}$, 436.1654. $C_{29}H_{24}O_4$ requires C, 79.8; H, 5.5%; $M^{+\cdot}$, 436.1675).



68 R = CH_2–

69 R = H

6-Benzyl-5,7-dihydroxyflavanone (**69**).— The previous flavanone (6-benzyl-7-benzyloxy-5-hydroxyflavanone) (**68**) was refluxed for *ca.* one week in 4% methanolic sulphuric acid, after which most of the solvent was removed *in vacuo*, and a large excess of water was added. The product was then extracted with ether, and the ether layer washed with 2 mol l^{-1} sodium hydroxide. The alkaline washings were acidified and the product extracted with ether. Subsequent removal of the solvent *in vacuo* followed by vacuum sublimation afforded a colourless oil with spectral characteristics corresponding to *5,7-dihydroxy-6-benzylflavanone* (**69**). This oil crystallised from chloroform as colourless crystals, m.p. 155-157°C; δ_H [90 MHz; $(CD_3)_2CO$] 2.98 (1 H, dd, $J_{3\text{-}H_{ax}}$ 17, $J_{2\text{-}H}$ 4 Hz, 3-H_{eq}), 3.31 (1 H, dd, $J_{3\text{-}H_{eq}}$ 17, $J_{2\text{-}H}$ 12 Hz, 3-H_{ax}), 4.06 (2 H, s, 6-CH_2Ar), 5.74 (1 H, dd, $J_{3\text{-}H_{ax}}$ 12, $J_{3\text{-}H_{eq}}$ 4 Hz, 2-H), 6.25 (1 H, s, 8-H), 7.2-7.8 (10 H, m, B-ring protons, 6-$CH_2C_6H_5$), 12.30 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 43.6 (2-C), 79.8 (2-C), 96.6 (8-C), 103.3 (10-C), 108.3 (6-C), 126.4, 127.1, 128.8, 129.3, 129.5 (2′-C, 6′-C, 3′-C, 5′-C, 4′-C, 2′′-C, 6′′-C, 3′′-C, 5′′-C, 4′′-C), 140.2 (1′-C)^a, 142.4 (1′′-C)^a, 161.1 (9-C)^b, 163.4 (5-C)^b, 165.2 (7-C)^b, 197.2 (C=O); *m/z* 347 (24%), 346

($M^{+\cdot}$, 100), 345 (37), 270 (10), 269 (60), 267 (11), 242 (40), 241 (38), 214 (47), 213 (75), 191 (25), 165 (18), 164 (13), 151 (15), 149 (14), 131 (18), 129 (10). (Found: $M^{+\cdot}$, 346.1199. $C_{22}H_{18}O_4$ requires $M^{+\cdot}$, 346.1205).

5-Hydroxyflavanone (*35*).— (*b*) To a solution of 5-methoxyflavanone (*80*; 0.15 g, 0.59 mmol) in dichloroethane (20 ml) at *ca.* -10°C, was added chilled boron trichloride (*ca.* 1 ml). The solution was then left in an ice bath for 30 minutes before a large excess of dilute hydrochloric acid (2 mol l^{-1}) was cautiously added. The organic phase was then separated and washed with water before removal of the solvent *in vacuo*. Recrystallisation of the residue from methanol and water afforded colourless crystals of 5-hydroxyflavanone (*35*) (0.06 g, 42%), identical by t.l.c., ^{1}H and ^{13}C n.m.r., and mass spectroscopy with the product obtained by cyclisation of 2′,6′-dihydroxychalcone (above).

5-Hydroxyflavanone (*35*).— (*c*) A solution of 6′-benzyloxy-2′-hydroxychalcone (*71*) in EtOH – CH_3CO_2H – conc. HCl (*ca.* 8:10:10) was refluxed for 48 hours. Most of the solvent was then removed *in vacuo*, and a large excess of water was added. The product was extracted with chloroform, which was in turn extracted with sodium hydroxide (2 mol l^{-1}). The alkaline extract was slowly neutralised, and the solid that precipitated was filtered at the pump. Recrystallisation of this solid from aqueous methanol afforded colourless crystals, identical by ^{1}H and ^{13}C n.m.r. spectroscopy with genuine 5-hydroxyflavanone (*35*) (above).

5-Hydroxy-3′,4′,7-trimethoxyflavanone (37).— To a suspension of 3′,5,7-trihydroxy-4′-methoxyflavanone (hesperetin) (*8*; 5.0 g, 1.5 mmol) in methanol (25 ml) and dimethyl sulphate (10 ml) was added methanolic potassium hydroxide (6.0 g KOH in 30 ml CH_3OH) over a period of six hours. The precipitated solid was collected and extracted with chloroform – water. The chloroform layer was separated, dried over magnesium sulphate, filtered, and the solvent removed *in vacuo*. Recrystallisation of the resultant solid from methanol gave 5-hydroxy-3′,4′,7-trimethoxyflavanone (**37**) (2.80g, 49%) as white needles, m.p. 132-134°C (lit.,[224,262] 136°C, 156°C); λ_{max} (EtOH) 287 nm (log ϵ 4.28) (lit.[262] 288 nm); δ_H (90 MHz; $CDCl_3$) 2.77 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 17.2, $J_{2\text{-}H}$ 4.0 Hz, 3-$H_{eq.}$), 3.14 (1 H, dd, $J_{3\text{-}H_{eq}}$ 17.2, $J_{2\text{-}H}$ 12.6 Hz, 3-$H_{ax.}$), 3.80 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 5.36 (1 H, dd, $J_{3\text{-}H_{ax}}$ 13, $J_{3\text{-}H_{eq}}$ 4 Hz, 2-H), 6.06 (2 H, s, 6-H, 8-H), 6.9-7.0 (3 H, m, 2′-H, 5′-H, 6′-H), 12.0 (1 H, br s, 5-OH); δ_C (22 MHz; $CDCl_3$) 43.3 (t, 3-C), 55.5, 55.9 (q, OCH_3), 79.1 (d, 2-C), 94.2 (d, 8-C)[a], 95.1 (d, 6-C)[a], 103.1 (s, 10-C), 109.4 (d, 2′-C)[b], 111.2 (d, 5′-C)[b], 118.8 (d, 6′-C), 130.8 (s, 1′-C), 149.2 (s, 3′-C)[c], 149.5 (s, 4′-C)[c], 162.8 (s, 5-C)[d], 164.1 (s, 9-C)[d], 167.9 (s, 7-C)[d], 195.9 (s, C=0); *m/z* 330 ($M^{+\cdot}$, 44%), 329 (14), 193 (10), 164 (52), 151 (100), 149 (23); (Found: C, 65.3; H 5.6. Calc. for $C_{18}H_{18}O_6$: C, 65.4; H, 5.5%).

5-Hydroxy-4′-methoxyflavanone (**39**).— A sample of 2′,6′-dihydroxy-4-methoxychalcone (*38*) was dissolved in ethanol – water (1:1), a little sodium bicarbonate was added, and the solution was left overnight, during which time it decolourised, depositing white crystals. The solution was acidified with HCl (2 mol l^{-1}) and the product recrystallised directly from the reaction solution to afford colourless crystals of *5-hydroxy-4-methoxyflavanone* (**39**), m.p. 87-88°C (lit.,[212] 86-87°C); λ_{max} (EtOH) 272 nm (log ϵ 4.00), 349 nm (log ϵ 3.51) (lit.,[212] 272, 350 nm); δ_H [90 MHz; $(CD_3)_2CO$] 3.00 (1 H, dd,

$J_{3\text{-Hax.}}$ 17.1, $J_{2\text{-H}}$ 3.5 Hz, 3-$H_{eq.}$), 3.47 (1 H, dd, $J_{3\text{-Heq.}}$ 17.1, $J_{2\text{-H}}$ 12.6 Hz, 3-$H_{ax.}$), 3.97 (3 H, s, OCH_3), 5.71 (1 H, dd, $J_{3\text{-Hax.}}$ 12.6, $J_{3\text{-Heq.}}$ 3.5 Hz, 2-H), 6.64 (2 H, d, J 8.2 Hz, 6-H, 8-H), 7.14 (2 H, d, J 8.8 Hz, 3′-H, 5′-H), 7.59 (1 H, t, J 8.2 Hz, 7-H), 7.60 (2 H, d, J 8.8 Hz, 2′-H, 6′-H), 11.95 (1 H, s, 5-OH); [200 MHz; solvent $(CD_3)_2CO$; standard Me_4Si] 2.86 (1 H, dd, $J_{3\text{-Hax.}}$ 17.2, $J_{2\text{-H}}$ 3.0 Hz, 3-$H_{eq.}$), 3.32 (1 H, dd, $J_{3\text{-Heq.}}$ 17.2, $J_{2\text{-H}}$ 13.0 Hz, 3-$H_{ax.}$), 5.58 (1 H, dd, $J_{3\text{-Hax.}}$ 13.0, $J_{3\text{-Heq.}}$ 3.0 Hz, 2-H); (200 MHz; solvent C_6D_6; standard Me_4Si) 2.27 (1 H, dd, $J_{3\text{-Hax.}}$ 17.1, $J_{2\text{-H}}$ 3.2 Hz, 3-$H_{eq.}$), 2.51 (1 H, dd, $J_{3\text{-Heq.}}$ 17.1, $J_{2\text{-H}}$ 13.0 Hz, 3-$H_{ax.}$), 4.67 (1 H, dd, $J_{3\text{-Hax}}$ 13.0, $J_{3\text{-Heq}}$ 3.2 Hz, 2-H), 6.36 (1 H, dd, J 8.2, 0.9 Hz, 6-H), 6.56 (1 H, dd, J 8.2, 0.9 Hz, 8-H), 6.72 (2 H, A_2X_2, J_{AX} 8.8, $J_{AA'}$, $J_{XX'}$ 3.0, 1.8 Hz, 3′-H, 5′-H), 6.94 (1 H, t, J 8.2 Hz, 7-H), 7.01 (2 H, A_2X_2, J_{AX} 8.8, $J_{AA'}$, $J_{XX'}$ 3.0, 1.8 Hz, 2′-H, 6′-H); δ_C [22 MHz; $(CD_3)_2CO$] 43.9 (3-C), 55.6 (OCH_3), 79.7 (2-C), 108.2 (8-C)^a, 108.9 (s, 10-C), 109.6 (6-C)^a, 114.8 (3′-C, 5′-C), 128.9 (2′-C, 6′-C), 131.7 (s, 1′-C), 139.1 (7-C), 161.0 (s, 4′-C)^b, 162.7 (s, 5-C)^b, 162.9 (s, 9-C)^b, 199.8 (s, C=O); m/z 270 ($M^{+\cdot}$, 33%), 269 (27), 163 (8), 136 (8), 135 (12), 134 (100), 121 (48), 119 (28), 108 (19); (Found C, 71.3; H, 5.3. Calc. for $C_{16}H_{14}O_4$: C, 71.1; H, 5.2%).

5-Hydroxy-3′,4′-dimethoxyflavanone (***41***).— A little 2′,6′-dihydroxy-3,4-dimethoxychalcone (***40***) was dissolved in ethanol – water (1:1) and a little sodium acetate was added. After standing overnight the ethanol was removed *in vacuo*, the colourless product was extracted into chloroform, the chloroform removed *in vacuo* and the residue recrystallised from aqueous methanol to give colourless crystals of *5-hydroxy-3′,4′-dimethoxyflavanone* (***41***), m.p. 153-155°C;

λ_{max} (EtOH) 275 nm (log ϵ 4.03), 350 nm (log ϵ 3.51); δ_H [90 MHz; $(CD_3)_2CO$] 3.01 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 17.2, $J_{2\text{-}H}$ 3.5 Hz, 3-$H_{eq.}$), 3.51 (1 H, dd, $J_{3\text{-}H_{eq.}}$ 17.2, $J_{2\text{-}H}$ 12.5 Hz, 3-$H_{ax.}$), 3.98 (3 H, s, 3'-OCH_3)^a, 4.00 (3 H, s, 4'-OCH_3)^a, 5.71 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 12.5, $J_{3\text{-}H_{eq.}}$ 3.4 Hz, 2-H), 6.64 (2 H, d, J 8.2 Hz, 6-H, 8-H), 7.2 and 7.3 (3 H, m, 2'-H, 5'-H, 6'-H), 7.60 (1 H, t, J 8.2 Hz, 7-H), 11.95 (br s, 5-OH); δ_C (22 MHz; $CDCl_3$) 43.8 (3-C), 56.0 (3-OCH_3, 4-OCH_3), 79.0 (2-C), 107.6 (8-C)^a, 108.2 (s, 10-C), 109.6 (6-C)^a, 109.6 (2'-C)^b, 111.4 (5'-C)^b, 118.8 (6'-C), 130.8 (s, 1'-C), 138.4 (7-C), 149.4 (s, 3'-C)^c, 149.7 (s, 4'-C)^c, 161.5 (s, 9-C)^d, 162.2 (s, 5-C)^d, 198.1 (s, C=O); *m/z* 300 ($M^{+\cdot}$, 37%), 299 (18), 165 (11), 164 (92), 163 (14), 152 (10), 151 (100), 149 (48), 138 (12), 121 (14), 119 (10), 108 (9), 103 (12); (Found C, 67.9; H, 5.5. $C_{17}H_{16}O_5$ requires C, 68.0; H, 5.4%).

5-Hydroxy-3',4',5'-trimethoxyflavanone (**43**).— A little 2',6'-dihydroxy-3',4',5'-trimethoxychalcone (*42*) (*ca.* 50 mg) was dissolved in methanol – water (1:1; *ca.* 2 ml), and left to stand at about 40°C overnight. The colourless needles of *5-hydroxy-3',4',5'-trimethoxyflavanone* (**43**) which had formed were then collected by filtration, without further purification, m.p. 144-145°C; λ_{max} (MeOH) 350 nm (log ϵ 3.44), 272 nm (3.89); δ_H [90 MHz; $(CD_3)_2CO$] 3.03 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 17.0 Hz, $J_{2\text{-}H}$ 3.2 Hz, 3-$H_{eq.}$), 3.14 (1 H, dd, $J_{3\text{-}H_{eq.}}$ 17.0 Hz, $J_{2\text{-}H}$ 12.6 Hz, 3-$H_{ax.}$), 3.90 (3 H, s, 4'-OCH_3), 4.01 (6 H, s, 3'-OCH_3, 5'-OCH_3), 5.71 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 12.6 Hz, $J_{3\text{-}H_{eq.}}$ 3.2 Hz, 2-H), 6.66 (2 H, dd, J 8.2, 2.3 Hz, 6-H, 8-H), 7.06 (2 H, s, 2'-H, 6'-H), 7.62 (1 H, t, J 8.2 Hz, 7-H), 11.94 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 44.2 (3-C), 56.6 (3'-OCH_3, 5'-OCH_3), 60.5 (4'-OCH_3), 80.1 (2-C), 105.1 (2'C, 6'-C), 108.2 (8-C)^a, 108.8 (10-C), 109.7 (6-C)^a, 135.2 (7-C), 139.0 (4'-C), 139.5 (1'-C), 154.5 (3'-C, 5'-C), 162.5 (9-C)^b, 162.9

(5-C)^b, 199.4 (C=O); *m/z* 331 (13%), 330 ($M^{+\cdot}$, 52), 329 (11), 299 (8), 194 (37), 182 (14), 181 (100), 179 (50), 163 (10), 151 (9), 136 (10); (Found C, 65.2; H, 5.4. $C_{18}H_{18}O_6$ requires C, 65.4; H, 5.5%).

5-Hydroxy-2',4',6'-trimethoxyflavanone (**45**).— A little 2',6'-dihydroxy-2,4,6-trimethoxychalcone (**44**) (*ca.* 50 mg) was dissolved in methanol – water (1:1, *ca.* 2 ml), and left to stand for 2 days at about 40°C. The buff-coloured needles of *5-hydroxy-2',4',6'-trimethoxyflavanone* (**45**) that had formed were then collected, without further purification, m.p. 188-190°C (with very long needles developing from 160°C): λ_{max} (acidic methanol) 350 nm (log ϵ 3.65), 272 nm (log ϵ 4.09); δ_H (90 MHz; solvent $CDCl_3$; standard Me_4Si) 2.57 (1 H, dd, $J_{3\text{-Hax.}}$ 17.3, $J_{2\text{-H}}$ 2.9 Hz, 3-H$_{eq.}$), 3.80 (6 H, s, 2'-OCH_3, 6'-OCH_3), 3.82 (3 H, s, 4'-OCH_3), 3.8-4.1 (m, partially obscured, 3-H$_{eq}$), 6.01 (1 H, dd, $J_{3\text{-Hax.}}$ 13.9, $J_{3\text{-Heq.}}$ 2.9 Hz, 2-H), 6.16 (2 H, s, 3'-H, 5'-H), 6.45 (2 H, dd, J 8.2, 2 Hz, 6-H, 8-H), 7.31 (1 H, t, J 8.2 Hz, 7-H), 11.91 (1 H, s, 5-OH); δ_H (90 MHz; C_6D_6) 2.77 (1 H, dd, $J_{3\text{-Hax.}}$ 17.4, $J_{2\text{-H}}$ 3.2 Hz, 3-H$_{eq.}$), 3.40 (6 H, s, 2'-OCH_3, 6'-OCH_3), 3.54 (3 H, s, 4'-OCH_3), 4.22 (1 H, dd, $J_{3\text{-Heq}}$ 17.4, $J_{2\text{-H}}$ 13.5 Hz, 3-H$_{ax.}$), 6.21 (2 H, s, 3'-H, 5'-H), 6.41 (1 H, dd, $J_{3\text{-Hax.}}$ 13.5, $J_{3\text{-Heq.}}$ 3.2 Hz, 2-H), 6.62 (1 H, dd, J 8.0, 1.2 Hz, 6-H)^a, 6.76 (1 H, dd, J 8.0, 1.2 Hz, 8-H)^a, 7.12 (1 H, t, J 8.0 Hz, 7-H), 12.83 (1 H, s, 5-OH); δ_C (22 MHz; $CDCl_3$) 40.7 (3-C), 55.4 (4'-OCH_3), 55.9 (2'-OCH_3, 6'-OCH_3), 71.3 (2-C), 91.2 (3'-C, 5'-C), 106.3 (10-C)^a, 107.6 (8-C)^b, 108.2 (1'-C)^a, 108.5 (6-C)^b, 137.7 (7-C), 160.2 (5-C, 9-C)^c, 162.3 (2'-C, 6'-C)^c, 163.0 (4'-C), 200.4 (C=O); *m/z* 330 ($M^{+\cdot}$, 31%), 299 (32), 194 (21), 182 (12), 181 (100), 180 (10), 179 (79), 168 (36), 151 (26), 149 (38), 139 (14), 137 (16), 121 (28); (Found C, 65.1; H, 5.5. $C_{18}H_{18}O_6$ requires C, 65.4; H, 5.5%).

4′-Chloro-5-hydroxyflavanone (**47**).— (*a*) A small amount of 4-chloro-2′,6′-dihydroxychalcone (**46**) was dissolved in methanol. After several days the solution was still yellow, although the u.v. spectrum showed that the chalcone was no longer present. The mass spectra of the product (which was not isolated) were consistent with the expected chloroflavanone (**47**). λ_{max} (MeOH) 336 nm, 273 nm; *m/z* 276 [$M^{+\cdot}$(^{37}Cl), 15%], 275 (17), 274 [$M^{+\cdot}$(^{35}Cl), 42], 273 (30), 256 (10), 163 (38), 162 (10), 138 (12), 137 (22), 136 (100), 108 (53), 103 (14); [Found: (average from 12 scans) $M^{+\cdot}$(^{37}Cl), 274.0386 (σ 0.0014); $M^{+\cdot}$(^{35}Cl), 276.0366 (σ 0.006). Calc. for $C_{15}H_{11}O_3Cl$: $M^{+\cdot}$(^{37}Cl), 274.0397; $M^{+\cdot}$(^{35}Cl), 276.0367].

4′-Chloro-5-hydroxyflavanone (**47**).— (*b*) A sample of 4-chloro-2′-hydroxy-6′-methoxychalcone (**90**) was refluxed in a solution containing dioxane and aqueous sodium bicarbonate (0.2 mol l^{-1}) (*ca.* 1:1) for two days. The product was extracted with chloroform (x2), the solvent removed *in vacuo*, and the resultant product refluxed for two days in a solution containing dioxane and sulphuric acid (2 mol l^{-1}) (*ca.* 1:1). After extraction in chloroform as above, the resultant solid was dissolved in acetic acid (10 ml), and concentrated hydroiodic acid (10 ml) added. The mixture was heated on a steam bath for 45 minutes, then added to a large excess of cold water and the product extracted with chloroform (x2). The organic phase was washed with dilute sodium thiosulphate solution, then water, and the solvent removed *in vacuo*. P.l.c. (dichloroethane as eluent) gave one major band at R_f ~ 0.5, which was removed. The product from this band gave a mass spectrum identical to that for 4-chloro-2′,6′-dihydroxychalcone (**46**), and the product was further purified by vacuum sublimation to afford a white crystalline substance, *4′-chloro-5-hydroxyflavanone*

(*47*), m.p. 101-102°C; λ_{max} (acidic methanol) 350 nm (log ϵ 3.26), 272 nm (log ϵ 3.67); δ_H [90 MHz; $(CD_3)_2CO$] 3.07 (1 H, dd, $J_{3\text{-H}_{ax}}$ 17.2, $J_{2\text{-H}}$ 3.7 Hz, 3-H_{eq}), 3.44 (1 H, dd, $J_{3\text{-H}_{eq}}$ 17.2, $J_{2\text{-H}}$ 12.2 Hz, 3-H_{ax}), 5.82 (1 H, dd, $J_{3\text{-H}_{ax}}$ 17.2, $J_{3\text{-H}_{eq}}$ 3.7 Hz, 2-H), 6.66 (2 H, dm, *J* 8.4 Hz, 6-H, 8-H), 7.5-7.9 (5 H, m, 2'-H, 3'-H, 7-H, 5'-H, 6'-H), 11.91 (1 H, s, 5-OH); δ_C [22 MHz; $(CD_3)_2CO$] 44.0 (t, 3-C), 79.1 (d, 2-C), 108.2 (d, 8-C)[a], 108.9 (s, 10-C), 110.0 (d, 6-C)[a], 129.0 (d, 2'-C, 6'-C)[b], 129.5 (d, 3'-C, 5'-C)[b], 134.7 (s, 1'-C), 138.8 (s, 4'-C), 139.1 (d, 7-C), 162.3 (s, 5-C)[c], 163.0 (s, 9-C)[c], 199.1 (s, C=O); *m/z* 276, 275, 274, 273, 256, 239, 163, 136 (100%), 108; (Found C, 65.6; H, 4.1; Cl, 13.2. $C_{15}H_{11}O_3Cl$ requires C, 65.6; H, 4.0; Cl, 12.9%). This flavanone isomerised in 1.00 mol l^{-1} NaOH, under the usual conditions used in chapter 2, with rate constants of 4.56 and 4.61×10^{-3} s^{-1} (in duplicate determinations). This is the same result as was obtained (chapter 2) using the same flavanone prepared *via* the isomeric chalcone.

5-Methoxyflavanone (*80*).— 2'-Hydroxy-6'-methoxychalcone (*88*) was refluxed in 4% v/v methanolic sulphuric acid for *ca*. 48 hours. Most of the solvent was then removed *in vacuo* and a large excess of water added. The product was then extracted into chloroform, and the organic layer washed once with 1 mol l^{-1} sodium hydroxide solution. Removal of the solvent *in vacuo* followed by recrystallisation from aqueous methanol gave colourless crystals of 5-methoxyflavanone (*80*), m.p. 141-142°C; δ_H (90 MHz; $CDCl_3$) 2.82 (1 H, dd, $J_{3\text{-H}_{ax}}$ 16.2, $J_{2\text{-H}}$ 3.5 Hz, 3-H_{eq}), 3.07 (1 H, dd, $J_{3\text{-H}_{eq}}$ 16.2, $J_{2\text{-H}}$ 12.5 Hz, 3-H_{ax}), 3.89 (3 H, s, OCH_3), 5.41 (1 H, dd, $J_{3\text{-H}_{ax}}$ 12.5, $J_{3\text{-H}_{eq}}$ 3.5 Hz, 2-H), 6.52 (1 H, d, *J* 9 Hz, 6-H)[a], 6.63 (1 H, d, *J* 9 Hz, 8-H)[a], 7.3-7.5 (6 H, m, 7-H, B-ring protons); δ_C (22 MHz;

$CDCl_3$) 48.9 (t, 3-C), 56.1 (q, OCH_3), 78.8 (d, 2-C), 104.8 (d, 8-C)^a, 111.1 (d, 6-C)^a, 111.3 (s, 10-C), 126.0 (d, 2′-C, 6′-C)^b, 128.5 (d, 4′-C), 128.6 (d, 3′-C, 5′-C)^b, 135.9 (d, 7-C), 138.6 (s, 1′-C), 160.6 (d, 9-C)^c, 163.0 (s, 5-C)^c, 190.4 (s, C=O); *m/z* 254 ($M^{+\cdot}$, 32%), 253 (27), 177 (25), 151 (12), 150 (100), 122 (21), 107 (21).

5,7-Dimethoxyflavanone (*81*).— To a *ca.* 0.25% w/v aqueous potassium hydroxide solution was added 2′-hydroxy-4′,6′-dimethoxychalcone (*91*; 0.5 g, 1.8 mmol), and the mixture stirred at room temperature for 60 hours. The mixture was then extracted with chloroform, then the solvent removed *in vacuo* and the resultant product recrystallised from methanol to afford colourless crystals (0.36 g, 72%) of 5,7-dimethoxyflavanone (*81*), m.p. 143-144°C (lit.,[263],[264],[178] 144°C, 141°C, 145-146°C). A second crop of colourless crystals (0.07 g, 14%) was obtained from the mother liquor. δ_H (90 MHz; $CDCl_3$) 2.74 (1 H, dd, $J_{3\text{-}H_{ax}}$ 17, $J_{2\text{-}H}$ 4.5 Hz, 3-$H_{eq.}$), 3.02 (1 H, dd, $J_{3\text{-}H_{eq.}}$ 17, $J_{2\text{-}H}$ 12.5 Hz, 3-H_{ax}), 3.79 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 5.38 (1 H, dd, $J_{3\text{-}H_{ax}}$ 12.5, $J_{3\text{-}H_{eq}}$ 4.5 Hz, 2-H), 6.06 (1 H, d, *J* 2.7 Hz, 6-H)^a, 6.13 (1 H, d, *J* 2.7 Hz, 8-H)^a, 7.3-7.5 (5 H, m, B-ring protons); δ_C (22 MHz; $CDCl_3$) 45.5 (t, 3-C), 55.5 (q, OCH_3), 56.1 (q, OCH_3), 79.1 (d, 2-C), 93.1 (d, 8-C)^a, 93.5 (d, 6-C)^a, 105.9 (s, 10-C), 126.0 (d, 2-C, 6-C)^b, 128.6 (d, 4-C), 128.7 (d, 3-C, 5-C)^b, 138.8 (s, 1′-C), 162.3 (s, 9-C)^c, 164.9 (s, 5-C)^c, 165.9 (s, 7-C)^c, 188.9 (s, C=O); *m/z* 284 ($M^{+\cdot}$, 70%), 207 (16), 181 (14), 180 (100), 152 (24), 137 (18).

5-Hydroxy-7-methoxyflavanone (*82*).— Aluminium chloride (2.3 g) was dissolved in diethyl ether (*ca.* 20 ml), and to this was added 5,7-dimethoxyflavanone (*81*; 0.27 g, 0.95 mmol). The solution, which went yellow upon addition of the flavanone, was allowed to stand for several hours, whereupon it was added to a large excess of hydrochloric acid (2 mol l^{-1}). The product was extracted with chloroform (×2), and the organic layer washed first with hydrochloric acid (2 mol l^{-1}), then with water. Finally the chloroform solution of the product was dried over magnesium sulphate and the solvent removed *in vacuo* to afford a colourless solid. The ^{13}C n.m.r. spectra of the product (figure 7-2) clearly indicated the presence of a mixture of approximately equal proportions of 5,7-dimethoxyflavanone (***81***) and the desired product, 5-hydroxy-7-methoxyflavanone (***82***). In particular note the appearance of: a sharp singlet at 12.00 ppm in the ^{1}H n.m.r. spectrum, typical of an intramolecularly hydrogen bonded O*H* group; and of a new signal in the ^{13}C n.m.r. spectrum at 195.7 ppm, a chemical shift typical of a *C*=O group of a flavanone which is intramolecularly hydrogen bonded to a 5-OH group. By comparing the ^{13}C n.m.r. spectra of 5,7-dimethoxyflavanone and of the demethylation product (shown in figure 7-2), it was possible to ascertain the ^{13}C n.m.r. spectrum of 5-hydroxy-7-methoxyflavanone (***82***) in $CDCl_3$: δ_C (90 MHz; $CDCl_3$) 43.4 (3-C), 55.6 (OCH_3), 79.2 (2-C), 94.2 (8-C), 95.1 (6-C), 103.1 (s, 10-C), 126.1 (2-C, 6-C)^a, 128.6 (4-C), 128.8 (3-C, 5-C)^a, 138.4 (s, 1′-C), 162.8 (s, 9-C), 164.1 (s, 5-C), 168.0 (s, 7-C), 195.7 (s, C=O).

5-Hydroxy-4′,7-bistetrahydropyranyloxyflavanone (***83***).— 4′,5,7-trihydroxyflavanone (***4***) and dihydropyran were reacted together in the same manner as for the preparation of 2′-hydroxy-6′-tetrahydropyranyloxyacetophenone (***65***). The bisTHP ether of the flavanone was isolated as a colourless oil in low yield from the product mixture, by p.l.c. The ^{13}C n.m.r. spectrum of the oil indicates three tetrahydropyranyl (THP) environments (two of which are *ca.* half the intensity of the third), although whether this represents an impurity, or a real steric or diastereoisomeric effect (each THP group contains a chiral centre, as does the pyranone ring of the flavanone) is unclear. However the spectral data do support 5-hydroxy-4',7-bis-tetrahydropyranyloxyflavanone (*83*) as at least the major constituent of the oil. δ_H (90 MHz; $CDCl_3$) 1·6, 1·8, 3·6, 3·8, 5·4 (br, tetra-hydropyranyl protons), 2·71 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 16, $J_{2\text{-}H}$ 4 Hz, 3-$H_{eq.}$), 2.95 (1 H, dd, $J_{3\text{-}H_{eq.}}$ 16, $J_{2\text{-}H}$ 12 Hz, 3-$H_{ax.}$), 5.15 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 12, $J_{3\text{-}H_{eq.}}$ 4 Hz, 2-H), 6.2 (2 H, br s, 6-H, 8-H), 7.1 (2 H, br d, 3'-H, 5'-H), 7.4 (2 H, br d, 2'-H, 6'-H), 11.93 (1 H, s, 5-OH); δ_C (22 MHz; $CDCl_3$) 18.0, 18.3, 19.2 (t, THP 4-C), 24.5, 24.8, 25.1 (t, THP 5-C), 29.4, 29.8, 30.2 (t, THP 3-C), 42.7 (t, 3-C), 61.4, 61.6, 62.1 (t, THP 6-C), 77.3 (d, 2-C), 93.9, 95.6, 95.8, 96.9 (d, 8-C, 6-C, THP 2-C), 103.1 (s, 10-C), 116.2 (d, 3'-C, 5'-C), 127.1 (d, 2'-C, 6'-C), 131.0 (s, 1'-C), 157.0 (s, 4'-C), 162.4 (s, 9-C)^a, 163.5 (s, 5-C)^a, 165.0 (s, 7-C)^a, 195.7 (s, C=O); *m/z* 440 ($M^{+\cdot}$, 1%), 356 ($M^{+\cdot}$ - 84, 6), 273 (15), 272 (356 - 84, 76), 271 (32), 179 (17), 166 (25), 153 (47), 120 (27), 107 (12), 101 (12), 85 (100).

5-Acetoxy-4',7-dimethoxyflavanone (*84*).— This substance was prepared by dissolving 5-hydroxy-4',7-dimethoxyflavanone (*33*) in 1:1 acetic anhydride and pyridine for *ca*. 3 hours at room temperature. Work-up followed by recrystallisation from methanol afforded white crystals of 5-acetoxy-4',7-dimethoxyflavanone (*84*), m.p. 160-162°C (lit.,[265a] 161°C); δ_H (90 MHz; solvent $CDCl_3$; standard Me_4Si) 2.38 (3 H, s, $OCOCH_3$), 2.68 (1 H, dd, $J_{3\text{-}H_{ax}}$ 16.7, $J_{2\text{-}H}$ 3.8 Hz, 3-$H_{eq.}$), 3.05 (1 H, dd, $J_{3\text{-}H_{eq.}}$ 16.7, $J_{2\text{-}H}$ 12.6 Hz, 3-$H_{ax.}$), 3.82 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 5.41 (1 H, dd, $J_{3\text{-}H_{ax.}}$ 12.6, $J_{3\text{-}H_{eq.}}$ 3.8 Hz, 2-H), 6.27 (1 H, d, J 2.6 Hz, 6-H)^a, 6.41 (1 H, d, J 2.6 Hz, 8-H)^a, 6.95 (2 H, d, J 8.5 Hz, 3'-H, 5'-H), 7.38 (2 H, d, *J* 8.5 Hz, 2′-H, 6′-H); δ_C (22 MHz; solvent $CDCl_3$; standard Me_4Si) 21.1 (q, $OCOCH_3$), 44.9 (t, 3-C), 55.3 (q, OCH_3), 55.8 (q, OCH_3), 79.3 (d, 3-C), 99.5 (d, 8-C)[a], 104.7 (d, 6-C)[a], 108.0 (s, 10-C), 114.3 (d, 3′-C, 5′-C), 127.8 (d, 2′-C, 6′-C), 130.5 (s, 1′-C), 151.8 (s, $OCOCH_3$), 160.0 (s, 4′-C), 164.3 (s, 9-C)[b], 165.4 (s, 5-C)[b], 169.5 (s, 7-C)[b], 189.0 (s, C=O); *m/z* 342 ($M^{+\cdot}$, 21%), 300 ($M^{+\cdot}$ - 42, 55), 299 (38), 193 (15), 166 (33), 135 (12), 134 (100), 121 (82), 119 (18); (Found: C, 66.6; H, 5.4. Calc. for $C_{19}H_{18}O_6$: C, 66.7; H, 5.3%).

7.4 Chalcones by Ring Opening of Flavanones

6a,12a-Dehydrorotenol (Rotenone-'chalcone") (*20*).— This compound, which is closely related to the chalcones, was prepared by ring opening rotenone (*21*) (Aldrich) with sodium in benzene (after the method of Crombie[109]). The product was purified by p.l.c. to afford a pale yellow solid. The ^1H n.m.r. spectrum of the product was virtually identical to that reported by Sasaki[105] for this substance. The ^{13}C n.m.r. spectrum of the product was also recorded, although most of the peaks were not assigned; δ_H (90 MHz: $CDCl_3$) 1.78 (3 H, s, $C\text{-}CH_3$), 3.10 (1 H, d, *J* 8.2 Hz), 3.32 (1 H, d, *J* 9.7 Hz, 4′-H_b), 3.73 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 4.84 (2 H, d, *J* 3.8 Hz, $C{=}CH\text{-}CH_2O$), 4.95 (1 H, br), 5.10 (1 H, br), 5.35 (1 H, t, *J* 8.8 Hz, 5′-H), 5.86 (1 H, t, *J* 4 Hz, 6_a-H), 6.25 (1 H, d, *J* 8.8 Hz, 10-H), 6.50 (1 H, s, 4-H), 6.71 (1 H, s, 1-H), 7.60 (1 H, d, *J* 8.8 Hz, 11-H), 12.57 (1 H, s, OH); δ_C (22 MHz; $CDCl_3$) 17.0 ($C\text{-}CH_3$), 31.0, 56.0 (OCH_3), 56.6 (OCH_3), 64.6, 88.2, 100.9, 102.0, 108.7, 112.1, 112.6, 113.2, 114.1, 121.7, 134.6, 135.9, 143.1, 143.8, 148.7, 150.7, 161.3, 167.7, 198.5 (C=O).

2',6'-Dihydroxy-4,4'-dimethoxychalcone (**32**).— 5-Hydroxy-4',7-dimethoxyflavanone (**33**; *ca*. 0.5 g, 1.7 mmol) was dissolved in methanolic KOH (*ca*. 5 ml; 30% w/w), and the solution was refluxed briefly (*ca*. 2 minutes). The resultant red solution was then poured into an excess of ice-cold hydrochloric acid (2 mol l^{-1}), and the orange solid that precipitated was filtered at the pump. Recrystallisation from chloroform – light petroleum (b.p. 40-60°C) (1:1) gave *2',6'-dihydroxy-4,4'-dimethoxychalcone* (**32**) as short orange needles, m.p. 156-158°C with decolourisation upon melting (lit.,[83] 157°C); λ_{max} (EtOH) 365 nm [lit.,[83,75] 360 nm, 355 nm (log ϵ 4.70)]; δ_H [90 MHz; $(CD_3)_2CO$] 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), 7.94 (1 H, d, *J* 16 Hz, α-H), 8.29 (1 H, d, *J* 16 Hz, β-H), 12.23 (2 H, br s, 2'-OH, 6'-OH); δ_C [22 MHz; $(CD_3)_2CO$] 55.8 (4-OCH_3, 4'-OCH_3), 94.6 (3'-C, 5'-C), 106.3 (s, 1'-C), 115.3 (3-C, 5-C), 125.9 (α-C), 129.1 (s, 1-C), 131.0 (2-C, 6-C), 143.2 (β-C), 162.6 (s, 4-C), 165.3 (s, 2'-C, 6'-C), 167.1 (s, 4'-C), 193.5 (s, C=O); *m/z* 301 (10%), 300 ($M^{+\cdot}$, 58), 299 (43), 193 (20), 167 (17), 166 (20), 138 (11), 135 (12), 134 (100), 121 (89), 119 (21); (Found: C, 67.6; H, 5.4. $C_{17}H_{16}O_5$ requires C, 68.0; H, 5.4%).

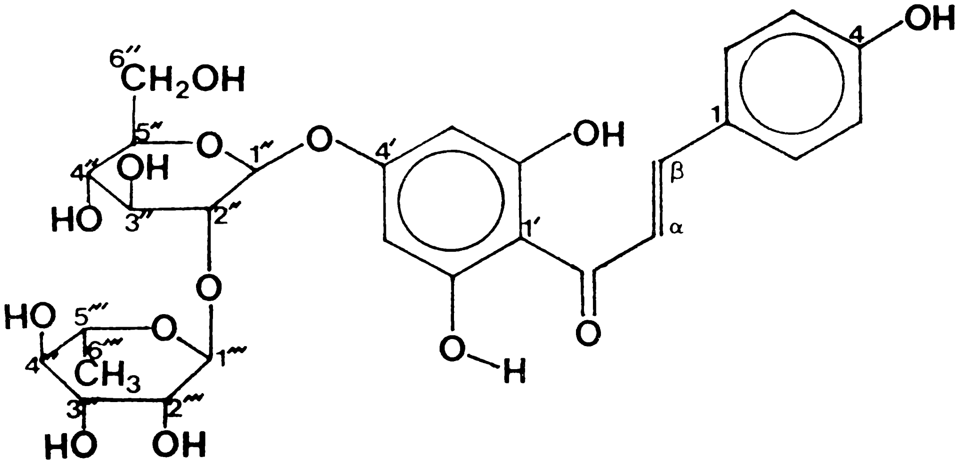
2',6'-Dihydroxy-3',4,4'-trimethoxychalcone (*36*).— 5-hydroxy-3',4',7-trimethoxyflavanone (*37*) (0.50 g, 1.5 mmol) was refluxed for 5 minutes in methanolic potassium hydroxide (5.0 g CH_3OH, 1.0 g KOH), and the resultant deep red solution was added slowly with stirring to an excess of ice-cold aqueous hydrochloric acid (2 mol l^{-1}). The resultant solid was extracted with diethyl ether (2×50 ml). The solvent was then removed *in vacuo* to give an orange solid which was purified by p.l.c. using ethyl acetate – chloroform (4:6) as eluent.

The product in the bright yellow band ($R_f \approx 0.6$) was taken up in diethyl ether, the solvent removed *in vacuo*, and the solid recrystallised from toluene – petroleum spirit (b.p. 60-80°C) to give *2′,6′-dihydroxy-3′,4,4′-trimethoxychalcone* (**36**) as an orange powder which gave one spot on t.l.c., m.p. 140-150°C, with the bulk melting at 147-150°C; λ_{max} (EtOH) 365 nm, λ_{max} (acidic methanol) 372 nm (log ϵ 4.35); δ_H [90 MHz, $(CD_3)_2CO$] 3.96 (3 H, s, OCH_3), 4.00 (3 H,s, OCH_3), 4.01 (3 H,s, OCH_3), 6.19 (2 H, s, 3′-H, 5′-H), 7.14 (1 H, d, *J* 8.8 Hz, 6-H)^a, 7.3-7.5 (2 H, m, 5-H, 2-H)^a, 7.92 (1 H, d, *J* 15.5 Hz, α-H), 8.28 (1 H, d, *J* 15.5 Hz, β-H), 12.3 (2 H, br s, 2′-OH, 6′-OH); δ_C [22 MHz, $(CD_3)_2CO$] 55.9, 56.1 (OCH_3), 94.6 (3′-C, 5′-C), 106.2 (1′-C), 111.9 (2-C)^a, 112.6 (5-C)^a, 123.6 (6-C), 126.0 (α-C), 129.3 (1-C), 143.5 (β-C), 150.5 (3-C)^b, 152.6 (4-C)^b, 165.4 (2′-C, 6′-C), 167.0 (4′-C), 193.4 (C=O); *m/z* 330 ($M^{+\cdot}$, 40%), 329 (14), 193 (11), 164 (53), 151 (100), 149 (26); (Found: C, 65.2; H, 5.3. $C_{18}H_{18}O_6$ requires C, 65.4; H, 5.5%).

4-Chloro-2′,6′-dihydroxychalcone (**46**).— To a solution of 4′-chloro-5-hydroxyflavanone [**47** (by method *b*); *ca.* 100 mg] in dichloromethane (*ca.* 3 ml) was added chlorotrimethylsilane (*ca.* 2 ml), DBU (*ca.* 1 ml) and A-4 molecular sieves. After 3 days the now red solution was poured into an excess of hydrochloric acid (1 mol l^{-1}), and the product was extracted with dichloromethane. Removal of the solvent *in vacuo*, followed by p.l.c. (dichloromethane as eluent) afforded *4-chloro-2′,6′-dihydroxychalcone* (**47**) as a red-orange solid, m.p. 121-127°C; λ_{max} (acidic methanol) 327 nm (log ϵ 3.67); δ_H [90 MHz; $(CD_3)_2CO$] 6.62 (2 H, d, *J* 8.2 Hz, 3′-H, 5′-H), 7.46 (1 H, t, *J* 8.2 Hz, 4′-H), 7.64 (2 H, d, *J* 8.5 Hz, 2-H, 6-H)^a, 7.92 (2 H, d, *J* 8.5 Hz, 3-H, 5-H)^a, 7.94 (1 H, d, *J* 15.5 Hz, α-H), 8.36 (1 H, d, *J* 15.5 Hz, β-H), 11.86 (2 H, br s, 2′-OH, 6′-OH); δ_C [22 MHz; $(CD_3)_2CO$] 108.7 (3-C, 5-C), 111.7 (1′-C), 129.3 (α-C), 130.1 (3-C, 5-C)^a, 130.9 (2-C, 6-C)^a, 135.3 (4-C), 136.5 (1-C), 137.3 (4′-C), 142.0 (β-C), 163.2 (2′-C, 6′-C), 195.4 (C=O); (Found: C, 65.0; H, 4.2. $C_{15}H_{11}O_3Cl$ requires C, 65.6; H, 4.0%). This chalcone isomerised in 1.00 mol l^{-1} NaOH at 30°C containing 4% v/v ethanol, with a rate constant of 4.45×10^{-3} s^{-1}. Under the same conditions (chapter 2) the isomeric flavanone [formed *via* isomerisation of chalcone ***46***, which was itself formed by the condensation reaction (section 7.5)] isomerised with rate constants of 4.42 and 4.62×10^{-3} s^{-1} (in duplicate determinations). (Found: $M^{+\cdot}(^{35}Cl)$, 274.0383; $M^{+\cdot}(^{37}Cl)$, 276.0375. $C_{15}H_{11}O_3Cl$ requires $M^{+\cdot}(^{35}Cl)$, 274.0397; $M^{+\cdot}(^{37}Cl)$, 276.0367).

Naringin-chalcone (***48***).— Naringin [***18***; 1 g (Sigma)] was added to a solution of potassium hydroxide (2 g) in ethanol-water (4 ml; 1:1) and heated on a steam bath for about 3 minutes. The deep red solution obtained was filtered immediately into an excess of ice-cold hydrochloric acid (2 mol l^{-1}). The precipitated yellow solid was extracted with diethyl ether. After drying over $MgSO_4$ the ether solution was filtered, and the solvent removed *in vacuo*. The solid residue was recrystallised from diethyl ether - chloroform - light petroleum (b.p. 40-60°C) (*ca*. 1:1:1) to give 4′-rhamnoglucosyloxy-2′,4,6′-trihydroxychalcone (naringin-chalcone) (***48***) as an orange powder, m.p. 170-183°C (lit.,[25,266] 185-200°C); λ_{max} (acidic methanol) 367 nm (log ε 4.46) [lit.,[25] (MeOH) 370 nm]; δ_H [90 MHz; $(CD_3)_2SO$] 1.30 (3 H, d, *J* 6 Hz, 6‴-CH_3), 3.4-3.8 (br m, other sugar protons), 5.20 (2 H, br s, anomeric 1″-H, 1‴-H), 6.16 (2 H, s, 3′-H, 5′-H), 6.95 (2 H, d, *J* 8 Hz, 3-H, 5-H), 7.65 (2 H, d, *J* 8 Hz, 2-H, 6-H), 7.70 (1 H, d,

J 16 Hz, α-H), 8.05 (1 H, d, J 16 Hz, β-H), 10.20 (1 H, br s, 4-OH), 12.60 (2 H, br s, 2'-OH, 6'-OH); δ_C [22 MHz; $(CD_3)_2SO$] 19.0 (q, 6'''-C), 61.3 (t, 6''-C), 69.3 (d, 5'''-C), 70.4, 71.3 (d, 2'''-C, 3'''-C, 4''-C), 72.8 (d, 4'''-C), 77.6, 78.1 (d, 2''-C, 3''-C, 5''-C), 95.9 (d, 3'-C, 5'-C), 98.2 (d, 1''-C), 101.5 (d, 1'''-C), 106.0 (s, 1-C), 117.0 (d, 3-C, 5-C), 124.7 (d, α-C), 126.9 (s, 1-C), 131.5 (d, 2-C, 6-C), 144.0 (d, β-C), 160.9 (s, 4-C), 163.8 (s, 4'-C), 164.6 (s, 2'-C, 6'-C), 193.4 (s, C=O); assignments of the sugar ^{13}C signals are based on those of Markham and Chari[251] for naringin (*18*). (Found: C, 53.4; H, 5.7. Calc. for $C_{27}H_{32}O_{14}$: C, 52.9; H, 5.3%).



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2',4,4',6'-Tetrahydroxychalcone (*85*).— (*a*) Naringenin (4',5,7-trihydroxyflavanone) (*4*; 0.5 g, 1.8 mmol) was reacted with an excess of 2,3-dihydropyran, as described above for 2'-hydroxy-6-tetrahydropyranyloxyacetophenone (*65*). The dioxane solution of the crude reactants was added directly to diethyl ether (50 ml), and shaken with aqueous potassium hydroxide (20 ml; 5 mol l^{-1}), then left to stand for five minutes. The aqueous layer was then run into an excess of aqueous hydrochloric acid (2 mol l^{-1}). The product was extracted with diethyl ether and purified by p.l.c., with chloroform – ethyl acetate (1:1) as eluent. The band at $R_f \approx 0.6$ was removed, the product extracted and recrystallised from diethyl ether – petroleum spirit (b.p. 40-60°C) to afford a crimson powder, 2',4,4',6'-tetrahydroxychalcone (*85*), m.p. 189-191°C (lit.[224,197,223] 205°C, 184°C, 173-174°C) [the solid decolourised upon melting, forming colourless crystals, which remelted at 243-246°C (lit.,[265b] m.p. of 4',5,7-trihydroxyflavanone (*4*) 250-251°C)]; λ_{max} (EtOH) 366 nm (lit.,[197] 362 nm) [a methanolic solution of this chalcone decolourised upon standing for several days, after which it exhibited a u.v. spectrum typical of a flavanone, with λ_{max} 288 nm (lit.,[267] 288 nm for 4',5,7-trihydroxyflavanone); the decolourisation reaction exhibited sharp isosbestic points when followed spectrophotometrically]; δ_H [90 MHz, $(CD_3)_2CO$] (*cf.* ref. 43) 6.12 (2 H, s, 3'-H, 5'-H), 7.05 (2 H, d, *J* 8.5 Hz, 3-H, 5-H), 7.71 (2 H, d, *J* 8.5 Hz, 2-H, 6-H), 7.89 (1 H, d, *J* 16.6 Hz, α-H), 8.25 (1 H, d, *J* 16.6 Hz, β-H); δ_C [22 MHz, $(CD_3)_2CO$] 105.6 (s, 1-C), 116.6 (d, 3-C, 5-C), 125.3 (d, α-C), 128.1 (s, 1'-C), 131.1 (d, 2-C, 6-C), 143.1 (d, β-C), 160.4, 165.2 (s, 2'-C, 4-C, 4'-C, 6'-C), 193.2 (s, C=O); *m/z* 273 (10%), 272 ($M^{+\cdot}$, 72), 271 (35), 179 (25), 166 (23), 153 (100), 152 (21), 124 (20), 121 (10), 120 (70), 119 (13), 107 (19) (a mass-spectrum of commercial 4',5,7-trihydroxyflavanone exhibited peaks at the same *m/z* values, and with essentially the same relative intensities, as this chalcone).

2',4,4',6'-Tetrahydroxychalcone (*85*).— (*b*) 4',5,7-Trihydroxyflavanone (naringenin) (*4*; 1.0 g) was dissolved in dioxane (5 ml), and pyridine (5 ml) and chlorotrimethylsilane (5 ml) added. After three days the solution went only pale yellow, and the u.v. spectrum indicated mainly the flavanone to be present. Approximately half the reaction solution was filtered off (from precipitated pyridine hydrochloride), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (*ca.* 1 ml) was added. After a further three days standing, the u.v. spectrum indicated that the solution contained chalcone and flavanone in the ratio of about 1:2. The reaction mixture was poured into hydrochloric acid (2 mol l^{-1}) and the product extracted into ether. T.l.c. indicated that deprotection had taken place during the work-up procedure, and the product was purified by p.l.c., to afford an orange solid, 2',4,4',6'-tetrahydroxychalcone (*85*), m.p. 248-250°C [with partial decomposition (to a red gum) occurring above 200°C] (lit.,[224,197,223] 205°C, 184°C, 173-174°C), (lit.[265b] m.p. for naringenin 250-251°C); λ_{max} (MeOH) 367 nm (log ϵ 4.3) (lit.,[197] 362 nm) [On standing in anhydrous methanol the solution decolourised, exhibiting clear isosbestic points, with a half-life of 103 min at 30°C (k_{obs} = 1.12×10^{-4} s^{-1}). The final product exhibited λ_{max} 288 nm (lit.,[265b] for naringenin 288 nm), and λ_{max} 292 nm (log ϵ 4.0) in methanol containing 0.1% v/v hydrochloric acid (1 mol l^{-1})]; δ_H [90 MHz; $(CD_3)_2CO$] 6.15 (2 H, s, 3'-H, 5'-H), 7.04 (2 H, d, *J* 8.5 Hz, 3-H, 5-H), 7.70 (2 H, d, *J* 8.5 Hz, 2-H, 6-H), 7.92 (1 H, d, *J* 15.5 Hz, α-H), 8.28 (1 H, d, *J* 15.5 Hz, β-H), 12.31 (2 H, br s, 2'-OH, 6'-OH); δ_C [22 MHz; $(CD_3)_2CO$] 96.0 (3'-C, 5'-C), 105.6 (1-C), 116.7 (3-C, 5-C), 125.2 (α-C), 128.0 (1'-C), 131.0 (2-C, 6-C), 143.2 (β-C), 160.3 (4-C), 165.2 (4'-C), 165.5 (2'-C, 6'-C), 193.1 (C=O) (Both the ^1H and ^{13}C n.m.r. spectra indicated the presence in this sample of approximately 10% of the isomeric flavanone, naringenin).

2',3,4',6'-Tetrahydroxy-4-methoxychalcone (*86*).— 3',5,7-Trihydroxy-4'-methoxyflavanone (hesperetin) (*8*; *ca.* 0.2 g) was reacted with excess chloro-t-butyldimethylsilane and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dioxane after the method[232] of Aizpurua. The reaction solution slowly went deep yellow, and after 3 days it was added to hydrochloric acid (2 mol l^{-1}) and extracted into chloroform. T.l.c. indicated that no significant deprotection had taken place during the work-up procedure. The product was then dissolved in methanol – dioxane (*ca.* 1:1; *ca.* 50 ml), and hydrochloric acid (2 mol l^{-1}; *ca.* 50 ml) added, and the deprotection was monitored by t.l.c. After 18 hours deprotection was complete. The solvent was removed *in vacuo* and the chalcone extracted into ether from dilute hydrochloric acid (*ca.* 1 mol l^{-1}) to afford a red solid, m.p. 130°C. This product was purified by p.l.c. to give pure *4-methoxy-2′,3,4′,6′-tetrahydroxychalcone* (**86**), m.p. 151-167°C; λ_{max} (MeOH) 372 nm; λ_{max} (acidic methanol) 371 nm (log ϵ 4.30) {The isomerisation reaction of this compound in anhydrous methanol [k_{obs} $3.22(\sigma = 0.01)\times10^{-5}$ s^{-1} at 30.0°C] showed sharp isosbestic points, and the final product had λ_{max} 288 nm (lit.,[267] hesperetin 289 nm)}; δ_H [90 MHz; solvent $(CD_3)_2CO$; standard Me_4Si] 3.90 (3 H, s, OCH_3), 5.98 (2 H, s, 3′-H, 5′-H), 6.99 (1 H, d, *J* 8 Hz, 5-H)^a, 7.09 (1 H, d, *J* 8 Hz, 6-H)^a, 7.21 (1 H, s, 2-H), 7.72 (1 H, d, *J* 15.5 Hz, α-H), 8.12 (1 H, d, *J* 15.5 Hz, β-H), 9.32 (br s, ArOH), 12.06 (2 H, s, 2′-OH, 6′-OH); δ_C [22 MHz; $(CD_3)_2CO$] 56.3 (q, OCH_3), 95.2 (d, 3′-C, 5′-C), 104.8 (s, 1′-C), 111.3 (d, 2-C)^a, 113.5 (d, 5-C)^a, 122.0 (d, 6-H), 125.1 (d, α-C), 128.8 (s, 1-C), 142.4 (d, β-C), 146.6 (s, 3-C)^b, 149.6 (s, 4-C)^b, 164.6 (s, 4′-C), 164.7 (s, 2′-C, 6′-C), 192.2 (s, C=O); (Upon standing in methanol for two days, this chalcone decolourised, and the ^{13}C n.m.r. spectrum of the product [m.p. 227-231 (lit.,[260] for hesperetin 226-228)] was the same as that for commercial hesperetin); (Found: C, 59.75; H, 4.9; $M^{+\cdot}$, 302.0791. $C_{16}H_{14}O_6\cdot H_2O$ requires C, 60.0; H, 5.0%; $M^{+\cdot}$, 302.0790).

7.5 Chalcones by Condensation Reactions

2',6'-Dihydroxychalcone (**34**).— To 2',6'-dihydroxyacetophenone (*73*; 1.00 g, 6.6 mmol) and 2,3-dihydropyran (3.0 g, 36 mmol) in dioxane (3 ml) were added a few drops of a solution of toluene-4-sulphonic acid (*ca.* 1 mol l^{-1}) in dioxane. The solution, which rapidly became warm to the touch, was left to stand 23 hours before being filtered into a separating funnel containing diethyl ether (75 ml) and 5% aqueous potassium carbonate (25 ml). After shaking, the ether layer was retained and extracted three further times with 5% aqueous potassium carbonate (3×25 ml) to remove unreacted acetophenone. The ether layer was then extracted with aqueous sodium hydroxide (2×20 ml; 2 mol l^{-1}), and the aqueous extracts were added to 10% aqueous potassium carbonate (50 ml). After careful acidification to pH 9-10 the now cloudy solution was extracted with chloroform (4×25 ml) and the solvent removed *in vacuo* to give a pale yellow oil, presumably 2'-hydroxy-6'-tetrahydropyranyloxyacetophenone (*65*), which gave one spot on t.l.c. (chloroform as eluent).

This oil was mixed with benzaldehyde (2.0 g, 19 mmol), and aqueous methanolic potassium hydroxide (5.0 g KOH; 5 ml H_2O; 10 ml CH_3OH) was added with stirring. Within one minute the solution changed from green to deep-red. After 3½ hours the reaction was stopped by pouring the mixture into a separating funnel containing diethyl ether (100 ml) and water (50 ml). After shaking, the aqueous layer was removed and the ether layer extracted twice with aqueous sodium hydroxide (2×10 ml; 2 mol l^{-1}). The three aqueous extracts were combined, acidified, extracted with chloroform (2×25 ml), and the solvent removed *in vacuo* to give a crystalline orange solid. T.l.c. indicated the product, presumably 2'-hydroxy-6'-tetrahydropyranyloxychalcone, to be fairly pure.

To the 2′-hydroxy-6′-*O*-THPchalcone in ethanol (25 ml) was added dilute hydrochloric acid (5 ml; 2 mol l^{-1}), t.l.c. showing deprotection to be complete after 10 minutes. The solution was added to water (150 ml), the products extracted with diethyl ether – chloroform (1:2; 2×50 ml) and the solvent removed *in vacuo*. Purification by p.l.c. (3:1 chloroform – ethyl acetate as eluent) and recrystallisation of the resultant orange solid from toluene – chloroform gave short orange needle-like crystals of *2′,6′-dihydroxychalcone* (**34**), m.p. 169-172°C (lit.,[197] 171°C); λ_{max} (acidic methanol) 325 nm (log ϵ 4.41) (lit.,[197] 323 nm, log ϵ 4.44); δ_H [90 MHz; $(CD_3)_2CO$] 6.63 (2 H, d, *J* 8.2 Hz, 3′-H, 5′-H), 7.34, 7.45, 7.5-7.6 and 7.8-7.9 (6 H, m, 2,3,4,4′,5,6-H), 7.99 (1 H, d, *J* 15.8 Hz, α-H), 8.37 (1 H, d, *J* 15.8 Hz, β-H), 11.68 (2 H, br s, 2′-OH, 6′-OH); δ_C [22 MHz; $(CD_3)_2CO$] 108.6 (3′-C, 5′-C), 111.7 (s, 1′-C), 128.4 (α-C), 129.3 (2-C, 6-C)[a], 129.8 (3-C, 5-C)[a], 131.2 (4-C), 136.2 (s, 1-C), 137.1 (4′-C), 143.6 (β-C), 163.1 (s, 2′-C, 6′-C), 195.4 (s, C=O); *m/z* 241 (17%), 240 ($M^{+\cdot}$, 71), 239 (75), 222 (22), 164 (18), 163 (84), 137 (34), 136 (100), 108 (42), 103 (21); (Found C, 75.2; H, 5.1. $C_{15}H_{12}O_3$ requires C, 75.0; H, 5.0%).

2′,6′-Dihydroxy-4-methoxychalcone (**38**).— Crude 2′-hydroxy-6′-tetrahydropyranyloxyacetophenone (**65**) was formed from 2′,6′-dihydroxyacetophenone (**73**; 1.00 g, 6.6 mmol) and 2,3-dihydropyran (3.0 g, 36 mmol) as described above for 2′,6′-dihydroxychalcone, and to the resulting oil was added 4-methoxybenzaldehyde (2.0 g, 15 mmol), followed by aqueous-methanolic potassium hydroxide (5.0 g KOH; 5 ml H_2O; 10 ml CH_3OH). The solution rapidly changed from green to deep red. It was stirred for $3\frac{1}{2}$ hours then added to water (50 ml), and the mono-*O*-THPchalcone obtained in the manner described above for

2′,6′-dihydroxychalcone, as a bright orange solid. To this solid was added ethanol (50 ml) and aqueous hydrochloric acid (10 ml; 2 mol l^{-1}). The mixture was refluxed for 10 minutes, filtered, and most of the solvent removed *in vacuo*. The residue was dissolved in diethyl ether (100 ml) and extracted with aqueous sodium hydroxide (2×50 ml; 2 mol l^{-1}). The extracts were then acidified, and extracted with diethyl ether. T.l.c. of this ether extract indicated the presence of the desired chalcone, with the isomeric flavanone as the major contaminant. Purification by p.l.c. with chloroform – ethyl acetate (6:4) as eluent gave a red solid which showed one spot on t.l.c. The solid was recrystallised from toluene – petroleum spirit (60-80°C) to afford fine red-orange crystals of *2′,6′-dihydroxy-4-methoxychalcone* (**38**), m.p. 142-152°C (decolourised during melting) (lit.,[200] 185-186°C); λ_{max} (acidic methanol) 356 nm (log ϵ 4.18); δ_H (*cf*. ref. 200) [90 MHz; $(CD_3)_2CO$] 3.98 (3 H, s, OCH_3), 6.63 (2 H, d, *J* 8.2 Hz, 3′-H, 5′-H), 7.13 (2 H, d, *J* 8.8 Hz, 3-H, 5-H), 7.42 (1 H, t, *J* 8.2 Hz, 4′-H), 7.81 (2 H, d, *J* 8.8 Hz, 2-H, 6-H), 7.98 (1 H, d, *J* 15.5 Hz, α-H), 8.27 (1 H, d, *J* 15.5 Hz, β-H), 11.7 (br s, 2′-OH, 6′-OH); δ_C [22 MHz; $(CD_3)_2CO$] 55.9 (OCH_3), 108.6 (3′-C, 5′-C), 111.7 (s, 1′-C), 115.4 (3-C, 5-C), 125.9 (α-C), 128.8 (s, 1-C), 131.2 (2-C, 6-C), 136.8 (4′-C), 144.0 (β-C), 162.8 (s, 4-C), 163.1 (s, 2′-C, 6′-C), 195.3 (s, C=O); *m/z* 270 ($M^{+\cdot}$, 29%), 269 (26), 136 (9), 135 (12), 134 (100), 121 (44), 119 (25), 108 (19); (Found C, 71.1; H, 5.2. $C_{16}H_{14}O_4$ requires C, 71.1; H, 5.2%).

2′,6′-Dihydroxy-3,4-dimethoxychalcone (**40**).— A solution of 2′,6′-dihydroxyacetophenone (**73**; 1.0 g, 6.6 mmol) and 2,3-dihydropyran (3.0 g, 36 mmol) was reacted as described above for 2′,6′-dihydroxychalcone (**34**), for 7½ hours, after which the reaction mixture was worked up as described for 2',6'-dihydroxychalcone above, to afford crude 2'-hydroxy-6'-*O*-THPacetophenone (**65**) [*m/z* 236 ($M^{+\cdot}$, 9%), 153 (21), 152 ($M^{+\cdot}$ - 84, 100), 137 (62)].

To this crude acetophenone (**65**) was added 3,4-dimethoxybenzaldehyde (2.0 g, 12 mmol), followed by methanolic potassium hydroxide (5.0 g KOH; 10 ml CH_3OH, 5 ml H_2O), and the mixture left to stand for about 3 hours before addition to diethyl ether. The hydroxychalcone was then extracted with aqueous sodium hydroxide (2 mol l^{-1}), the extract acidified, the chalcone extracted into chloroform and the solvent removed *in vacuo*. Ethanol containing a little aqueous HCl (2 mol l^{-1}) was added to the chalcone, which after 10 minutes standing, followed by p.l.c. (chloroform – ethyl acetate as eluent) and recrystallisation of the resultant orange solid from toluene – petroleum spirit (b.p. 40-60°C) afforded yellow crystals of *2',6'-dihydroxy-3,4-dimethoxychalcone* (**40**), m.p. 163-167°C (the crimson crystals went colourless and opaque at 148°C) (lit.,[200] 187-188°C): λ_{max} (EtOH) 367 nm, λ_{max} (acidic methanol) 373 nm (log ϵ 4.32); δ_H (*cf.* ref. 200) [90 MHz; $(CD_3)_2CO$] 4.02 (3 H, s, OCH_3), 6.62 (2 H, d, *J* 7.9 Hz, 3'-H, 5'-H), 7.16 (1 H, d, *J* 8.8 Hz, 5-H)[a], 7.3-7.5 (3 H, m, 2-H, 4'-H, 6-H)[a], 7.96 (1 H, d, *J* 15.5 Hz, α-H), 8.25 (1 H, d, *J* 15.5 Hz, β-H), 11.69 (2 H, s, 2'-OH, 6'-OH); δ_C [22 MHz; $(CD_3)_2CO$] 56.1 (3-OCH_3, 4-OCH_3), 108.5 (3'-C, 5'-C), 111.9 (s, 1'-C), 111.9 (2-C)[a], 112.6 (5-C)[a], 123.9 (6-C), 126.0 (α-C), 129.0 (s, 1-C), 136.7 (4'-C), 144.4 (β-C), 150.5 (s, 3-C)[b], 152.8 (s, 4-C)[b], 163.0 (s, 2'-C, 6'-C), 195.2 (s, C=O); *m/z* 300 ($M^{+\cdot}$, 51%), 299 (23), 283 (10), 165 (12), 164 (92), 163 (17), 152 (11), 151 (100), 149 (50), 138 (13), 137 (10), 136 (10), 121 (16), 108 (11), 103 (13); (Found C, 68.3; H, 5.4. $C_{17}H_{16}O_5$ requires C, 68.0; H, 5.4%).

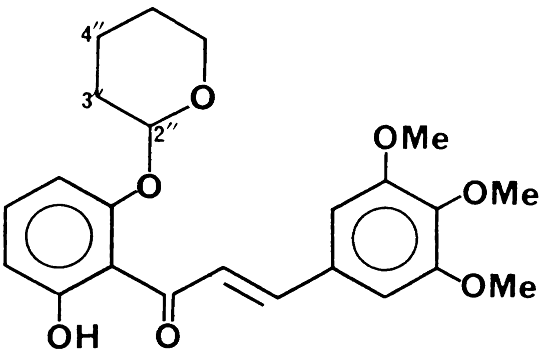
2',6'-Dihydroxy-3,4,5-trimethoxychalcone (**42**).— 2'-Hydroxy-6'-tetrahydropyranyloxy-3,4,5-trimethoxychalcone (**70**; 0.20 g 0.48 mmol) was dissolved in ethanol (20 ml) and aqueous hydrochloric acid (5 ml; 2 mol l^{-1}) was added. After warming for about 5 minutes t.l.c. showed deprotection to be complete. Most of the solvent was then removed *in vacuo*, and the product was added to water (200 ml) and extracted with diethyl ether (2x20 ml). The ether-soluble fraction was dried over magnesium sulphate, filtered, and the solvent removed *in vacuo*. Recrystallisation of the residue from petroleum spirit (60-80°C) – toluene – chloroform afforded a bright orange powder, *2',6'-dihydroxy-3,4,5-trimethoxychalcone* (**42**) (0.13 g, 81%), m.p. 159-168°C; λ_{max} (acidic methanol) 353 nm (log ϵ 4.22). δ_H [90 MHz; (CD$_3$)$_2$CO] 3.93 (3 H, s, 4-OCH$_3$), 4.04 (6 H, s, 3-OCH$_3$, 5-OCH$_3$), 6.63 (2 H, d, *J* 8.2 Hz, 3'-H, 5'-H), 7.20 (2 H, s, 2-H, 6-H), 7.44 (1 H, t, *J* 8.2 Hz, 4'-H), 7.92 (1 H, d, *J* 15.5 Hz, α-H), 8.27 (1 H, d, *J* 15.5 Hz, β-H), 11.7 (2 H, br s, 2'-OH, 6'- OH); δ_C [22 MHz; (CD$_3$)$_2$CO] 56.5 (q, 3-OCH$_3$, 5-OCH$_3$), 60.7 (q, 4-OCH$_3$), 107.0 (d, 2-C, 6-C), 108.5 (d, 3'-C, 5'-C), 111.7 (s, 1'-C), 127.6 (d, α-C), 131.7 (s, 1-C), 136.9 (d, 4'-C), 141.6 (s, 4-C), 144.1 (d, β-C), 154.6 (s, 3-C, 5-C), 163.0 (s, 2'-C, 6'-C), 195.2 (s, C=O); *m/z* 331 (14%), 330 ($M^{+\cdot}$, 63), 329 (14), 208 (11), 194 (44), 182 (11), 181 (100), 179 (53), 163 (11); (Found C, 65.4; H, 5.3. $C_{18}H_{18}O_6$ requires C, 65.4, H, 5.5%).

2',6'-Dihydroxy-2,4,6-trimethoxychalcone (**44**).— 2',6'-Dihydroxyacetophenone (**73**; 0.5 g, 3.3 mmol) was reacted with 2,3-dihydropyran (2 ml, 20 mmol) and the resulting 2'-hydroxy-6'-tetrahydropyranyloxyacetophenone purified, as described above for 2',6'-dihydroxychalcone (**34**). This acetophenone was condensed with 2,4,6-trimethoxybenzaldehyde (1.0 g, 5.1 mmol), and the resulting chalcone deprotected, as described above for 2',6'-dihydroxy-3,4,5-trimethoxychalcone (***42***), to afford a red solid. P.l.c. (chloroform – ethyl acetate; 3:1) followed by recrystallisation from chloroform – petroleum spirit gave *2',6'-dihydroxy-2,4,6-trimethoxychalcone* (***44***) as crimson prisms, m.p. 142-150°C (the crimson prisms form colourless needles, which become very long needles at about 175°C, melting at 187-189°C); λ_{max} (MeOH) 363 nm; λ_{max} (acidic methanol) 385 nm (log ϵ 4.18), 258 nm (log ϵ 3.74); δ_H [90 MHz; $(CD_3)_2CO$] 4.01 (3 H, s, 4-OCH_3), 4.07 (6 H, s, 2-OCH_3, 6-OCH_3), 6.43 (2 H, s, 3-H, 5-H), 6.58 (2 H, d, *J* 8.1 Hz, 3'-H, 5'-H), 7.37 (1 H, t, *J* 8.1 Hz, 4'-H), 8.51 (1 H, d, *J* 15.8 Hz, α-H), 8.75 (1H, d, *J* 15.8 Hz, β-H); δ_C [22 MHz; $(CD_3)_2CO$] 55.9 (q, 4-OCH_3), 56.3 (q, 2-OCH_3, 6-OCH_3), 91.6 (d, 3-C, 5-C), 107.3 (s, 1-C), 108.4 (d, 3'-C, 5'-C), 112.1 (s, 1'-C), 127.6 (d, α-C), 135.7 (d, 4'-C), 136.1 (d, β-C), 162.8 (s, 2'-C, 6'-C)^a, 163.0 (s, 2-C, 6-C)^a, 164.6 (s, 4-C)^a, 196.7 (s, C=O); *m/z* 331 (13%), 330 ($M^{+\cdot}$, 65), 299 (50), 194 (55), 181 (100), 180 (10), 179 (94), 168 (47), 165 (31), 163 (11), 151 (29), 149 (14), 146 (11), 137 (22), 121 (33), 118 (13), 108 (14); The crystal structure determination of this compound is described in chapter 4.

4-Chloro-2',6'-dihydroxychalcone (***46***).— The 4-chloro-6'-*O*-THP-chalcone (*87*) was deprotected in ethanol with dilute aqueous hydrochloric acid (2 mol l^{-1}) as described above for 2',6'-dihydroxy-3,4,5-trimethoxychalcone (*42*), then added to water. The chalcone was then extracted with diethyl ether, the solvent removed *in vacuo* and the product purified by p.l.c. to afford a red-orange solid, *2',6'-dihydroxy-4-chlorochalcone* (***46***), λ_{max} (water) 325 nm; *m/z* 276 [$M^{+\cdot}$(^{37}Cl), 11%], 275 (15), 274 [$M^{+\cdot}$(^{35}Cl), 31], 273 (28), 256

(10), 163 (44), 162 (12), 149 (14), 138 (11), 137 (31), 136 (100), 108 (45), 107 (11), 103 (12), 102 (11), 101 (10). The kinetics of isomerisation of this compound have been investigated in the range pH4-14 (chapter 2), and the reaction exhibited the usual[130] pseudo first-order kinetics and isosbestic points in this pH range.

2'-Hydroxy-6'-tetrahydropyranyloxy-3,4,5-trimethoxychalcone (*70*). — To 2'-hydroxy-6'-tetrahydropyranyloxyacetophenone (*65*; 0.35 g, 1.48 mmol) and 3,4,5-trimethoxybenzaldehyde (1.0 g, 5.1 mmol) was added with stirring a solution of aqueous-methanolic potassium hydroxide (2.5 g KOH; 2.5 g H_2O; 5 ml CH_3OH). The solution rapidly went red, and was stirred for 5½ hours before being poured into a separating funnel containing diethyl ether - petroleum spirit (1:1; 50 ml) and aqueous sodium hydroxide (10 ml; 2 mol l^{-1}) and the alkali-soluble fraction extracted. A second extraction with NaOH 10 ml; 2 mol l^{-1}) was performed, and the combined extracts added to 5% aqueous sodium bicarbonate, which was then carefully acidified to *ca*. pH 9, whereupon the chalcone precipitated. Extraction of the suspension with chloroform (2x30 ml) gave an orange oil after concentration *in vacuo*. Purification by p.l.c. using chloroform - ethyl acetate (4:1) as eluent afforded an orange oil (0.50 g, 81%) which recrystallised from petroleum spirit (60-80°C) and toluene (after seeding) as a yellow powder (0.35 g, 57%) (seed crystals were formed in the joints of the separating funnel), which was *2'-hydroxy-6'-tetrahydropyranyloxy-3,4,5-trimethoxychalcone* (*70*), m.p. 119-124°C; λ_{max} (EtOH) 367 nm, λ_{max} (MeOH) 345 nm (log ϵ 4.29); δ_H [90 MHz; $(CD_3)_2CO$] 1.8, 3.8, 5.8 (br, tetrahydropyranyl protons), 3.94 (3 H, s, 4-OCH_3), 4.05 (6 H, s, 3-OCH_3, 5-OCH_3), 6.72 (1 H, dd, *J* 8.2, 0.9 Hz, 3'-H), 6.92 (1 H, dd, *J* 8.2, 0.9 Hz, 5'-H), 7.23 (2 H, s, 2-H, 6-H), 7.54 (1 H, t, *J* 8.2 Hz, 4'-H), 7.90-7.96 (2 H, m, α-H, β-H), 12.60 (1 H, br s, 2'-OH); δ_C (22 MHz; $CDCl_3$) 18.8 (t, 4''-C), 24.8 (t, 5''-C), 30.4 (t, 3''-C), 55.9 (q, 3-OCH_3, 5-OCH_3), 60.8 (q, 4-OCH_3), 62.2 (t, 6''-C), 97.1 (d, 2''-C), 105.2 (d, 3'-C)[a], 105.6 (d, 2-C, 6-C), 111.2 (d, 5'-C)[a], 112.6 (s, 1'-C), 126.7 (d, α-C), 130.5 (s, 1-C), 135.7 (d, 4'-C), 140.2 (s, 4-C), 142.9 (d, β-C), 153.3 (s, 3-C, 5-C), 158.0 (s, 6'-C)[b], 164.1 (s, 2'-C)[b], 194.1 (s, C=O); *m/z* 414 ($M^{+\cdot}$, 4%), 331 (13), 330 ($M^{+\cdot}$ - 84, 61), 329 (11), 194 (36), 182 (11), 181 (100), 179 (37), 163 (11); (Found C, 66.4; H, 6.25. $C_{23}H_{26}O_7$ requires C, 66.7; H, 6.3%).



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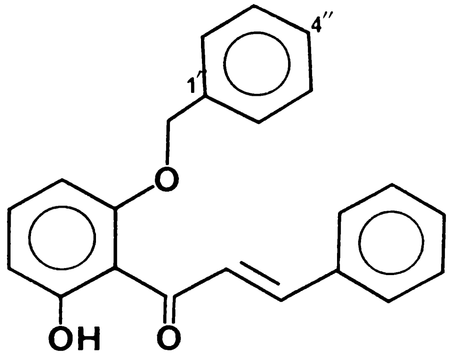
6'-Benzyloxy-2'-hydroxychalcone (*71*).— To a solution of potassium hydroxide (10 g) in water (10 g) was added a solution containing benzaldehyde (6.0 g, 57 mmol) and 6'-benzyloxy-2'-hydroxyacetophenone (*66*; 2.00 g, 8.26 mmol) in ethanol (20 ml). The mixture was allowed to stand for 24 hours before being acidified, and the product was extracted into chloroform. The chloroform was then removed *in vacuo* and the resultant orange solid was recrystallised from methanol to afford the chalcone (*71*; 2.10 g, 77%) as orange needles, m.p. 128-130°C; δ_H (90 MHz; solvent $CDCl_3$; standard Me_4Si) 5.13 (2 H, s, OCH_2Ar), 6.55 (1 H, d, *J* 8.4 Hz, 3'-H)[a], 6.65 (1 H, d, *J* 8.4 Hz, 5'-H)[a], 7.1-7.5 (11 H, m, 4'-H, $OCH_2C_6H_5$, B-ring protons), 7.75 (1 H, d, *J* 16 Hz, α-H)^b, 7.88 (1 H, d, *J* 16 Hz, β-H)^b, 13.48 (1 H, s, 2'-OH); [90 MHz; $(CD_3)_2CO$)] 5.41 (2 H, s, OCH_2Ar), 6.75 (1 H, d, *J* 8 Hz, 3'-H)^a, 6.93 (1 H, d, *J* 8 Hz, 5'-H)^a, 7.4-7.8 (11 H, m, 4'-H, $OCH_2C_6H_5$, B-ring protons), 7.86 (1 H, d, *J* 16 Hz, α-H), 8.09 (1 H, d, *J* 16 Hz, β-H); δ_C (22 MHz; $CDCl_3$) 71.5 (OCH_2Ar), 102.4 (5'-C), 111.5 (3'-C), 111.9 (s, 1'-C), 127.7 (α-C), 128.6-128.7 (2-C, 6-C, 3-C, 5-C, 2''-C, 6''-C, 3''-C, 5''-C), 128.9 (4''-C), 130.1 (4-C), 135.1 (s, 1''-C)^a, 135.7 (s, 1-C)^a, 136.0 (4'-C), 143.3 (β-C), 160.3 (s, 6'-C), 165.6 (s, 2'-C), 194.5 (C=O); *m/z* 330 ($M^{+\cdot}$, 27%), 239 (48), 137 (11), 131 (20), 92 (18), 91 (100).



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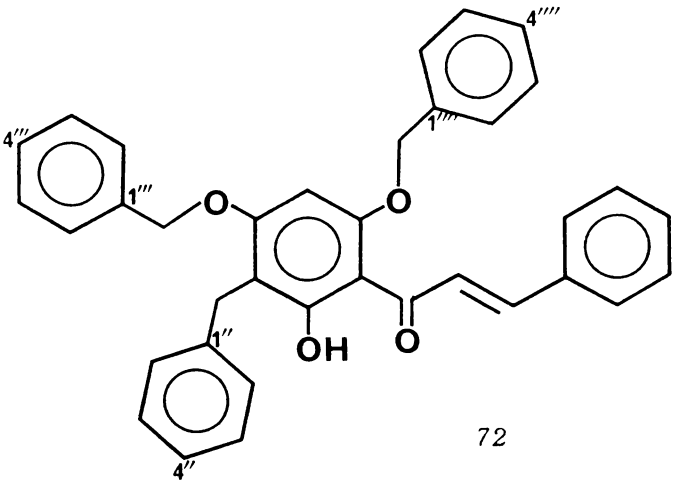
3'-Benzyl-4',6'-dibenzyloxy-2'-hydroxychalcone (*72*).— Benzaldehyde was condensed with 3'-benzyl-4',6'-dibenzyloxy-2'-hydroxyacetophenone (*78*) in the usual manner (as for *89*), with a reaction time of *ca.* 10 minutes. The red solution was then poured into an excess of hydrochloric acid (2 mol l^{-1}), and the product was extracted with chloroform. Removal of the chloroform *in vacuo*, followed by recrystallisation from aqueous methanol afforded ***2'-hydroxy-3'-benzyl-4',6'-dibenzyloxychalcone* (*72*)** as orange needles, m.p. 154-156°C; λ_{max} (MeOH) 336 nm (log ε 4.31); δ_H [90 MHz; solvent $(CD_3)_2CO/CDCl_3$ (1:1); standard Me_4Si]] 4.00 (2 H, s, 3'-CH_2Ar), 5.12

(2 H, s, OCH_2Ar), 5.21 (2 H, s, OCH_2Ar), 6.34 (1 H, s, 5'-H), 7.0-7.5 (20 H, m, B-ring protons, $3\times CH_2C_6H_5$), 7.67 (1 H, d, *J* 15.8 Hz, α-H), 7.89 (1 H, d, *J* 15.8 Hz, β-H), 14.53 (1 H, s, 2'-OH); δ_C [90 MHz; solvent $(CD_3)_2CO/CDCl_3$ (1:1); standard Me_4Si]] 69.6 (t, OCH_2Ar), 70.8 (t, OCH_2Ar), 88.4 (5'-C), 105.6 (s, 1'-C), 109.5 (s, 3'-C), 124.7 (α-C), 126.7, 127.3, 127.4, 127.7, 128.0, 128.2, 129.2 (2-C, 6-C, 3-C, 5-C, 4-C, 2''-C, 6''-C, 3,''-C, 5''-C, 4''-C, 2'''-C, 6'''-C, 3'''-C, 5'''-C, 4'''-C, 2''''-C, 6''''-C, 3''''-C, 5''''-C, 4''''-C), 134.7 (s, 1''-C)[a], 135.0 (s, 1'''-C)[a], 135.8 (s, 1-C)[a], 141.0 (s, 1''''-C), 141.8 (β-C), 160.3 (s, 6'-C)[b], 162.2 (s, 4'-C)[b], 164.2 (s, 2'-C)[b], 192.2 (s, C=O); (22 MHz; $CDCl_3$) 28.3 (t, 3'-CH_2Ar), 70.3 (t, OCH_2Ar), 71.4 (t, OCH_2Ar) (*i.e.* three triplets were present in the spectrum, one of which was obscured by the CD_3COCD_2H signal in the previous ^{13}C n.m.r. spectrum); *m/z* 526 ($M^{+\cdot}$, 9%), 435 (10), 345 (5), 241 (4), 180 (3), 131 (18), 92 (10), 91 (100); (Found: C, 81.9; H, 5.5; $M^{+\cdot}$, 526.2148. $C_{36}O_4H_{30}$ requires C, 82.1; H, 5.7; $M^{+\cdot}$, 526.2144).



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4-Chloro-2'-hydroxy-6'-tetrahydropyranyloxychalcone (***87***).— 2'-hydroxy-6'-tetrahydropyranyloxyacetophenone (*65*; 0.25 g, 1.06 mmol) was reacted with 4-chlorobenzaldehyde (0.75 g, 5.34 mmol) in aqueous-methanolic potassium hydroxide as described above for 2′-hydroxy-6′-tetrahydropyranyloxy-3,4,5-trimethoxychalcone (*70*). A large amount of red gum formed during the reaction. The reaction mixture was added to diethyl ether, and the crude chalcone was extracted into aqueous sodium hydroxide (2 mol l^{-1}). The alkaline extracts were then carefully acidified to pH 8 as described above for 2′-hydroxy-6′-tetrahydropyranyloxy-3,4,5-trimethoxychalcone (*70*) and extracted with chloroform. The chloroform was dried and the solvent evaporated *in vacuo*, and the residue purified by p.l.c. to afford orange crystals of 4-chloro-2′-hydroxy-6′-tetrahydropyranyloxychalcone (*87*) (0.08 g, 21%). (numbering scheme as *per* *70* above), δ_C (22 MHz; $CDCl_3$) 18.7 (4′′-C), 24.9 (5′′-C), 30.4 (3′′-C), 62.4 (6′′-C), 97.4 (2′′-C), 105.4 (3′-C)^{a}, 111.4 (5′-C)^{a}, 112.7 (1′-C), 128.4 (α-C), 129.2 (2-C, 6-C)^{b}, 129.5 (3-C, 5-C)^{b}, 133.8 (1-C), 136.0 (4-C), 136.2 (4′-C), 141.0 (β-C), 158.4 (2′-C)^{c}, 164.2 (6′-C)^{c}, 194.4 (C=O); *m/z* 358 ($M^{+\cdot}$, 4%), 276 (35), 275 (31), 274 (100), 273 (39), 256 (10), 163 (41), 162 (11), 137 (27), 136 (67), 108 (17), 101 (10). [Found: $M^{+\cdot}$(^{37}Cl), 360.0934; $M^{+\cdot}$(^{35}Cl), 358.0964. Calc. for $C_{20}H_{19}O_4Cl$: $M^{+\cdot}$(^{37}Cl), 360.0942; $M^{+\cdot}$(^{35}Cl), 358.0972].

2′-Hydroxy-6′-methoxychalcone (*88*).— To a stirred aqueous solution of potassium hydroxide (2.5 ml; 50% w/w) was added a solution consisting of 2′-hydroxy-6′-methoxyacetophenone (*75*; 0.13 g, 0.8 mmol) and benzaldehyde (0.47 g, 4.4 mmol) in methanol (2.5 ml). The mixture rapidly became warm and red in colour. After standing for 48 hours the mixture was added to ether in a separating funnel, and extracted with 2 mol l^{-1} sodium hydroxide. The basic extract was acidified, extracted with chloroform, then the chloroform removed *in vacuo*. The resultant orange solid was then recrystallised from methanol and water to afford bright orange crystals (0.20 g, 55%) of 2'-hydroxy-6'-methoxychalcone (*88*), m.p. 63-64°C (lit.,[236] 65°C); δ_H [90 MHz; (CD$_3$)$_2$CO] 4.15 (3 H, s, OCH$_3$), 6.71 (1 H, d, *J* 8.2 Hz, 3'-H)[a], 6.76 (1 H, d, *J* 8.2 Hz, 5'-H)[a], 7.59 (1 H, t, *J* 8.2 Hz, 4'-H), 7.5-7.6 (3 H, m, B-ring protons), 7.8-7.9 (2 H, m, B-ring protons), 7.93 (1 H, d, *J* 15.8 Hz, α-H), 8.08 (1 H, d, *J* 15.8 Hz, β-H), 13.01 (1 H, s, 2'-OH); (90 MHz; solvent CDCl$_3$; standard Me$_4$Si) 3.89 (3 H, s, OCH$_3$), 6.38 (1 H, d, *J* 8.2 Hz, 3'-H)[a], 6.59 (1 H, d, *J* 8.2 Hz, 5'-H)[a]), 7.3-7.6 (6 H, m, 4'-H, B-ring protons), 7.80 (2 H, br s, α-H, β-H), 13.17 (1 H, s, 2'-OH); δ_C (22 MHz; CDCl$_3$) 56.0 (q, OCH$_3$), 101.6 (d, 5'-C), 110.9 (d, 3'-C), 112.1 (s, 1'-C), 127.6 (d, α-C), 128.4 (d, 2-C, 6-C)[a], 128.9 (d, 3-C, 5-C)[a], 130.3 (d, 4-C), 135.3 (s, 1-C), 135.9 (d, 4'-C), 142.9 (d, β-C), 161.0 (s, 6'-C), 164.9 (s, 2'-C), 194.5 (s, C=O); *m/z* 255 (10%), 254 ($M^{+\cdot}$, 62), 253 (79), 238 (12), 237 (12), 178 (12), 177 (100), 162 (11), 151 (29), 150 (30), 137 (10), 136 (10), 103 (20); (Found: C, 75.7; H, 5.7. Calc. for C$_{16}$H$_{14}$O$_3$: C, 75.6; H, 5.55%).

2'-Hydroxy-4,6'-dimethoxychalcone (*89*).— A solution of 4-methoxybenzaldehyde (2.00 g, 14.7 mmol) and 2'-hydroxy-6'-methoxyacetophenone (*75*; 1.00 g, 6.02 mmol) in methanol (10 ml) was added to a solution of potassium hydroxide (5.0 g) in water (5.0 g). The solution was allowed to stand for six hours before being acidified and the product extracted into chloroform. The chloroform solution was then extracted with aqueous sodium hydroxide solution (4×20 ml; 2 mol l^{-1}), and the alkaline washings acidified. Extraction of the acidified extract with chloroform, followed by removal of the solvent *in vacuo* and recrystallisation from ethanol and water afforded bright orange needles (*89*; 1.26 g, 74%) of the desired chalcone, m.p. 116-117°C. A second crop of the product was obtained from the mother liquor (0.07 g, 3%). δ_H [90 MHz; $(CD_3)_2CO$] 4.00 (3 H, s, 4-OCH_3), 4.13 (3 H, s, 6'-OCH_3), 6.71 (1 H, d, *J* 8.2 Hz, 3'-H)[a], 6.73 (1 H, d, *J* 8.2 Hz, 5'-H)[a], 7.14 (2 H, d, *J* 9.1 Hz, 3-H, 5-H), 7.56 (1 H, t, *J* 8.2 Hz, 4'-H), 7.84 (2 H, d, *J* 9.1 Hz, 2-H, 6-H), 7.95 (2 H, br s, α-H, β-H), 13.19 (1 H, s, 2'-OH); (90 MHz; solvent $CDCl_3$; standard Me_4Si) 3.82 (3 H, s, 4-OCH_3)[a], 3.91 (3 H, s, 6'-OCH_3)[a], 6.40 (1 H, d, *J* 8.4 Hz, 3'-H)[b], 6.61 (1 H, d, *J* 8.4 Hz, 5'-H)[b], 6.92 (2 H, d, *J* 9 Hz, 3-H, 5-H), 7.32 (1 H, t, *J* 8.4 Hz, 4'-H), 7.56 (2 H, d, *J* 9 Hz, 2-H, 6-H), 7.76 (2 H, br s, α-H, β-H), 13.29 (1 H, s, 2'-OH); δ_C (22 MHz; $CDCl_3$) 55.3 (4-OCH_3), 55.9 (6'-OCH_3), 101.5 (5'-C), 110.9 (3'-C), 112.0 (s, 1'-C), 114.4 (3-C, 5-C), 125.1 (α-C), 128.0 (s, 1-C), 130.2 (2-C, 6-C), 135.6 (4'-C), 143.1 (β-C), 160.9 (s, 4-C), 161.5 (s, 2'-C), 164.8 (s, 6'-C), 194.2 (s, C=O); *m/z* 284 ($M^{+\cdot}$, 53%), 283 (74), 268 (10), 267 (13), 177 (24), 151 (20), 150 (26), 135 (16), 134 (100), 133 (14), 122 (14), 121 (55), 119 (14), 108 (16); (Found: C, 71.9; H, 5.8. Calc. for $C_{17}H_{16}O_4$: C, 71.8; H, 5.7%).

4-Chloro-2'-hydroxy-6'-methoxychalcone (*90*).— Exactly the same procedure was used in the preparation of this chalcone as was used in preparing 2'-hydroxy-4,6'-dimethoxychalcone (*89*) (above), except that 4-chlorobenzaldehyde (2.00 g, 14.2 mmol) was used in place of 4-methoxybenzaldehyde. The product was obtained as silky yellow needles (0.75 g, 43%), m.p. 125-126°C.

A much better yield was obtained using an alternative method where the reaction time for the condensation was very much shorter. A solution containing 4-chlorobenzaldehyde (0.60 g, 4.3 mmol) and 2'-hydroxy-6'-methoxyacetophenone (*75*; 0.50 g, 3.0 mmol) in methanol (7 ml) was added to aqueous potassium hydroxide (10 ml; 8.9 mol l^{-1}).

The solution was then left to stand for 10 minutes before being acidified with a large excess of sulphuric acid (2 mol l^{-1}), and the resultant precipitate was filtered at the pump. Recrystallisation of the precipitate from methanol and water gave the expected chalcone (***90***) (0.68 g, 78%) as silky yellow needles, m.p. 125-126°C; δ_H [90 MHz; $(CD_3)_2CO$] 4.15 (3 H, s, OCH_3), 6.71 (1 H, d J 8 Hz, 3'-H)^a, 6.77 (1 H, d, J 8 Hz, 5'-H)^a, 7.60 (1 H, t, J 8 Hz, 4'-H), 7.63 (2 H, d, J 8.5 Hz, 3-H, 5-H), 7.90 (1 H, d, J 15.8 Hz, α-H), 7.94 (2 H, d, J 8.5 Hz, 2-H, 6-H), 8.08 (1 H, d, J 15.8 Hz, β-H), 12.91 (1 H, s, 2'-OH); (90 MHz; solvent $CDCl_3$; standard Me_4Si) 3.95 (3 H, s, OCH_3), 6.43 (1 H, dd, $J_{4'-H}$ 8.4, J 0.7 Hz, 3'-H), 6.62 (1 H, dd, $J_{4'-H}$ 8.4, J 0.7 Hz, 5'-H), 7.37 (1 H, t, J = 8.4 Hz, 4'-H), 7.37 (2 H, d, J 8.8 Hz, 2-H, 6-H)^a, 7.55 (2 H, d, J 8.8 Hz, 3-H, 5-H)^a, 7.74 (1 H, d, J 16 Hz, α-H)^b, 7.81 (1 H, d, J 16 Hz, β-H)^b, 13.04 (1 H, s, 2'-OH); δ_C [22 MHz; $(CD_3)_2CO$] 56.6 (OCH_3), 102.9 (d, 5'-C), 111.2 (d, 3'-C), 112.8 (s, 1'-C), 129.3 (d, α-C), 130.0 (d, 2-C, 6-C)^a, 130.9 (d, 3-C, 5-C)^a, 135.1 (s, 4-C)^b, 136.5 (s, 1-C)^b, 137.1 (4'-C), 142.0 (d, β-C), 162.1 (s, 6'-C), 165.2 (s, 2'-C), 195.2 (s, C=O); m/z 290 [$M^{+\cdot}$(^{37}Cl), 19%], 289 (35), 288 [$M^{+\cdot}$(^{35}Cl), 58], 287 (83), 272 (10), 271 (12), 178 (11), 177 (100), 176 (18), 165 (12), 162 (10), 151 (46), 150 (61), 137 (19), 136 (17), 125 (10), 122 (19), 108 (11), 107 (14); (Found: C, 66.5; H, 4.6; Cl, 12.3. Calc. for $C_{16}H_{13}O_3Cl$: C, 66.6 H, 4.5; Cl, 12.3%).

2'-Hydroxy-4',6'-dimethoxychalcone (***91***).— A solution of benzaldehyde (4.5 g, 42 mmol) and 2'-hydroxy-4',6'-dimethoxyacetophenone (***76***; 1.50 g, 7.65 mmol) in methanol (20 ml) was added to a solution of potassium hydroxide (10 g) in water (15 g), and the mixture was allowed to stand for 21 hours. The mixture was then acidified, the product extracted into chloroform, and the chloroform removed *in vacuo*. The resultant orange solid was then recrystallised from methanol and water to afford orange-yellow crystals of the chalcone (***91***; 1.81 g, 83%), m.p. 85-88°C (lit.,[60],[246] 91-92°C, 90-91°C); δ_H [90 MHz; (CD$_3$)$_2$CO] 4.02 (3 H, s, 4'-OCH$_3$), 4.15 (3 H, s, 6'-OCH$_3$), 6.26 (2 H, s, 3'-H, 5'-H), 7.5-7.6 (3 H, m, B-ring protons), 7.8-8.0 (2 H, m, B-ring protons), 7.92 (1 H, d, *J* 15.8 Hz, α-H), 8.16 (1 H, d, *J* 15.8 Hz, β-H), 14.34 (1 H, s, 2'-OH); (90 MHz; solvent CDCl$_3$; standard Me$_4$Si), 3.82 (3 H, s, OCH$_3$), 3.91 (3 H, s, OCH$_3$), 5.96 (1 H, d, *J* 2.2 Hz, 3'-H)^a, 6.11 (1 H, d, *J* 2.2 Hz, 5'-H)^a, 7.3-7.7 (6 H, m, 4'-H, B-ring protons), 7.78 (1 H, d, *J* 15.8 Hz, α-H)^b, 7.89 (1 H, d, *J* 15.8 Hz, β-H)^b, 14.27 (1 H, s, 2'-OH); δ_C (22 MHz; CDCl$_3$) 55.6 (OCH$_3$), 55.9 (OCH$_3$), 91.3 (d, 5'-C)^a, 93.8 (d, 3'-C)^a, 106.4 (s, 1'-C), 127.6 (d, α-C), 128.4 (d, 2-C, 6-C)^b, 128.9 (d, 3-C, 5-C)^b, 130.1 (d, 4-C), 135.6 (s, 1-C), 142.3 (d, β-C), 162.5 (s, 6'-C), 166.3 (s, 4'-C)^c, 168.4 (s, 2'-C)^c, 192.6 (s, C=O); *m/z* 285 (12%), 284 ($M^{+\cdot}$, 67), 283 (58), 208 (12), 207 (100), 181 (24), 103 (11).

2'-Hydroxy-4',6'-bistetrahydropyranyloxychalcone (***92***).— Benzaldehyde and 2'-hydroxy-4',6'-bis-*O*-THPacetophenone (***79***) were condensed in the usual manner (*e.g.* see ***70***), and the crude chalcone purified by p.l.c. to afford a yellow oil whose n.m.r. and mass-spectral properties were consistent with the desired bis-*O*-THPchalcone (***92***). δ_H (90 MHz; CDCl$_3$) 1.7, 1.9, 3.7, 4.2, 5.5 (br, pyran protons), 6.25 (2 H, s, 3'-H, 5'-H), 7.3-7.6 (6 H, m, 4'-H, B-ring protons), 7.72 (1 H, d, *J* 16 Hz, α-H), 7.98 (1 H, d, *J* 16 Hz, β-H), 14.38 (1 H, s, 2'-OH); δ_C (22 MHz; CDCl$_3$) 18.9 (t, 4''-C)^a, 23.3 (t, 4'''-C)^a, 24.9 (t, 5''-C)^b, 26.0 (t, 5'''-C)^b, 30.4 (t, 3''-C)^c, 31.4 (t, 3'''-C)^c, 62.4 (t, 6''-C)^d, 69.7 (t, 6'''-C)^d, 95.5 (d, 2''-C)^e, 95.7 (d, 5'-C)[e], 97.3 (d, 2'''-C)[e], 97.5 (d, 3'-C)[e], 105.9 (s, 1'-C), 128.0 (d, α-C), 128.2 (d, 2-C, 6-C)[f], 128.8 (d, 3-C, 5-C)[f], 130.0 (d, 4-C), 135.6 (s, 1-C), 141.8 (d, β-C), 159.5 (s, 4'-C)[g], 163.2 (s, 2'-C)[g], 163.4 (s, 6'-C)[g], 192.9 (s, C=O); *m/z* 424 ($M^{+\cdot}$, 4%), 341 (17), 340 ($M^{+\cdot}$ - 84, 74), 322 (14), 295 (13), 270 (12), 269 (52), 263 (12), 257 (22), 218 (10), 208 (25), 207 (17), 205 (12), 191 (36), 190 (12), 189 (12), 181 (13), 179 (25), 167 (10), 166 (13), 165 (43), 163 (14), 152 (15), 149 (24), 137 (11), 131 (22), 129 (11), 105 (11), 104 (16), 103 (19).

2'-Hydroxy-4',6'-dimethoxy-3'-methylchalcone (**93**).— Benzaldehyde was condensed with 2'-hydroxy-4',6'-dimethoxy-3'-methylacetophenone (*77*) in the usual manner (as for *89*), with a reaction time of *ca.* 10 minutes. The red solution was then poured into an excess of hydrochloric acid (2 mol l^{-1}), and then extracted with chloroform. Removal of the chloroform *in vacuo*, followed by recrystallisation from aqueous methanol afforded *2'-hydroxy-4',6'-dimethoxy-3'-methylchalcone* (**93**) as crimson prisms, m.p. 122-123°C (the solidified melt remelted at 141-142°C) (lit.,[226] 140-141°C); λ_{max} (MeOH) 353 nm (log ε 4.17); δ_H [90 MHz; $(CD_3)_2CO$] 2.12 (3 H, s, 3'-CH_3), 4.10 (3 H, s, OCH_3), 4.18 (3 H, s, OCH_3), 6.43 (1 H, s, 5'-H), 7.5-7.65, 7.75-7.9 (5 H, m, B-ring protons), 7.90 (1 H, d, *J* 15.8 Hz, α-H), 8.15 (1 H, d, *J* 15.8 Hz, β-H), 14.30 (1 H, s, 2'-OH); δ_C [22 MHz; $(CD_3)_2CO$] 7.5 (3'-CH_3), 56.2 (OCH_3), 56.5 (OCH_3), 87.9 (5'-C), 105.9 (s, 1'-C)[a], 106.8 (s, 3'-C)[a], 128.8 (α-C), 129.2 (2-C, 6-C)[b], 129.9 (3-C, 5-C)[b], 131.0 (4-C), 136.5 (s, 1-C), 142.6 (β-C), 162.4 (s, 6'-C), 165.0 (s, 2'-C, 4'-C), 193.7 (s, C=O); *m/z* 298 ($M^{+\cdot}$, 100%), 221 (72), 195 (20), 194 (14), 137 (10), 136 (14), 103 (17), 77 (20); (Found: C, 72.2; H, 6.0; $M^{+\cdot}$, 298.1211. $C_{18}H_{18}O_4$ requires C, 72.5; H, 6.1%; $M^{+\cdot}$, 298.1205).

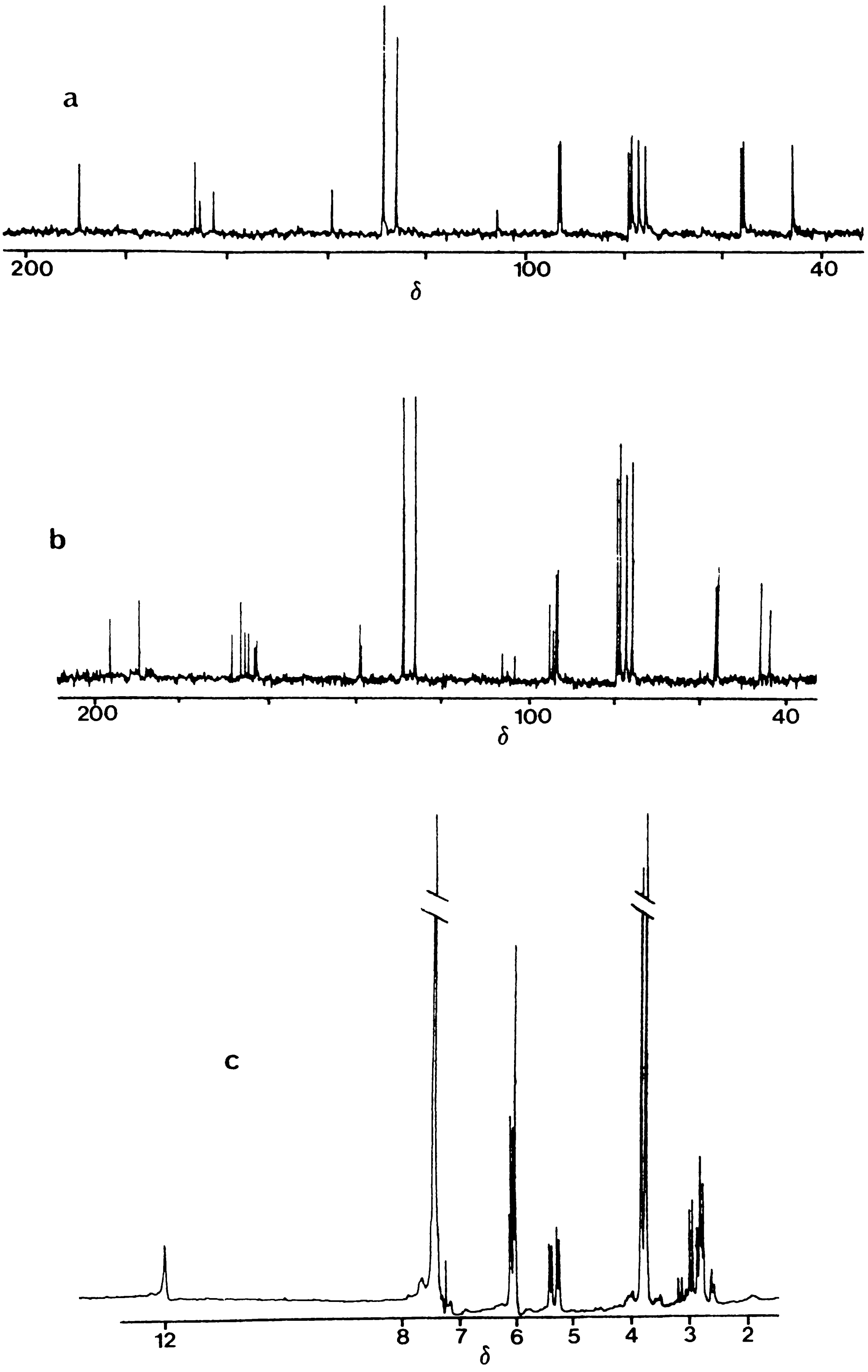
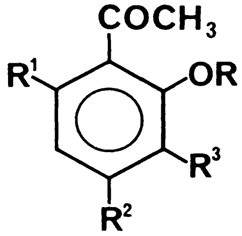


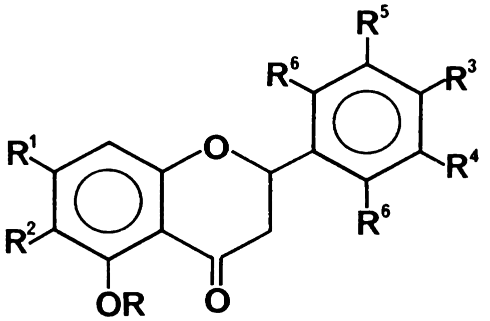
FIGURE 7-2 ^{13}C n.m.r. spectra of (a) 5,7-Dimethoxyflavanone (*81*); (b) the Product from the AlCl$_3$ Demethylation of 5,7-Dimethoxyflavanone; (c) the ^{1}H n.m.r. spectrum of the Demethylation Product

TABLE 7-1 Important Acetophenones Used in this Study



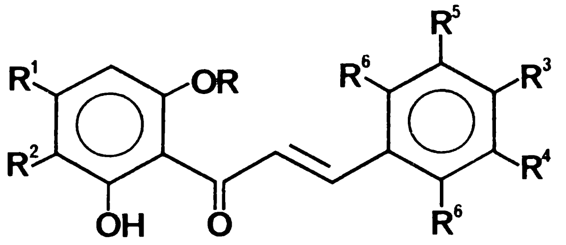
	R	R^1	R^2	R^3
65	H	*O*-THP	H	H
66	H	OC_7H_7	H	H
67	$OCOC_6H_5$	$OCOC_6H_5$	H	H
73	H	OH	H	H
74	H	OH	OH	H
75	H	OCH_3	H	H
76	H	OCH_3	OCH_3	H
77	H	OCH_3	OCH_3	CH_3
78	H	OC_7H_7	OC_7H_7	C_7H_7
79	H	*O*-THP	*O*-THP	H

TABLE 7-2 Flavanones Used in this Study



	R	R^1	R^2	R^3	R^4	R^5	R^6
4	H	OH	H	OH	H	H	H
8	H	OH	H	OCH_3	OH	H	H
18	H	*O*-GlRh	H	OH	H	H	H
33	H	OCH_3	H	OCH_3	H	H	H
35	H	H	H	H	H	H	H
37	H	OCH_3	H	OCH_3	OCH_3	H	H
39	H	H	H	OCH_3	H	H	H
41	H	H	H	OCH_3	OCH_3	H	H
43	H	H	H	OCH_3	OCH_3	OCH_3	H
45	H	H	H	OCH_3	H	H	OCH_3
47	H	H	H	Cl	H	H	H
68	H	OC_7H_7	C_7H_7	H	H	H	H
69	H	OH	C_7H_7	H	H	H	H
80	CH_3	H	H	H	H	H	H
81	CH_3	OCH_3	H	H	H	H	H
82	H	OCH_3	H	H	H	H	H
83	H	*O*-THP	H	*O*-THP	H	H	H
84	$COCH_3$	OCH_3	H	OCH_3	H	H	H

TABLE 7-3 Chalcones Used in this Study



	R	R^1	R^2	R^3	R^4	R^5	R^6
32	H	OCH_3	H	OCH_3	H	H	H
34	H	H	H	H	H	H	H
36	H	OCH_3	H	OCH_3	OCH_3	H	H
38	H	H	H	OCH_3	H	H	H
40	H	H	H	OCH_3	OCH_3	H	H
42	H	H	H	OCH_3	OCH_3	OCH_3	H
44	H	H	H	OCH_3	H	H	OCH_3
46	H	H	H	Cl	H	H	H
48	H	*O*-GlRh	H	OH	H	H	H
70	THP	H	H	OCH_3	OCH_3	OCH_3	H
71	C_7H_7	H	H	H	H	H	H
72	C_7H_7	OC_7H_7	C_7H_7	H	H	H	H
85	H	OH	H	OH	H	H	H
86	H	OH	H	OCH_3	OH	H	H
87	THP	H	H	Cl	H	H	H
88	CH_3	H	H	H	H	H	H
89	CH_3	H	H	OCH_3	H	H	H
90	CH_3	H	H	Cl	H	H	H
91	CH_3	OCH_3	H	H	H	H	H
92	THP	*O*-THP	H	H	H	H	H
93	CH_3	OCH_3	CH_3	H	H	H	H

APPENDIX 1

COMPUTER PROGRAMME FOR ANALYSIS OF THE pH-RATE PROFILES OF CHAPTER 2

A computer programme was written (in Pascal) to assist in the analysis of the pH-rate profiles for the chalcone – flavanone isomerisation (see chapter 2). The programme calculated values for k_{obs} (at either designated pH values or pH increments) from input pK_a values and rate coefficients, which were then compared with the experimental pH-rate data, and the input data (pK_a values and rate coefficients) adjusted accordingly. The calculations of k_{obs} were based on equation 2-19 (see chapter 2):

$$k_{obs} = k_{H^+}f_{CHH}[H^+] + k_{CHH}f_{CHH} + k_{CH^-}f_{CH^-} + k_{C^{2-}}f_{C^{2-}} + k_{OH^-}[OH^-] \quad \ldots 2\text{-}19$$

The meaning of the symbols is given in chapter 2. The fraction of the chalcone in any given state of ionisation (*e.g.* mono- or di-anion) depends only on the pH, and the two pK_a values of the chalcone. It was found necessary to take into account in the calculations of k_{obs}, the fact that the two pK_a values of the chalcone may not be sufficiently separated to be treated independently. Thus, for the equilibrium:

$$\mathrm{CHH} \overset{K_{a1}}{\rightleftharpoons} \mathrm{CH^-} + \mathrm{H^+} \quad \text{and} \quad \mathrm{CH^-} \overset{K_{a2}}{\rightleftharpoons} \mathrm{C^{2-}} + \mathrm{H^+}$$

$$K_{a1} = \frac{[CH^-][H^+]}{[CHH]} \quad \text{and} \quad K_{a2} = \frac{[C^{2-}][H^+]}{[CH^-]}$$

So $$[C^{2-}] = \frac{[CH^-]}{[H^+]K_{a2}} \quad \ldots\ldots A1\text{-}1$$

and $$[CH^-] = \frac{K_{a1}[CHH]}{[H^+]} \quad \ldots\ldots A1\text{-}2$$

Rearranging A1-1 and A1-2 we get

$$[CH^-] = \frac{[C^{2-}][H^+]}{K_{a2}} \qquad \text{....A1-3}$$

and

$$[CHH] = \frac{[CH^-][H^+]}{K_{a1}} \qquad \text{....A1-4}$$

substituting A1-3 into A1-4, we get

$$[CHH] = \frac{[C^{2-}][H^+]^2}{K_{a1}K_{a2}} \qquad \text{....A1-5}$$

Since $[CHH] + [CH^-] + [C^{2-}] = [C_{tot}]$, we see that

$$\frac{[CHH]}{[C_{tot}]} + \frac{[CH^-]}{[C_{tot}]} + \frac{[C^{2-}]}{[C_{tot}]} = 1 = f_{CHH} + f_{CH-} + f_{C2-}$$

So if we divide both sides of equations A1-1 to A1-5 by $[C_{tot}]$, then these equations will then be in terms of the fraction of the total chalcone present in the specified state of ionisation, rather than in terms of actual concentrations. The resultant forms of equations A1-1 to A1-5 were used in the derivations of equations A1-7 to A1-9.

$$f_{CHH} + f_{CH-} + f_{C2-} = 1 \qquad \text{....A1-6}$$

by substituting A1-1 and A1-5 into A1-6:

$$f_{CHH} + \frac{K_{a1}f_{CHH}}{[H^+]} + \frac{K_{a1}K_{a2}f_{CHH}}{[H^+]^2} = 1$$

thus $$f_{CHH} = \frac{[H^+]^2}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \qquad \text{....A1-7}$$

by substituting A1-1 and A1-4 into A1-6:

$$\frac{f_{CH-}[H^+]}{K_{a1}} + f_{CH-} + \frac{f_{CH-}K_{a2}}{[H^+]} = 1$$

thus $$f_{CH-} = \frac{K_{a1}[H^+]}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \qquad \text{....A1-8}$$

by substituting A1-3 and A1-5 into A1-6:

$$\frac{f_{C2-}[H^+]^2}{K_{a1}K_{a2}} + \frac{f_{C2-}[H^+]}{K_{a2}} + f_{C2-} = 1$$

thus $$f_{C^{2-}} = \frac{K_{a1}K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad \ldots.A1\text{-}9$$

How to use the Programme

(i) At any pH, the programme assigns the value 10^{-pH} to $[H^+]$, and $10^{(pH-pK_w)} = 10^{(pH-13.833)}$ (at 30°C) to $[OH^-]$. When run, the programme first asks for values for pK_{a1} and pK_{a2}. The values of K_{a1} and K_{a2} in the equations (A1-7, A1-8 and A1-9) used in the calculation of k_{obs} are assigned values of $10^{-pK_{a1}}$ and $10^{-pK_{a2}}$ respectively.

(ii) After the pK_a values have been entered, it then asks whether you require calculated values of k_{obs} at irregular (*i.e.* at specified) pH values, rather than at regular pH increments.

Regular increments of pH [answer *no* (*i.e.* *N* or *0*) at (ii) above] are useful when it is desired to generate a predicted pH-rate profile from supplied pK_a values and rate constants. The programme will interrogate for the initial and final pH, and the increments at which calculated values of k_{obs} are required.

Irregular increments [answer *yes* (*i.e.* *Y* or *1*) at (ii) above] are useful when comparing experimental pH-rate data with pH-rate data calculated from estimated rate coefficients and pK_a values. The programme will interrogate for the pH values at which calculated values of k_{obs} are required. When the pH values have been entered, data entry is terminated by entering a pH value > 100.

(iii) The programme will then ask for values for the rate constants [k(H+), k(CH2), k(CH-), k(C2-) and k(OH-)]. These may be entered in normal or in exponential form *e.g.* 0.0345 *or* 3.45E-02.

(iv) The values of k_{obs} are now calculated, and the results (along with the pK_a and rate coefficient values used in the calculation) are displayed in tabulated form, and automatically appended to a file called *KOBS.DAT*, which is created when the programme is run.

(v) The programme then asks if you wish to loop the programme. If you answer *no*, the programme is exited, and all the results of the calculations performed are stored in the file *KOBS.DAT*. If, however, you answer *yes*, the programme asks if calculations with a different set of pK_a values are desired. If you answer *yes* [if *no*, the programme goes to step (vi)], the programme asks whether you wish to change pKa1. If *yes*, it asks for the new value; if *no*, it repeats this process for pKa2.

(vi) The programme then asks if you wish to use new values for the rate coefficients. If *no*, the programme loops back to step (iv). If *yes*. the programme goes through all the rate constants, one at a time, asking if you want to change its value, and if so, what the new value is. When this process is finished, the programme loops back to step (iv).

The step (vi) - step (iv) loop, in conjunction with the irregular pH-value facility of the programme, makes it particularly useful for analysing experimental pH-rate data for rate and acidity constants. The programme is just asked to calculate values for k_{obs} at pH values for which experimental values are available. Then estimated values for pK_as and rate constants are entered, and the calculated and experimental values of k_{obs} compared. The rate coefficients and pK_a values are then adjusted accordingly, and the process repeated until satisfactory agreement between the calculated and experimental values of k_{obs} is reached.

The programme can also be used for simple cases, where only one ionisable acidic group is present. All that is necessary is to assign a very high value to the second pK_a (*e.g.* 25), so that it has virtually no effect on the calculated neutral and mono-anion concentrations (it is also advisable to assign a value of 0.0 to k_{C2-}). The pH-rate profile for 2'-hydroxy-6'-methoxychalcone (see figure 1-6) was generated in this manner, from the data of Old.[46,71]

The Programme

```
Program pk(input,output,outfile);

const    lntolog = 2.302585;

var      fraction: ARRAY [1..140,1..6] of REAL;
         pk1, pk2, ph, phstart, phstop, phincrement: REAL;
         kch2, kch, kc2, koh, kh: REAL;
         outfile: TEXT;
         ans: CHAR;
         fc, x, y: INTEGER;

procedure headings;
begin
   writeln(outfile);
   writeln(outfile);
   writeln(outfile,'  pH      [H+]          f of CH-      f of C2-      f of Ch2      k(tot)');
   writeln('  pH      [H+]          f of CH-      f of C2-      f of Ch2      k(tot)');
   writeln('----------------------------------------------------------------------------');
   writeln(outfile,'----------------------------------------------------------------------------');
end;

Procedure alter_ks;

var     store: REAL;

begin
        writeln('Do you wish to change the k values of ');
        writeln('k(H+) = ',kh:1,'   k(CH2) = ',kch2:1,'   k(CH-) = ',kch:1);
        write('k(C2-) = ',kc2:1,'   k(OH-) = ',koh:1,'   (y/n) ?  ');
        readln(ans);
        if ((ans = 'y') or (ans = 'Y')) then begin
                write('Enter new k(H+), or 0 for no change  ');
                readln(store);
                if store <> 0.0 then kh := store;
                write('Enter new k(CH2), or 0 for no change  ');
                readln(store);
                if store <> 0.0 then kch2 := store;
                write('Enter new k(CH-), or 0 for no change  ');
                readln(store);
                if store <> 0.0 then kch := store;
                write('Enter new k(C2-), or 0 for no change  ');
                readln(store);
                if store <> 0.0 then kc2 := store;
                write('Enter new k(OH-), or 0 for no change  ');
                readln(store);
                if store <> 0.0 then koh := store;
                end;
        writeln(outfile,'New values of ');
        writeln(outfile,'k(H+) = ',kh,'  k(CH2) = ',kch2,'  k(CH-) = ',kch);
        writeln(outfile,'k(C2-) = ',kc2,'  k(OH-) = ',koh);
        writeln(outfile);
        writeln(outfile);
end;
```

```
Procedure alter_pKas;

var     store: REAL;

begin
        write('Do you wish to change the pKa values of ',pk1:1,' and ',pk2:1,' ? ');
        readln(ans);
        if ((ans = 'y') or (ans = 'Y')) then begin
                write('Enter new pKa1, or 0 for no change  ');
                readln(store);
                if store <> 0.0 then pk1 := store;
                write('Enter new pKa2, or 0 for no change  ');
                readln(store);
                if store <> 0.0 then pk2 := store;
                end;
        writeln(outfile);
        writeln(outfile);
        writeln;
        writeln(outfile,'new values of pKa1 = ',pk1,'   pKa2 = ',pk2);

        writeln(outfile);
        writeln(outfile);
end;

Procedure eqn(fc:integer);

var      store, largek1, largek2: REAL;

begin
      fraction[fc,2] := exp( - fraction[fc,1] * lntolog );
      largek1 := exp( - pk1 * lntolog );
      largek2 := exp( - pk2 * lntolog );
      store := 1.0 + ((fraction[fc,2] / largek2) * (1.0 + (fraction[fc,2] / largek1)));
      fraction[fc,4] := 1.0 / store;
      fraction[fc,3] := fraction[fc,2] / (largek2 * store);
      fraction[fc,5] := (fraction[fc,2] ** 2) / ( largek1 * largek2 * store );
end;

Procedure intake;
begin
   writeln('Give your values of k(H+), k(CH2), k(CH-), k(C2-), k(OH-) please  ');
   readln(kh, kch2, kch, kc2, koh);
   writeln(outfile,'k(H+) = ',kh,'  k(CH2) = ',kch2,'  k(CH-) = ',kch);
   writeln(outfile,'k(C2-) = ',kc2,'  k(OH-) = ',koh);
end;

Procedure ktot(w:integer);
begin
      fraction[w,6] := fraction[w,2] * kh * fraction[w,5] +  kch2 * fraction[w,5] + kch * fraction[w,3];
      fraction[w,6] := fraction[w,6] +  kc2 * fraction[w,4]  + koh * exp((fraction[w,1] - 13.833) * lntolog);
         write(fraction[w,1]:6:3,'  ');
         write(outfile,fraction[w,1]:6:3,'  ');
         for y := 2 to 6 do write(outfile,fraction[w,y],'  ');
         for y := 2 to 6 do write(fraction[w,y],'  ');
         writeln;
         writeln(outfile);
end;
begin

open(outfile,'kobs.dat');
rewrite(outfile);

writeln('enter pKa1 and pKa2 ');
readln(pk1,pk2);
writeln(outfile,'pKa1 = ',pk1,'   pKa2 = ',pk2);
writeln(outfile);
writeln(outfile);

write('Do you want irregular values rather than regular increments? ');
readln(ans);

If ((ans = 'y') or (ans = 'Y')) THEN BEGIN
      fc := 0;
      writeln('Enter ph > 100 to finish data');
      repeat
         write('pH value please');
         fc := fc + 1;
         readln(ph);
         fraction[fc,1]  := ph;
      UNTIL ph > 99;
      fc := fc - 1;
   end
```

```
  ELSE
      begin
      writeln('Enter the starting pH, finishing pH and pH increment.');
      readln(phstart,phstop,phincrement);
      ph := phstart;
      fc := 0;
      repeat
         fc := fc + 1;
         fraction[fc,1] := ph;
         ph := ph + phincrement;
         until ph > phstop;
   END;
ans := '~';
repeat
        if ans = '~' then intake else begin
                alter_pKas;
                alter_ks;
                end;
        for x := 1 to fc do  eqn(x);
        headings;
        for x := 1 to fc do ktot(x);
        write('Do you wish to loop the program (y/n) ?  ');
        readln(ans);
until ((ans <> 'y') and (ans <> 'Y'));
end.
```

APPENDIX 2

CRYSTAL STRUCTURE POSITIONAL AND THERMAL PARAMETERS

TABLE A2-1 Final Positional Parameters for 2',6'-dihydroxy-2,4,6-trimethoxychalcone

ATOM	X/A	Y/B	Z/C
C(1)	0.4880(3)	0.0739(2)	0.1886(2)
C(2)	0.6085(3)	0.0126(2)	0.2448(2)
C(3)	0.7326(3)	-0.0132(2)	0.2067(2)
C(4)	0.7418(4)	0.0251(2)	0.1114(2)
C(5)	0.6317(3)	0.0894(2)	0.0555(2)
C(6)	0.5073(3)	0.1129(2)	0.0938(2)
C(7)	0.3565(3)	0.0888(2)	0.2304(2)
C(8)	0.2271(3)	0.1412(2)	0.1936(2)
C(9)	0.1089(3)	0.1408(2)	0.2503(2)
C(11)	-0.0282(3)	0.2032(2)	0.2252(2)
C(12)	-0.0727(3)	0.2631(2)	0.1356(2)
C(13)	-0.1980(4)	0.3239(2)	0.1217(3)
C(14)	-0.2849(4)	0.3249(2)	0.1940(3)
C(15)	-0.2508(4)	0.2656(2)	0.2798(3)
C(16)	-0.1244(3)	0.2054(2)	0.2953(2)
C(21)	0.7156(4)	-0.0752(3)	0.4047(3)
C(41)	0.9643(4)	-0.0690(2)	0.1118(3)
C(61)	0.4134(4)	0.2211(2)	-0.0489(2)
O(2)	0.5934(2)	-0.0183(2)	0.3397(2)
O(4)	0.8577(3)	0.0051(1)	0.0664(2)

TABLE A2-1 Continued

ATOM	X/A	Y/B	Z/C
O(6)	0.3958(2)	0.1752(1)	0.0438(2)
O(9)	0.1251(2)	0.0857(2)	0.3286(2)
O(12)	0.0086(3)	0.2593(2)	0.0626(2)
O(16)	-0.0935(3)	0.1479(2)	0.3810(2)
H(16)	-0.004(5)	0.114(3)	0.373(3)
H(12)	-0.040(4)	0.298(2)	0.002(3)

TABLE A2-2 Final Thermal Parameters for 2',6'-Dihydroxy-2,4,6-trimethoxychalcone

ATOM	U11	U22	U33	U23	U13	U12
C(1)	0.0290(7)					
C(2)	0.0301(7)					
C(3)	0.0316(7)					
C(4)	0.0315(7)					
C(5)	0.0310(7)					
C(6)	0.0288(7)					
C(7)	0.0311(7)					
C(8)	0.0315(7)					
C(9)	0.0296(7)					
C(11)	0.0288(7)					
C(12)	0.0309(7)					
C(13)	0.0372(8)					
C(14)	0.0437(9)					
C(15)	0.0413(8)					
C(16)	0.0327(7)					
C(21)	0.041(2)	0.058(2)	0.055(2)	0.029(2)	0.009(2)	0.011(2)

TABLE A2-2 Continued

ATOM	U11	U22	U33	U23	U13	U12
C(41)	0.038(2)	0.040(2)	0.057(2)	0.005(2)	0.016(2)	0.012(2)
C(61)	0.040(2)	0.040(2)	0.031(2)	0.007(1)	0.007(1)	0.004(2)
O(2)	0.034(1)	0.047(1)	0.041(1)	0.017(1)	0.010(1)	0.008(1)
O(4)	0.038(1)	0.042(1)	0.051(1)	0.007(1)	0.020(1)	0.012(1)
O(6)	0.033(1)	0.040(1)	0.036(1)	0.012(1)	0.0100(9)	0.010(1)
O(9)	0.0407(6)					
O(12)	0.037(1)	0.051(1)	0.035(1)	0.015(1)	0.012(1)	0.012(1)
O(16)	0.039(1)	0.046(1)	0.030(1)	-0.006(1)	0.012(1)	-0.007(1)
H(16)	0.060					
H(12)	0.060					

TABLE A2-3 Final Positional and Thermal Parameters of Calculated Hydrogen Atoms for 2',6'-dihydroxy-2,4,6-trimethoxychalcone

ATOM	X/A	Y/B	Z/C	U11
H(3)	0.8202(3)	-0.0620(2)	0.2499(2)	0.060
H(5)	0.6437(3)	0.1204(2)	-0.0169(2)	0.060
H(7)	0.3629(3)	0.0518(2)	0.3034(2)	0.050
H(8)	0.2115(3)	0.1830(2)	0.1228(2)	0.060
H(13)	-0.2278(4)	0.3705(2)	0.0542(3)	0.060
H(14)	-0.3817(4)	0.3733(2)	0.1829(3)	0.060
H(15)	-0.3219(4)	0.2660(2)	0.3345(3)	0.060
H(211)	0.6836(4)	-0.0871(3)	0.4772(3)	0.060
H(212)	0.8269(4)	-0.0390(3)	0.4227(3)	0.060
H(213)	0.7256(4)	-0.1413(3)	0.3676(3)	0.060
H(411)	1.0419(4)	-0.0756(2)	0.0617(3)	0.060
H(412)	0.9041(4)	-0.1344(2)	0.1129(3)	0.060

TABLE A2-3 Continued

ATOM	X/A	Y/B	Z/C	U11
H(413)	1.0322(4)	-0.0513(2)	0.1916(3)	0.060
H(611)	0.3133(4)	0.2671(2)	-0.0747(2)	0.060
H(612)	0.4121(4)	0.1712(2)	-0.1110(2)	0.060
H(613)	0.5204(4)	0.2616(2)	-0.0318(2)	0.060

TABLE A2-4 Final Positional Parameters for 5-hydroxy-4',7-dimethoxy-flavanone

ATOM	X/A	Y/B	Z/C
O(1)	0.1461(3)	0.024(1)	0.8358(1)
C(2)*	0.1289(9)	-0.195(3)	0.8123(5)
C(2')*	0.0888(8)	-0.085(3)	0.8055(4)
C(3)	0.0561(4)	-0.327(1)	0.8186(2)
C(4)	0.0361(4)	-0.311(1)	0.8635(2)
O(4)	-0.0130(3)	-0.459(1)	0.8755(2)
C(5)	0.0571(4)	-0.081(2)	0.9351(2)
O(5)	0.0047(3)	-0.234(1)	0.9509(2)
C(6)	0.0917(4)	0.105(1)	0.9620(2)
C(7)	0.1459(4)	0.257(2)	0.9455(2)
O(7)	0.1851(3)	0.446(1)	0.9694(2)
C(71)	0.1679(5)	0.493(2)	1.0122(2)
C(8)	0.1652(4)	0.227(2)	0.9035(2)
C(9)	0.1285(4)	0.042(1)	0.8774(2)
C(10)	0.0736(4)	-0.118(1)	0.8921(2)
C(11)	0.1288(6)	-0.120(2)	0.7638(3)
C(12)	0.1723(5)	-0.297(2)	0.7459(3)
C(13)	0.1872(4)	-0.277(2)	0.7031(2)

TABLE A2-4 Continued

ATOM	X/A	Y/B	Z/C
C(14)	0.1580(4)	-0.076(2)	0.6774(2)
O(14)	0.1763(3)	-0.076(1)	0.6357(2)
C(141)	0.1495(5)	0.132(2)	0.6083(3)
C(15)	0.1150(4)	0.110(2)	0.6934(2)
C(16)	0.1014(5)	0.082(2)	0.7376(3)
H(5)	-0.009(4)	-0.38(1)	0.926(2)

TABLE A2-5 Final Thermal Parameters for 5-hydroxy-4',7-dimethoxyflavanone

ATOM	U11	U22	U33	U23	U13	U12
O(1)	0.074(4)	0.065(4)	0.037(3)	-0.009(3)	0.020(3)	-0.037(3)
C(2)*	0.040(4)					
C(2')*	0.034(4)					
C(3)	0.066(6)	0.048(5)	0.028(4)	0.002(4)	0.015(4)	-0.024(4)
C(4)	0.042(2)					
O(4)	0.066(4)	0.058(4)	0.052(3)	0.005(3)	0.012(3)	-0.027(3)
C(5)	0.049(2)					
O(5)	0.071(4)	0.065(4)	0.058(4)	-0.002(3)	0.027(3)	-0.034(3)
C(6)	0.045(2)					
C(7)	0.054(5)	0.053(6)	0.034(4)	-0.014(4)	0.005(4)	-0.024(5)
O(7)	0.080(4)	0.065(4)	0.045(3)	-0.015(3)	0.022(3)	-0.032(4)
C(71)	0.071(6)	0.064(7)	0.046(5)	-0.010(5)	0.013(4)	-0.017(5)
C(8)	0.047(2)					
C(9)	0.040(2)					
C(10)	0.040(2)					
C(11)	0.095(8)	0.086(9)	0.048(6)	-0.022(6)	0.023(5)	-0.068(7)

TABLE A2-5 Continued

ATOM	U11	U22	U33	U23	U13	U12
C(12)	0.075(7)	0.076(8)	0.048(6)	0.005(6)	-0.002(5)	-0.028(6)
C(13)	0.046(5)	0.067(6)	0.047(6)	0.011(5)	0.007(4)	0.009(5)
C(14)	0.040(5)	0.059(6)	0.031(4)	0.000(4)	0.014(4)	0.002(5)
O(14)	0.074(4)	0.068(4)	0.041(3)	0.010(3)	0.023(3)	0.027(3)
C(141)	0.096(7)	0.075(7)	0.044(5)	0.020(5)	0.015(5)	0.031(6)
C(15)	0.051(5)	0.064(7)	0.054(6)	-0.010(5)	0.021(4)	0.002(5)
C(16)	0.067(7)	0.068(7)	0.079(7)	-0.032(6)	0.035(5)	-0.030(6)
H(5)	0.060					

TABLE A2-6 Final Positional and Thermal Parameters of Calculated Hydrogen Atoms for 5-hydroxy-4',7-dimethoxyflavanone

ATOM	X/A	Y/B	Z/C	U11
H(6)	0.0776(4)	0.132(1)	0.9948(2)	0.110
H(711)	0.2062(5)	0.646(2)	1.0240(2)	0.080
H(712)	0.1783(5)	0.336(2)	1.0345(2)	0.080
H(713)	0.1082(5)	0.553(2)	1.0105(2)	0.080
H(8)	0.2081(4)	0.346(2)	0.8918(2)	0.110
H(12)	0.1956(5)	-0.454(2)	0.7657(3)	0.080
H(13)	0.2216(4)	-0.419(2)	0.6897(2)	0.080
H(141)	0.1664(5)	0.121(2)	0.5759(3)	0.080
H(142)	0.1752(5)	0.297(2)	0.6250(3)	0.080
H(143)	0.0869(5)	0.144(2)	0.6055(3)	0.080
H(15)	0.0926(4)	0.268(2)	0.6735(2)	0.080
H(16)	0.0682(5)	0.225(2)	0.7515(3)	0.080

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