KINETIC STUDIES OF CYCLISATIONS OF 2′-HYDROXYCHALCONES AND DERIVATIVES

A thesis

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ABSTRACT

The rates of cyclisation of various 2'-hydroxychalcones to the equilibrium mixture of chalcone and flavanone have been determined over the pH range in which the chalcones undergo ionisation. The compounds studied were 2'-hydroxy-, 2'-hydroxy-5',6'-benzo-, 2',4'-dihydroxy-, 2'-hydroxy-4'-methoxy-, 2'-hydroxy-6'-methoxy-, and 2'-hydroxy-4',6'-dimethylchalcone. In each case the observed first order rate constant has been fitted to a theoretical pH-rate profile based on the equation

$$k_{obs} = kfCH + k'fC^- + k''\{OH^-\}$$

where k is the rate constant for the cyclisation of neutral chalcone (fCH is the fraction of neutral chalcone), k' is the rate constant for the cyclisation of the ionised chalcone (fC^- is the fraction of ionised chalcone), and k'' is a measure of the rate in the reverse reaction. The expression implies that the reaction in the forward direction can proceed from neutral or ionised chalcone, and in the reverse direction through the reaction of flavanone with hydroxide ion. In all cases the forward reaction of the ionised chalcone (k') is very much faster than that of the neutral species (k). Weak catalysis by some buffer species has been detected but the origin of this catalysis has not been pursued in detail. Comparisons are made between the forward and reverse rate constants for the different chalcones in order to see how they are affected by different A-ring substituents. It is observed that electron releasing groups at the 4'- or 6'- positions decrease the rate. This diminution in the observed rate is particularly pronounced with 6'-substituents, and is considered as possibly due to the steric and/or electrostatic

interaction of this substituent with the carbonyl group, an interaction that could force the carbonyl group to lie out of the plane of the A-ring thereby changing rates of reactions by electronic or steric effects.

The first kinetic study of the reactivity of a 2'-hydroxychalcone epoxide is reported. The variation of the rate of cyclisation of the parent epoxide, 2'-hydroxychalcone epoxide, to 3-hydroxyflavanone in water, over the range pH 1 - 6.5, is shown to be consistent with the rate equation.

$$\text{Rate} = 0.235\ [\text{Epoxide}]\{H^+\} + 1.85 \times 10^6\ [\text{Epoxide}]\ \{OH^-\}\ \text{mol}\ l^{-1}s^{-1}$$

This shows the epoxide to be very unstable at or above neutral pH, and suggests that such compounds, if they do occur, as has often been suggested, as intermediates in flavanoid biosynthesis would probably be very difficult to isolate. Their detection under the basic conditions of either the Algar-Flynn-Oyamada oxidation of 2'-hydroxychalcones, or the formation of aurones from certain 2'-hydroxychalcone dibromides, both reactions in which they have been suggested as intermediates, would be equally unlikely : based on a suggested mechanism, the parent epoxide of this study can be predicted to have a half-life at pH 10 of the order of a few milliseconds.

The first determination of the ratio of E- and Z-2'-hydroxy-α-bromochalcones formed directly from a 2'-hydroxychalcone dibromide is described. Combined kinetic and spectrophotometric measurements give the yield (%) of E- and Z-2'-hydroxy-α-bromochalcone as 35 ± 2 and 63 ± 8 respectively for the reaction of *erythro*-2'-hydroxychalcone dibromide in 4:1 ethanol-water buffered at pH 7.88 with N-ethylmorpholine. Direct cyclisation of the dibromo compound, if it occurs at all, is

only a very minor route to 3-bromoflavanone. The lack of any detected dependence on buffer concentrations of the rate constants or of the proportions of the E- and Z-isomers formed suggests an El_{cB} mechanism rather than an E2. However, with the particular elimination involved (i.e. a double bond being formed α,β to a carbonyl group) there is precedence for small catalysis by N-alkylmorpholines and therefore, it is not certain that the lack of measurable buffer catalysis does in fact mean a total lack of catalysis. Further, if the elimination is in fact by an El_{cB} type mechanism, a carbanion is not necessarily involved, as the elimination could proceed from the phenolate ion as conjugate base, the phenoxide group acting in place of an external base. An El mechanism is excluded on kinetic grounds.

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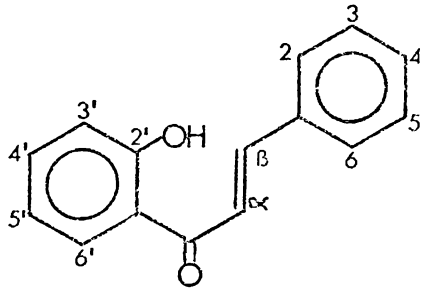
The University Grants Committee for financial assistance.

CONTENTS

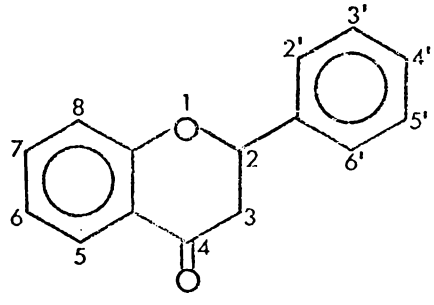
NOMENCLATURE OF FLAVANOIDS

Shown below are the major compounds referred to in the text with their common and systematic names. The common name (with the numbering shown) has, in the main been used throughout this thesis.



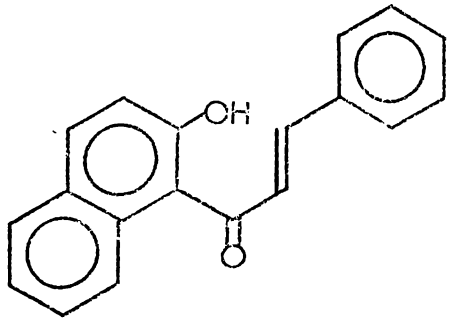
2'-Hydroxychalcone

1-(2-Hydroxyphenyl)-3-phenyl-2-propen-1-one



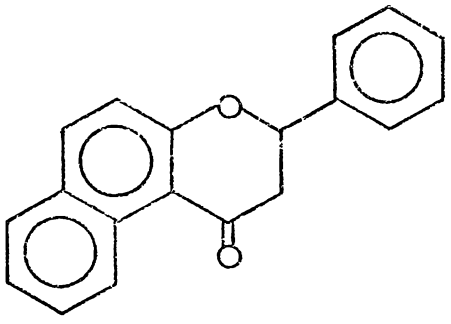
Flavanone

2,3-Dihydro-2-phenyl-4H-1-benzopyran-4-ones



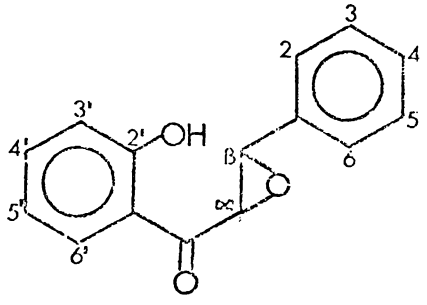
2'-Hydroxy-5',6'-benzochalcone

1-(2-Hydroxynaphthalenyl)-3-phenyl-2-propen-1-one



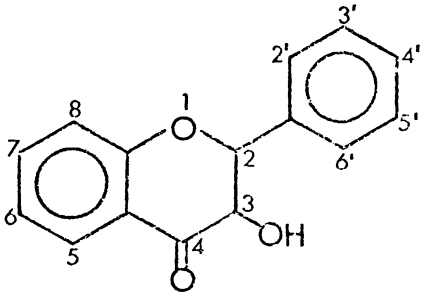
5,6-benzoflavanone

2,3-Dihydro-3-phenyl-1H-naphtho-[2,1-b] pyran-1-one



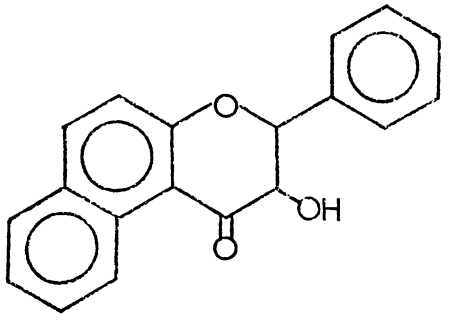
2'-Hydroxychalcone epoxide

2-Hydroxyphenyl (3-phenyl oxiranyl) methanone



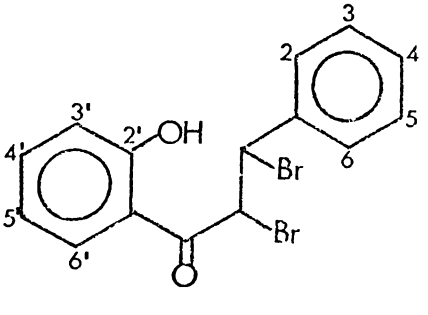
3-Hydroxyflavanone

2,3-Dihydro-3-hydroxy-2-phenyl-4H-1-benzopyran -4-one



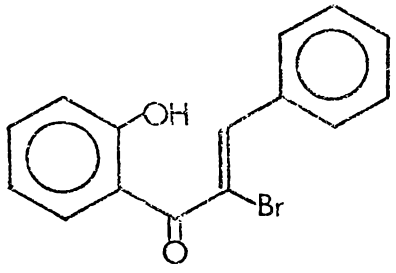
3-Hydroxy-benzoflavanone

2,3-Dihydro-2-hydroxy-3-phenyl-1-H-naphtho [2,1-b] pyran-1-one



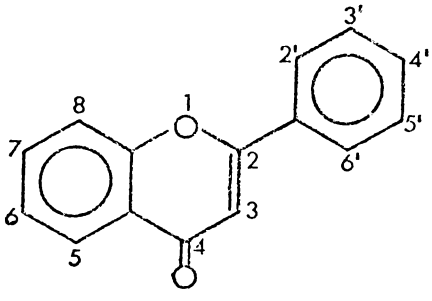
2'-Hydroxychalcone dibromide

2,3-Dibromo-1-(2-hydroxyphenyl)-3-phenyl-propan-1-one



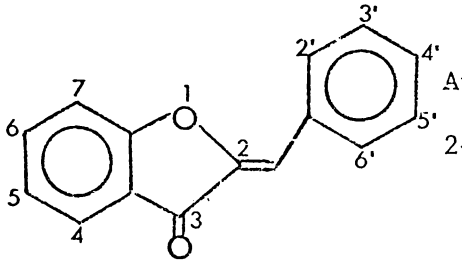
2'-Hydroxy-α-bromochalcone

2-Bromo-1(2-hydroxyphenyl)-3-phenyl-2-propen-1-one



Flavone

2-Phenyl-4H-benzo [b] pyran-4-one

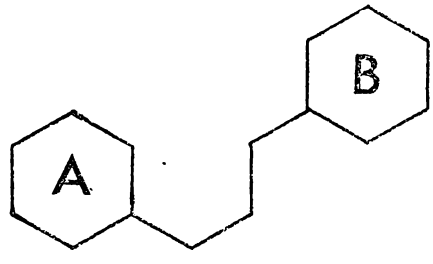


Aurone

2-(Phenylmethylene)-3(2H)-benzofuranone

1. INTRODUCTION

Flavonoids constitute one of the most numerous and widespread groups of natural products and are ubiquitous in higher plants. They often occur as their glycosides, but have the basic C_6-C_3-C_6 skeleton below which is regarded as being made up of two distinct units: the A-ring; and the B-ring with the C_3 chain.



By isotopic labelling experiments it has been established that ring A is formed, biosynthetically, by head to tail condensation of three "acetate units", while ring B with the three carbon chain is formed from a phenylpropanoid precursor derived via the shikimic acid pathway (Geissman & Swain, 1957; Neish, 1964; Grisebach, 1968; Nakanishi, 1975) (Fig. 1.1). The ring A substitution pattern of naturally occurring flavonoids is determined by the nature of the "acetate units", and is usually based on the phloroglucinol pattern (2', 4', 6'-trihydroxy-using chalcone numbering) (See Nomenclature of Flavonoids).

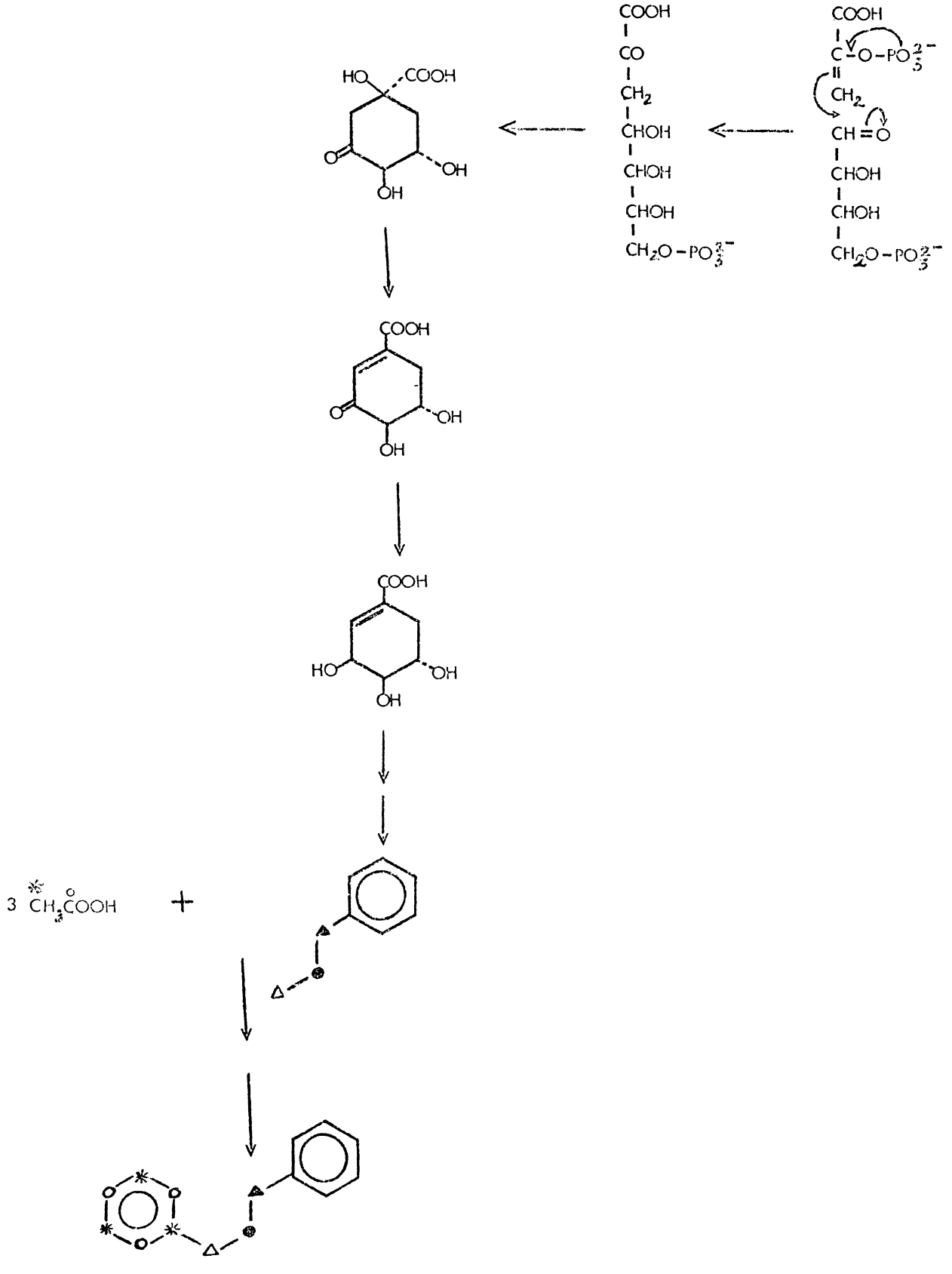


Figure 1.1. Origin of the carbon atoms in flavonoid compounds (Neish, 1964; Grisebach, 1968)

The origin of the substitution pattern in ring B is less certain and may arise from the substitution pattern of the phenylpropanoid precursor (often a cinnamic acid or L-phenylalanine), or may be the result of further substitution of the ring after the formation of the flavonoid intermediate (Ebel et al, 1970). Considerable evidence has been advanced from tracer and enzymic studies to support the theory that modifications of the ring B substitution pattern can often occur subsequent to the formation of the flavonoid intermediate (Grisebach, 1967, 1968). Hess (1964) and others, however, have demonstrated that in certain cases at least, the substitution pattern is determined at the cinnamic acid stage resulting in Hess's "cinnamic acid starter hypothesis". This hypothesis postulates that in the condensation between activated cinnamic acid and possibly malonyl CoA (see Fig. 1.2.) the enzyme selects with a high degree of specificity from a given cinnamic acid pool. It appears, therefore, that as with other cases of secondary plant metabolism, there may be more than one pathway leading to flavonoids with different substitution patterns in ring-B.

1-1 THE CHALCONE (FLAVANONE) INTERMEDIATE

It has been postulated that the first specific reaction in flavonoid biosynthesis is the enzyme-catalysed condensation of an activated cinnamic acid with three molecules of malonyl CoA to give a chalcone or flavanone (Grisebach, 1967) (Fig. 1.2.).

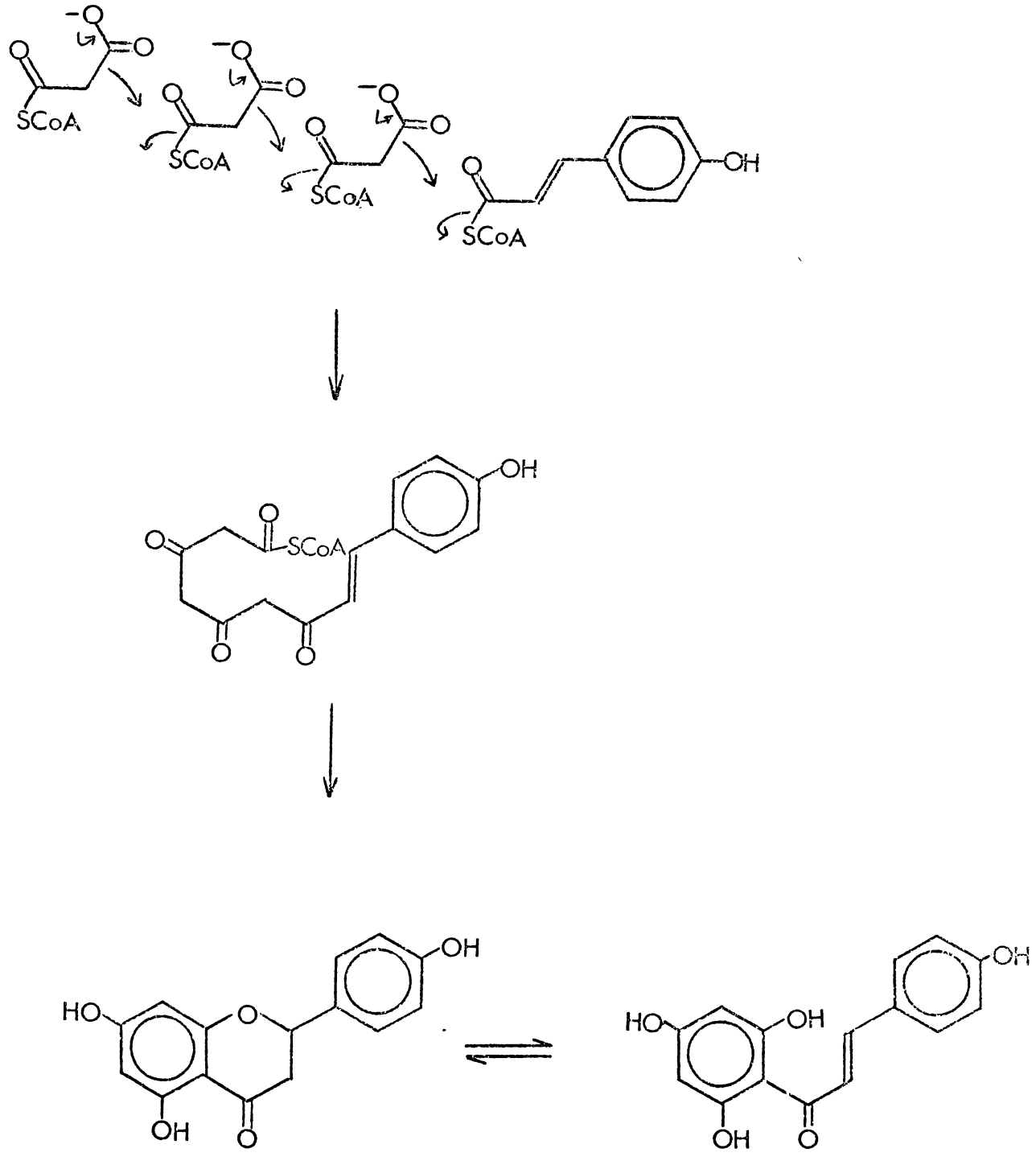


Figure 1.2. Possible mechanism for the formation of a flavanone (chalcone) from p-coumaryl CoA and malonyl CoA (Grisebach, 1967).

1-1.1 Chalcone-Flavanone Synthetase

The enzyme chalcone-flavanone synthetase (presumably as a multienzyme complex) catalyses the reaction in Fig. 1.2. and labelling experiments have confirmed the overall mechanism (Grisebach, 1974). However, no experimental evidence has been obtained concerning possible intermediates in the formation of the A-ring. The enzyme does not require any cofactors and has an optimum activity around pH 8. There seems to be uncertainty as to whether chalcone or flavanone is the more immediate product of this enzyme. A synthetase which catalyses the conversion of 10% p-coumaroyl CoA to naringenin (5, 7, 4'-trihydroxyflavanone) was found, upon partial purification, to contain no chalcone, and since no chalcone-flavanone isomerase (see section 1-1.2.) activity was detected it was assumed that the flavanone was the immediate product of this synthetase reaction. The chalcone equivalent to this flavanone would be 2',4',6',4-tetrahydroxy-chalcone and it should be noted that any chalcone containing both a 2'-hydroxyl group and a 6'-hydroxyl group would be extremely difficult to isolate without it cyclising. Thus the present evidence does not inexorably prove which form is the original product.

Suggestions have been made regarding possible immediate precursors to the chalcone (flavanone) intermediate. Ramakrishnan and Kagan (1970a) have proposed that the photochemical conversion of substituted phenyl cinnamates to 2'-hydroxychalcone, may be a possible model for the biological reaction (Fig. 1.3.).

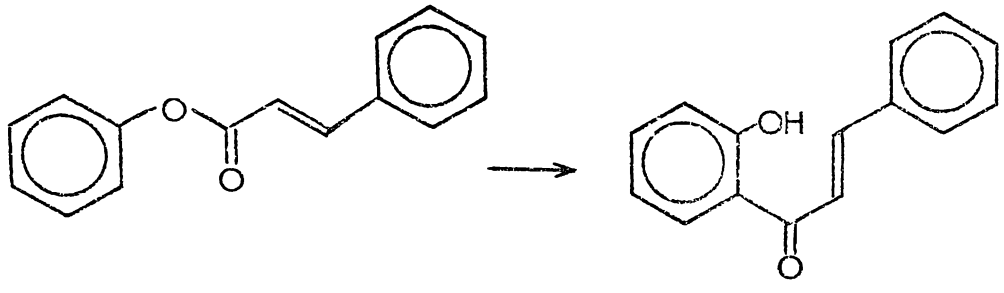


Figure 1.3. Photo-Fries conversion of phenylcinnamate to 2'-hydroxychalcone

1-1.2. Chalcone-Flavanone Isomerase

This enzyme catalyses the equilibrium of the chalcone with the corresponding flavanone. Although at physiological pH the equilibrium lies well to the flavanone side, there is no certainty as to the direction of the enzyme-catalysed isomerisation in vivo. Preliminary evidence for the presence of such an enzyme was provided by Shimokoriyama (1957), but it was Wong and Moustafa (1966) who first authenticated its existence, making it the first enzyme reported to catalyse a reaction specifically involved in the biosynthesis of flavonoids. (Moustafa and Wong, 1967). The results from experiments with several chalcone-flavanone isomerases suggest that there is a correlation between their substrate specificity and the substitution patterns of flavonoid compounds in a particular plant. For example, the parsley isomerase has a high substrate specificity for ring A substitution, and only chalcones with a phloroglucinol

type of substitution in ring A were found to serve as substrate (Hahlbrock et al, 1970). This is consistent with the finding that only flavonoids with this ring A substitution pattern have been found in parsley. Similarly the fact that both phloroglucinol- and resorcinol-type substituted flavonoids occur in Mung beans and Garbanzo beans is consistent with the rather low specificities of the isomerases isolated from these plants.

The stereochemistry of the Mung bean-isomerase reaction has been investigated by following the cyclisation of 4,2',4'-trihydroxychalcone to the (-)(2S)-7,4'-dihydroxyflavanone (Grisebach and Hahlbrock, 1974). Following the reactions of the deuterium-labelled [α^2H]trihydroxychalcone in H_2O and of unlabelled trihydroxychalcone in ^2H_2O (Fig. 1.4.) showed that the hydrogen (or deuterium) provided by the solvent was preferentially incorporated into the axial position at C3 in the flavanone.

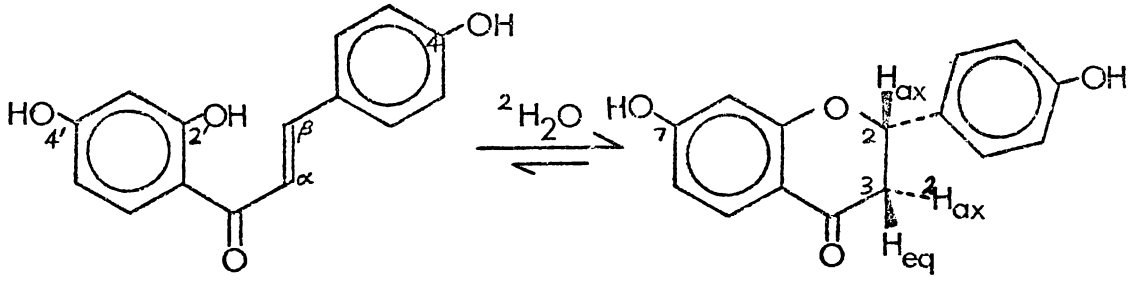


Figure 1.4. Stereospecific incorporation of deuterium into 7,4'-dihydroxyflavanone.

A mechanism consistent with these results would be an acid-base-catalysed reaction (Fig. 1.5.) leading to a flav-3-en-4-ol intermediate which undergoes a stereospecific proton transfer to the axial site to form the flavanone.

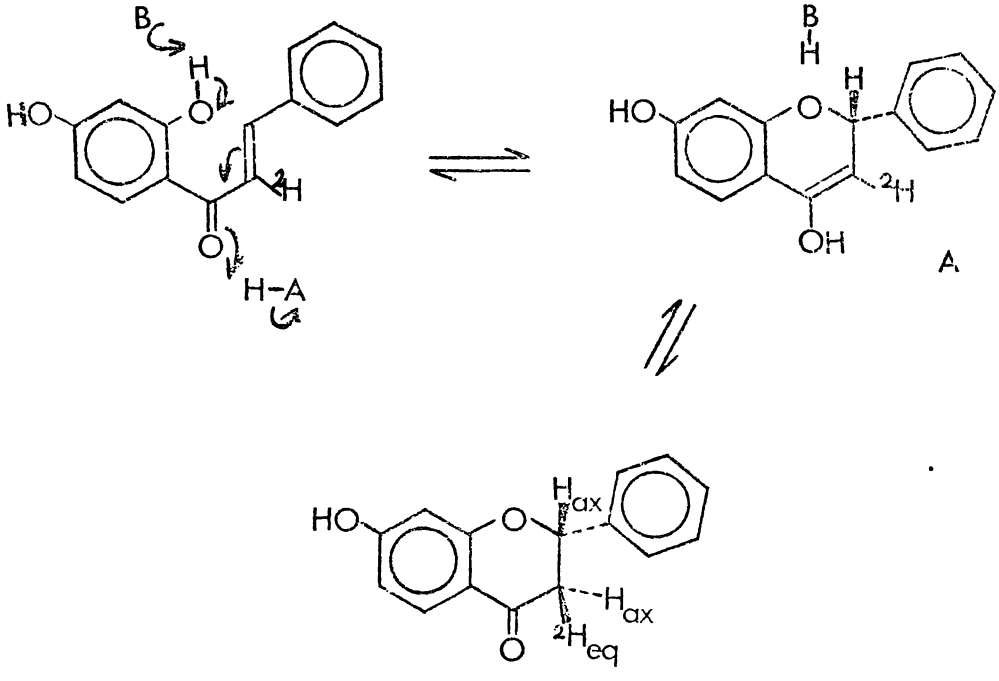


Figure 1.5. Hypothetical mechanism for chalcone-flavanone isomerase reaction. B = Basic group on enzyme
AH= Acidic group on enzyme

1-2 THE CHALCONE-FLAVANONE EQUILIBRIUM

Reichel et al (1941) performed some of the early studies on the 2'-hydroxychalcone-flavanone isomerisation reaction. They obtained no kinetic data, but found that 1g of 2'-hydroxychalcone in 80mls ethanol and 10mls of a citrate-HCl solution (pH 4.5) at 37°C gave 40% flavanone after 30 days and 65% flavanone after 60 days.

Their postulated mechanism is shown in Figure 1.6.

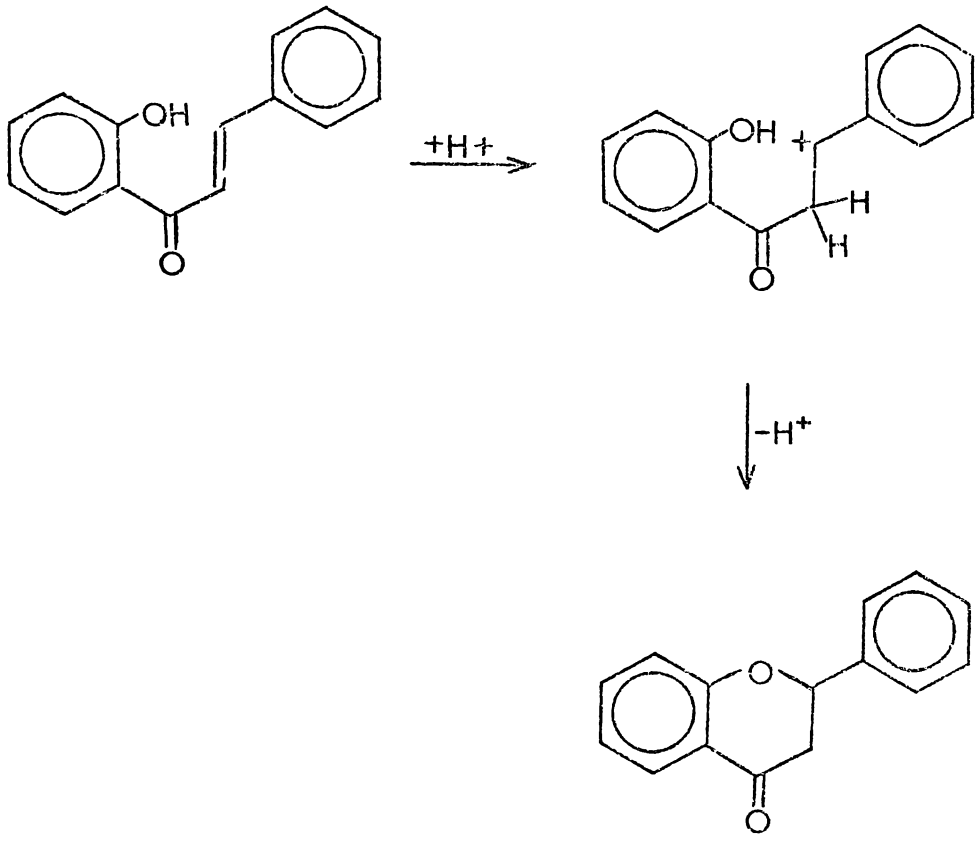
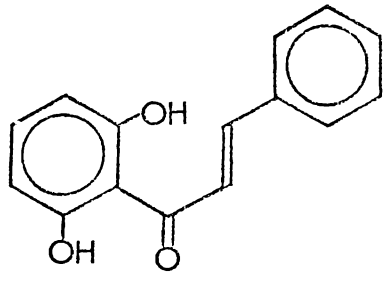


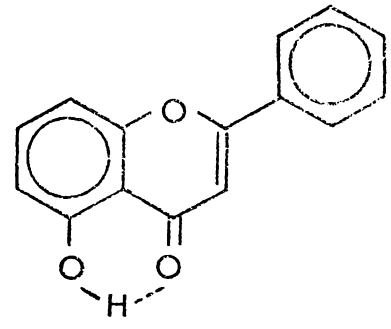
Figure 1.6. Mechanism proposed by Reichel et al for the cyclisation of 2'-hydroxychalcone to flavanone.

Narasimhachari et al (1948) investigated the effect that a 6'-hydroxyl group (chalcone numbering) had on the position of the equilibrium. In their work with chalcone glycosides they observed two related facts. Firstly, chalcone glycosides containing a 6'-hydroxyl group gave, on boiling with 7% sulphuric acid, only the corresponding flavanone, whereas those without a 6'-hydroxyl group gave some flavanone and residual uncyclised chalcone. Secondly, that 5-hydroxyflavanones dissolved readily with cold 10% NaOH and were precipitated unchanged by acidification, whereas 5-methoxyflavanones dissolved only on warming and gave only the corresponding 2'-hydroxychalcone on acidification. From these experiments they showed

that the presence of a 6'-hydroxy group in the chalcone shifts the equilibrium in the chalcone-flavanone isomerisation in favour of the flavanone, because of the hydrogen-bonding stabilisation between the 5-hydroxyl group and the carbonyl in the flavanone.



2',6'-dihydroxychalcone



5-hydroxyflavanone

Figure 1.7. Isomerisation of a chalcone with a 6'-hydroxyl group, showing the possibility of hydrogen-bonding in the flavanone.

Shimokoriyama (1957) tested the stability of several chalcone glycosides at various pH values by measuring the time required for the complete decolourisation of the solutions. For naringin chalcone (i.e. a 4'-neohesperidoside of 2',4',6',4-tetrahydroxychalcone) he also followed the isomerisation reaction by measuring the change in absorption at various time intervals. His conclusions were that the cyclisation was monomolecular and that the reaction took place rapidly in alkaline and neutral media, the chalcone being fairly stable in weak acid and extremely stable at pH3.

The rate of cyclisation of the parent compound 2'-hydroxychalcone to flavanone has been looked at by David et al (1961), who found that the rate increased with increasing temperature and with increasing pH. They showed the reaction to be first order and submitted a mechanism (Fig. 1.8.) to account for the cyclisation in basic solution (c.f. the mechanism of Reichel et al Fig. 1.6. for acid solution). No reasons were put forward to indicate why this mechanism was preferred over a simple cyclisation via conjugate addition.

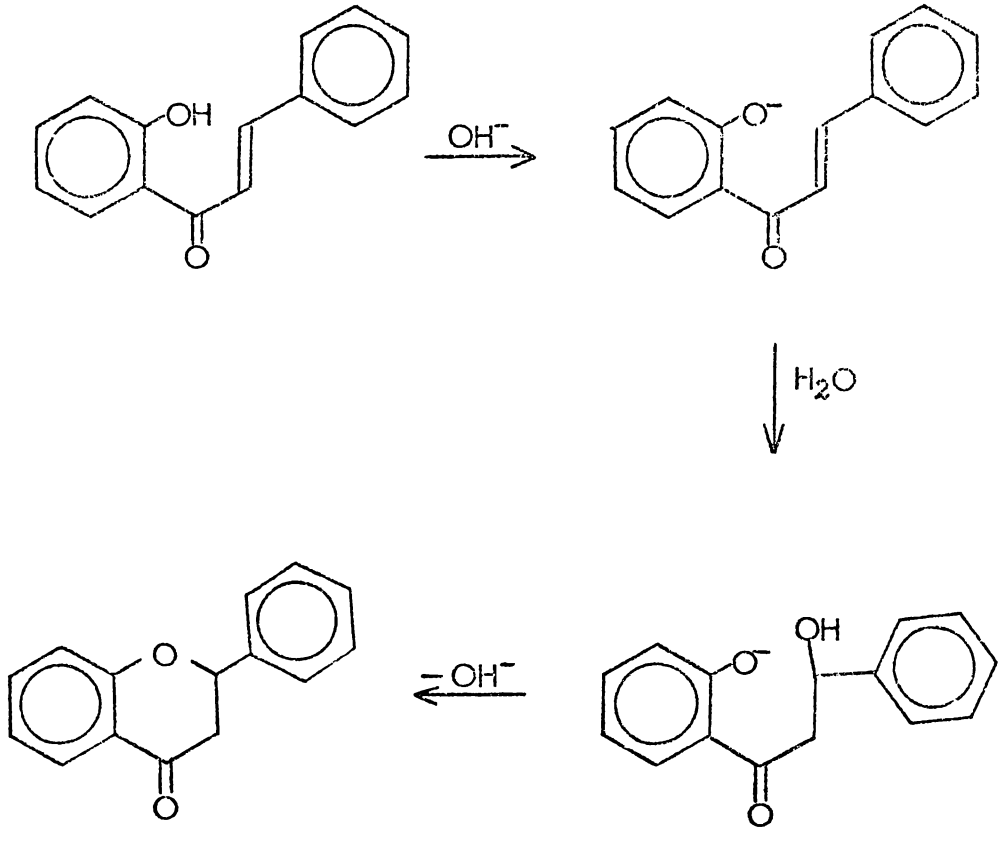


Figure 1.8. Mechanism proposed by David et al for the cyclisation of 2'-hydroxychalcone to flavanone.

In some later work this same group observed that the decisive factor in the rate of oxidation reactions of chalcones (flavanones) was the rate of the chalcone-flavanone isomerisation (Litkei et al 1973). The pH-dependence of the rate of isomerisation determined by them is shown in Fig. 1.9.

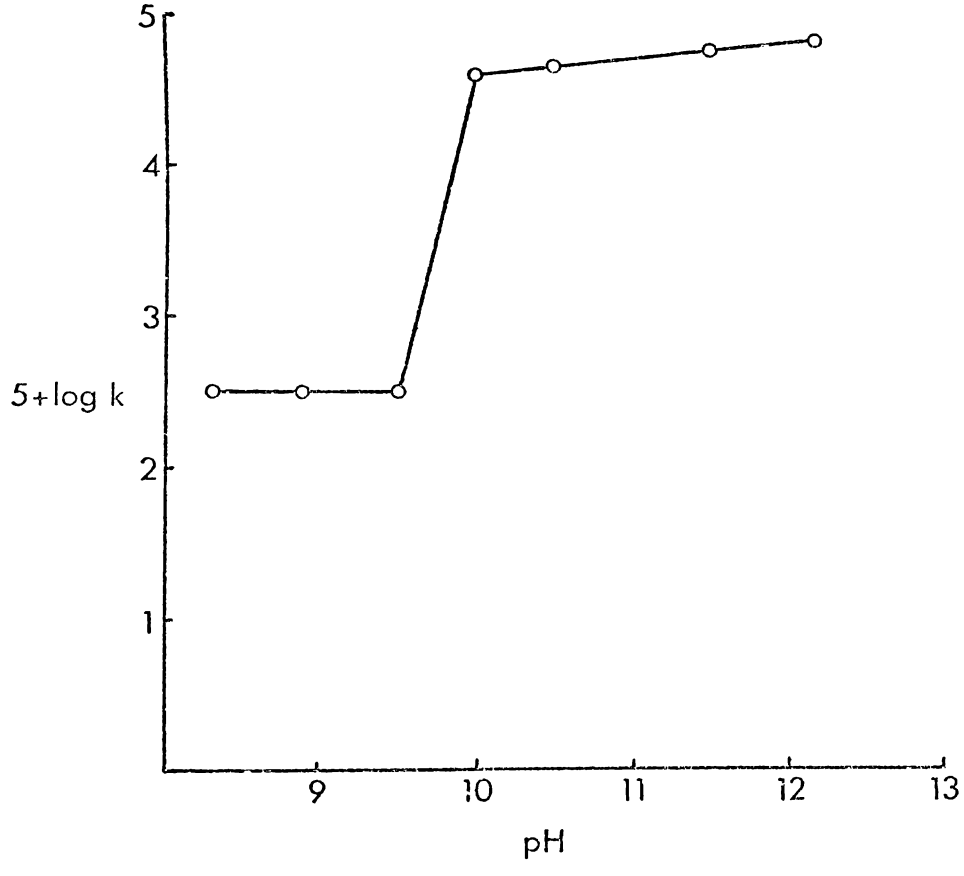


Figure 1.9. Flavanone -2'-hydroxychalcone isomerisation as a function of pH (Litkei et al, 1973).

The only quantitative study on the effect of substituents on cyclisation has been a preliminary investigation by Grouiller et al (1973). They did not measure any rate constants, but for several monosubstituted chalcones they determined the equilibrium constant by measuring the residual quantity of chalcone at equilibrium. All reactions were performed at 80°C and pH 7, in ethanol-water (1:3), and resulted in the conclusion that cyclisation, as measured by the equilibrium constant,

was favoured by electron-withdrawing substituents at position 4 or 4', and by electron-donating substituents at the 5'-position. In accord with this they noted that in the synthesis of chalcones with 5'-amino, 5'-hydroxy or 5'-methoxy groups, the flavanone was obtained directly, and not the chalcone.

Kinetic determinations have been made of the rate of formation of 7-hydroxyflavanone from 2',4'-dihydroxychalcone in an alkaline medium (Panasenko et al, 1975). It was shown that the process was reversible and that by decreasing the alkali concentration the equilibrium was shifted to the side of the flavanone, although this decreased its rate of formation. Sodium hydroxide concentrations from 0.0859M - 5.08M (i.e. pH > 12) were used and it was found that the best yield of the flavanone (~96%) was obtained with approximately 0.09M NaOH. The mechanism proposed involved the formation of a diphenoxide anion, and ring closure via a cyclic transition state (Fig. 1.10).

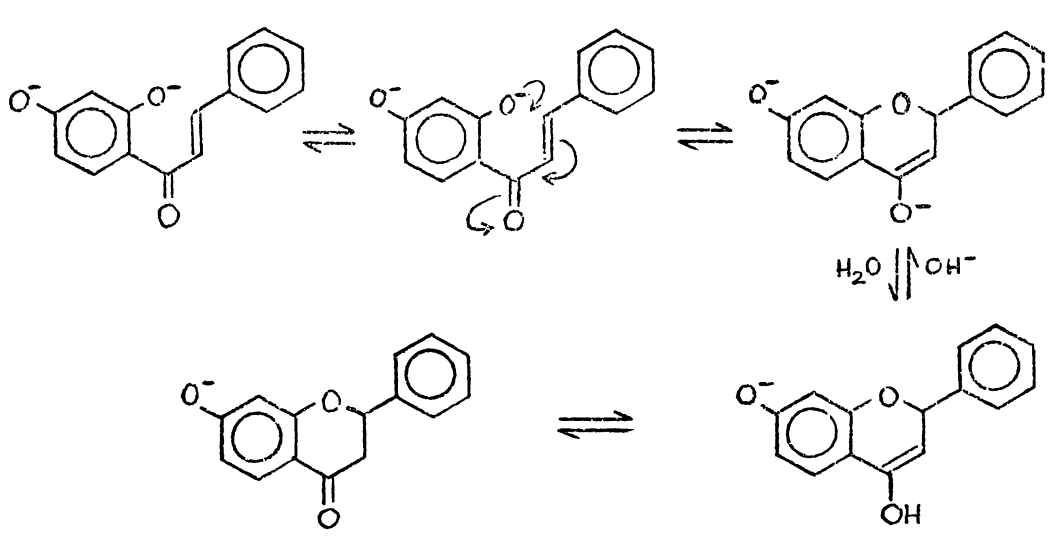


Figure 1.10. Mechanism proposed by Panasenko et al (1975) for the formation of 7-hydroxyflavanone from 2',4'-dihydroxychalcone in an alkaline medium.

1-3 BIOSYNTHETIC CONVERSIONS OF CHALCONES(FLAVANONES) TO OTHER FLAVANOIDS

It has been established using specifically labelled chalcones and flavanones that most, if not all, flavonoids have these compounds as central intermediates (Fig. 1.11). However, the mechanism by which they are converted to other flavonoids remains controversial.

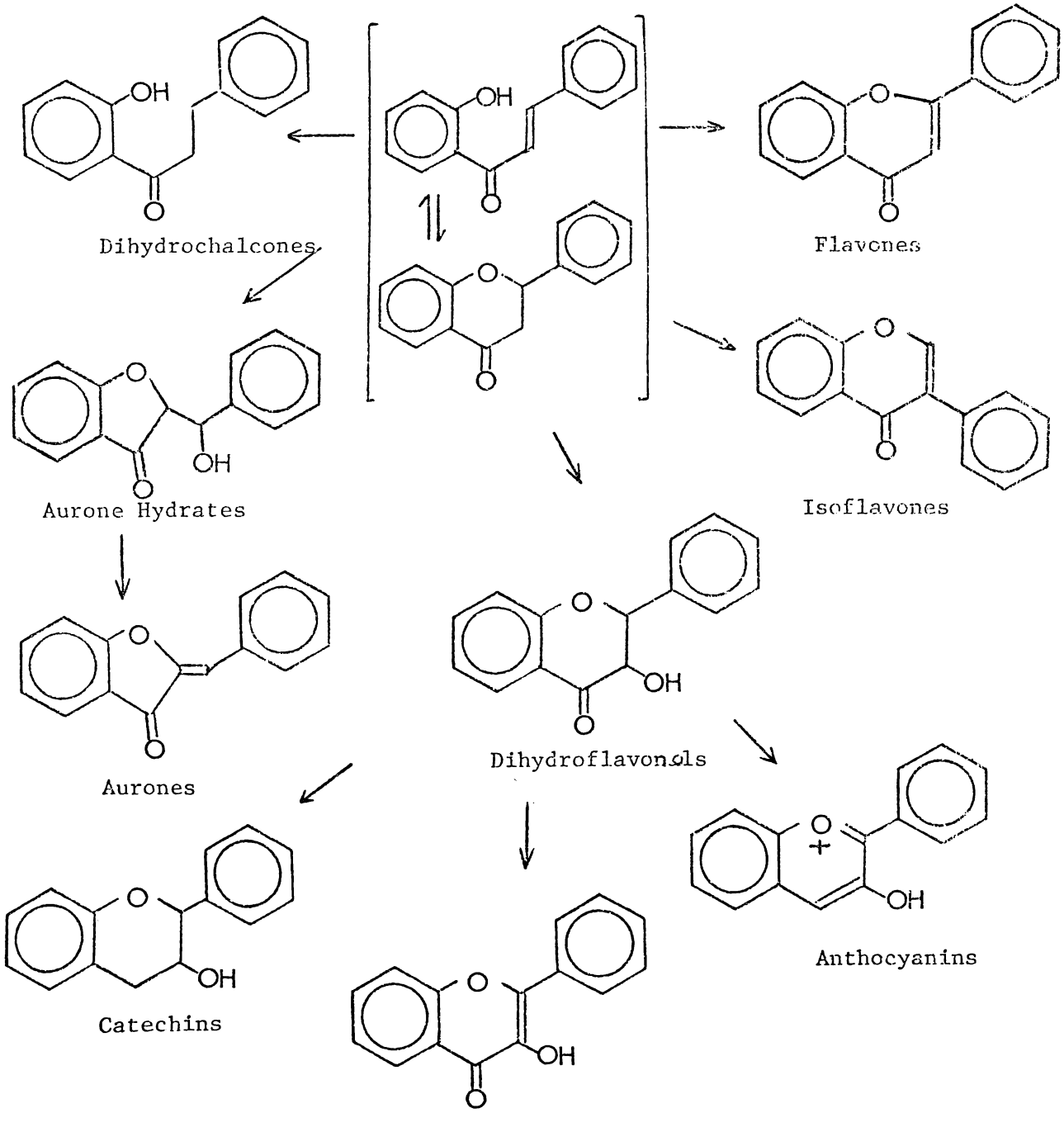
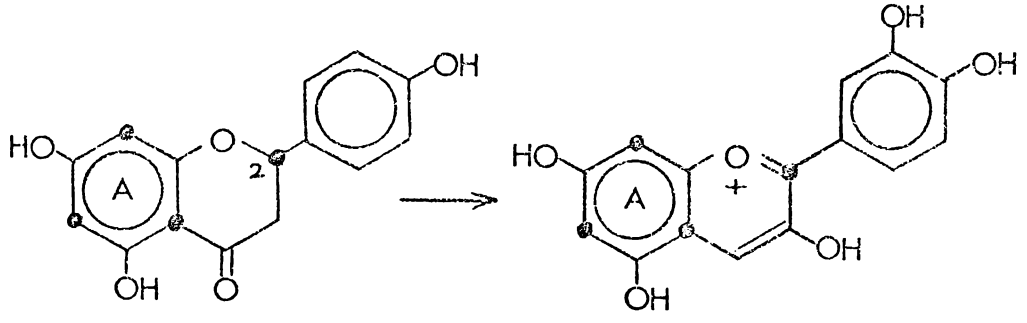


Figure 1.11. Probable biosynthetic route to some flavonoids

One of the first questions to be asked was whether the chalcone (flavanone) was incorporated intact into the flavonoids or whether it was degraded to phenylpropanoid compounds prior to incorporation. The proof of intact incorporation was provided by administering a chalcone (flavanone) labelled in both the A-ring and the β-position (5,7,4'-trihydroxyflavanone- [2,6,8,10-$^{14}C_4$])* to red cabbage seedlings (Grisebach, 1968). The activity ratio A-ring/C2 in the isolated cyanidin remained unchanged, thus proving the intact conversion of the flavanone into cyanidin (Fig. 1.12).



A-ring/C2 = 1:2.04 A-ring/C2 = 1:2.00

Figure 1.12. Proof of the intact incorporation of 5,7,4'-trihydroxy-flavanone [2,6,8,10-$^{14}C_4$] (4,2',4',6'-tetrahydroxychalcone [1,3,5,β-$^{14}C_4$]) into cyanidin. (Grisebach, 1968)

*In this case the equilibrium in aqueous solution is well to the side of the flavanone because the latter is stabilised by hydrogen bonding between the 5-hydroxyl group and the carbonyl group (see Fig. 1.7.)

The incorporation of a chalcone (flavanone) into other flavonoids appears always to occur with retention of the hydroxylation pattern of ring A. Thus, 2',4',6'-trihydroxychalcones form only 5,7-dihydroxy-flavonoids, for instance (Hahlbrock and Grisebach, 1975). However, no such specificity exists regarding the substitution pattern of ring B. Chalcones with a 4-hydroxyl group are not only incorporated into 4'-hydroxy and 4'-methoxy flavonoids, but also into flavonoids further substituted in this ring. The fact that 4,2',4',6'-tetrahydroxychalcone is incorporated into both cyanidin (see Fig. 1.12.) and quercetin (3',4',5,7-tetrahydroxyflavonol) indicates that a change in the oxidation state of the C_3 chain as well as introduction of an additional hydroxyl group ortho to the one already present in ring B can take place at the level of the C_6-C_3-C_6 intermediate. This prompted Grisebach (1967) to suggest that flavonoids with different substitution patterns in ring B could all be derived from a chalcone (flavanone) intermediate containing only a 4-hydroxyl group in the B-ring. The work of Pelter et al (1971) also suggests the importance of a 4-(or 2-) hydroxyl group in the chalcone B-ring for the formation of flavonoids containing B-ring hydroxyl groups. The free-radical mechanisms (see Fig. 1.15.) they propose for the formation of various flavonoids require a 4-(or 2-) hydroxyl group in the B-ring, but do not explain the production of flavonoids without B-ring hydroxyls. They suggest for these compounds that they may be formed either by a totally different route, or, if the general biosynthetic mode is the same, by B-ring hydroxyl groups being lost after flavanoid formation.

Since the majority of flavonoids contain a central heterocylic ring it may seem reasonable to believe that flavanones are the more direct precursors of the various flavonoids. This opinion was widely held until Wong et al (1969) performed a double-labelling experiment using (4-^{14}C)-4,2',4'-trihydroxychalcone and (3',5'-$^{3}H_2$)-7,4'-dihydroxyflavanone. A mixture containing equal amounts of the ^{14}C-labelled chalcone and tritium labelled flavanone was fed as precursor, and the ^{14}C/T ratio of the flavonoid product was used to indicate whether the chalcone or the flavanone had been the more direct precursor (Fig. 1.13). The results showed that in fact the chalcone was the more immediate precursor (i.e. ^{14}C/T > 1).

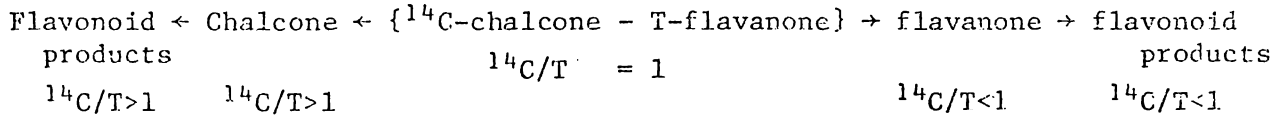


Figure 1.13. General plan of the double labelling used by Wong et al (1969) showing the expected results depending on whether chalcone or flavanone is the more immediate precursor in the biosynthesis of flavanoids.

1-3.1 Conversion of Chalcones (Flavanones) to 3-Hydroxyflavanones

Since a significant proportion of flavonoids contain a 3-hydroxyl group the conversion of chalcones (flavanones) to 3-hydroxyflavanones (dihydroflavonols) has been the subject of considerable interest. However, there is still no agreement regarding the origin of the oxygen atom in the 3-position. The addition of H_2O to the 2,3-double bond of a flavone and the pathway flavanone → flavone → flavanol → dihydroflavanol were among the earlier considered pathways, but labelling experiments (Grisebach, 1967) have eliminated them as a possibility. Mechanisms based on the chalcone-flavanone equilibrium (I ⇌ II) include the following (Fig.1.14):

(i) Direct oxidation of a flavanone to give a cation at C-3 followed by attack by OH^- (Grisebach 1968) (II → IIa → III)

(ii) Enolisation of the flavanone followed by direct reaction with the equivalent of OH^+ (Pelter et al, 1971) (II → IIb → III)

(iii) Attack by water or hydroxyl radical at the α-position of a chalcone radical formed by electron transfer to an enzyme. (Pelter et al 1971) (I → Ia → III).

(iv) Epoxidation of a chalcone as proposed for the Algar-Flynn-Oyamada (AFO) reaction. (Geissman 1948) (I → Ib → III).

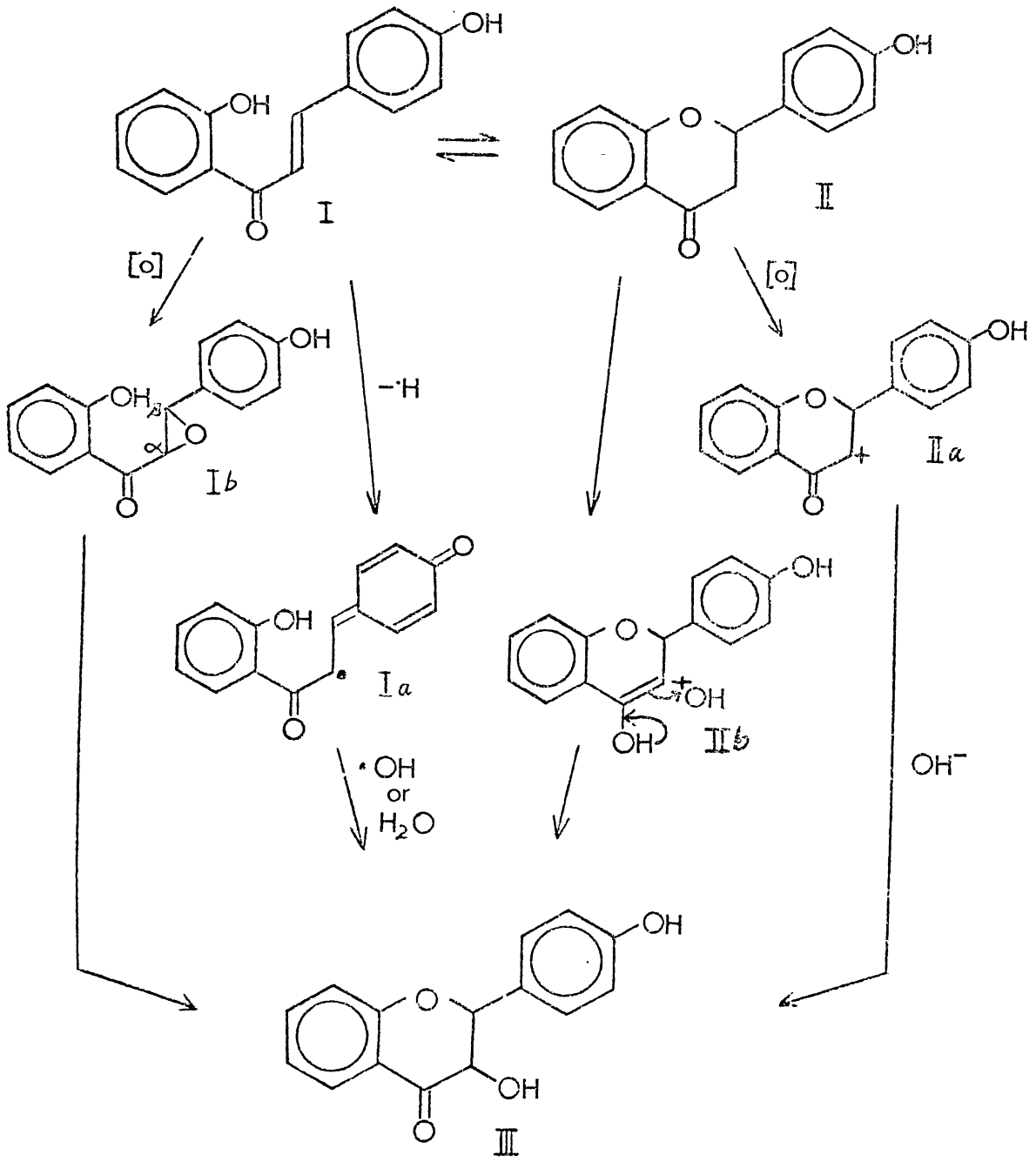


Figure 1.14. Possible pathways for the conversion of chalcones (flavanones) to 3-hydroxyflavanones.

The results of Wong et al (1969), which indicated that chalcones rather than flavanones were the more direct precursors of flavonoids ostensibly excludes routes (i) and (ii). There has been no speculation

about the precise chemical nature of the oxidants involved in the above suggested mechanisms.

1-3.2 Free Radical Mechanisms

Route I → Ia → III (Fig. 1.14.) was proposed by Pelter et al (1971) as a result of studies on the oxidation of chalcones by potassium ferricyanide. They have also proposed further free radical mechanisms which could account for the formation of a large section of flavonoids (Fig. 1.15.), but it should be reiterated that they require the chalcone (flavanone) intermediate to have a 4' (or 2') - hydroxyl group on the B-ring, and do not account for flavonoids that do not bear a hydroxyl group on the B-ring. The overall conclusions were that biosynthetically these reactions could be catalysed by peroxidases via free radicals as intermediates.

A free radical route to 3-hydroxyflavanones is also supported by the work of Chawla et al (1978) on the photochemical conversion of 2'-hydroxy-4',6',3,4-tetramethoxychalcone to 5,7,3',4'-tetramethoxyflavanone. They suggested that the source of the 3-hydroxyl group in the 3-hydroxyflavanone was probably water and since the chalcone studied contained no hydroxyl groups in the B-ring they proposed that such hydroxyl groups are not necessarily involved in the biosynthesis.

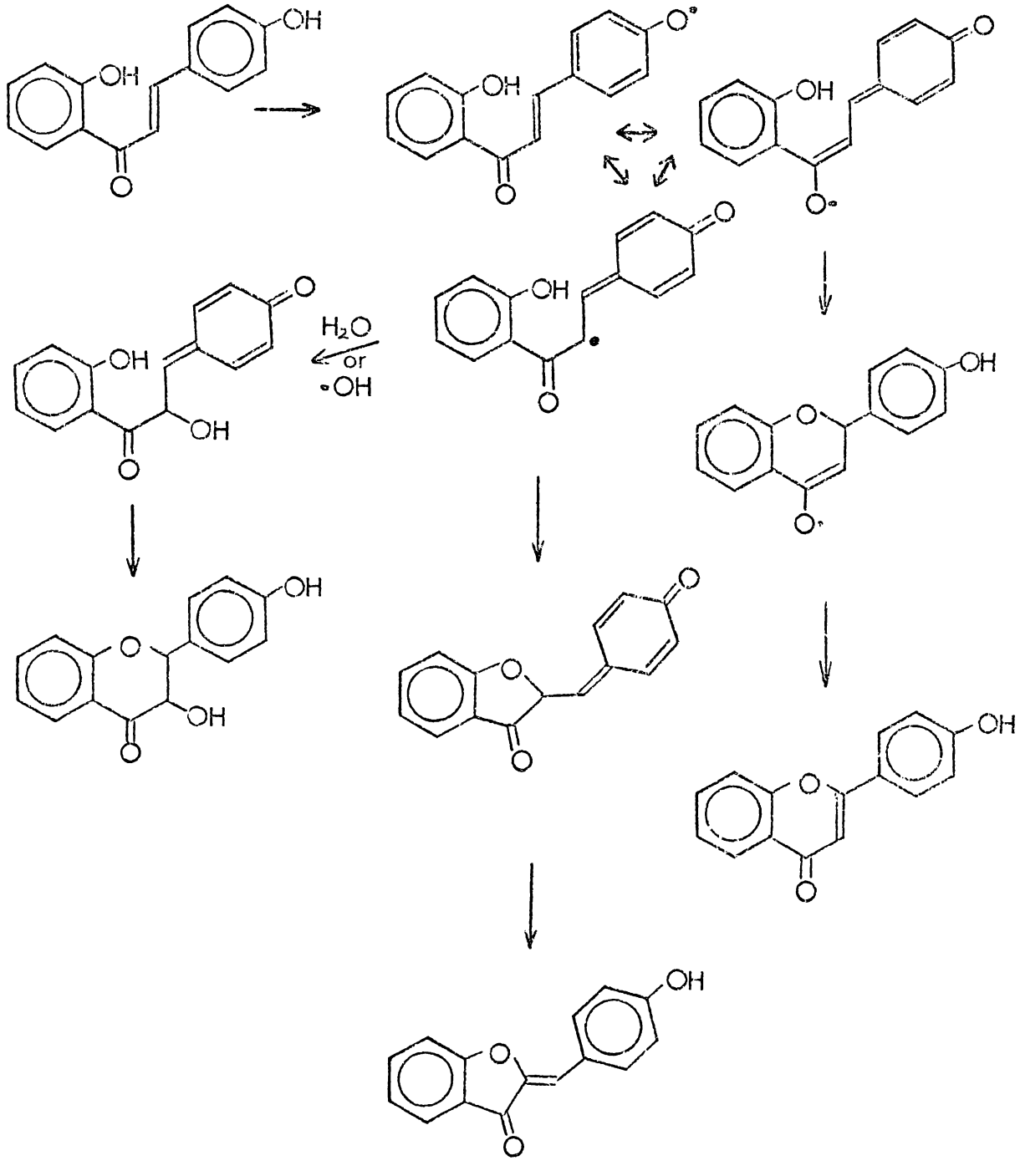


Figure 1.15. Mechanisms proposed by Pelter et al (1971) for the conversion of chalcones to 3-hydroxyflavanones, aurones and flavones.

1-3.3. Chalcone-Flavanone Oxidase

There have been very few enzymic studies on chalcone (flavanone) oxidations, but an enzyme which catalyses the transformation of naringenin (5,7,4'-trihydroxyflavanone) to apigenin (5,7,4'-trihydroxyflavone) has been located in parsley (Grisebach et al, 1974). The enzyme had a pH optimum between 7 and 7.5 and its reaction was dependent on a co-factor, probably Fe^{2+}/Fe^{3+}. Data indicated that this particular enzyme could not be a peroxidase since the addition of catalase to the incubation mixture had no influence on the yield of apigenin. Chalcone-flavanone isomerase was active in the crude extract so it was not possible to determine whether the chalcone or the flavanone was the actual substrate for the enzyme.

1-3.4. The Possibility of an Epoxide Intermediate

The suggestion that 3-hydroxyflavanones may be formed via an epoxide mechanism, as outlined in Fig. 1.14. (I → Ib → III), has arisen mainly as a result of work on the Algar-Flynn-Oyamada (AFO) reaction (see section 1-4.1). The formation of a 3-hydroxyflavanone would require cyclisation to the β-carbon of a chalcone epoxide but, as will be shown in Section 1-4.1, such an epoxide could also cyclise to the α-carbon resulting in the formation of aurone hydrates and aurones (see Fig. 1.16.)

Wong (1967, 1971) suggests that since other flavonoids are derived from chalcone rather than flavanone, and since they only differ from the chalcone in the state of oxidation of the central C_3 chain, then biochemical oxidation (and reduction) reactions involving the chalcone nucleus would appear to be key steps in the elaboration of these compounds. On this basis he proposed the epoxide mechanism shown in Fig. 1.16 for the biosynthesis of 4',6-dihydroxyaurone.

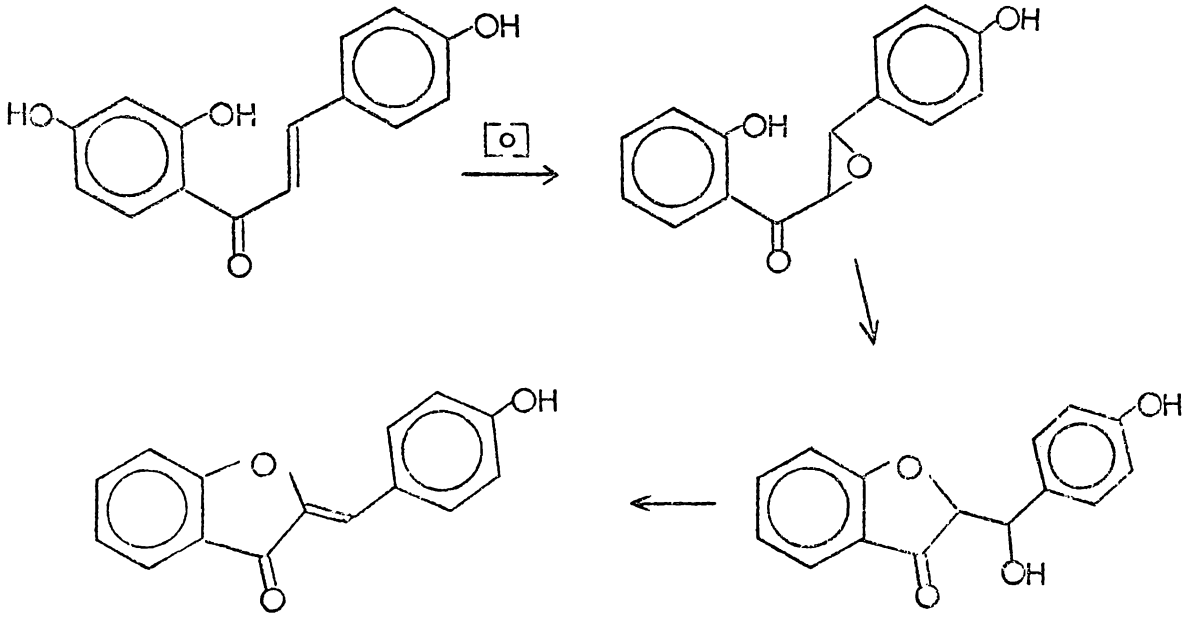


Figure 1.16. Possible mechanism for aurone biosynthesis. Wong (1971).

Biochemically such transformations would involve enzymes of the hydroxylase and isomerase types, and although the dehydration of the aurone hydrate to aurone would occur readily it is probable that in vivo this step is also enzyme catalysed.

Jain (1965) has indicated the possible central role of chalcone epoxides in the formation of many flavonoids, including dibenzoylmethanes, flavones, isoflavones, aurone hydrates, aurones, 3-hydroxyflavanones and flavonols.

1-4 CHEMICAL CONVERSION OF CHALCONES (FLAVANONES) TO OTHER FLAVONOIDS

1-4.1 The Algar-Flynn-Oyamada (AFO) Reaction

This reaction involves oxidation of 2'-hydroxychalcones by means of alkaline hydrogen peroxide and was first described by Algar and Flynn (1934) and Oyamada (1934). Originally it was thought that flavonols were the only product of the reaction, but it is now established that, depending upon ring substituents and the reaction conditions, 3-hydroxyflavanones, aurones, 2-benzyl-2-hydroxy-dihydro-benzofuran-3-ones, and 2-arylbenzofuran-3-carboxylic acids can all be formed (Cummins et al, 1963). The mechanisms for the formation of 3-hydroxyflavanones (I), flavonols(II), (route [A]) and aurones (III), (route [B]) as proposed by Geissman et al (1948) and supported by Cummins et al (1963) are shown in Fig. 1.17.

Chalcones with a hydroxyl group in position 2 or 4 and lacking a 6'-substituent cyclise predominantly to the β-position (flavonol formation) whereas chalcones with a 6'-substituent and lacking a 2- or 4-OH group can cyclise to either the α-position (aurone formation) or the β-position. It has been determined by Philbin et al (1956) from their study of 2'-hydroxy-6'-methoxychalcones, that the formation of aurones is favoured by oxidation in the cold, whereas high termperatures favour flavonol production.

The intermediacy of an epoxide in the AFO reaction does not receive universal support. Dean et al (1965) proposed that the course of the reaction of 2'-hydroxychalcones to flavonols does not involve epoxides.

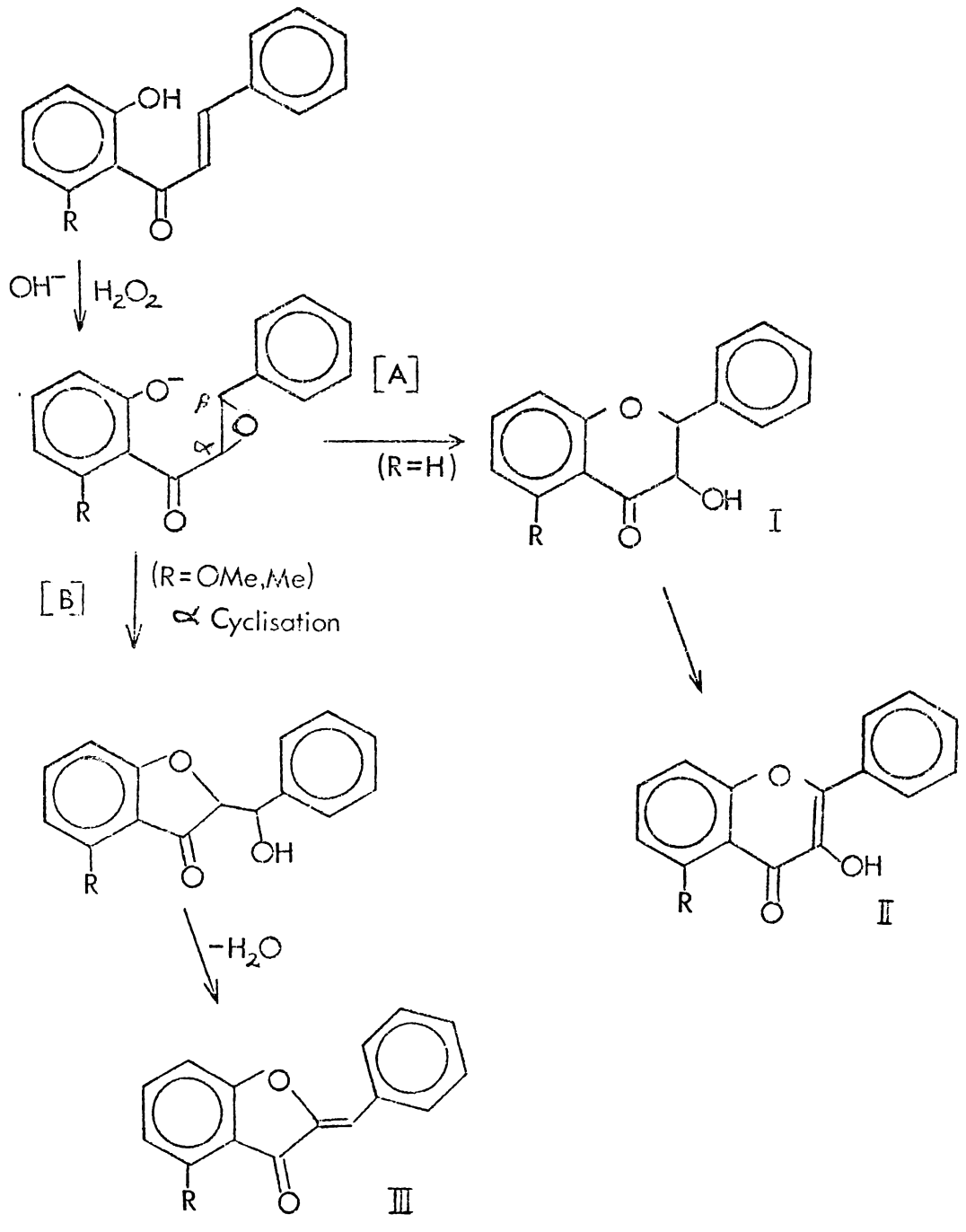


Figure 1.17. Mechanism for epoxide cyclisation in the formation of flavonoids in the AFO reaction.

Instead, they suggested that the pyrone ring is produced either before oxidation (route [C] Fig. 1.18.) or concurrently with it (route [D] Fig. 1.18).

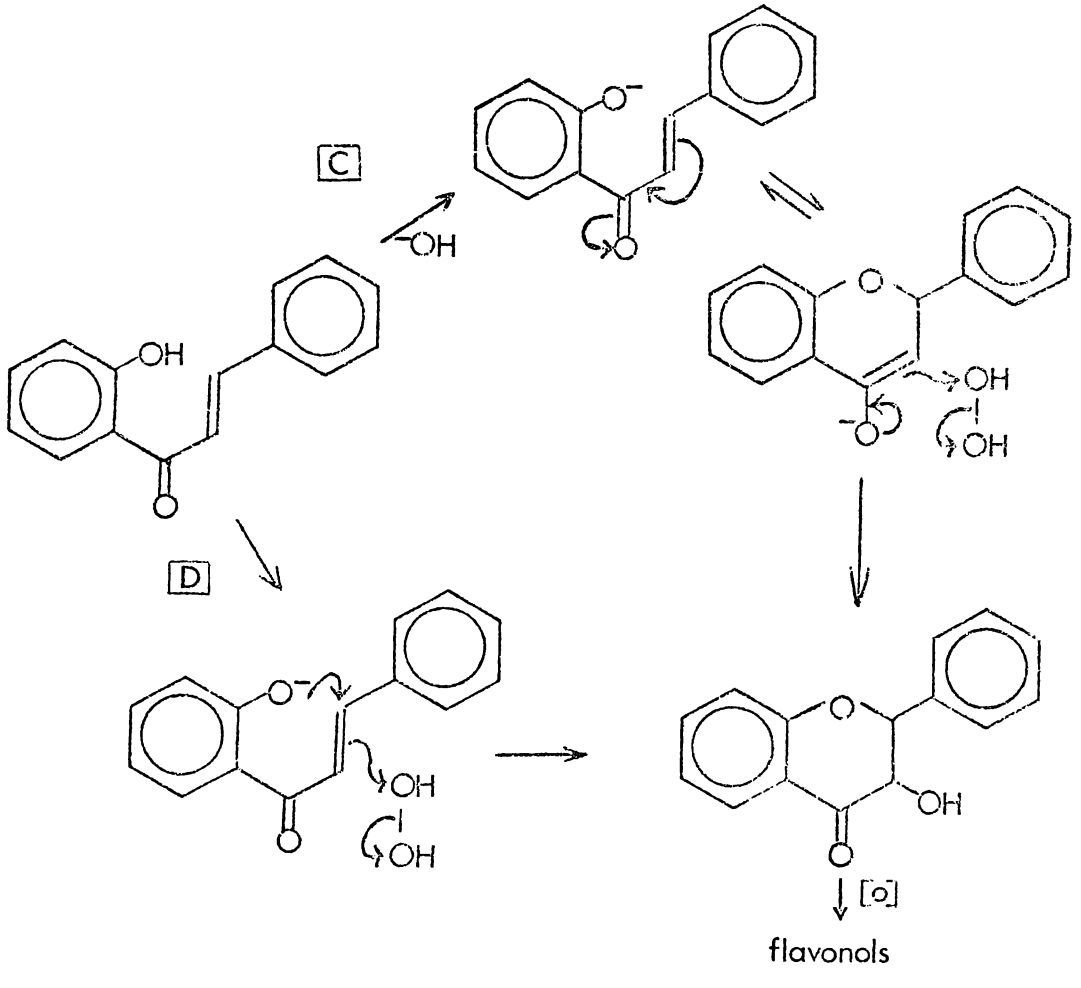
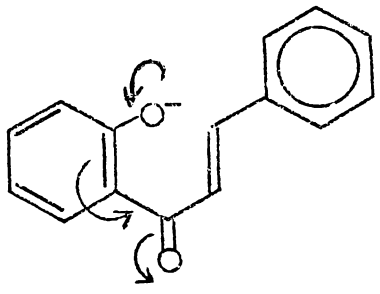


Figure 1.18. Routes proposed by Dean et al (1965) for the conversion of 2'-hydroxychalcones to flavonols in the AFO reaction.

Their conclusions evolved from the work of Bunton et al (1949), who showed that epoxidation of α, β-unsaturated ketones required an attack at the β-position by hydroperoxide anion. Dean et al (1965) considered this would be very difficult in 2'-hydroxychalcones because the strongly alkaline conditions would convert them into anions and consequently there would be coulombic repulsion of the reagent as well as internal electronic inactivation as indicated in the structure below.



The formation of aurones from chalcones with 6'-substituents in route [B] (Fig. 1.17.) is supported by these workers. They reason that although there would still be coulombic repulsion, the carbonyl group would be protected from an inactivating interaction because it is prevented for steric reasons from obtaining coplanarity with the phenolic ring.

There have been numerous explanations for the effect of a 6'-substituent in directing ring closure to the α-position. One consideration was that the steric repulsion between the 6'-substituent and the carbonyl oxygen would favour the product with the smaller heterocyclic ring (Barr et al, 1959). Dean et al (1965) suggest that displacement of the carbonyl group out of the plane of the phenolic ring by steric interaction with the 6'-substituent increases the distance of the phenolic oxygen from the β-position more than from the α-position. They also suggest that these steric effects are not large and diminish rapidly as the temperature rises leading to flavonol formation and hence providing an explanation for the results of Philbin et al (1956). In certain limited circumstances (i.e. high temperature oxidations of chalcones with 6'-substituents), therefore, they accept route [A] in Fig. 1.17. as being probable. However, more

recent work by Gormley et al (1973) on the reactions of 2'-tosyloxychalcone epoxide and 6'-methoxy-2'-tosyloxychalcone epoxide under AFO conditions, has shown that once an intermediate chalcone epoxide is formed, the 6'-methoxyl-substituent directs cyclisation exclusively to the α-position over a range of temperatures. Their explanation for flavonol formation on the oxidation of 2'-hydroxy-6'-methoxychalcones at temperatures greater than 20°C is that the inhibition to resonance between the 2'-O^- ion and the carbonyl group, caused by steric interaction between the 6'-methoxyl group and the carbonyl group, which facilitates epoxide formation, is overcome at higher temperatures. The reaction then proceeds by routes [C] or [D] (Fig. 1.18.) without the formation of an intermediate epoxide.

Litkei et al (1973) reject the intermediacy of a chalcone epoxide in the AFO reaction and Dean's proposal that oxidation could take place simultaneously with the cyclisation.

Dean et al (1965) also provide a reason why 6'-substituted 2'-hydroxychalcones with a 2- or 4-hydroxyl group form only flavonols. They regard coulombic repulsions as the decisive factor. These dihydroxychalcones would exist mainly as doubly charged anions, strongly repelling attack by hydroperoxide anions and route [B](Fig. 1.17) would be unfavourable. In contrast their alternative routes ([C] and [D] Fig. 1.18.) require only the approach of a neutral hydrogen peroxide molecule, and, further, the dianion would eliminate one of its charges as an integral part of the process, especially in route [D].

Thus, although the AFO reaction is regarded as a good chemical analogy for the biosynthetic pathway, there is little agreement as to its mechanism.

1-4.2. 2'-Hydroxychalcone epoxide and derivatives

Chemical studies on 2'-hydroxychalcone epoxides have been performed almost exclusively on epoxides in which the hydroxyl group in the 2'-position is substituted. The first preparation of a 2'-hydroxychalcone epoxide derivative was reported by Marathey (1955). 2'-Acetoxy-3',4'-benzo-4-methoxychalcone epoxide was formed by treating the bromohydrin derived from the corresponding chalcone dibromide with sodium acetate in acetone. It was found that heating this epoxide with acetone and 10% sodium carbonate gave 3-hydroxy-4'-methoxy-7,8-benzoflavanone.

Bognar and Stefanovsky (1962) established that epoxidation of 2'-hydroxychalcone derivatives was possible with alkaline hydrogen peroxide provided that the hydroxyl group was substituted. By studying the reactions of 2'-benzyloxy-,2'-p-nitrobenzyloxy- and 2'-methoxychalcone epoxide they found that the reaction products depended on the 2'-substituent. 2'-Benzyloxychalcone epoxide in ethereal hydrogen chloride yielded 3-hydroxyflavanone. Similar results were obtained using halogen acids in acetic acid or boiling with iodic acid in acetone, but by reacting the epoxide with stannic tetrachloride in benzene the corresponding chlorohydrin was formed (See Fig. 1.19). In the case of 2'-p-nitrobenzyloxy- and 2'-methoxychalcone epoxide the only product obtained from any of the above reactions was the chlorohydrin.

The effect of substituents in the B-ring of the epoxide was researched by Bhara et al (1964) by looking at the reactions of epoxides with a 4-methoxyl group. They found that from 2'-benzyloxy-4-methoxy-, 2',4'-dibenzyloxy-4-methoxy- and 4,2'-4'-

-trimethoxychalcone epoxide any of the following could be formed, depending on the reaction conditions: chlorohydrin, glycol monoacetate, 1,2-diketone and isoflavone (formed by cyclisation of an α-formyldeoxybenzoin), but no 3-hydroxyflavanone (see Fig. 1.19) Bognar and Stefanovsky (1962) had found that 2'-benzyloxychalcone epoxide in ethereal hydrogen chloride afforded 3-hydroxyflavanone and their result was confirmed by these workers. A further experiment using 2'-benzyloxy-4'-methoxychalcone epoxide under the same conditions also gave only the 3-hydroxyflavanone, a result verified by Chopin and Durual (11963, 1965). From these results it was assumed that substituents in the A-ring of the chalcone epoxide did not hinder ring closure, but that substituents in the B-ring could alter the course of the reaction.

Three epoxides with a 2'-methoxymethyl protecting group were found to give the same product irrespective of what B-ring substituents were present in the epoxide. (Aubry & Chopin, 1971). That is 2'-methoxymethyloxy-4',6'-dimethoxy-, 2'-methoxymethyloxy-4',6'-dimethoxy-4-methoxy-, and 2'-methoxymethyloxy-4',6'-dimethoxy-3-methoxychalcone epoxide all yielded 3-hydroxyflavanones when treated with sulphuric acid. Litkei et al (1972) suggest that the 2'-methoxymethyl protecting group will increase the nucleophilic character of the ether oxygen and so promote direct cyclisation, leading to 3-hydroxyflavanones. By analogous reasoning they propose that epoxides with a p-nitrobenzyl protecting group give solely chlorohydrins (Bognar & Stefanovsky, 1962) because the p-nitro group decreases the nucleophilic character of the ether oxygen and hence hinders direct cyclisation. This principle was employed by Clark-Lewis and Tucker (1976) to form *trans*-3-hydroxy-7,4'-dimethoxyflavanone. 2'-Benzyloxy-4,4'-dimethoxychalcone epoxide with

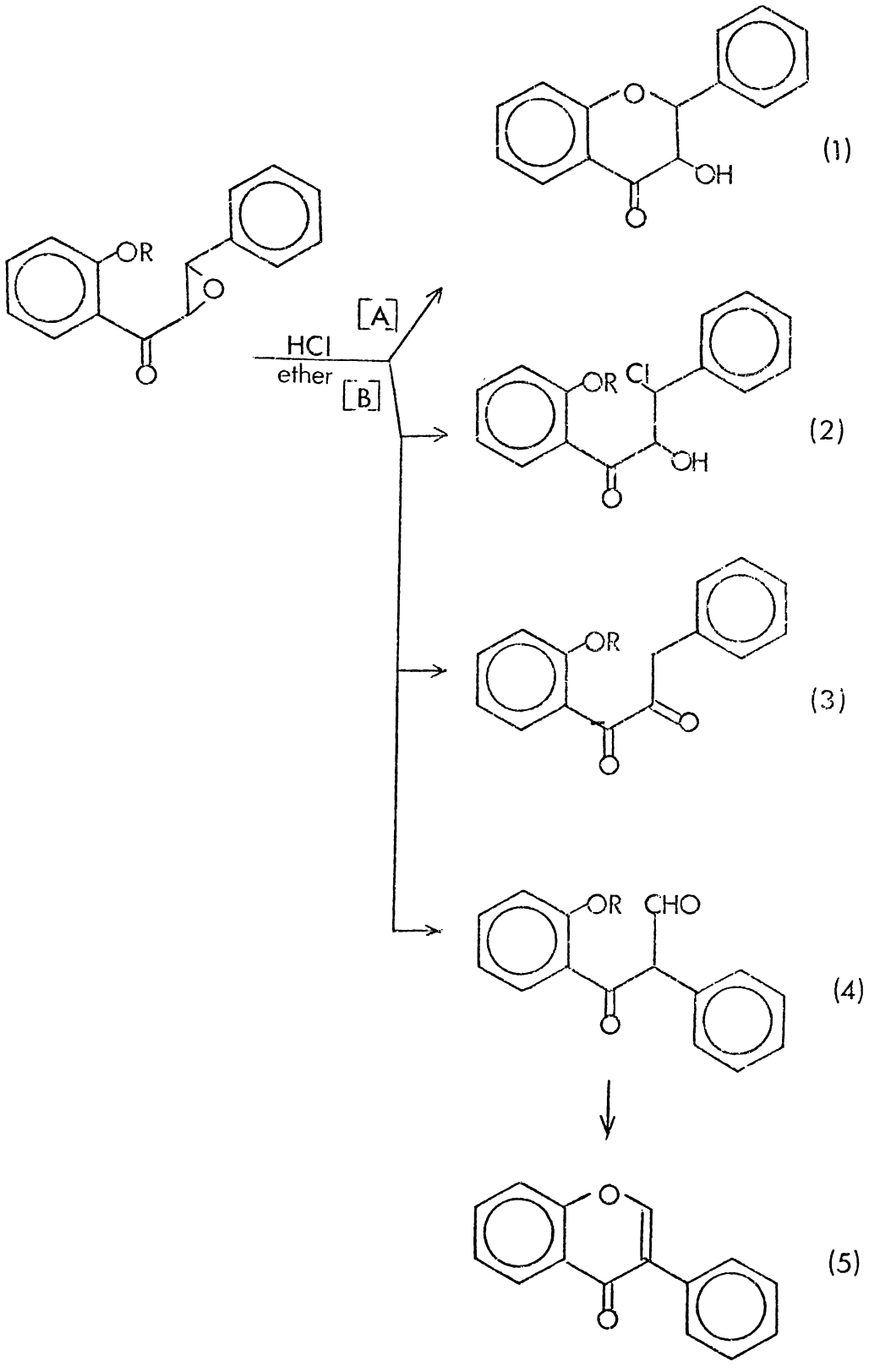


Figure 1.19. Summary of the possible reaction products from chalcone epoxides. Route [A] leading to 3-hydroxyflavanones (1) is favoured by electron-withdrawing substituents in ring B and electron-releasing 2'-protecting groups. Route [B] leading to chlorohydrins (2), diketones (3) α-formyldeoxybenzoins (4) and isoflavones (5) is favoured by electron-releasing substituents in ring B and electron-withdrawing 2'-protecting groups.

hydrochloric acid in acetic acid gave no 3-hydroxyflavanone, but the corresponding 2'-(4-methoxybenzyloxy) epoxide gave the required product, presumably due to the electron-releasing substituent in the benzylic protecting group facilitating cyclisation by increasing the nucleophilicity of the ether oxygen.

Litkei et al (1972) have compared the reactions of a large number of substituted 2'-benzyloxychalcone epoxides in ethereal hydrogen chloride and in boron trifluoride etherate, and have related the effect of the B-ring substituents on the course of the reaction to their Hammett σ value. In epoxides with electron-withdrawing substituents ($\sigma>0$) in ring B the electron density of the β-carbon is decreased, thus favouring debenzylation and internal nucleophilic attack of the ether oxygen leading to 3-hydroxyflavanones. For epoxides with electron-releasing substituents ($\sigma<0$) in ring B the electron density is increased on the β-carbon and direct cyclisation is hindered. Instead, the oxirane ring is cleaved generating a carbonium ion which can undergo nucleophilic attack leading to chlorohydrins, or can rearrange by aroyl migration to α-formyldeoxybenzoin and then cyclise to the corresponding isoflavone derivative (Fig. 1.19). If the Hammett σ value is about zero (± 0.2) a mixture of chlorohydrins and 3-hydroxy flavanones can be formed. A further interesting result was that 2'-benzyloxy-5',6'-benzochalcone epoxide in ethereal hydrogen chloride formed the chlorohydrin and not the 3-hydroxyflavanone as had been observed for all previous reactions of 2'-benzyloxychalcone epoxides lacking B-ring substituents. Therefore, contrary to the previous literature (Chopin, 1963, 1965; Bhara, 1964) which stated that substituents in the A-ring had no directing effect, the results of Litkei et al (1972) indicate that the cleavage of the oxirane ring

and the direction of its conversion may depend not only on substituents in the B-ring but also on those in ring A.

There have been only two reported preparations of chalcone epoxides with a free 2'-hydroxyl group. Verma and Bokadia (1965) described the formation of 2'-hydroxy-5'-methyl-4-methoxychalcone epoxide by the action of monoperphthalic acid on the corresponding chalcone, and Ramakrishnan and Kagan (1970) formed the parent epoxide using m-chloroperoxybenzoic acid. The epoxide was well characterised in the latter case, but in the former report the chemical data did not completely exclude the possibility that their "epoxide" was in fact the corresponding 3'-hydroxyflavanone or a mixture of the two compounds. It was shown that 2'-hydroxychalcone epoxide converted to 3-hydroxyflavanone in base, but no further reactions were studied.

As well as being postulated as intermediates in the AFO reaction (see Section 1-4.1), 2'-hydroxychalcone epoxides have also been implicated (Donnelly et al, 1975) in an analogous reaction involving the ring closure of 2'-hydroxychalcone dibromides (see section 1-4.3) with alkali (the Emilewicz-von Kostanecki reaction). In this reaction, too, the number and position of substituents in the two aromatic rings influence the course of the reaction and determine whether a flavone or an aurone is formed. The authors reported that 2'-hydroxychalcone dibromides with a 6'-methoxyl group (Class 2A, see section 1-4.3) formed little or no aurone in dilute ethanolic alkali. However, the proportion of aurone to flavone was found to increase with increasing hydroxide concentration, and a mechanism involving chalcone epoxides was proposed to account for the effect of base on aurone formation (Fig. 1.20).

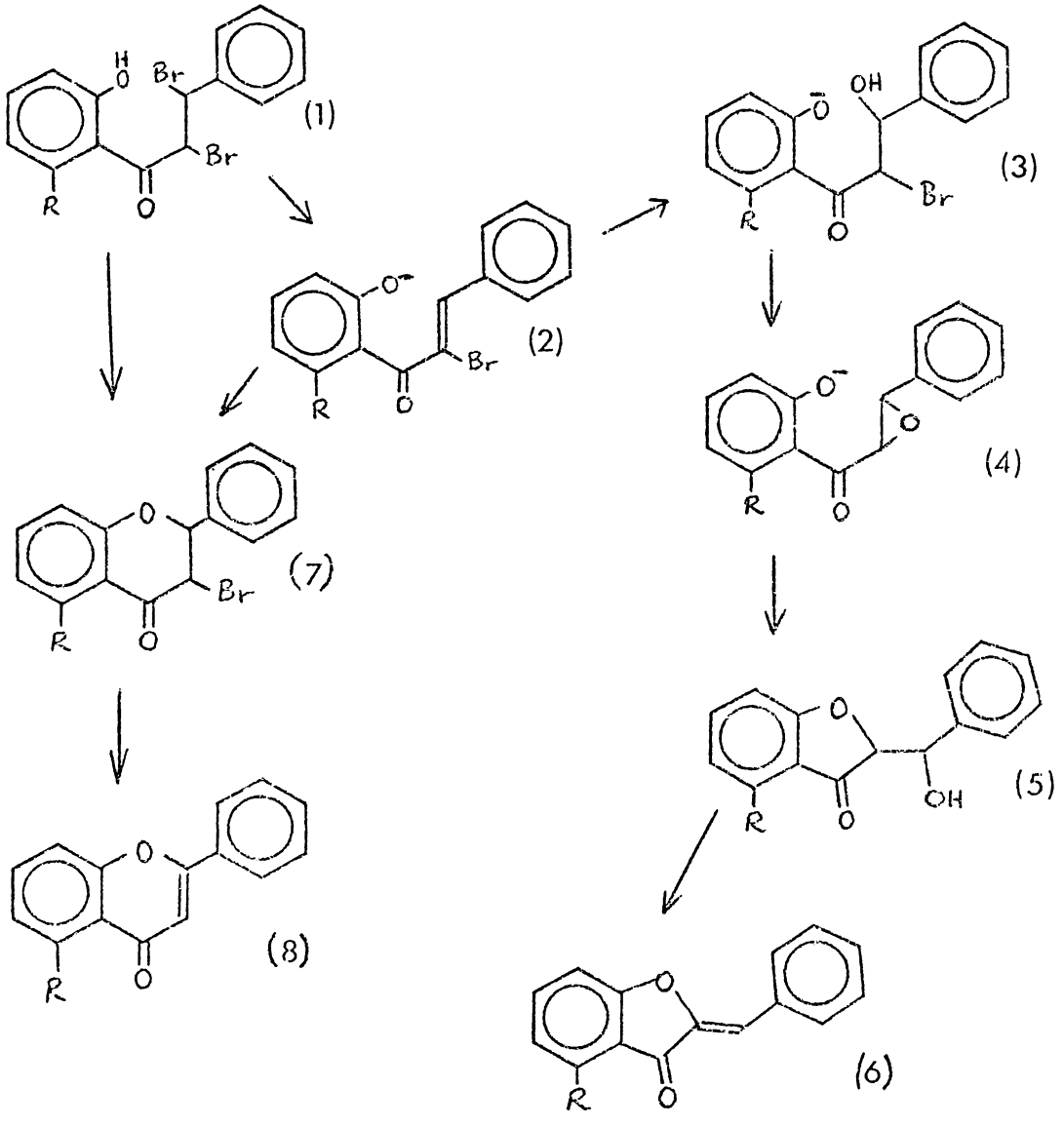


Figure 1.20. Mechanism proposed by Donnelly et al (1975) to account for the formation of flavones and aurones from 2'-hydroxychalcone dibromides (1).

They have suggested that the higher the hydroxide concentration the more favourably intermolecular attack by hydroxide on the double bond of the α-bromochalcone (2) competes with intramolecular attack by phenoxide. The resulting bromohydrin (3) can then form an epoxide (4) which can cyclise to the aurone hydrate(5), leading finally to aurone (6). The observation that 3'-bromo-4'-hydroxy-2',6'-

dimethoxychalcone dibromide, under identical reaction conditions, yielded the corresponding epoxide, gave support to the intermediacy of epoxides in aurone formation from Class 2A chalcone dibromides.

1-4.3 Conversion of 2'-hydroxychalcone dibromides to flavones and/or aurones

2'-Hydroxychalcone dibromides are conveniently formed by the reaction of 2'-hydroxychalcones with a range of brominating agents, and under a variety of conditions (Litkei et al 1971, 1972). A lot of the interest in these compounds is due to the ease with which they can be converted to flavones and/or aurones in aqueous alcoholic alkali (Emilewicz and von Kostanecki, 1898) (see Fig. 1.20). The ratio of flavone to aurone in the final product is dependent upon the nature and position of the substituents in the A and B rings and on the conditions of the reaction. Although studies have been performed on the Emilewicz-von Kostanecki reaction for many years, most of the recent work on determining its course and mechanism is recorded within a series of reports by Donnelly et al (1966-1977).

2'-Hydroxy (or acetoxy) chalcone dihalides have been divided into two classes (Donnelly et al, 1973) depending on the products of cyclisation with alcoholic alkali : those that yield flavones exclusively (Class 1) and those that yield some aurone (Class 2). The latter class has been further subdivided as follows: Class 2A being those with a 6'-methoxyl substituent in the A-ring, Class 2B those with a 2- or 4-alkoxyl group in the B-ring, and Class 2C those having a 2- or 4-nitro group in the B-ring. Most studies have been performed on chalcone dihalides of Class 2 as many of these yield naturally occurring flavonoids.

Since von Kostanecki and Tambor (1899) formed an α-bromochalcone by brominating 2'-acetoxy-4',6'-dimethoxy-3,4-methylenedioxychalcone and found that it cyclised in alcoholic potassium hydroxide to give the aurone, it has been often suggested that α-bromochalcones might be intermediates in the cyclisation of Class 2A 2'-hydroxychalcone dibromides to aurones in the Emilewicz-von Kostanecki reaction. (Auwer and Anschutz, 1921; Hutchins and Wheeler, 1939). Donnelly et al (1973) added support to the possible role of α-bromochalcone intermediates by showing that α-bromo and α-chloro derivatives of 2'-acetoxy-3'-bromo-4',6'-dimethoxychalcone, when cyclised with alcoholic potassium hydroxide gave aurone in similar amounts to those obtained from the corresponding chalcone dihalides.

Further work on Class 2A chalcone dibromides (Donnelly et al 1974, 1975) established that the formation of aurone decreased as the base concentration was decreased, and the mechanism proposed to account for this effect is shown in Fig 1.20. In order to gain additional support for the intermediacy of the 2'-hydroxy-α-bromochalcones as proposed in Fig. 1.20 Donnelly et al (1975a) formed the E- and Z-2'-hydroxy -α-bromochalcones from several 2'-hydroxychalcone dibromides under mildly basic conditions, and studied their cyclisation to determine whether or not they yielded flavone and aurone and whether the product composition was dependent on base concentration. Both the E- and the Z-isomers yielded flavone and aurone, and the proportion of aurone was greater at the higher hydroxide concentration, this being in agreement with the results expected if they were in fact intermediates in the reaction.

The E- and Z-isomers of 2'-hydroxy-α-bromo-4-nitrochalcone were prepared by the action of 2'-hydroxy-4-nitrochalcone dibromide (Class 2C)

with ethanolic potassium acetate in small yield (Donnelly, 1977) and their intermediacy in the Emilewicz-von Kostanecki reaction was tested by following their cyclisation in aqueous ethanolic potassium hydroxide (1.0 mol l^{-1}). Both isomers yielded flavone and aurone, but in quite different ratios. The E-isomer gave aurone to flavone in a ratio of 6.7:1 while the Z-isomer gave aurone to flavone in a ratio of 1:2. If the cyclisation of 2'-hydroxy-4-nitrochalcone dibromide occurred entirely through the E- and Z-isomers they would have had to be formed in the ratio 30:70 in order that the aurone/flavone ratio of ≈ 2:1 be observed. NMR data on the E-isomer/Z-isomer ratio for the elimination products from 2'-acetoxy-4-nitrochalcone dibromide indicated a value of 25:75, which is very close to that required considering that the data were not from the 2'-hydroxy-4-nitrochalcone dibromide but from its acetate.

2'-Hydroxy-α-bromochalcones clearly have an important role in determining the ratio of aurone to flavone formed in the above examples, but for 2'-hydroxychalcone dibromides which form no aurone (Class 1) the possible role of 2'-hydroxy-α-bromochalcones has been little considered and not explored. The failure to isolate them in an early attempt (Auwer & Anschutz, 1921) led to the view that in the formation of flavones the first formed intermediate was a 3-bromoflavanone (Fig. 1.21.)

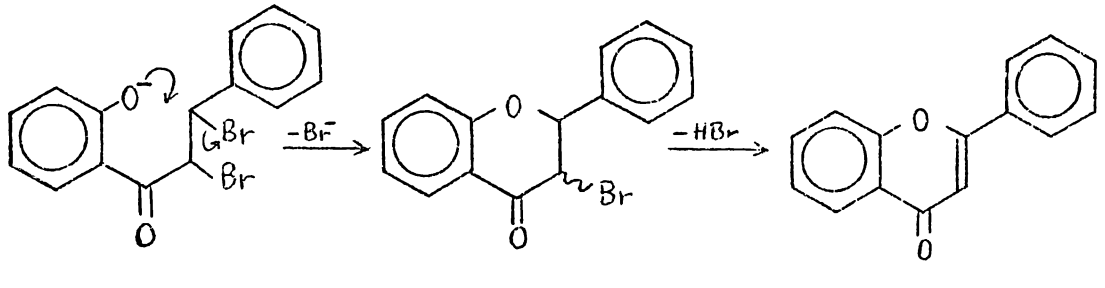


Figure 1.21. Possible direct cyclisation of the dibromide anion, leading through 3-bromoflavanone to flavone.

Although this mechanism has frequently been considered for the cyclisation of 2'-hydroxychalcone dibromides which lead ultimately to flavones, it has never been established whether this route does occur or whether elimination-addition sequences (Fig. 1.22.) occur in competition or even exclusively. The course of the reaction is therefore worthy of study.

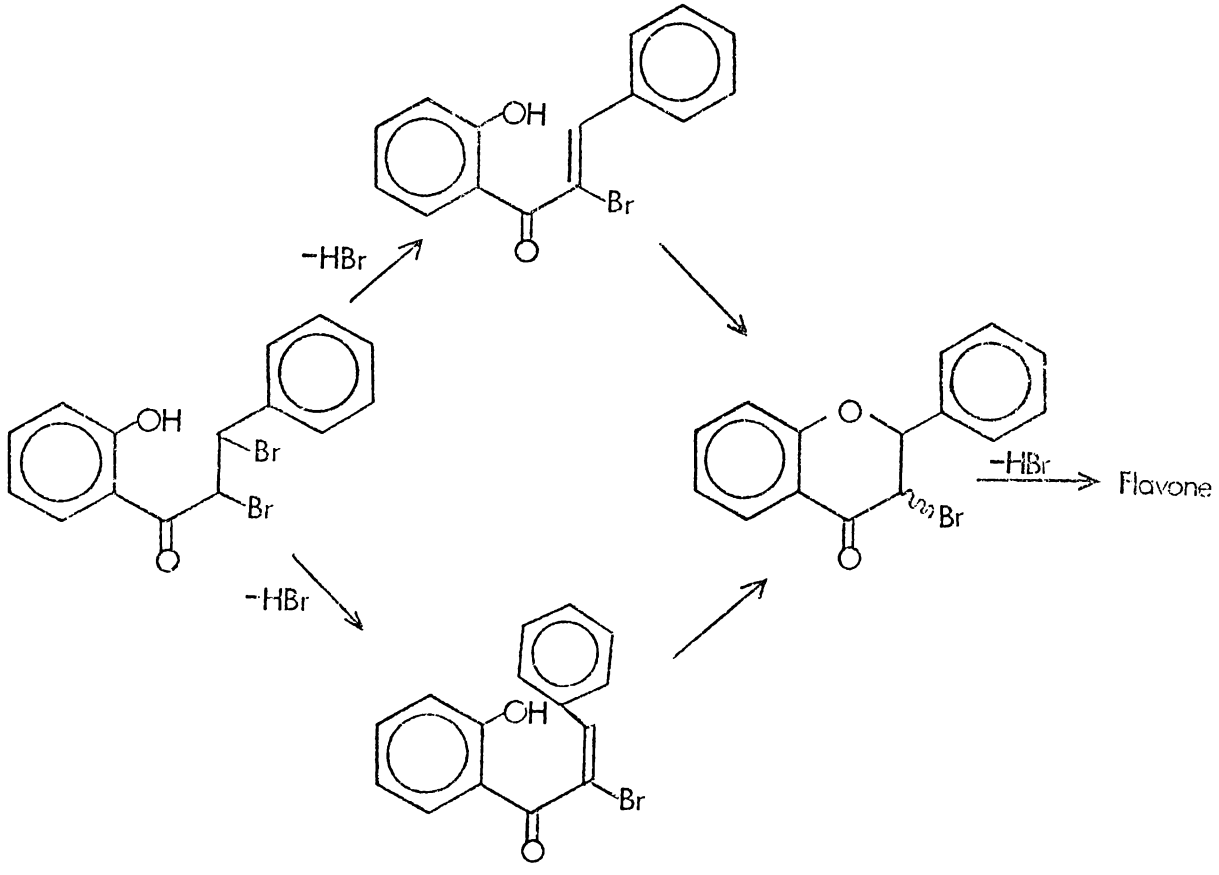


Figure 1.22. Possible elimination-addition sequences for the formation of flavones from Class 1 chalcone dibromides.

1-5 OUTLINE OF AIMS

The following is a summary of the main aims of this study.

(i) Although the 2'-hydroxychalcone-flavanone isomerisation reaction represents an important step in the biosynthesis of flavanoids, a systematic study of factors that affect the rate of this reaction has not been performed. We therefore set out to prepare a series of 2'-hydroxychalcones, so that kinetic studies could be carried out in order to determine the effect that substituents in the A-ring have on the rate of attainment of the 2'-hydroxychalcone-flavanone equilibrium. Of particular interest was the reaction of 2'-hydroxychalcones with 6'-substituents, as it is possible that such substituents could force the carbonyl group to lie out of the plane of the aromatic ring, an effect which could change the rate of the reaction.

(ii) Considering the controversy over the role, if any, of 2'-hydroxychalcone epoxides in the biosynthesis of flavanoids and in the AFO reaction, an investigation into the reactivity of these compounds was of interest. Thus, the objective was to prepare a 2'-hydroxychalcone epoxide and study the rate of its cyclisation in aqueous solution.

(iii) The possible role of 2'-hydroxy-α-bromochalcones in the cyclisation of class 1 chalcone dibromides has not been researched, and it was therefore our intention to study the cyclisation of *erythro*-2'-hydroxychalcone dibromide in an effort to determine whether E- and Z-2'-hydroxy-α-bromochalcone are involved as intermediates on the route to flavone or whether the cyclisation to 3-bromoflavanone occurs directly.

2. SYNTHESIS OF 2'-HYDROXYCHALCONES AND DERIVATIVES

In theory there are at least four ways of forming the $C_6-C_3-C_6$ flavonoid skeleton (Gripenberg, 1962), but only the following two methods are of practical importance: (i) Condensation of an *o*-hydroxyacetophenone with an aromatic aldehyde; (ii) Acylation of phenols with a cinnamic acid derivative. The preparation of 2'-hydroxychalcones is confined almost entirely to the first of these methods.

2-1 PRELIMINARY EXPERIMENTAL DETAILS

2-1.1. Starting Materials

The following chemicals were obtained from the named company and used without further purification (Analytical grade reagents are shown as AR, and Laboratory grade reagents as LR).

(i) *o*-hydroxyacetophenone (LR), Aldrich and BDH
(ii) benzaldehyde (AR), BDH
(iii) acetic anhydride (LR), May and Baker
(iv) anhydrous aluminium chloride (LR), BDH
(v) methyl iodide (LR), Fisons
(vi) tetra-n-butylammoniumhydroxide (LR), BDH
(vii) m-chlorobenzoyl chloride (LR), BDH
(viii) 2,6-di-t-butyl-4-methylphenol (99%), Aldrich
(ix) 3-t-butyl-4-hydroxy-5-methylphenylsulphide (99%), Aldrich
(x) diethyl ether (AR), BDH
(xi) petroleum ether (AR), BDH
(xii) ethanol (AR), Ajax
(xiii) bromine (AR), Hopkin & Williams

The following materials were further purified.

(i) 2,4-dihydroxyacetophenone (LR), BDH;
2-hydroxy-4-methoxyacetophenone (99%), Aldrich;
2-hydroxy-4,6-dimethylacetophenone (97%), Aldrich;
2,6-dihydroxyacetophenone (97%), Aldrich; and
β-naphthol (AR), BDH, were all recrystallised at least twice from ethanol before use.

(ii) 1,2-dichloroethane (LR), May and Baker, was purified by washing with concentrated sulphuric acid, followed in succession by 5% sodium hydroxide, water, and a solution of saturated sodium chloride. It was dried over anhydrous calcium chloride and then fractionally distilled using a Vigreux fractionating column fitted with a drying tube. Since 1,2-dichloroethane (b.p. 83.5°C) and water (b.p. 100°C) form an azeotropic mixture that boils at 72°C, and contains 80.5% 1,2-dichloroethane and 19.5% water, any residual water would be removed from the solution in the early stages of distillation. For this reason only a middle fraction was retained.

(iii) m-chloroperoxybenzoic acid (85%), Aldrich, has as a contaminant m-chlorobenzoic acid and since this is more strongly acidic than the peracid it was removed by washing the 85% material with a phosphate buffer of pH 7.5 and drying the residue at reduced pressure (Nefkens, 1962). The above procedure was not always employed as, under the epoxidation conditions used in this study, the 85% material proved to be quite satisfactory without further purification. The m-chloroperoxybenzoic acid used in some of the earlier attempted epoxidations was made by the reaction of m-chlorobenzoyl chloride in alkaline peroxide (McDonald, 1970).

2-1.2. Spectroscopic and Chromatographic Methods

Ultraviolet-visible (UV) spectra were recorded on either a Cary 17 or a Pye Unicam SP 1800 spectrophotometer.
Proton nuclear magnetic resonance spectra (NMR) were recorded on a Jeol C-60HL instrument using either $CDCl_3$ or CCl_4 as solvent, and tetramethylsilane as the internal reference.
Infra-red spectra were recorded on either a Shimadzu IR-27G or a Perkin-Elmer 180 spectrophotometer.
Mass spectra were run at Ruakura Agricultural Research Centre on a Varian MAT CH5 instrument.
The silica gel used for thin layer chromatography (t.l.c.) was Kieselgel G, Type 60 (Merck), and for preparative layer chromatography (p.l.c.) Kieselgel 60 $PF_{254+366}$ (Merck).

It should be noted that the main objective in this synthetic work was to prepare a product of high purity, in sufficient quantity for future kinetic studies, and generally no attempts were made to maximise the yields.

2-2 THE PREPARATION OF 2'-HYDROXYCHALCONES

2-2.1. The Preparation of 2'-hydroxychalcone

The method used to prepare the parent 2'-hydroxychalcone was based on that originally described by Emilewicz and von Kostanecki (1898), and involves the condensation of *o*-hydroxyacetophenone with benzaldehyde in 50% sodium hydroxide solution.

Equimolar quantities of *o*-hydroxyacetophenone and benzaldehyde in absolute ethanol were stirred vigorously while aqueous sodium hydroxide

(50% 1.5 molar equivalents) was added very slowly. Stirring was continued until the mixture became solid (≈60 min). After the product had been kept at 0°C for 24 hours it was poured into an excess of ice-concentrated HCl (3:1) to precipitate the chalcone, which was then recrystallised from ethanol. The melting point of the resultant yellow crystals was 88°C which is in agreement with that determined by Emilewicz and von Kostanecki (89°C) for *trans*-2'-hydroxychalcone. The UV spectrum in ethanol (λ_{max} at 365 (inflection), 317, 220 nm) compares well with that determined by Jurd & Horowitz (1961), and the finding of the phenolic proton in the ^{1}H-nmr spectrum at 12.75δ is consistent with a hydrogen-bonded OH. The yield by this method was better than 60%.

Two slight variations were tried on the above method.

(i) Instead of the reaction being performed at room temperature and cooling the product at 0°C for 24 hours, the mixture was refluxed for one hour and immediately poured into the ice : HCl solution. (Cullen et al, 1971). Difficulties were experienced in recrystallising the product due to oil formation, but eventually yellow crystals were obtained. However, the melting point was only 63°C and it was later found that the product contained a significant amount of flavanone.

(ii) In this preparation the mixture was again stirred at room temperature until solid, but instead of cooling at 0°C for 24 hours it was immediately added to the ice : HCl solution. The chalcone separated as a yellow oil which was difficult to crystallise, but ultimately yellow crystals were obtained with a melting point of 87°C. The yield in this case was only ≈20%, and this fact combined with the difficulties

in purifying the product made this method impractical.

2-2.2. The Preparation of 2'-hydroxy-5',6'-benzochalcone

(i) β-Naphthyl acetate was prepared by the method of Vogel (1970) in which β-naphthol is dissolved in 10% sodium hydroxide, and to this is added ice and acetic anhydride. The mixture is shaken for ≈20 mins and the filtered product recrystallised from petroleum ether (b.p. 60-80°C). It had m.p. 71°C (lit. 71°C, Vogel, 1970).

(ii) 2-Hydroxy-5,6-benzoacetophenone was prepared from β-naphthyl acetate via a Fries rearrangement (Imoto, 1937). β-Naphthyl acetate (19g) and anhydrous $AlCl_3$ (20g) were finely ground together and heated slowly to 120°C (≈0.5 hr), this temperature being maintained for a further 0.5 hr. After decomposing the excess $AlCl_3$ with concentrated HCl : ice (1:10) the product was extracted into diethyl ether. It was then extracted into a 10% sodium hydroxide solution, acidified with concentrated HCl and taken up again in diethyl ether. This ether extract was then washed with water until neutral, dried with $CaSO_4$ and rotary evaporated. A red oil was obtained which was partially extracted into petroleum ether (b.p. 30°C - 40°C). On evaporation this yielded a yellow oil which was crystallised from ethanol-water, m.p. 62°C, (lit. 64°C, Imoto, 1937).

(iii) The preparation of 2'-hydroxy-5',6'-benzochalcone was first attempted using the same method as described in the first part of section 3-2.1. The product this time was, however, a reddy-brown mass which finally gave white crystals after

repeated recrystallisations. The melting point of this compound was 115°C which compares closely with the value reported by Tambor et al (1926) of 117°C for the corresponding flavanone, 5,6-benzoflavanone. The UV spectrum was also in agreement with the compound being 5,6-benzoflavanone (Lin et al, 1958). Several variations to the method were tried, but all gave 5,6-benzoflavanone on "purification". The method which utlimately proved the most satisfactory was to first form and purify 5,6-benzo-flavanone, then dissolve it in the minimum quantity of ethanol and open the ring by the addition of a 10% sodium hydroxide solution. The red solution was filtered to remove any unchanged 5,6-benzoflavanone and was reacidified with concentrated HCl:water (1:3) at 0°C. The now yellow solution was partially evaporated to remove the ethanol and then extracted into diethyl ether and evaporated to dryness, resulting in orange crystals. Recrystallisation was effected by dissolving the sample in the minimum amount of petroleum ether at room-temperature and allowing crystallisation to take place at -15°C. Bright yellow needles resulted (m.p. 79°C). There is considerable confusion within the literature regarding this compound and previously quoted melting points are as follows:

Fujise and Suzuki (195i), 88°C (dark orange);
Joshi and Shah (1952), 112-13°C (no colour quoted); and
Rao et al (1972), 109°C (no colour quoted).

We feel that in the latter two cases at least, their samples could have been predominantly 5,6-benzoflavanone (m.p. 115-117°C). Joshi and Shah quote analysis figures but these are of course no help in differentiating between the isomeric chalcones and flavanones. Rao et al report no additional data, but have brominated their product and report analysis figures for the dibromide (m.p. 154°C). On brominating our chalcone by the method of Litkei et al (1971, 1972) we obtained very pale green (almost colourless) needles with a m.p. 166°C. Its mass spectrum was consistent with the compound being the dibromide, showing a molecular ion at m/e 434, and a base peak at m/e 170 as a result of cleavage α to the carbonyl group. The spectrum was typical for compounds with two bromine atoms, showing the expected isotopic patterns. It is possible therefore that Rao et al were in fact brominating a compound with a high content of 5,6-benzoflavanone, a reaction that is well known (Chang, 1962) and can result in a mixture of mono- and dibromoflavanones, which would account for their analysis figures. Fujise and Suzuki used the same method in their preparation that gave us a very impure product and since they do not report any further chemical data it is also possible that their sample contained significant amounts of the flavanone.

Support for the authenticity of our product is as follows: t.l.c. shows only one (yellow) compound is present; a brown colour is produced with ethanolic $FeCl_3$, indicating a phenolic-OH; the ^{1}H-nmr is almost identical, apart from aromatic proton signals, to that of 2'-hydroxychalcone

showing a signal at 12.76, indicative of a hydrogen-bonded -OH, and on integration revealing a proton ratio of 1:13 when comparing the -OH signal with the rest of the spectrum; the UV spectrum shows that the compound converts readily to 5,6-benzoflavanone in weakly basic conditions (see Figs. 3.6 and 3.7); and the mass spectrum shows a molecular ion at m/e 274, and major fragment ions at m/e 197 and m/e 171 which would result from α-cleavage either side of the carbonyl group. Unfortunately the mass spectra of chalcones bearing a 2'-hydroxyl group are known to be almost identical to those of the isomeric flavanones (Van de Sande, 1972), so mass spectral studies alone would fail to differentiate between 2'-hydroxy-5',6'-benzochalcone and 5,6-benzoflavanone, but combined with the other evidence it provides good support for the sample being the required 2'-hydroxy-5',6'-benzo-chalcone.

2-2.3. The Preparation of 2',4'-dihydroxychalcone

There was confusion in the early literature regarding the preparation of this compound (see Bargellini and Marantonio, 1908; Mahal et al, 1935; Ellison, 1927) but it now a well documented chalcone (Saiyad et al, 1937; Mittal, 1946).

The major difference in the preparation of this compound compared with those previously mentioned is in the amount of sodium hydroxide needed to perform the synthesis. A mole ratio of sodium hydroxide to the acetophenone of ≈10:1 (c.f. 1.5:1,previously) was necessary in order to gain any satisfactory product. Most quoted preparations have involved condensation at room termperature (Saiyad et al, 1937) or below (Adityachaudhury, 1971) and keeping the reaction flask stoppered

for ≈7 days before work-up. These methods, and others involving hot condensations (Geissman & Clinton, 1946) were tried in order to determine the method which most readily yielded a pure product. It was considered a possibility that under these strongly basic conditions the benzaldehyde could undergo a simultaneous oxidation and reduction reaction to yield benzyl alcohol and benzoic acid (under the reaction conditions this would be as the sodium salt, but, if present, it would convert to the acid during work-up). This reaction is well known for aldehydes without α-hydrogen atoms (the Cannizzaro reaction), and in an attempt to lessen the extent of this reaction it was decided that the benzaldehyde should be added dropwise to the basic solution of the acetophenone (rather than adding the base in last as is usual) as an aid to getting the benzaldehyde to react with the acetophenone rather than with itself. A new work-up procedure was also devised which involved extracting the reaction mixture with ether before acidification, to remove unreacted benzaldehyde and any benzyl alcohol. After acidification the product was extracted into ether and washed with 5% $NaHCO_3$ solution (to remove any benzoic acid), then with water and finally with saturated sodium chloride. The ether solution was further dried over anhydrous $CaCl_2$, and evaporated to dryness. In summary, the most successful method was as follows:

(i) the mole ratio of the reactants 2,4-dihydroxyacetophenone, benzaldehyde and sodium hydroxide was 1:1.5:10.

(ii) the 2,4-dihydroxyacetophenone was dissolved in ethanol and then added to the reaction flask containing the 50% sodium hydroxide solution (at times more water needed to be added to redissolve the salt of the acetophenone). The solution was then heated to ≈55°C at which time the benzaldehyde was added (dropwise with stirring).

(iii) After ≈18 hours at ≈55°C the reaction mixture was worked up as described earlier.

(iv) The final product was easily recrystallised from ethanol-water to yield yellow needles of m.p. 148°C (lit. 147 - 149°C Adityachaudhury 1971; 150°C, Saiyad et al, 1937). The UV spectrum in ethanol (345(inflection) 318, 268 nm) was almost identical to that reported by Jurd and Horowitz (1961), 345, 317, 267 nm.

This method is significantly faster than those quoted in the literature and results in a good yield of the pure product.

2-2.4. The Preparation of 2'-hydroxy-4'-methoxychalcone

The method used for the preparation of this chalcone was very similar to that used to prepare 2'-hydroxychalcone (section 3-2.1), but a pure product was more easily obtained in this case by adding the benzaldehyde to the basic acetophenone solution (as discussed in the previous section). The method used was as follows:

(i) The mole ratio of the reactants 2-hydroxy-4-methoxyacetophenone, benzaldehyde and sodium hydroxide was 1:1:1.5.

(ii) The acetophenone was dissolved in ethanol and then added to the 50% sodium hydroxide solution. The mixture was cooled to 0°C, and the benzaldehyde added dropwise, stirring continuously.

(iii) The reaction flask was stoppered and kept at room temperature for ≈2 days, after which time the mixture was acidified, and the resultant precipitate recrystallised from ethanol. Yellow needles were

formed of m.p. 108°C (lit. 106 - 8°C, Bargellini & Monji, 1914). The UV spectrum in ethanol showed λ_{max} at 343 (inflection), 318, 253 nm (lit. 342 (inflection), 317 252 nm, Mabry et al, 1970).

2-2.5. The Attempted Preparation of 2',6'-dihydroxychalcone

The preparation of this chalcone was attempted using all the previously mentioned methods, but all proved to be unsuccessful. The problems encountered in the preparation were probably associated with the fact that this chalcone would exist preferentially as the isomeric flavanone, since this form would be stabilised by hydrogen-bonding between the 5-hydroxyl group and the carbonyl group (see Fig. 1.7.).

It appears that this compound has only ever been formed as a minor byproduct in a photochemical reaction. Ramakrishnan and Kagan (1970a) report its formation (in low yield) as a result of irradiating resorcinol monocinnamate in benzene.

2-2.6. The Preparation of 2'-hydroxy-6'-methoxychalcone

(i) To prepare this compound it was first necessary to form 2-hydroxy-6-methoxyacetophenone. The work of Adityachaudhury et al (1971) suggested that methylation of 2,6-dihydroxyacetophenone should result in only one of the hydroxyl groups being substituted, and the required preparation was thus accomplished by refluxing equimolar quantities of 2,6-dihydroxyacetophenone and methyl iodide in dry acetone, in the presence of anhydrous potassium carbonate.

(ii) The condensation of 2-hydroxy-6-methoxychalcone with benzaldehyde, to form the chalcone, was performed in the same manner as that described for 2'-hydroxy-4'-methoxy-

chalcone (section 2-2.4.), but it was found to be advantageous for the reaction mixture to be kept at room temperature for up to 14 days. In this case the work up procedure was that described for the preparation of 2',4'-dihydroxychalcone (section 2-2.3). The yellow crystals that formed as a result of re-crystallisation from ethanol had a melting point of 65°C (lit. 65°C, Cummins et al, 1963; 64°C, Oliverio & Schiavello, 1950; 127-9°C, Seshadri & Venkateswarlu, 1947). The chalcone prepared by Seshadri & Venkateswarlu (1947) was thought to be identical with the others even though its melting point differed significantly. Oliverio and Schiavello (1950) consider the chalcone is dimorphic.

2-2.7. The Preparation of 2'-hydroxy-4',6'-dimethylchalcone

All attempts to form pure 2'-hydroxy-4'.6'-dimethylchalcone directly from the base condensation of 2-hydroxy-4,6-dimethylacetophenone and benzaldehyde were unsuccessful. In all cases an oil was formed which, after purification by p.l.c. (20% diethyl ether/petroleum ether (b.p. 30°C - 40°C)) could still not be crystallised, even though t.l.c. showed that it contained a high percentage of one (yellow/orange) compound. However, the preparation was accomplished by a method similar to that described for the formation of 2'-hydroxy-5',6'-benzochalcone (section 2-2.2). That is, the isomeric 5,7-dimethylflavanone was formed first and this, after purification by recrystallisation from ethanol, was converted to the chalcone by the method described previously (section 2-2.2.).

The condensation reaction was carried out as for 2'-hydroxy-4'-methoxychalcone (section 2-2.4 parts (i) and (ii)), but after the reaction mixture had been stirred for ≈2 hr, water was added to the solution until it became only weakly basic. The reaction vessel was then kept at room temperature for ≈12 hours, during which time a pale yellow precipitate had formed. This was filtered from the solution and recrystallisation from ethanol resulted in white crystals. The 5,7-dimethylflavanone, so formed, was then converted to the chalcone, which after recrystallisation from petroleum ether b.p. ($30^{o}C$ - $40^{o}C$), by cooling to $-15^{o}C$, yielded orange crystals with a melting point of $47^{o}C$. The yield of recrystallised 5,7-dimethyl-flavanone by this method was ≈74%, and this was converted to the chalcone in ≈70% yield.

2'-hydroxy-4',6'-dimethylchalcone does not appear to have been reported in the literature, although several of these chalcones have been formed with substituents in the B-ring (Takatori & Fujise, 1957). The following data are in support of this compound being the required 2'-hydroxy-4',6'-dimethylchalcone.

(i) A brown colour is produced in ethanolic ferric chloride indicating a phenolic -OH.

(ii) The UV spectrum in ethanol shows λ_{max} at 298, 220 nm which is characteristic for chalcones, and the compound is seen (Figs. 3.20 & 3.21) to cyclise in aqueous conditions to a compound with a typically flavanone-type UV spectrum.

(iii) The mass spectrum has a base peak at m/e 252 (which would represent the required molecular ion) and the following peaks in order of relative abundance: m/e 148, 161, 175 and 149. All

these peaks are consistent with the compound being 2'-hydroxy-4',6'-dimethylchalcone. An analysis is awaited, but as mentioned previously this will not differentiate between the chalcone and the isomeric flavanone.

2-3 THE PREPARATION OF 2'-HYDROXYCHALCONE EPOXIDES

2-3.1. The Preparation of 2'-hydroxychalcone Epoxide

It was unfortunate that we were unaware of the one report on the preparation of this compound (Ramakrishnan & Kagan, 1970a), until after we had successfully completed the synthesis, as considerable time was spent determining favourable reaction conditions and characterising the compound. There have been many reports of unsuccessful attempts to prepare this compound (Baker & Robinson, 1932; Murakami & Irie, 1935; Geissman & Fukushima, 1948) and since 2'-hydroxychalcone epoxides have often been postulated as important intermediates in the biosynthesis of flavonoids, its preparation and study was one of our original aims.

Our method, as it eventuated, was very similar to that of Ramakrishnan and Kagan, and is outlined below.

(i) 2'-hydroxychalcone and m-chloroperoxybenzoic acid (mole ratio 1:1.5) were refluxed in 1,2-dichloroethane for ≈2 hours.

(ii) The reaction flask was cooled and a white precipitate of m-chlorobenzoic acid was removed by filtration. The solution was then washed successively with 10% sodium bisulphite, 5% sodium bicarbonate, water, and saturated sodium chloride. (Since the stability of the epoxide was suspect in all except totally non-polar environments, a t.l.c. was run between each

washing to ensure that the reaction mixture remained unchanged).

(iii) The solution was then concentrated on a rotary evaporator (no heat) and applied, via a syringe, to p.l.c. plates containing a 1.5 mm layer of silica gel 60 PF_{254} + 366

(iv) Two developments using 10% diethyl ether/petroleum ether (b.p. 30°C - 40°C) resulted in a good separation of the compounds. The epoxide band was located using a UV lamp and eluted from the silica gel immediately with diethyl ether. (It was found that the epoxide cyclised to 3-hydroxyflavanone on the p.l.c. plates after the solvent had evaporated. The rationalisation for this is probably that in the low polarity solvent the molecule exists in the hydrogen-bonded configuration, but once the solvent is removed it exists to a greater extent in the open conformation which allows cyclisation, probably catalysed by the silica gel).

(v) The ether solution was evaporated to dryness and the resultant solid was dissolved in petroleum ether (b.p. 40°C - 60°C). That which would not dissolve, and that which precipitated on initial cooling was filtered from the solution (white crystals of 3-hydroxyflavanone). The remaining solution was partially evaporated and then cooled, resulting in pale yellow crystals. Several recrystallisations were carried out using petroleum ether (b.p. 40°C - 60°C), resulting in almost colourless crystals of m.p. 77°C (lit. 78°C, Ramakrishnan & Kagan, 1970a). The ^{1}H-nmr spectrum in $CDCl_3$ showed the following signals at δ4.13 (d, J = $2H_Z$, 1H), 4.33 (d, J = $2H_Z$, 1H), 6.8 - 8.0 (m, 9H), 11.90 (S, 1H, hydrogen-

bonded OH). The protons adjacent to the epoxide group appear as the pair of doublets at δ4.13 and 4.33, and the small coupling constant (2Hz) indicates that the compound is *trans*-2'-hydroxychalcone epoxide. The mass spectrum shows the molecular ion at m/e 240, but the base peak is at m/e 121. This latter fragment ion is characteristically intense in chalcone epoxides and indicates that the α-fission leading to the benzoyl ion ($HOC_6H_4 - C \equiv O^+$) receives a "push" by the simultaneous epoxide ring opening (Porter and Baldas, 1971). These spectral data are in complete agreement with those of Ramakrishnan and Kagan. Our method differed from the published preparation in that they refluxed in chloroform for 15 hr and separated the product using column chromatography on deactivated silica gel, with benzene as the eluting solvent. In the course of determining our reaction conditions numerous tests were made on the stability of the peracid at elevated temperatures. The peracid was refluxed in 1,2-dichloroethane without the chalcone present, samples were removed at various time intervals and the peracid content determined iodimetrically. The results showed that after 2hr ≈15% peracid remained, but the same test with the 2'-hydroxychalcone present (even with peracid in excess) showed that no peracid was left after 2 hr. Thus although the reaction temperature used by Ramakrishnan and Kagan was lower than ours, it would still seem highly probable that the productive reaction time would be very much less than the 15 hr they used.

Work by Kishi et al (1972) on epoxidations at elevated temperatures indicated that the presence of a radical inhibitor in the reaction solution would slow the thermal decomposition of the peracid considerably. Several compounds were tested to determine their effectiveness at preventing the peracid decomposition, and the best of those tried was 3-t-butyl-4-hydroxy-5-methylphenylsulphide. Refluxing the peracid in 1,2-dichloroethane in the presence of 1% (by weight) of this compound resulted in there being ≈90% peracid remaining after 2 hr and ≈57% after 18 hr. However, when epoxidising 2'-hydroxychalcone it proved to have no effect whatsoever on the thermal stability of the peracid.

In the search for a more efficient method of preparing 2'-hydroxychalcone epoxides, epoxidation via phase transfer catalysis was attempted. Recent reports indicate that many compounds can be epoxidised using a range of epoxidising agents (but in particular sodium hypochlorite) in the presence of a phase transfer catalyst (Krishnan et al, 1977; Lee & Freedman, 1976; Helder et al, 1976; Starks et al, 1971, 1973). The epoxidation of 2'-hydroxychalcone was attempted using sodium hypochlorite, with tetra-n-butylammonium chloride as the phase transfer catalyst in a two phase system (water: 1,2-dichloroethane). The reaction was tried at a variety of pH values, but the only product that formed in good yield was the flavanone.

2-3.2. Attempted Epoxidations of 2'-hydroxy-5',6'-benzochalcone and of 2'-hydroxy-4'-methoxychalcone

The peracid epoxidations of the above compounds were attempted, but were unsuccessful. In the former case a compound was isolated, the spectral data for which suggested it was the corresponding 3-hydroxy--5,6-benzoflavanone (from the ^{1}H-nmr, by comparison with the spectrum of 3-hydroxyflavanone; and from the mass spectrum which showed the required

molecular ion at m/e 290, the expected base peak at m/e 171 due to the retro-Diels-Alder reaction with hydrogen transfer, and a prominent ion at m/e 261 which is characteristic of 3-hydroxyflavanones and is as a result of the loss of 29 mass units, CHO).

The ^{1}H-nmr spectrum of a p.l.c. separated product from the attempted epoxidation of 2'-hydroxy-4'-methoxychalcone showed cursory evidence for the formation of the required epoxide. A weak doublet appeared in the region expected for the protons adjacent to the epoxide group, but the sample was clearly impure.

3. THE RATE OF ATTAINMENT OF THE 2'-HYDROXYCHALCONE-FLAVANONE EQUILIBRIUM

In an attempt to understand the effect that some A-ring substituents have on the rate of attainment of the 2'-hydroxychalcone-flavanone equilibrium, the rate constants for the conversion of 2'-hydroxychalcone to the 2'-hydroxychalcone-flavanone equilibrium mixture have been determined for the following six chalcones: 2'-hydroxychalcone; 2'-hydroxy-5',6'-benzochalcone; 2',4'-dihydroxychalcone; 2'-hydroxy-4'-methoxychalcone; 2'-hydroxy-6'-methoxychalcone; 2'-hydroxy-4',6'-dimethylchalcone. The effect of pH on the rate has, except for 2',4'-dihydroxychalcone, led to the calculation of the pK_a and the rate constants for the forward and the reverse reactions for the chalcones studied.

3-1 EXPERIMENTAL

The determination of reaction rates requires that the change in concentration of a reactant (or product) can be measured with time, and this was found to be conveniently effected by following the change in the absorption spectrum of the chalcone with time by UV spectroscopy. The ability to take *in situ* measurements of the concentrations of reactants or products during a reaction also affords the opportunity to follow the production and disappearance of any intermediate species that may be formed during the reaction.

The rate of attainment of the 2'-hydroxychalcone-flavanone equilibrium was measured by following the decrease in absorbance of 2'-hydroxychalcone at a fixed wavelength, this wavelength being one at which there was a large change in the absorbance reading between the chalcone and the final reaction product. In theory the reaction could

equally well have been followed by starting with the flavanone, but it was found that under most of the pH conditions involved, the equilibrium lies well to the side of the flavanone, and thus there was very little change in absorbance for the conversion of flavanone to the 2'-hydroxychalcone-flavanone equilibrium mixture. The determination of rate constants by following the reaction of the flavanone would, therefore, have been either impossible or have been subject to considerable error.

3-1.1. Buffer Solutions

The buffer tables described by Perrin (1974) were used as the basis for making the buffer solutions. In all cases calculated amounts of potassium chloride were added to maintain a constant ionic strength of 0.5 mol l^{-1}. This ionic strength resulted in the measured pH values being quite different from those given in the tables since the figures given result in an overall ionic strength of 0.01 mol l^{-1}. In most cases the addition of potassium chloride lowered the pH, in some cases by up to 0.5 pH units. The addition of this amount of neutral salt was an attempt to minimise the differences between different types of buffers that might arise due to variations in the activity coefficients of the different buffer species.

The buffers that were to be used for the calibration of the pH meter (see section 3-1.2) were prepared using doubly-distilled water which had been boiled, and then cooled whilst passing a stream of oxygen-free nitrogen through it. This procedure removed the bulk of the dissolved carbon dioxide from the water and thereby prevented it from affecting the measured value of the pH standard. All other buffer solutions were prepared using doubly-distilled water without deaeratingit. In all cases analytical grade reagents were used for

the preparation of buffer solutions.

3-1.2. pH measurements

Since most pH determinations were to be performed on small samples in the neutral to basic region precautions were necessary to ensure minimal carbon dioxide absorption, and therefore all measurements were made in a partly closed system through which a slow, surface flow of nitrogen was passed. The pH values were measured at 30°C, to an accuracy of ±0.001 pH units, using a Radiometer Model 26 pH meter fitted with either a G202B glass electrode and a K401 calomel electrode, or, for small samples, with a GK2401B combination electrode. The pH meter was calibrated daily using the pH standards 0.01 mol l^{-1} sodium borate (pH = 9.139 at 30°C) and 0.05 mol l^{-1} potassium hydrogen phthalate (pH = 4.015 at 30°C) (Perrin, 1974).

Since the pH measurements were made at relatively high ionic strengths, and in some cases in mixed solvents, no attempt has been made to relate the measured pH to the hydrogen ion concentration, and in all cases it will be the hydrogen ion activity that is quoted (and shown as {}). In fact, the experimental pH can never be an exact measure of either the concentration or of the activity of the hydrogen ion (Bates, 1973), and it is only in dilute aqueous solutions (where the residual liquid junction potential is very small) that a quantitative interpretation of measured pH values has any real significance. This means that probably the most useful application of pH measurements outside the dilute aqueous medium is in the determination of relative acidities in a solvent of fixed composition.

The calculation of the hydroxide ion activity from the pH has been accomplished using the relationship, pH + pOH = pK_W where pK_W = 13.833 at 30°C. (Weast, 1975).

3-1.3. Kinetic Methods

The kinetic measurements were carried out using either a Cary 17 Spectrophotometer equipped with a 1729 programmer and a 1718 digital repetitive scan unit or, using a Pye-Unicam SP 1800 spectrophotometer fitted with a Unicam SP 1805 program controller and an AR 55 linear recorder. The cell blocks of the spectrophotometers were maintained at constant temperature (30°C) by circulation of water from an external thermostatted water bath.

The required amount (either 2.375 ml or 2.25 ml, see section 3-1.5) of the aqueous buffer solution was transferred by pipette into a silica cuvette, which was then stoppered and allowed to equilibrate at 30°C in the cell block of the spectrophotometer for approximately 15 minutes. The reaction was started by the addition of the chalcone, via a syringe, as a dioxan solution (either .125 mls or .250 mls), also at 30°C. The decrease in absorbance was then recorded at a wavelength determined by which chalcone was under study. The reaction was normally run for ten half-lives to obtain a good infinity reading, and at this time a UV spectrum was run of the final product to ensure that it was similar to that of the flavanone.

The pH of the reaction solution was measured after the completion of each kinetic run, it having been established that there was no significant change in the pH during the course of the reaction.

The absorption-time data obtained from the kinetic runs were analysed by one (or more) of the following methods:

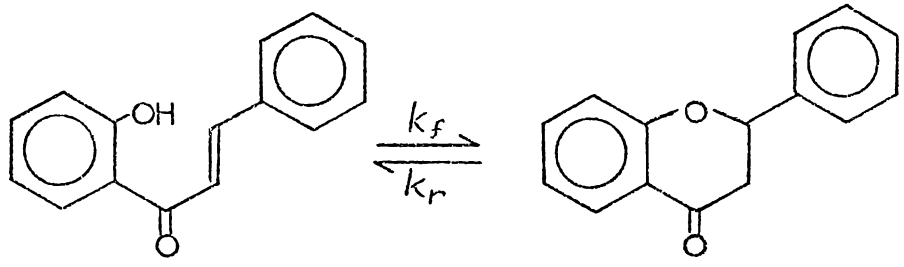
(i) The standard infinity plot method for first order or pseudo first order reactions in which a plot of the logarithm of $(A_\infty - A)$ against time is used to determine the rate constant. The line of best fit was determined using a linear least-squares fit program.

(ii) The Guggenheim (1926) method which requires the readings to be taken at constant, and fairly large, time intervals, but does not require the equilibrium or final reading to be made. The line of best fit for the plot of $\log(A_t - A_{t+\Delta t})$ versus time (where Δt is a constant time interval) was also determined using a least squares fit program.

(iii) The non-linear method for unknown initial and final readings devised by Moore (1972). This method has the added advantage that readings do not need to be taken at regular intervals or for any particular length of time. The data were treated by running a non-linear least squares computer program, prepared by Gumbley (1976), on a Digital PDP 11/05 computer.

3-1.4. The kinetic form of a reversible first order reaction

Using the 2'-hydroxychalcone-flavanone equilibrium (shown below) as an example of a reversible first order reaction (Szabo, 1969)



CH F

the rate expression is

$$\frac{-d[CH]}{dt} = k_f[CH] - k_r[F] \qquad \ldots\ldots\ldots \qquad (1)$$

where k_f is the rate constant for the conversion of chalcone (CH) to flavanone (F) and k_r is that for the reverse reaction. If $[CH]_o$ is the concentration of chalcone initially then $[F] = [CH]_o - [CH]$ and equation (1) can be rewritten as

$$-\frac{d[CH]}{dt} = k_f[CH] - k_r([CH]_o - [CH])$$

$$= (k_f + k_r)[CH] - k_r[CH]_o \qquad \ldots\ldots\ldots \qquad (2)$$

At equilibrium the rates of the two opposing reactions are equal, and thus

$$k_f\,[CH]_e = k_r[F]_e = k_r([CH]_o - [CH]_e) \qquad \ldots\ldots\ldots \qquad (3)$$

OR

$$[CH]_o = \frac{(k_f + k_r)\,[CH]_e}{k_f} \qquad \ldots\ldots\ldots \qquad (4)$$

where $[CH]_e$ is the concentration of chalcone at equilibrium. Substituting this value for $[CH]_o$ into equation (2) we get

$$-\frac{d[CH]}{dt} = (k_f + k_r)([CH] - [CH]_e) \qquad \ldots\ldots\ldots \qquad (5)$$

The result is that the approach to equilibrium is a first order process with the observed rate constant being the sum of the rate constants for the forward and reverse reactions.

$$\text{i.e. } k_{obs} = k_f + k_r \qquad \ldots\ldots\ldots \quad (6)$$

3-1.5. Establishing Reaction Conditions

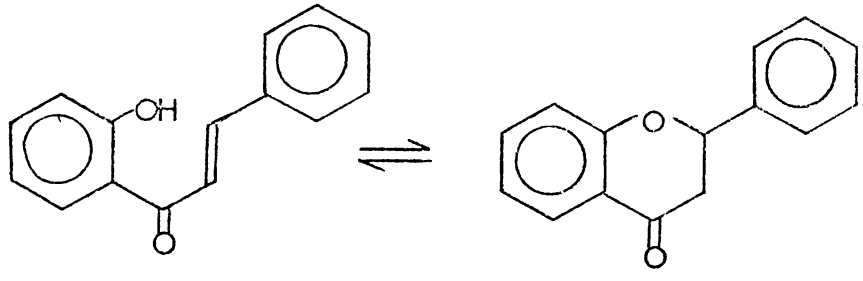
Some preliminary kinetic runs were performed, on 2'-hydroxychalcone and 2'-hydroxy-5',6'-benzochalcone at constant pH and fixed wavelength in which the chalcone was injected into the buffer solution as a methanol solution. The standard infinity plot method yielded good linear plots for the 2'-hydroxychalcone but not for the 2'-hydroxy-5',6'-benzochalcone. Following the reaction for the latter compound by repeatedly scanning the spectrum at known time intervals showed that the chalcone was not converting cleanly to flavanone as expected, but was in fact forming a "compound" that had very little absorption in the ultraviolet region of the spectrum. Closer inspection of the reaction cuvette and the spectrum, revealed that the flavanone was forming, but was then precipitating from the solution. Higher methanol concentrations were tried but these were also unsuccessful and the solvent which proved most able to keep the flavanone in solution was dioxan. Most of the problems occurred at the lower pH values and it was ultimately found than to ensure continuous solubilisation of the chalcone-flavanone, a 10% dioxan solution needed to be used, combined with an absorbance range of 0-0.2.

To avoid any problems with the other chalcones their solubility, and that of the corresponding flavanone, was studied in order to determine the minimum concentration of dioxan necessary to avoid precipitation problems. It was found that for the other chalcones the reaction

could be safely followed in a solution containing 5% dioxan. The 0-0.5 absorbance range was also able to be used except for 2'-hydroxy-4'-methoxychalcone which required the use of the 0-0.2 absorbance range.

In summary, the reaction of 2'-hydroxy-5',6'-benzochalcone was studied in 10% dioxan solution (2.25 mls buffer and 0.25 mls dioxan) but all other reactions were followed in 5% dioxan solution (2.375 mls buffer and 0.125 mls dioxan). The dioxan used was BDH 1,4-dioxan "special for spectroscopy." It was packed under nitrogen and was purged before and after use with nitrogen.

3-2 THE 2'-HYDROXYCHALCONE-FLAVANONE EQUILIBRIUM



The rate of conversion of 2'-hydroxychalcone to its equilibrium mixture was measured at 30°C, in 5% dioxan solution, over the range pH 6.78 - 11.41. Repetitive wavelength scans at pH 8.55 (Fig. 3.1.) and pH 10.26 (Fig. 3.2.) show that under these conditions the reaction proceeds cleanly to equilibrium, and that the reaction product is predominantly flavanone. By following the decrease in absorbance at 320 nm as the chalcone reacted, first order rate constants were determined.

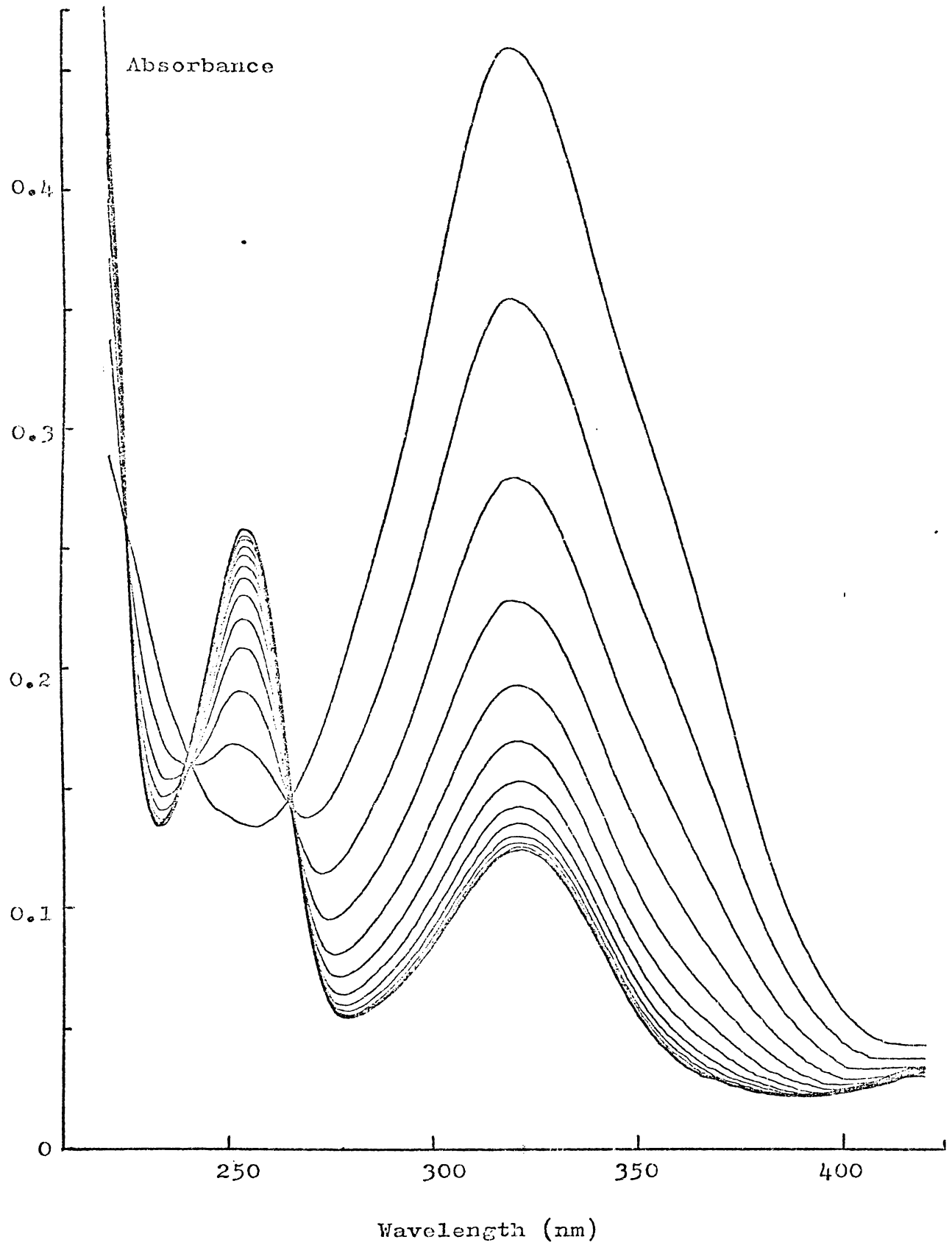


Figure 3.1. Repetitive wavelength scans at 10 min. intervals and pH 8.55 showing the course of the cyclisation reaction of 2'-hydroxychalcone to flavanone.

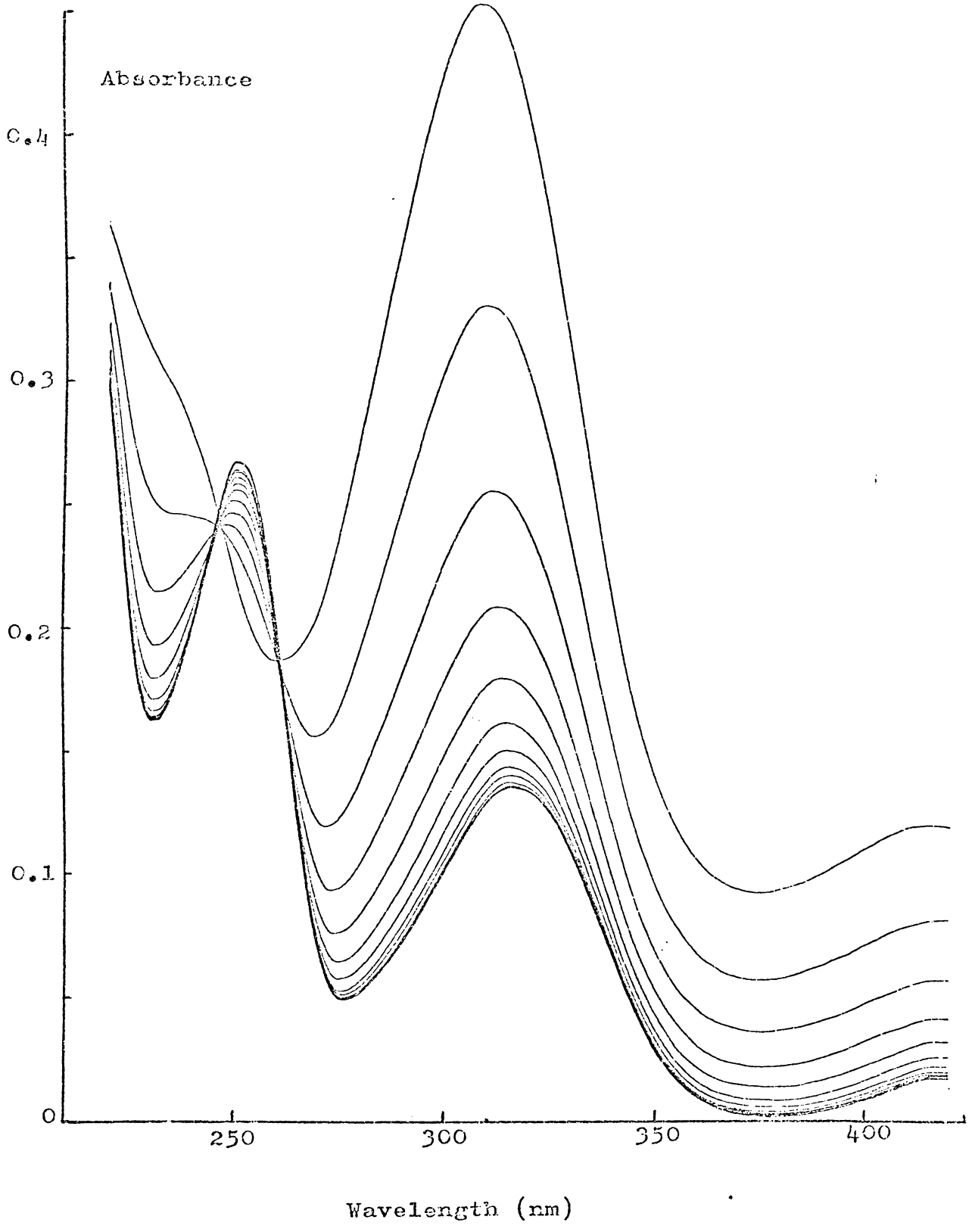


Figure 3.2. Repetitive wavelength scans at 1 min. intervals and pH 10.26 showing the course of the cyclisation reaction of 2'-hydroxychalcone to flavanone.

3-2.1 Buffer Dilution Studies

The effect of the buffer concentration on the observed rate constant was measured for a range of buffers and the results are recorded in Table 3-1, and presented graphically in Fig. 3.3. It had been anticipated that any buffer catalysis that was observed could have been as a result of the basic buffer species catalysing the reverse reaction. Had this been the case more catalysis would have been expected by the carbonate buffer at pH 10.55 than by the pH 9.52 carbonate buffer. In fact the slope of the two graphs in Fig. 3.3. (a) and (b) is very similar (and very small). There was also very little variation in k_{obs} with changes in the buffer concentration for phosphate at pH 8.14 and negligible changes for phosphate at pH 6.78. Maintaining the ionic strength at 0.5 mol l^{-1} necessitated the addition of varying amounts of potassium chloride and it is possible that this caused changes in the nature of the solvent medium sufficient to explain the results observed for the carbonate and phosphate buffers. Even in moderately concentrated solutions specific ion effects and solvent effects can change the activity coefficients of the reactants or the transition state (Jencks, 1969) and hence affect the observed reaction rate.

From the graphs in Fig. 3.3. (a), (b) and (c) it can be seen that the difference in k_{obs} between 0.02 mol l^{-1} buffer concentration and zero buffer concentration is, for the carbonate buffer at pH 10.55 approximately 1.5%, for the carbonate buffer at pH 9.52 approximately 3%, and for the phosphate buffer at pH 8.14 approximately 3.5%. Since the k_{obs} values can only be determined to an accuracy of 3% it was decided that irrespective of whether the observed changes in k_{obs} with buffer concentration were due to "true" buffer catalysis or were a result of changes in the reaction medium, the rate constants could

TABLE 3-1 Effect on k_{obs} of changing the total buffer concentration at constant pH, and constant ionic strength

Buffer	Concentration (mol l^{-1})	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$
Carbonate (pH = 10.55)	0.10	85.2 86.5 83.8	85.2 ± 2.6
	0.07	83.0 84.6 83.0	83.5 ± 2.5
	0.04	80.3 81.3 81.5	81.0 ± 2.4
	0.02	78.7 79.2 79.8	79.2 ± 2.4
Carbonate (pH = 9.52)	0.10	42.2 42.7	42.5 ± 1.3
	0.06	39.5 40.3	39.9 ± 1.2
	0.02	37.2 36.8	37.0 ± 1.1
Phosphate	0.10	3.34	3.34 ± 0.10
(pH = 8.14)	0.06	3.16	3.16 ± 0.09
	0.03	2.98	2.98 ± 0.09
Phosphate (pH = 6.78)	0.10	0.227 0.228	0.228 ± 0.007
	0.06	0.232 0.230	0.231 ± 0.007
	0.02	0.227 0.227	0.227 ± 0.007
Diethanolamine	0.05	52.2	52.2 ± 1.6
(pH = 9.42)	0.04	52.0	52.0 ± 1.6
	0.03	51.2	51.0 ± 1.6
	0.02	49.3	49.3 ± 1.5
	0.01	41.7	41.7 ± 1.3

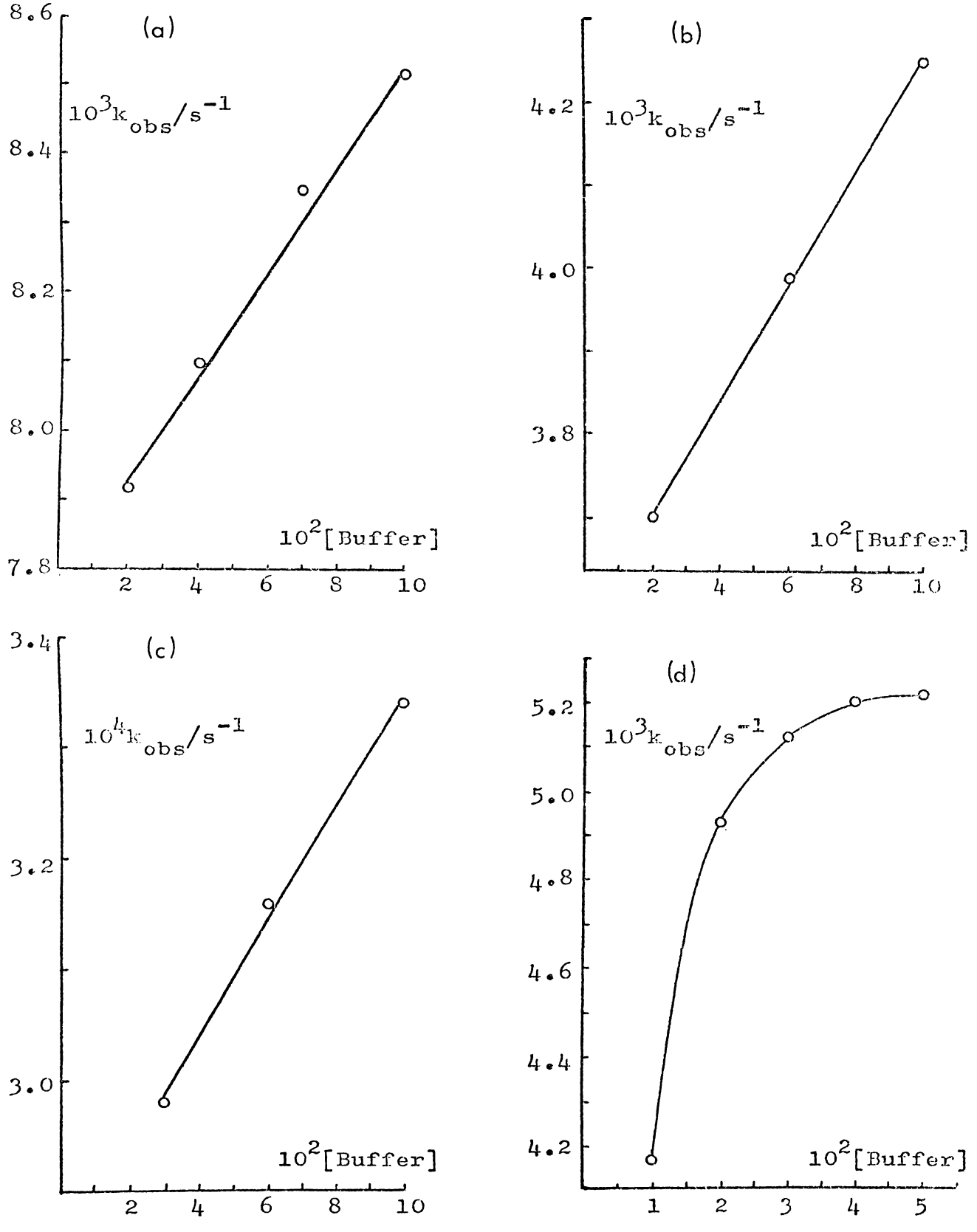


Figure 3.3. Effect of buffer concentration on the observed rate constant.
(a) Carbonate pH 10.55
(b) Carbonate pH 9.52
(c) Phosphate pH 8.14
(d) Diethanolamine pH 9.42

be reliably determined using carbonate and phosphate buffers with a concentration of 0.02 mol l^{-1}.

The non-linear dependence of k_{obs} on the concentration of diethanolamine is shown in Fig. 3.3. (d). This type of downward deviation may be caused either by some sort of complex formation of the reactants or by a change in the rate-determining step (see section 3-3). The determination of rate constants for zero buffer concentration is almost impossible if this type of relationship exists, and therefore this buffer was not used for further kinetic measurements.

The buffers used in this study were: from pH 6.78 - 8.14, phosphate; from pH 8.55 - 10.86, carbonate; and for the higher pH values, orthophosphate (which also showed negligible variation in k_{obs} with changing buffer concentration). In all cases the buffer concentration was 0.02 mol l^{-1}.

3-2.2. The Effect of pH on the Rate of Attainment of the 2'-hydroxy-chalcone-flavanone Equilibrium

The pH dependence of the rate of isomerisation of 2'-hydroxychalcone is shown in Table 3-2. The mechanism proposed to account for the observed kinetics is shown in Fig 3.4.

TABLE 3-2. Effect of pH on the observed rate constant for the attainment of the 2'-hydroxychalcone-flavanone equilibrium at 30°C and μ = 0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$*
6.78	0.227 0.227	0.227 ± 0.007
7.42	0.645 0.640	0.643 ± 0.019
8.14	2.82 2.95	2.89 ± 0.09
8.55	7.07 7.03	7.05 ± 0.21
8.77	10.8 10.5	10.7 ± 0.3
9.20	23.7 24.0	23.9 ± 0.7
9.39	33.3 31.7	32.5 ± 1.0
9.67	44.0 45.7	44.9 ± 1.3
9.86	53.2 53.0	53.1 ± 1.6
10.10	62.2 61.2	61.7 ± 1.9
10.26	68.2 69.5	68.9 ± 2.1
10.57	82.3 82.8	82.6 ± 2.5
10.86	96.2 96.8	96.5 ± 2.9
11.11	117 116	117 ± 4
11.41	158 163	161 ± 5

*Mean values taking 3% experimental error in k_{obs} into account (See appendix 1 (a)).

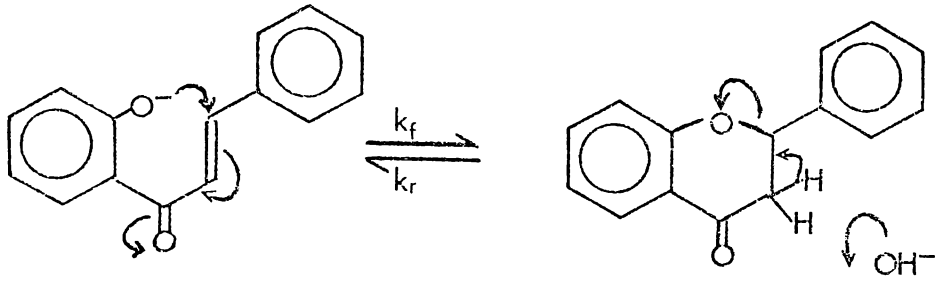


Figure 3.4. Mechanism proposed for the isomerisation of 2'-hydroxychalcone.

As determined in section 3-1.5 (equation (6)) the observed rate constants represent the sum of the rate constants for the forward and the reverse reactions.

$$\text{i.e.} \quad k_{obs} = k_f + k_r$$

It is proposed that the rate constant in the forward direction can be represented as

$$k_f = kfCH + k'fC^- \qquad \ldots\ldots \quad (7)$$

where k represents the rate constant for cyclisation of the neutral chalcone (this would make a small contribution to the overall rate and is not depicted in the above mechanism), fCH represents the fraction of neutral chalcone, k' represents the rate constant for the cyclisation of the ionised chalcone, and fC^- represents the fraction of ionised chalcone. Making the assumption that rate of the reverse reaction is proportional to the hydroxide ion activity, k_r can be expressed as

$$k_r = k''\{OH^-\} \qquad \ldots\ldots (8)$$

If this assumption is correct it should therefore be possible to fit k_{obs} to the overall expression

$$k_{obs} = kfCH + k'fC^- + k''\{OH^-\} \qquad \ldots\ldots (9)$$

The method of determining the values of best fit is explained in appendix 2, but briefly it involved an initial estimate of the pK_a of the chalcone (which therefore defined fCH and fC^-) by a visual inspection of the pH-rate profile (shown in Fig. 3.5.) This allowed an estimate of k' to be made (as $\leq$ half the value of k_{obs} at the pKa chosen) which in turn allowed initially estimates of k'' and k. Although the data given in appendix 2 are for 2'-hydroxy-6'-methoxychalcone the same procedure for optimising the fit was used for all the chalcones. In the case 2'-hydroxychalcone the values obtained were as follows:

$$pK_a = 9.55$$
$$k = 10 \times 10^{-6} s^{-1}$$
$$k' = 75 \times 10^{-4} s^{-1}$$
$$k'' = 22.5 \times 10^{-1} \text{ activity}^{-1}\ s^{-1}$$

The overall rate expression is therefore,

$$\text{Rate} = (10 \times 10^{-6} fCH + 75 \times 10^{-4} fC^- + 22.5 \times 10^{-1}\{OH^-\})\ ([\text{Chalcone}] - [\text{Chalcone}]_{\text{equilibrium}})$$

In Fig. 3.5. the experimentally determined rate constants are plotted as points, while the line is theoretical, being based on the above rate expression. The agreement between the experimental points and the

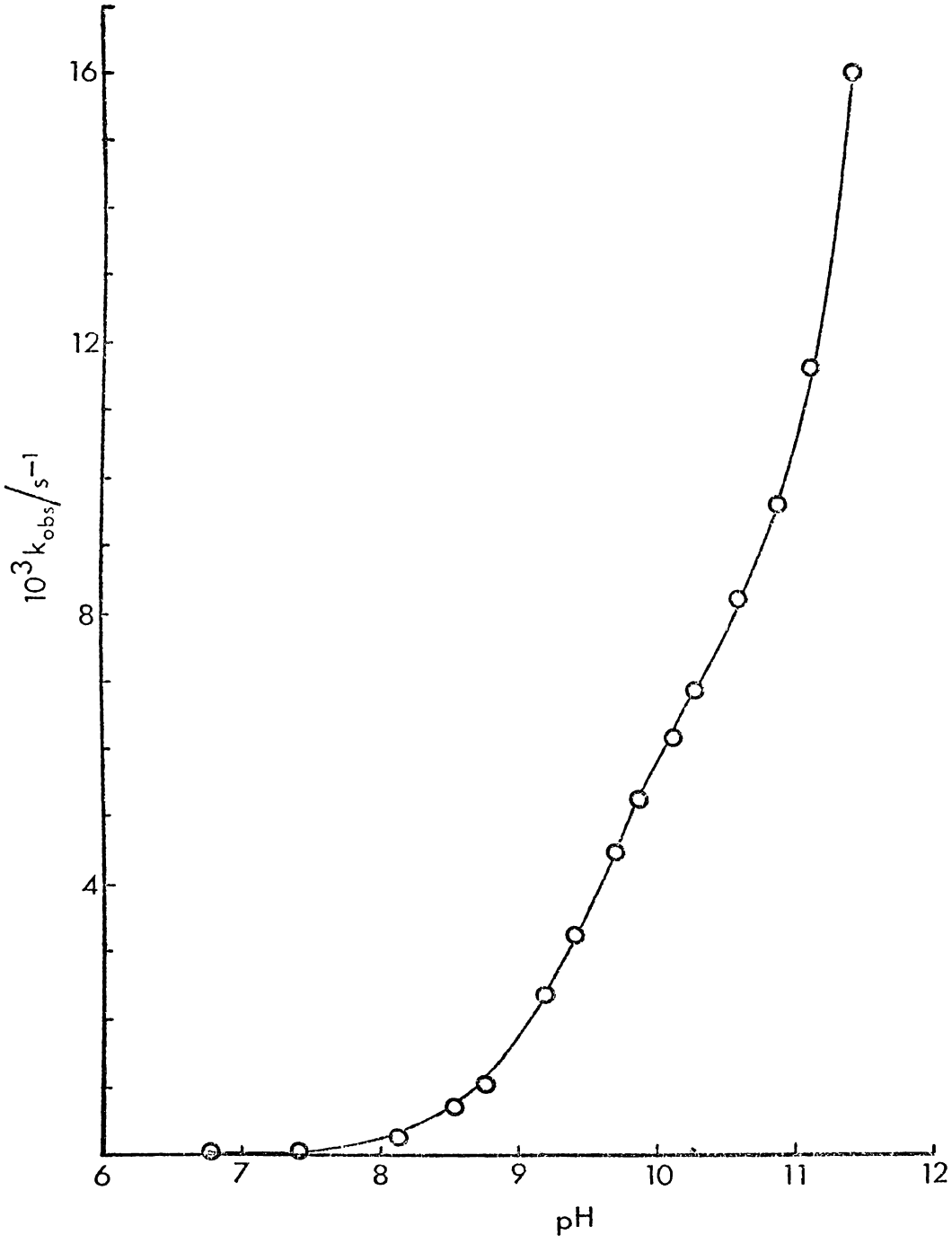
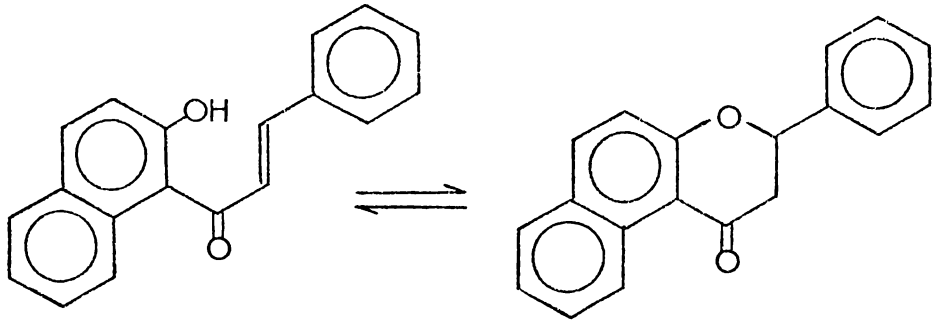


Figure 3.5. pH-rate profile for the attainment of the 2'-hydroxychalcone-flavanone equilibrium at 30°C, μ= 0.5 mol l^{-1}

theoretical line is within 3%, indicating that the kinetic data are consistent with the above rate expression at the pH values employed, thus supporting the assumptions made in deriving the rate expression.

It should be noted that the shape of this pH-rate profile is different from that determined by Litkei et al (1973) (see Fig. 1.9.) in which it is implied that the rate is invariant between pH 8 - 9.5, and increases quite slowly above pH 10.

3-3 THE 2'-HYDROXY-5',6'-BENZOCHALCONE ⟷ 5,6-BENZOFLAVANONE EQUILIBRIUM



The rate of conversion of 2'-hydroxy-5',6'-benzochalcone to its equilibrium mixture was measured at 30°C, in 10% dioxan solution, over the range pH 6.96 - 10.67. Repetitive wavelength scans at pH 7.80 (Fig. 3.6.) and pH 10.23 (Fig. 3.7.) show that within this pH range the reaction studied represents cyclisation of the chalcone to the corresponding flavanone. First order rate constants were determined

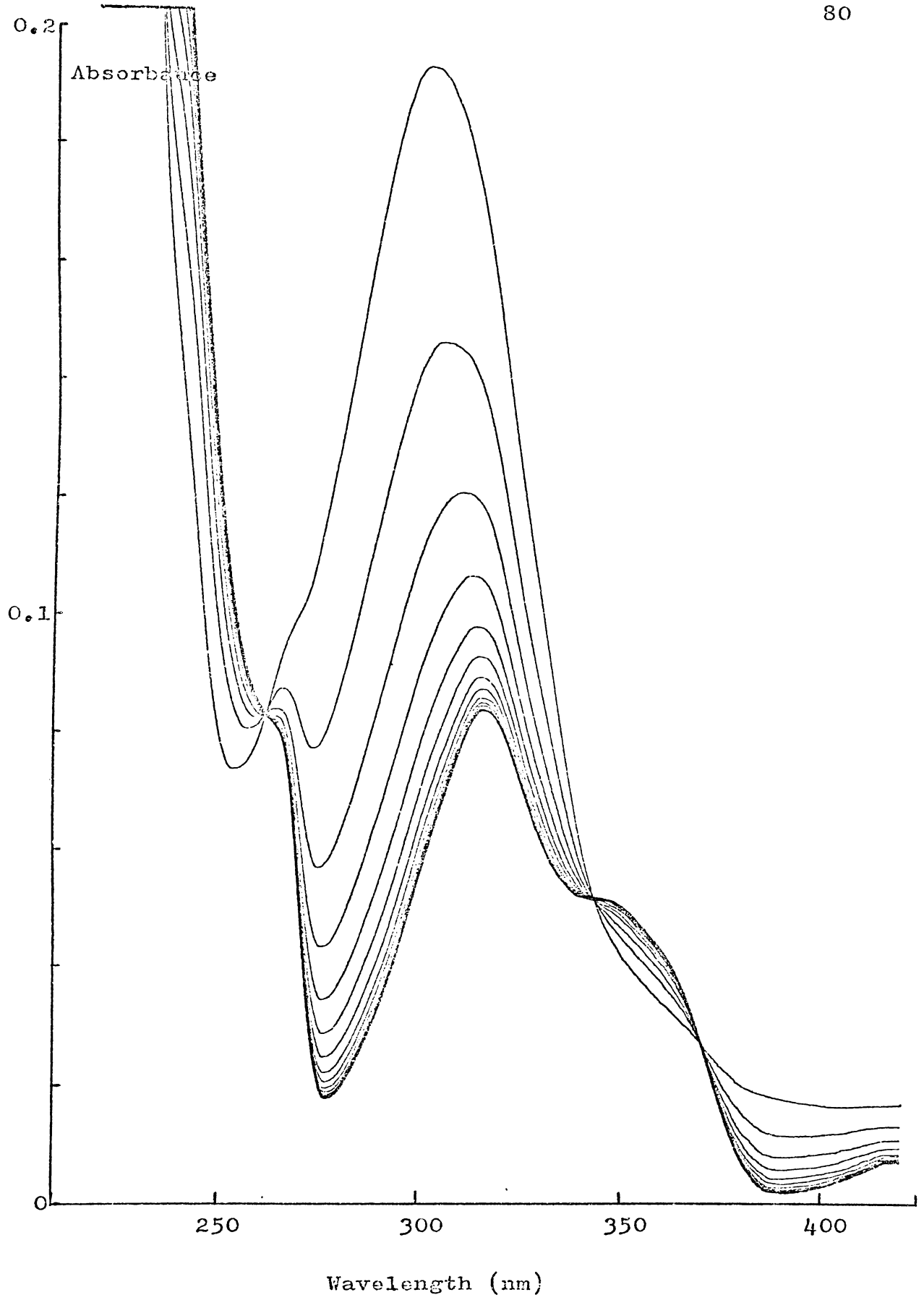


Figure 3.6. Repetitive wavelength scans at 120 min. intervals and pH 7.80 showing the course of the cyclisation reaction of 2'-hydroxy-5',6'-benzochalcone to 5,6-benzoflavanone.

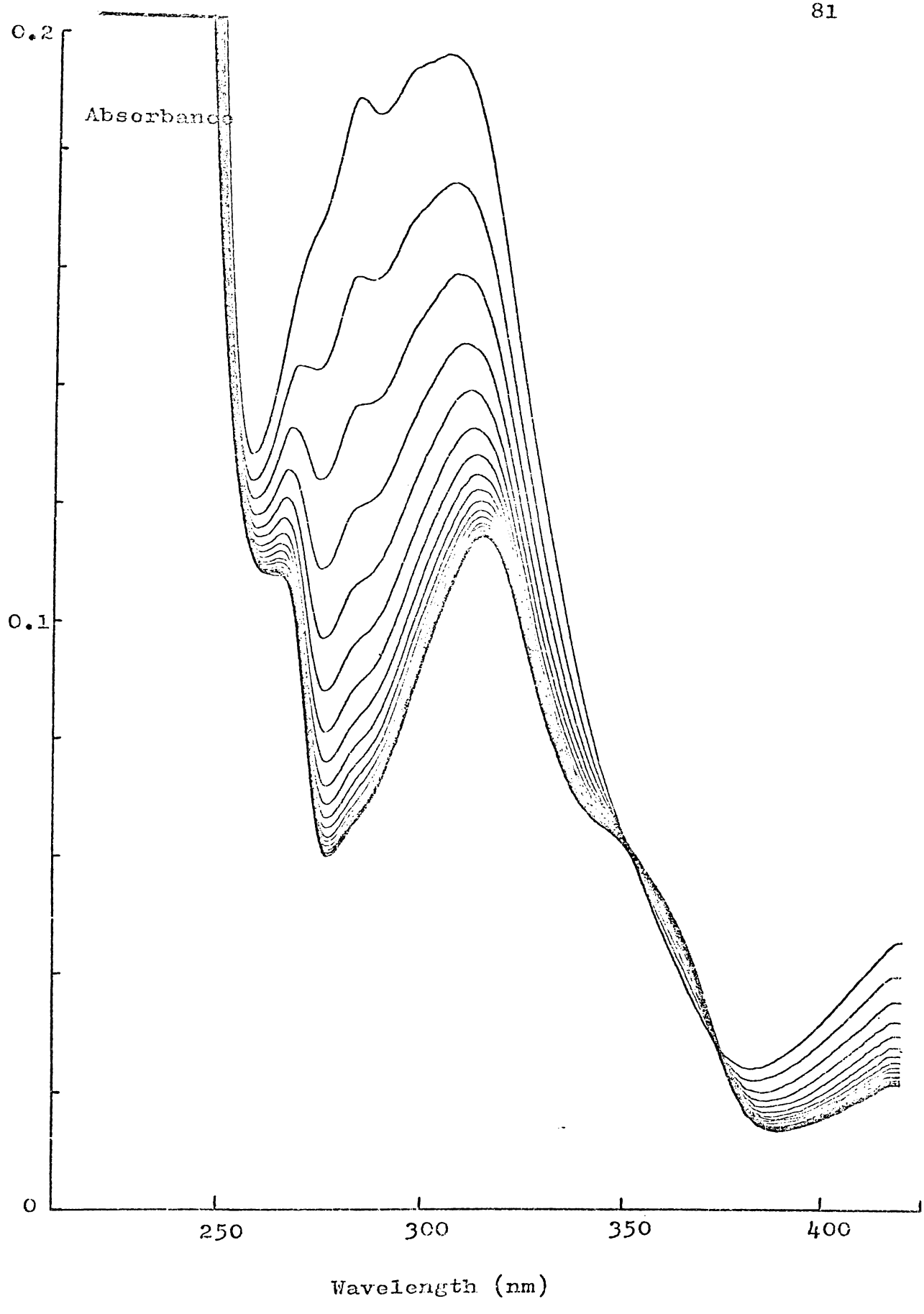


Figure 3.7. Repetitive wavelength scans at 8 min. intervals and pH 10.23 showing the course of the cyclisation reaction of 2'-hydroxy-5',6'-benzochalcone to 5,6-benzoflavanone

TABLE 3-3 Effect on k_{obs} of changing the total buffer concentration at constant pH, and constant ionic strength

Buffer	Concentration (mol l^{-1})	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$
Carbonate (pH = 10.64)	0.10	6.63 6.67	6.65 ± 0.40
	0.07	6.42 6.30	6.36 ± 0.38
	0.04	6.32 6.03	6.18 ± 0.37
Carbonate (pH = 9.60)	0.10	6.45 6.43	6.44 ± 0.39
	0.06	5.83 5.88	5.86 ± 0.35
	0.02	5.33 5.13	5.23 ± 0.31
Phosphate (pH = 8.05)	0.10	3.38 3.43	3.41 ± 0.20
	0.06	2.80 2.77	2.79 ± 0.17
	0.03	2.27 2.28	2.28 ± 0.14
Pyrophosphate (pH = 8.82)	0.054	3.87 3.90	3.89 ± 0.23
	0.036	3.82 3.80	3.81 ± 0.23
	0.022	3.67 3.67	3.67 ± 0.22

by following the decrease in the chalcone absorption at 300 nm.

3-3.1. Buffer Dilution Studies

The effect of the buffer concentration on the observed rate constant was measured for a range of buffers as shown in Table 3-3. Plotting the values for the carbonate buffer at pH 10.64 and pH 9.60 showed that the differences between k_{obs} at 0.02 mol l^{-1} buffer concentration and k_{obs} at zero buffer concentration obtained by extrapolation were small (approximately 3% and 4% respectively), as had been found in the previous section, but for the phosphate buffer at pH 8.05 the difference was found to be approximately 14%. The phosphate buffer was therefore considered unsuitable for the kinetic studies, and since the carbonate buffer was not particularly effective at maintaining a constant pH below ≈pH 9 for these slower reactions, an alternative buffer in the range pH 7 - 9 was sought. Diethanolamine, tris (hydroxymethyl) aminomethane and N-ethylmorpholine were tried as buffers for this pH region, but in all cases a non-linear dependence of k_{obs} on the concentration of the amine was observed, making the use of these buffers impracticable. An example of the data obtained for N-ethylmorpholine at pH 8.27 is presented in Table 3-4 and shown graphically in Fig. 3.8.

TABLE 3-4. Effect on k_{obs} of changing the total buffer concentration at constant ionic strength for N-ethylmorpholine at pH 8.27

Buffer Concentration (mol l^{-1})	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$
0.1	14.12	
	14.03	
	14.18	14.11 ± 0.85
0.07	13.60	
	13.13	
	12.76	13.16 ± 0.79
0.04	12.07	
	11.30	
	11.68	11.68 ± 0.70
0.01	6.83	
	7.00	
	6.58	6.80 ± 0.41

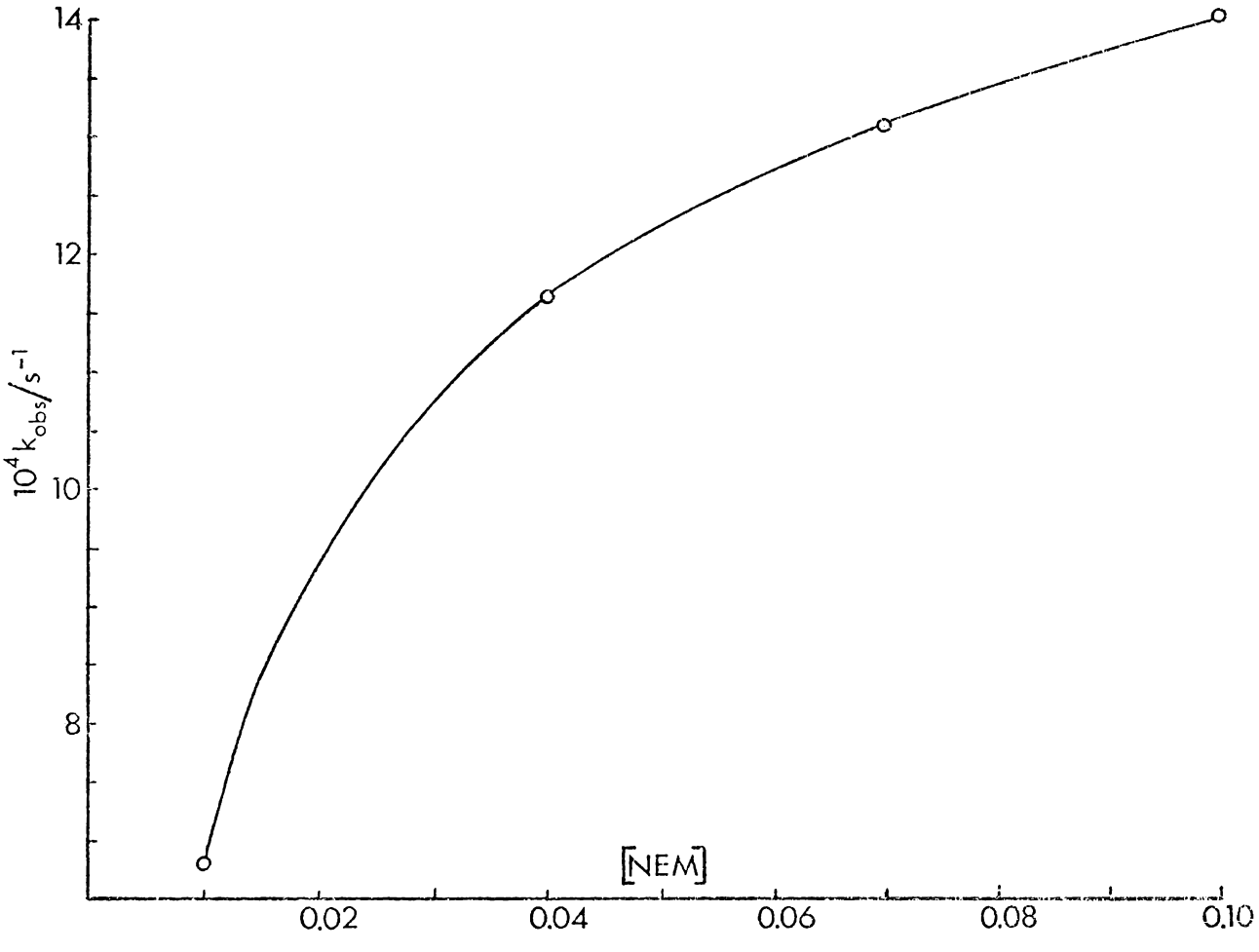


Figure 3.8. A plot of k_{obs} versus total buffer concentration for N-ethylmorpholine at pH 8.27

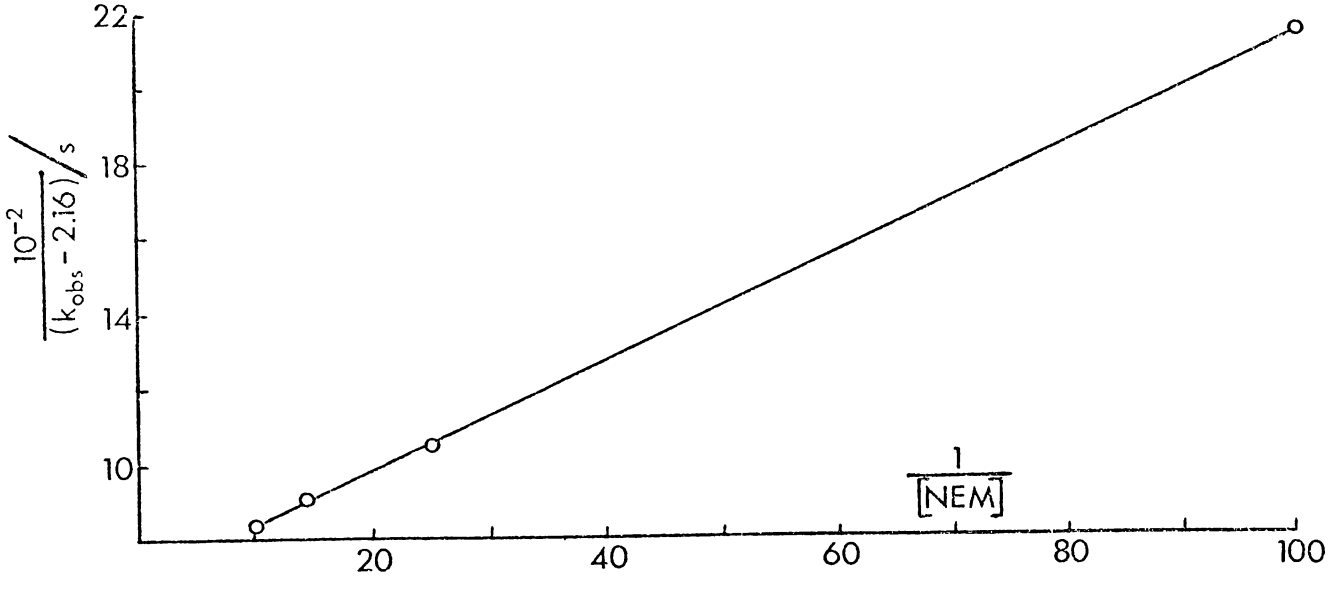


Figure 3.9. A plot of $\frac{1}{(k_{obs} - 2.16)}$ versus $\frac{1}{[NEM]}$

To test if this negative deviation could have been caused by some type of complex formation between the amine and the chalcone, a plot of the reciprocal of $k_{obs} - k_{true}$ (where k_{true} is the "true rate constant" at this pH as determined by non-catalysing buffers) versus the reciprocal of the N-ethylmorpholine concentration was made (Fig. 3.9.). The linear relationship observed indicates the possibility of complexation but considerably more work would be necessary to determine the true significance of these results.

The buffer finally chosen as being suitable for the range pH 7 - 9 was pyrophosphate which, as seen in Table 3-3 has very little catalytic effect. Thus, in the range pH 6.96 - 8.80, 0.02 mol l^{-1} pyrophosphate buffers were used and in the range pH 8.9 - 10.67, 0.02 mol l^{-1} carbonate buffers were used.

3-3.2. The Effect of pH on the Rate of Attainment of the 2'-hydroxy-5',6'-benzochalcone——5,6-benzoflavanone equilibrium

The pH dependence of the rate of isomerisation of 2'-hydroxy-5',6'-benzochalcone is shown in table 3-5. Using the method described in appendix 2 the best fit of the data to the overall expression for k_{obs} derived in section 3-2.2 (equation (9)) was obtained by using the following values:

$$pK_a = 8.5$$

$$k = 16 \times 10^{-6} s^{-1}$$

$$k' = 5.0 \times 10^{-4} s^{-1}$$

$$k'' = 1.7 \times 10^{-1} \text{activity}^{-1} s^{-1}$$

The overall expression for k_{obs} is therefore,

$$k_{obs} = 16 \times 10^{-6} fCH + 5.0 \times 10^{-4} fC^{-} + 1.7 \times 10^{-1} \{OH^{-}\}\ s^{-1}$$

TABLE 3-5. Effect of pH on the observed rate constant for the attainment of the 2'-hydroxy-5',6'-benzochalcone —— 5,6'-benzoflavanone equilibrium at 30°C and μ= 0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$
6.96	0.298 0.285	0.292 ± 0.017
7.32	0.482 0.490	0.486 ± 0.029
7.80	1.02 1.03	1.03 ± .06
8.12	1.75 1.75	1.75 ± 0.11
8.44	2.43 2.43	2.43 ± 0.15
8.80	3.37 3.33	3.35 ± 0.20
8.90	3.57 3.52	3.55 ± 0.21
9.30	4.28 4.58	4.43 ± 0.27
9.51	4.60 4.53	4.57 ± 0.27
9.59	4.73 4.82	4.78 ± 0.29
9.97	5.22 5.18	5.20 ± 0.31
10.23	5.52 5.53	5.53 ± 0.33
10.67	5.92 6.02	5.97 ± 0.36

*Mean values taking 6% experimental error in k_{obs} into account (See appendix 1 (b)).

In Fig 3.10. the experimentally determined rate constants are plotted as points, while the line is theoretical, being based on the above expression. In this case the agreement is not as good as for the parent chalcone and thus there is more uncertainty in the values calculated above. In fact, a fit almost equally good (except at pH 10.67) can be obtained using the following values: $_{p}K_{a}$ = 8.5, k = 13 x $10^{-6}s^{-1}$, k' = 4.7 x $10^{-4}s^{-1}$, k" = 3.3 x 10^{-1}activity$^{-1}s^{-1}$. Thus although the value of $_{p}K_{a}$, k and k' are reasonably well defined, the value derived for k" is very uncertain. Factors contributing to this problem were the need for solubility reasons, to work in 10% dioxan solution and the need to use the very low absorbance range 0 - 0.2. The most important factor was, however, the difficulty in obtaining reliable k_{obs} values at pH values greater than 10.5. At these high pH values, very small changes in optical density occurred during the reaction, which led to large errors in k_{obs}. Had k_{obs} been measured reliably at higher pH values the results obtained (particularly in the case of k") would have been far more meaningful. Even so the results do indicate that the presence of benzo group in the 5',6'-position of the chalcone slows the reaction considerably in both the forward and the reverse directions, compared with the parent 2'-hydroxychalcone.

Values for k_{obs} were also measured in the range pH 0.12 - 6.5 for the benzochalcone, and, as had been observed by Shimokoriyama (1957) for chalcones prepared from phloroglucinol-type flavanone 7-glucosides, the reaction was slowest at ≈pH 3. Below pH 3 the reaction rate increased very slowly and k_{obs} at pH 0.12 was still less than k_{obs} at pH 6.5.

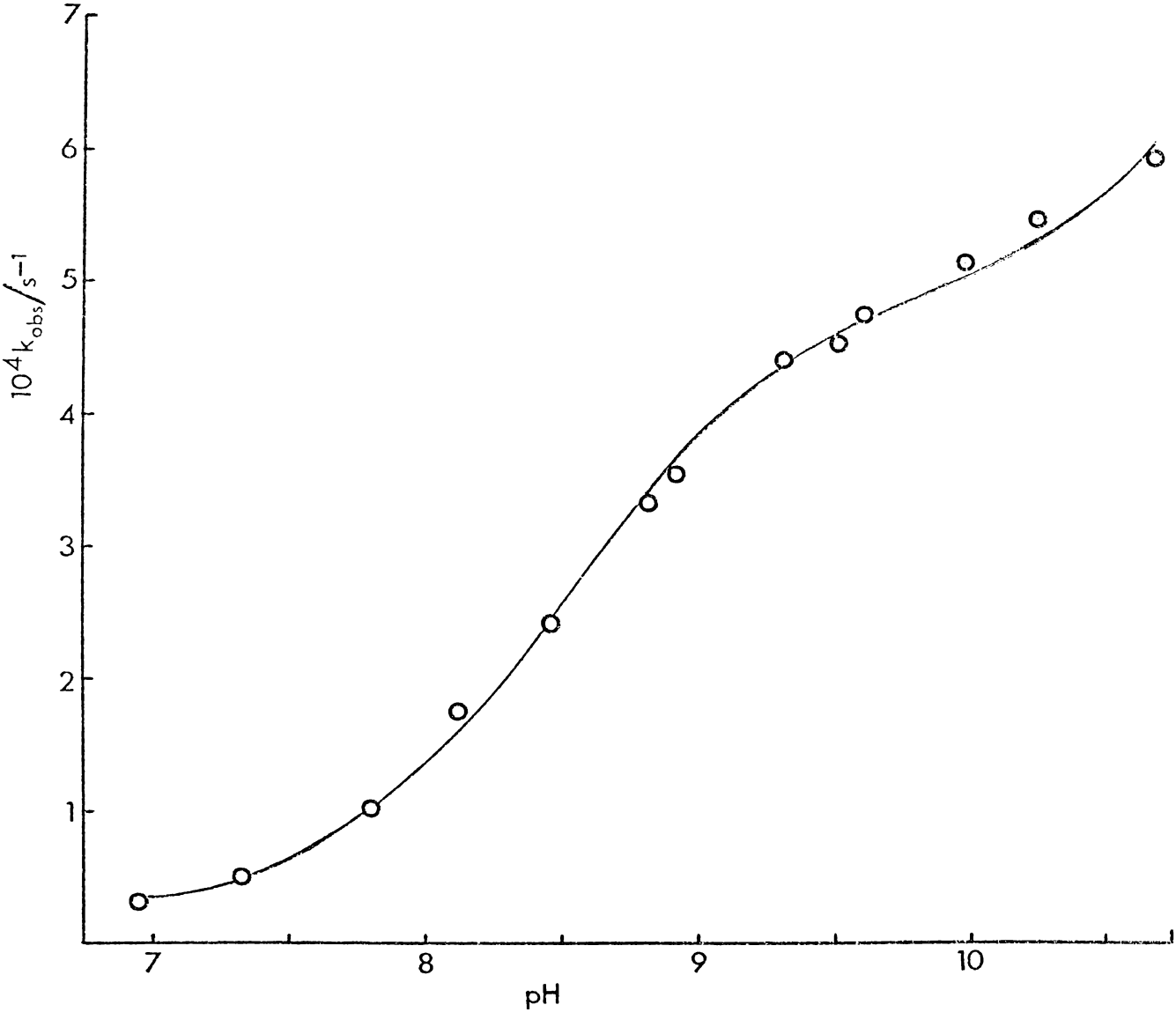
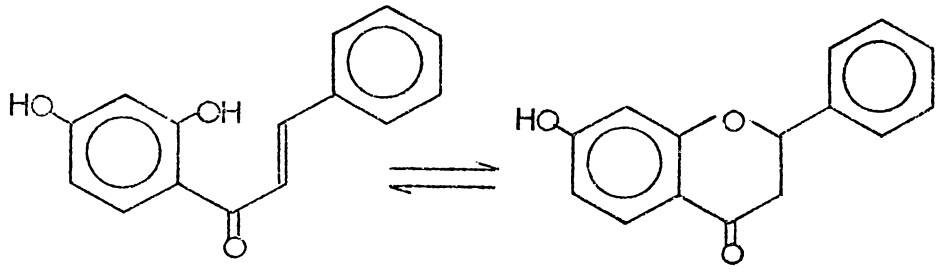


Figure 3.10. pH-rate profile for the attainment of the 2'-hydroxy-5',6'-benzochalcone — 5,6-benzoflavanone equilibrium at 30°C, μ = 0.5 mol l^{-1}

3-4 THE 2',4'-DIHYDROXYCHALCONE——7-HYDROXYFLAVANONE EQUILIBRIUM



The rate of cyclisation of 2',4'-dihydroxychalcone to 7-hydroxyflavanone was measured at 30°C, in 5% dioxan solution using the following buffers: pH 8.9, pyrophosphate; pH 9.38 - 10.81, carbonate; pH 11.07 - 11.40, orthophosphate; and at higher pH values sodium hydroxide solutions. The first order rate constants for the reaction were determined by following the decrease in the chalcone absorbance at 400 nm, and the results are shown in Table 3-6, and presented graphically in Fig. 3.11.

TABLE 3-6 Effect of pH on the observed rate constant for the attainment of the 2',4'-dihydroxychalcone——7-hydroxyflavanone equilibrium at 30°C and μ=0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$*
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

*Mean values taking 3% experimental error in kobs into account (see appendix 1(a

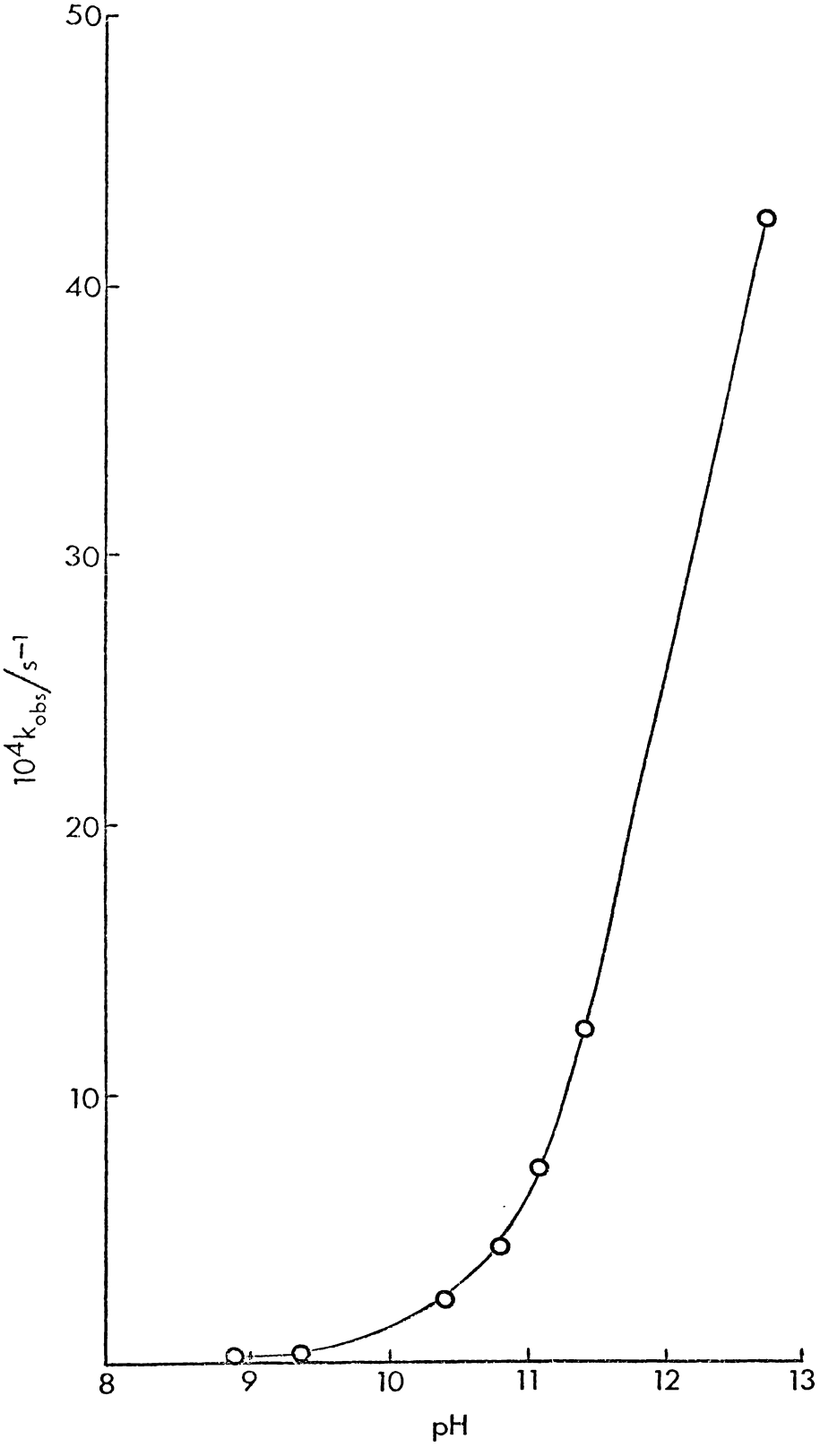


Figure 3.11. pH-rate profile for the attainment of the 2',4'-dihydroxychalcone — 7-hydroxyflavanone equilibrium at 30°C, μ = 0.5 mol l^{-1}

The forward reaction for this chalcone could involve contributions from the neutral, monoanionic and dianionic species and the reverse reaction contributions from the reaction of base with both neutral and anionic flavanone. As a consequence, analysis of the data by the method used for the other chalcones is not possible, and no attempt has been made to fit the data to the much more complicated kinetic form which would apply. Nevertheless, the important observation is made that, unlike the reactions of the other chalcones, the position of the equilibrium is still well towards flavanone even at pH as high as 12.7. A comparison of the repetitive wavelength scans at pH 10.40 (Fig. 3.12). and pH 12.7 (Fig. 3.13.) shows that in both cases the final product is predominantly flavanone. Reaction is still observable at ≈pH 14 and the rate constant determined at this pH was ≈1.67 x $10^{-2}s^{-1}$, which is very similar to that measured for the parent chalcone at pH 11.41 (see Table 3-2). Comparing the value of k_{obs} ($=k_f + k_r$) for 2',4'-dihydroxychalcone at pH 12.7 (42.8 x $10^{-4}s^{-1}$) with the rate constant in the reverse direction only ($k_r = k''\{OH^-\}$) for 2'-hydroxychalcone (1650 x $10^{-4}s^{-1}$) and 2'-hydroxy-4'-methoxychalcone (860 x $10^{-4}s^{-1}$) shows that the rate constant for the reverse reaction of 2',4'-dihydroxychalcone must be less than one twentieth that of kr for 2'-hydroxy-4'-methoxychalcone, and less than one fortieth that of k_r for 2'-hydroxychalcone. That the reverse reaction is significantly slower at high pH for 2',4'-dihydroxychalcone than for the other chalcones studied, is as would be expected for the reaction of hydroxide ion with anionic flavanone.

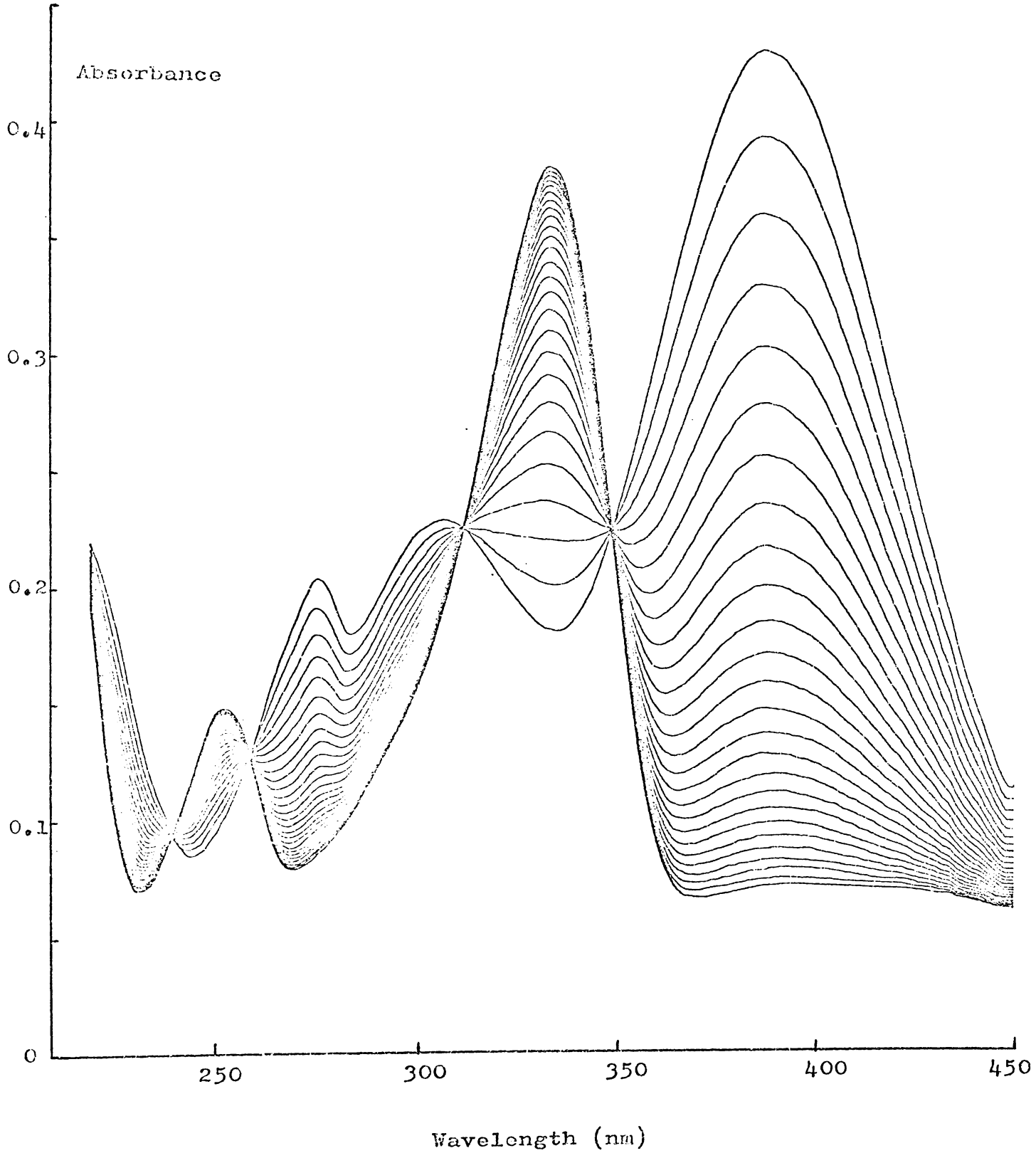


Figure 3.12. Repetitive wavelength scans at 10 min. intervals and pH 10.40 showing the course of the cyclisation reaction of 2',4'-dihydroxychalcone to 7-hydroxyflavanone.

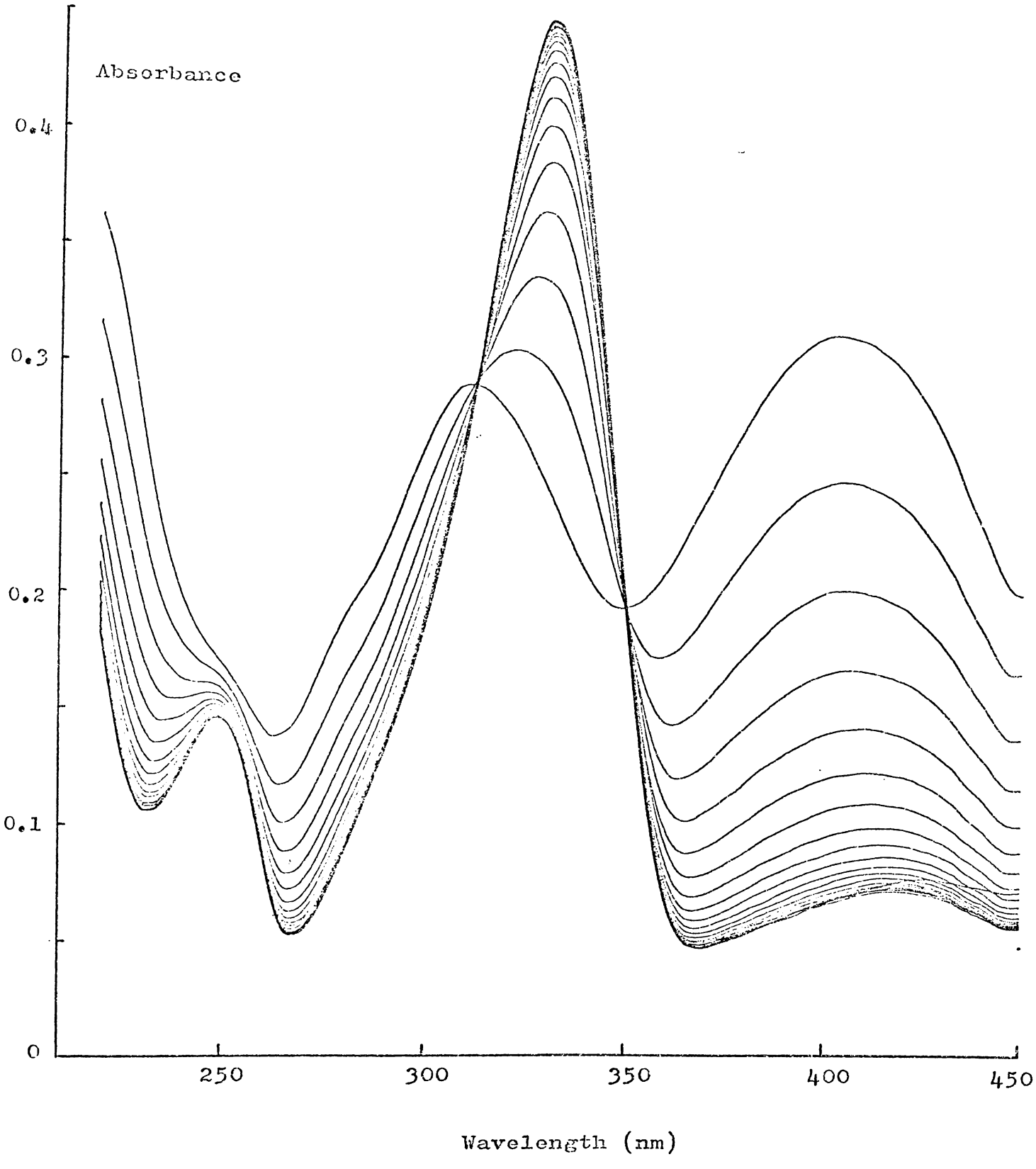
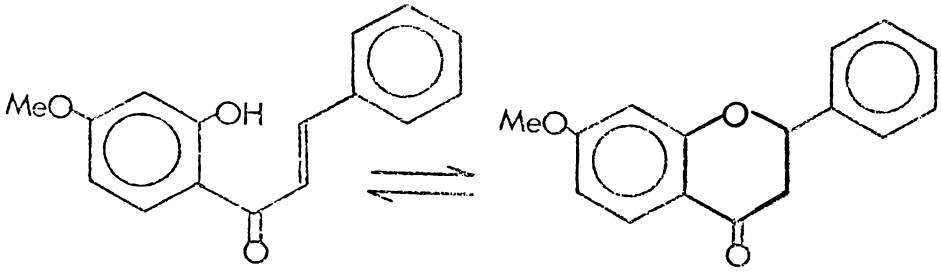


Figure 3.13. Repetitive wavelength scans at 1.2 min. intervals and pH 12.7 showing the course of the cyclisation reaction of 2',4'-dihydroxychalcone to 7-hydroxyflavanone.

3-5 THE 2'-HYDROXY-4'METHOXYCHALCONE——7--METHOXYFLAVANONE EQUILIBRIUM



The rate of conversion of 2'-hydroxy-4'-methoxychalcone to its equilibrium mixture was measured at 30°C, in 5% dioxan solution, over the range pH 7.29 - 11.40. The repetitive wavelength scans at pH 8.40 (Fig. 3.14) and pH 10.22 (Fig. 3.15) determined that, in order to measure the largest possible absorbance change, the reaction should be studied by following the decrease in the chalcone absorbance at 350 nm for pH values less than 9.5 and at 310 nm for pH values greater than 9.5.

3-5.1. Buffer Dilution Studies

The effect of buffer concentration on k_{obs} for the carbonate buffer at pH 10.50 and for the pyrophosphate buffer at pH 8.76 is shown in Table 3-7. The catalysis was again small, and on the basis of these results the following buffers were considered suitable for studying this reaction: from pH 7.29 - 8.9, 0.02 mol l^{-1} pyrophosphate; from pH 9.18 - 10.83, 0.02 mol l^{-1} carbonate; and for higher pH values, 0.02 mol l^{-1} orthophosphate.

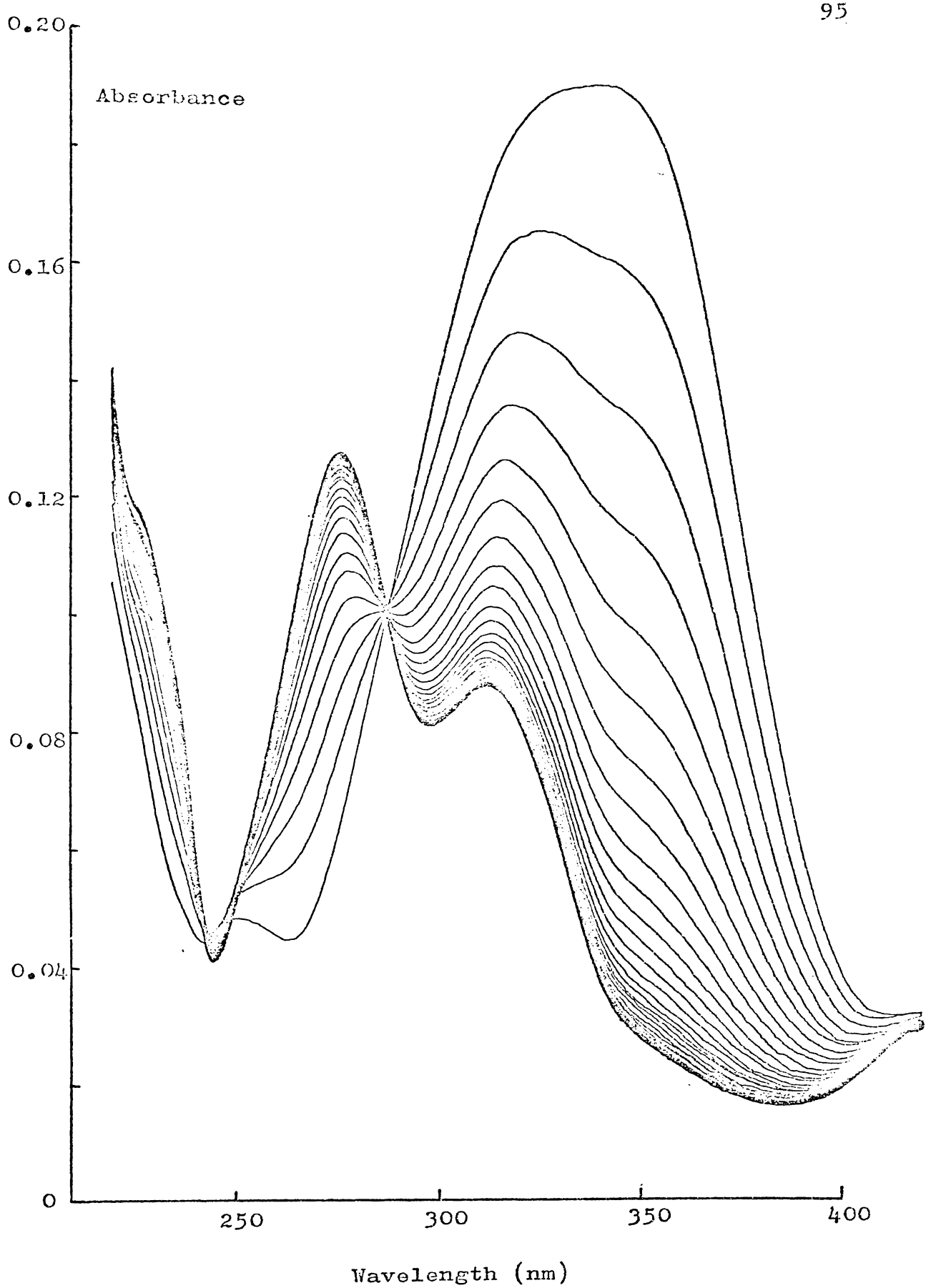


Figure 3.14. Repetitive wavelength scans at 30 min. intervals and pH 8.40 showing the course of the cyclisation reaction of 2'-hydroxy-4'-methoxychalcone to 7-methoxyflavanone.

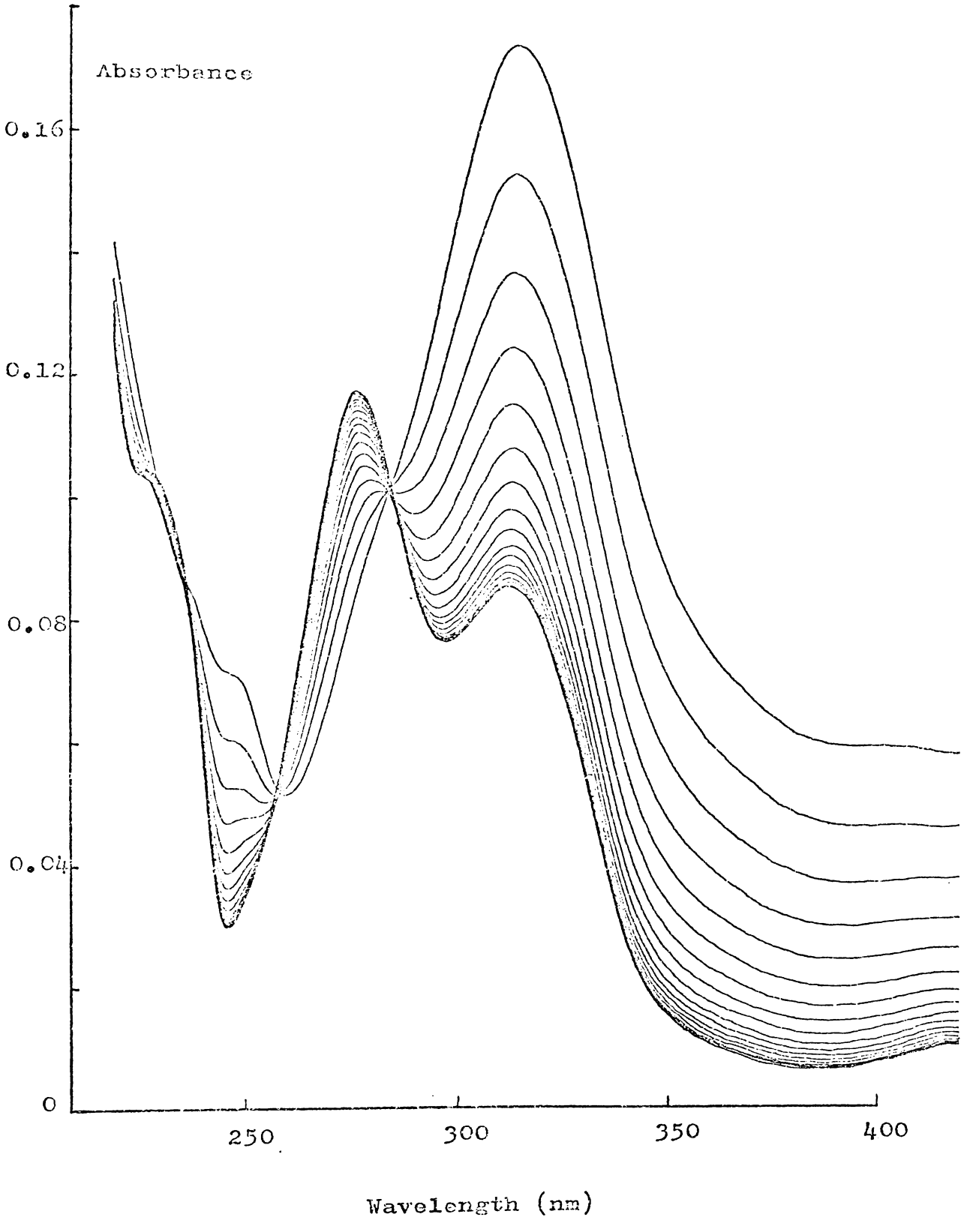


Figure 3.15. Repetitive wavelength scans at 2 min. intervals and pH 10.22 showing the course of the cyclisation reaction of 2'-hydroxy-4'-methoxychalcone to 7-methoxyflavanone.

TABLE 3-7 Effect of Buffer Concentration on k_{obs}

Buffer	Concentration (mol l^{-1})	$10^4 k_{obs}/s^{-1}$
Carbonate (pH = 10.50)	0.1	30.1 ± 1.8
	0.07	29.2 ± 1.8
	0.04	28.3 ± 1.7
Pyrophosphate (pH = 8.76)	0.054	4.28 ± 0.26
	0.036	4.10 ± 0.25
	0.022	3.93 ± 0.24

3-5.2. The effect of pH on the rate of attainment of the 2'-hydroxy-4'-methoxychalcone⟷7-methoxyflavanone equilibrium

The pH dependence of the rate of cyclisation of 2'-hydroxy-4'-methoxychalcone to 7-methoxyflavanone is recorded in Table 3-8. The method described in appendix 2 was used to fit the data to the derived expression for k_{obs} (equation (9), section 3-2.2). The best fit values were:

$$pK_a = 9.55$$

$$k = 7 \times 10^{-6} s^{-1}$$

$$k' = 27 \times 10^{-4} s^{-1}$$

$$k'' = 11.7 \times 10^{-1} \text{activity}^{-1} s^{-1}$$

The overall expression for k_{obs} is therefore,

$$k_{obs} = 7 \times 10^{-6} fCH + 27 \times 10^{-4} fC^- + 11.7 \times 10^{-1} \{OH^-\}\ s^{-1}$$

In Fig. 3.16. the experimentally determined k_{obs} values are plotted as points, while the line is theoretical, being based on the above expression. The agreement between the experimental points and the theoretical line is within 6% indicating that the kinetic data are consistent with the above rate expression over the pH range studied.

TABLE 3-8. Effect of pH on the observed rate constant for the attainment of the 2'-hydroxy-4'-methoxychalcone —— 7-methoxyflavanone equilibrium at 30°C and μ= 0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$
7.29	0.222 0.228	0.225 ± 0.014
7.72	0.448 0.445	0.447 ± 0.027
7.80	0.550 0.548	0.549 ± 0.033
8.12	1.14 1.12	1.13 ± 0.07
8.40	1.99 1.98	1.99 ± 0.12
8.76	3.85 3.77	3.81 ± 0.23
8.90	5.08 5.05	5.07 ± 0.30
9.18	8.25 8.38	8.32 ± 0.50
9.38	11.62 11.55	11.6 ± 0.7
9.48	13.3 12.9	13.1 ± 0.8
9.84	19.6 19.3	19.5 ± 1.2
10.10	22.9 23.0	23.0 ± 1.4
10.22	24.7 24.7	24.7 ± 1.5
10.46	28.3 27.1	27.7 ± 1.7
10.54	29.3 28.7	29.0 ± 1.7
10.83	35.3 35.6	35.5 ± 2.1
11.07	46.8 47.2	47.0 ± 2.8
11.40	69.5 71.3	70.4 ± 4.2

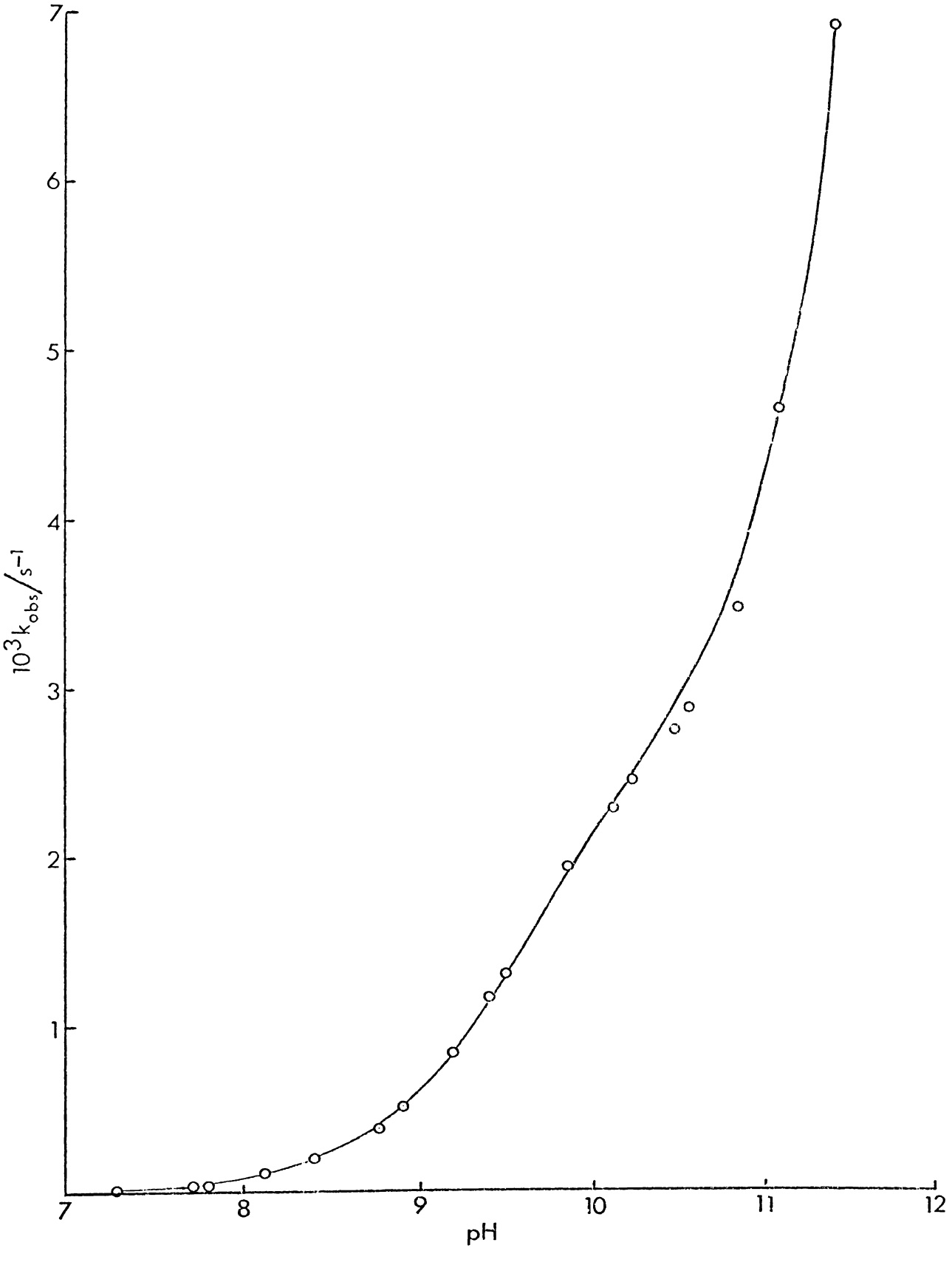
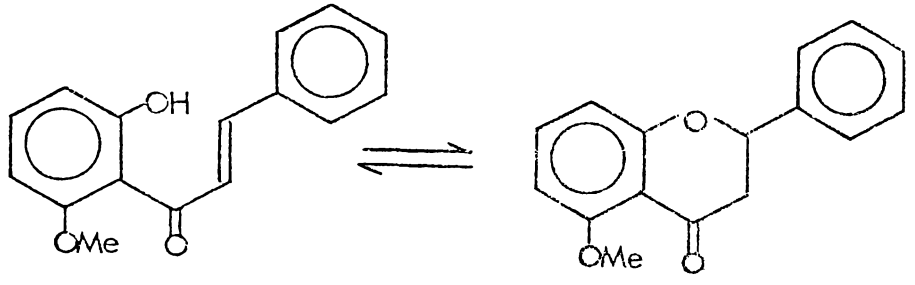


Figure 3.16. pH-rate profile for the attainment of the 2'-hydroxy-4'-methoxychalcone— 7 methoxyflavanone equilibrium at 30°C, μ= 0.5 mol l^{-1}

3-6 THE 2'-HYDROXY-6'-METHOXYCHALCONE——5 -METHOXYFLAVANONE EQUILIBRIUM



The rate of conversion of 2'-hydroxy-6'-methoxychalcone to its equilibrium mixture was measured at 30°C, in 5% dioxan solution, over the pH range 6.94 - 10.83. The repetitive wavelength scans at pH 7.79 (Fig. 3.17.) and pH 10.23 (Fig. 3.18). show the clean conversion of the chalcone to the chalcone-flavanone equilibrium mixture, and reveal that at both these pH values the largest possible absorbance change occurs at 300 nm, thereby determining the wavelength at which the reaction should be followed.

3-6.1. Buffer Dilution Studies

The effect of buffer concentration on k_{obs} was again measured for the carbonate buffer at pH 10.50 and for the pyrophosphate buffer at pH 8.76. The results confirmed that little error would be introduced into the observed rate constants by the use of 0.02 mol l^{-1} carbonate and pyrophosphate buffers.

3-6.2. The Effect of pH on the Rate of Attainment of the 2'-hydroxy-6'-methoxychalcone——5-methoxyflavanone Equilibrium

The pH dependence of the rate of cyclisation of 2'-hydroxy-6'-methoxychalcone to 5-methoxyflavanone is recorded in Table 3-9. The method used to fit the data to the derived expression for k_{obs}

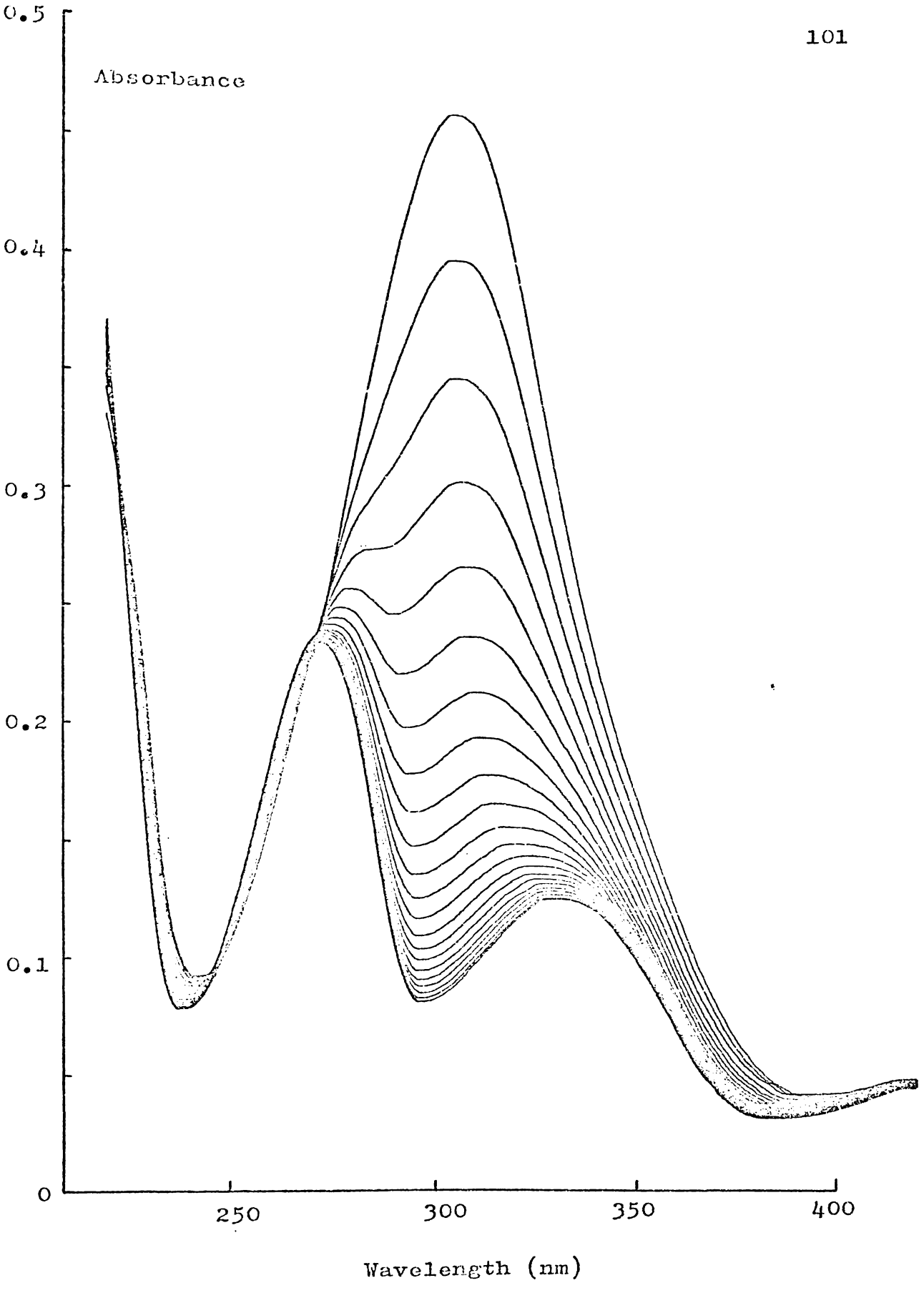


Figure 3.17. Repetitive wavelength scans at 60 min. intervals and pH 7.79 showing the course of the cyclisation reaction of 2'-hydroxy-6'-methoxychalcone to 5-methoxyflavanone.

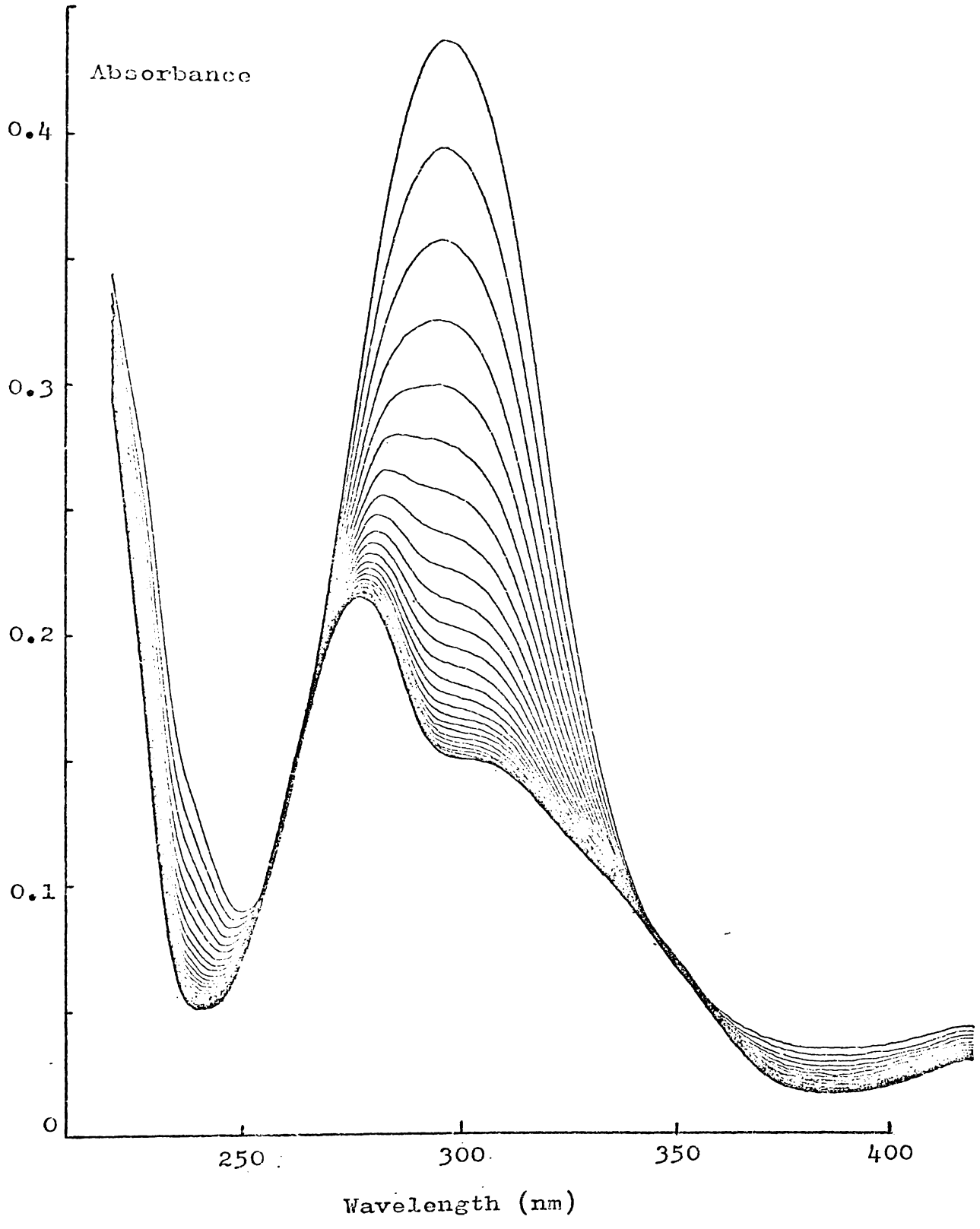


Figure 3.18. Repetitive wavelength scans at 4 min. intervals and pH 10.23 showing the course of the cyclisation reaction of 2'-hydroxy-6'-methoxychalcone to 5-methoxyflavanone.

TABLE 3-9. Effect of pH on the observed rate constant for the attainment of the 2'-hydroxy-6'-methoxychalcone ⟷ 5-methoxyflavanone equilibrium at 30°C and μ= 0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$*
6.94	0.135 0.130	0.133 ± 0.004
7.29	0.198 0.202	0.200 ± 0.006
7.79	0.436 0.430	0.433 ± 0.013
8.14	0.802 0.798	0.800 ± 0.024
8.43	1.34 1.34	1.34 ± 0.04
8.77	2.25 2.27	2.26 ± 0.07
8.90	2.60 2.60	2.60 ± 0.08
9.25	3.58 3.58	3.58 ± 0.10
9.39	4.15 4.17	4.16 ± 0.12
9.85	5.35 5.35	5.35 ± 0.16
10.12	6.13 6.13	6.13 ± 0.18
10.23	6.57 6.58	6.58 ± 0.20
10.50	7.95 7.83	7.89 ± 0.24
10.83	11.45 11.57	11.51 ± 0.35

*Mean values taking 3% experimental error in k_{obs} into account. (See appendix 1 (a)).

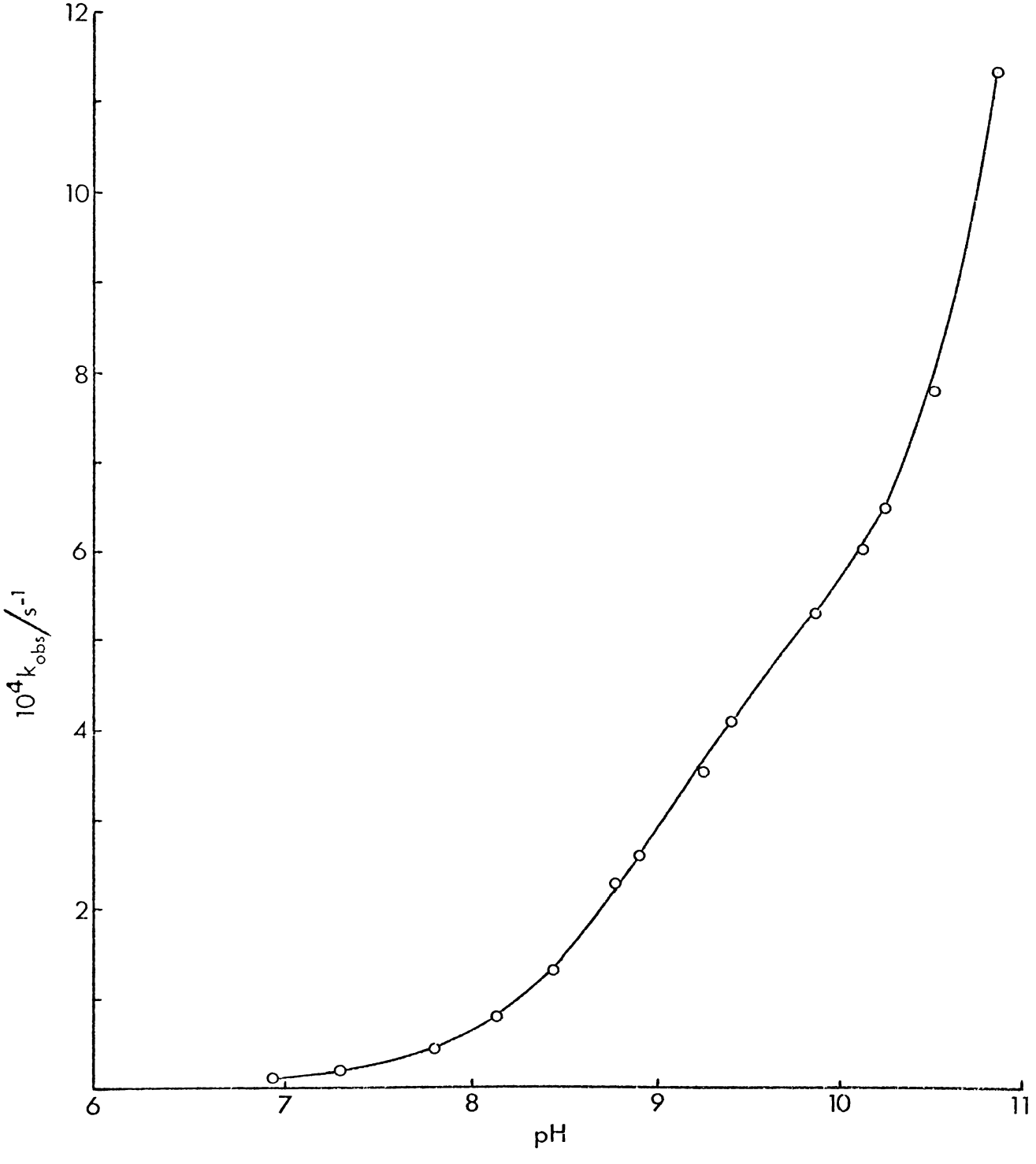


Figure 3.19. pH-rate profile for the attainment of the 2'-hydroxy-6'-methoxychalcone —— 5-methoxyflavanone equilbrium at 30°C, μ= 0.5 mol l^{-1}

(equation (9), section 3-2.2) is explained in detail for this compound in appendix 2. The best fit values were:

$$pK_a = 8.95$$

$$k = 8 \times 10^{-6} s^{-1}$$

$$k' = 5.3 \times 10^{-4} s^{-1}$$

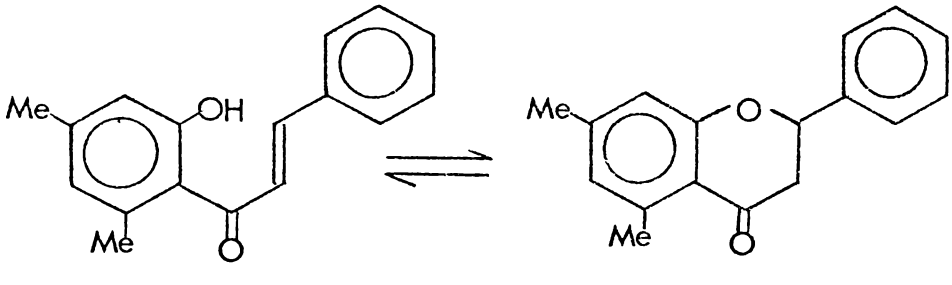
$$k'' = 6.33 \times 10^{-1} \text{activity}^{-1} s^{-1}$$

The overall expression for k_{obs} is therefore,

$$k_{obs} = 8 \times 10^{-6} fCH + 5.3 \times 10^{-4} fC^{-} + 6.33 \times 10^{-1} \{OH^{-}\}\ s^{-1}$$

In Fig. 3.19. the points are experimental and the line is based on the above expression for k_{obs}. The agreement between the experimental points and the theoretical line is within 3% for this chalcone, indicating that the kinetic data are consistent with the above rate expression over the pH range studied.

3-7 THE 2'-HYDROXY-4',6'-DIMETHYLCHALCONE——5,7-DIMETHYLFLAVANONE EQUILIBRIUM



The rate of conversion of 2'-hydroxy-4',6'-dimethylchalcone to its equilibrium mixture was measured at 30°C, in 5% dioxan solution, over the range pH 7.29 - 11.42. The repetitive wavelength scans for this chalcone are shown at pH 7.80 (Fig. 3.20.) and pH 10.24 (Fig. 3.21.).

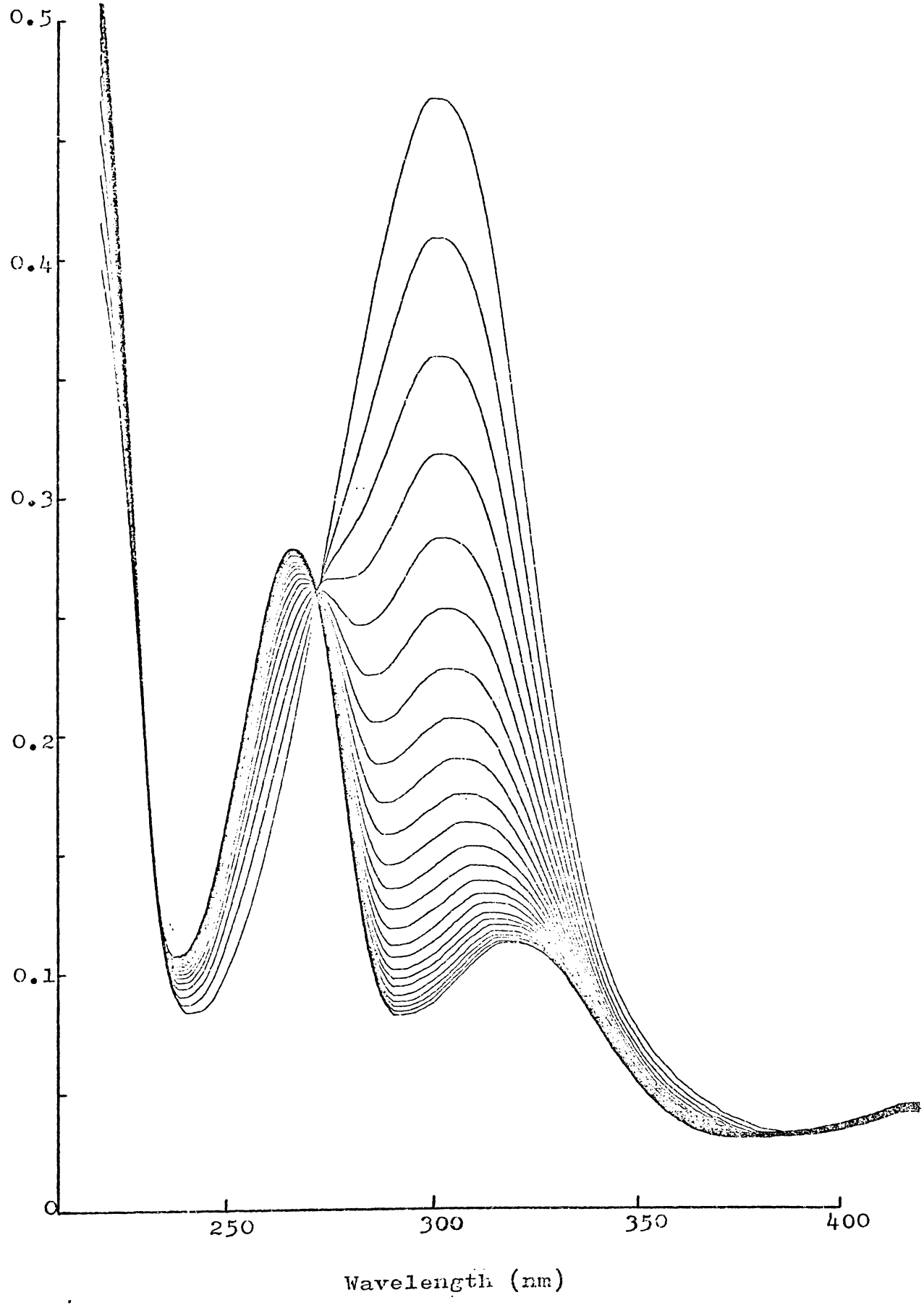


Figure 3.20. Repetitive wavelength scans at 60 min. intervals and pH 7.80 showing the course of the cyclisation reaction of 2'-hydroxy-4',6'-dimethylchalcone to 5,7-dimethylflavanone.

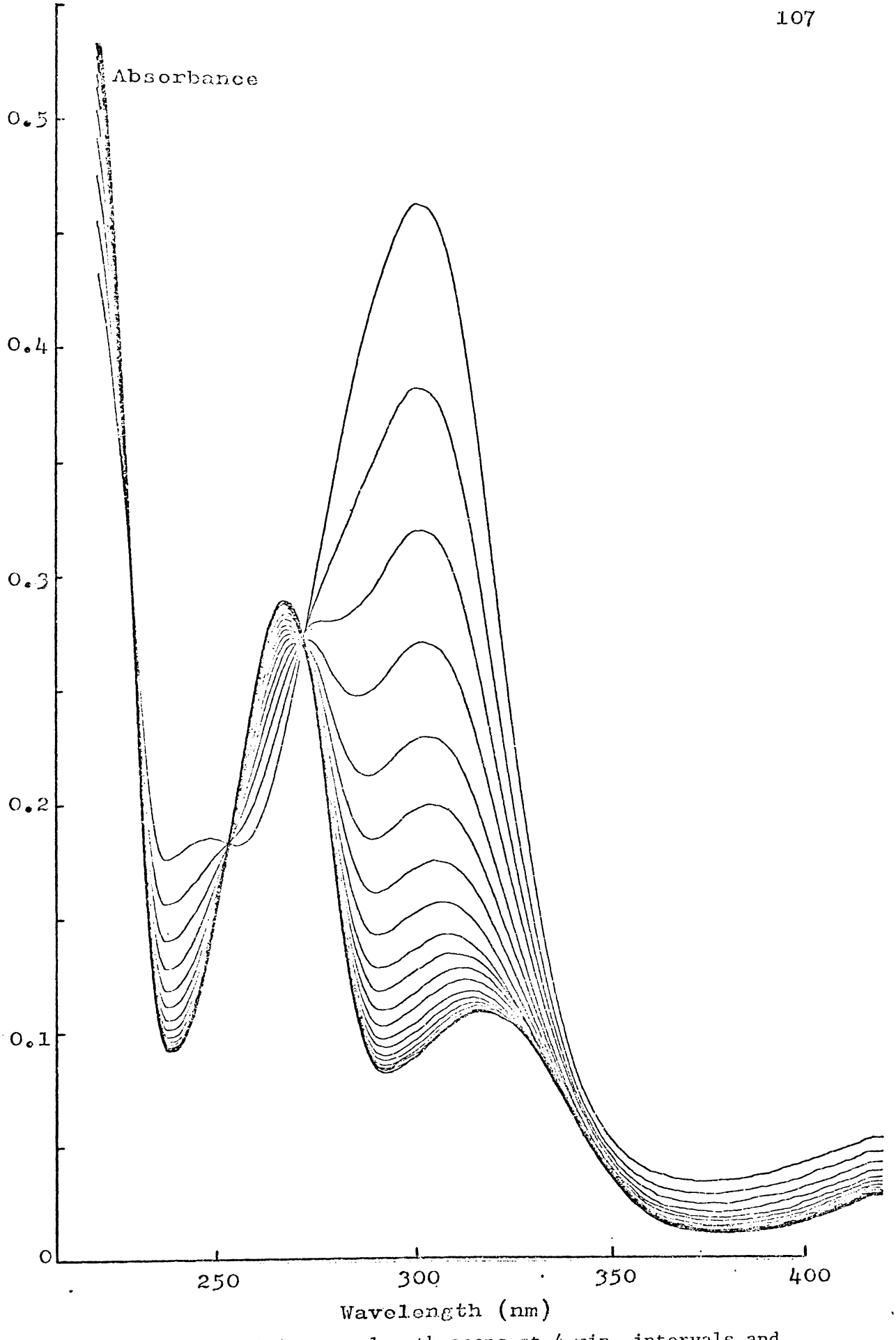


Figure 3.21. Repetitive wavelength scans at 4 min. intervals and pH 10.24 showing the course of the cyclisation reaction of 2'-hydroxy-4',6'-dimethylchalcone to 5,7-dimethylflavanone.

The rate constants were determined by following the decrease in the chalcone absorbance at 300 nm.

3-7.1. Buffer Dilution Studies

Buffer catalysis was essentially undetectable for the buffers used in this study, which were from pH 7.29 - 8.89, 0.02 mol l^{-1} pyrophosphate; from pH 9.18 - 10.85, 0.02 mol l^{-1} carbonate; and from pH 11.10 - 11.42, 0.02 mol l^{-1} orthophosphate.

3-7.2. The Effect of pH on the Rate of Attainment of the 2'-hydroxy-4',6'-dimethylchalcone⟷5,7-dimethylflavanone Equilibrium

The pH dependence of the rate of cyclisation of 2'-hydroxy-4',6'-dimethylchalcone to 5,7-dimethylflavanone is recorded in Table 3-10. The method used to fit this data to the derived expression for k_{obs} (equation (9), section 3-2.2.) is described in appendix 2. The best fit values were:

$$pK_a = 9.25$$
$$k = 4 \times 10^{-6} s^{-1}$$
$$k' = 10.0 \times 10^{-4} s^{-1}$$
$$k'' = 1.9 \times 10^{-1} \text{activity}^{-1} s^{-1}$$

The overall expression for k_{obs} is therefore,

$$k_{obs} = 4 \times 10^{-6} fCH + 10.0 \times 10^{-4} fC^- + 1.9 \times 10^{-1} \{OH^-\}\ s^{-1}$$

The experimentally determined k_{obs} values are shown as points in Fig. 3.22. and the line is theoretical, being based on the above expression for k_{obs}. Apart from the values at pH 7.29 which differ by ≃ 4% all other values agree to within 3%, indicating consistency between the kinetic data and above rate expression for the pH range studied.

TABLE 3-10. Effect of pH on the observed rate constant for the attainment of the 2'-hydroxy-4',6'-dimethylchalcone ⟷ 5,7-dimethylflavanone equilibrium at 30°C and μ= 0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$*
7.29	0.142 0.144	0.143 ± 0.004
7.80	0.393 0.385	0.389 ± 0.012
8.14	0.765 0.770	0.768 ± 0.023
8.42	1.33 1.33	1.33 ± 0.04
8.76	2.48 2.55	2.52 ± 0.08
8.89	3.13 3.13	3.13 ± 0.09
9.18	4.62 4.62	4.62 ± 0.14
9.38	5.78 5.82	5.80 ± 0.17
9.48	6.40 6.43	6.42 ± 0.19
9.84	8.12 8.25	8.19 ± 0.25
10.10	9.12 9.08	9.10 ± 0.27
10.24	9.37 9.33	9.35 ± 0.28
10.50	10.03 10.08	10.06 ± 0.30
10.85	11.5 11.6	11.6 ± 0.3
11.10	13.3 13.5	13.4 ± 0.4
11.42	17.7 17.3	17.5 ± 0.5

*Mean values taking 3% experimental error in k_{obs} into account (see appendix 1 (a))

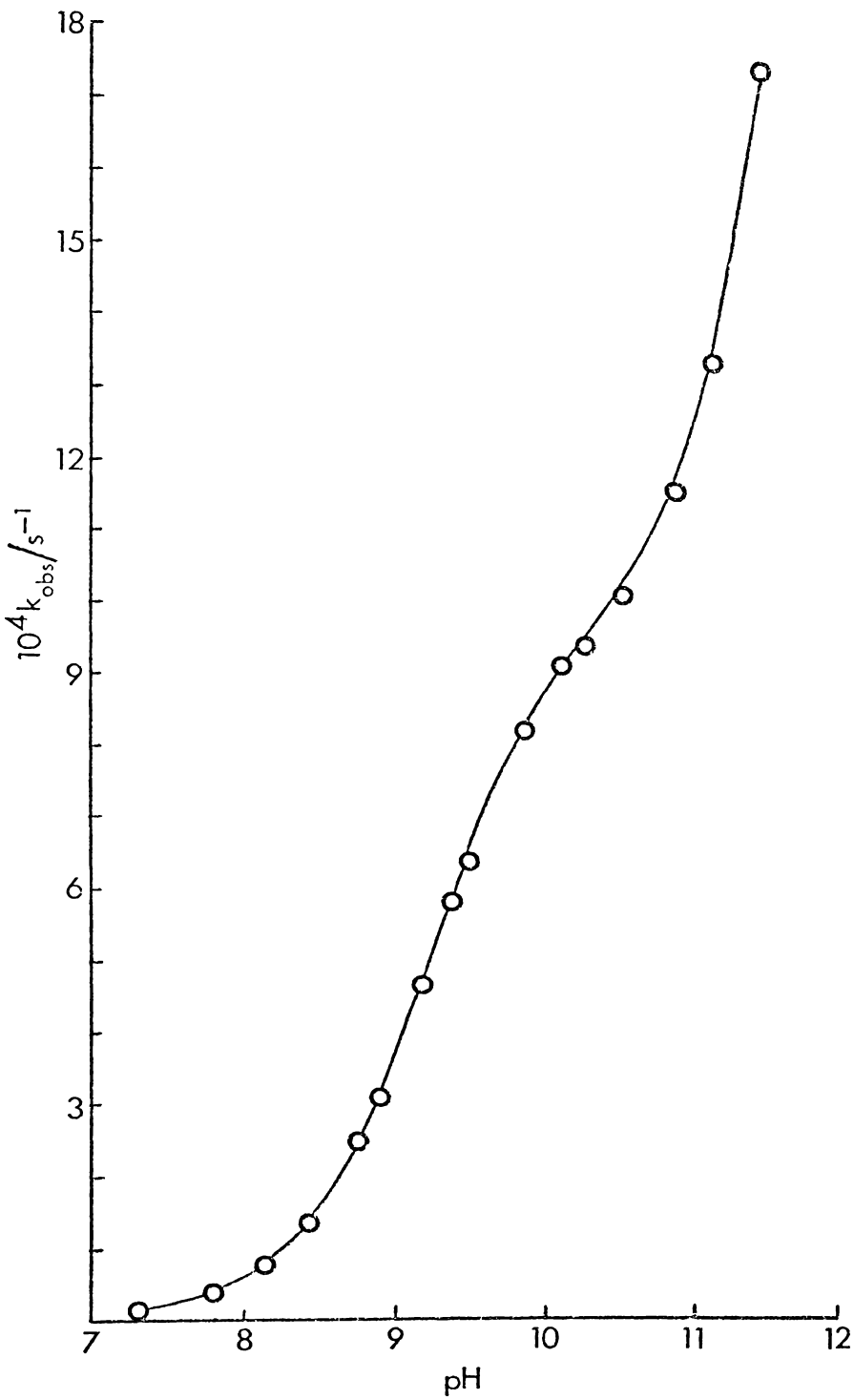


Figure 3.22. pH-rate profile for the attainment of the 2'-hydroxy-4', 6'-dimethylchalcone — 5,7-dimethylflavanone equilibrium at 30°C, μ= 0.5 mol l^{-1}

3-8 DISCUSSION

3-8.1. Mechanism

A slightly expanded form of the most probable mechanism proposed to apply in the forward direction for the chalcone anion is shown in Fig. 3.23. The protonation of the enolate ion would probably be mainly at the oxygen leading to the enol which tautomerises. The mechanism is therefore an intramolecular case of 1,4 nucleophilic addition to the α,β-unsaturated carbonyl system, followed by protolytic rearrangement.

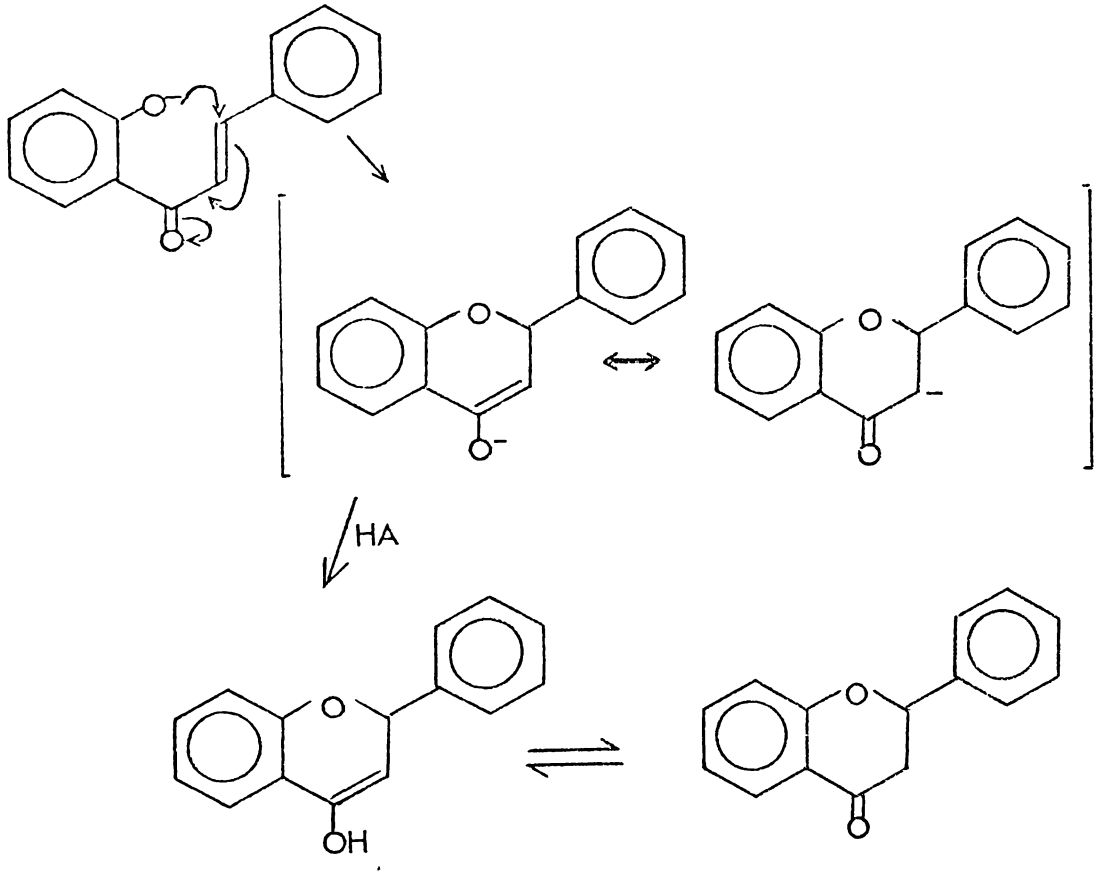


Figure 3.23. Mechanism showing cyclisation of ionised 2'-hydroxychalcone

The cyclisation of the neutral chalcone can be similarly depicted (Fig 3.24.).

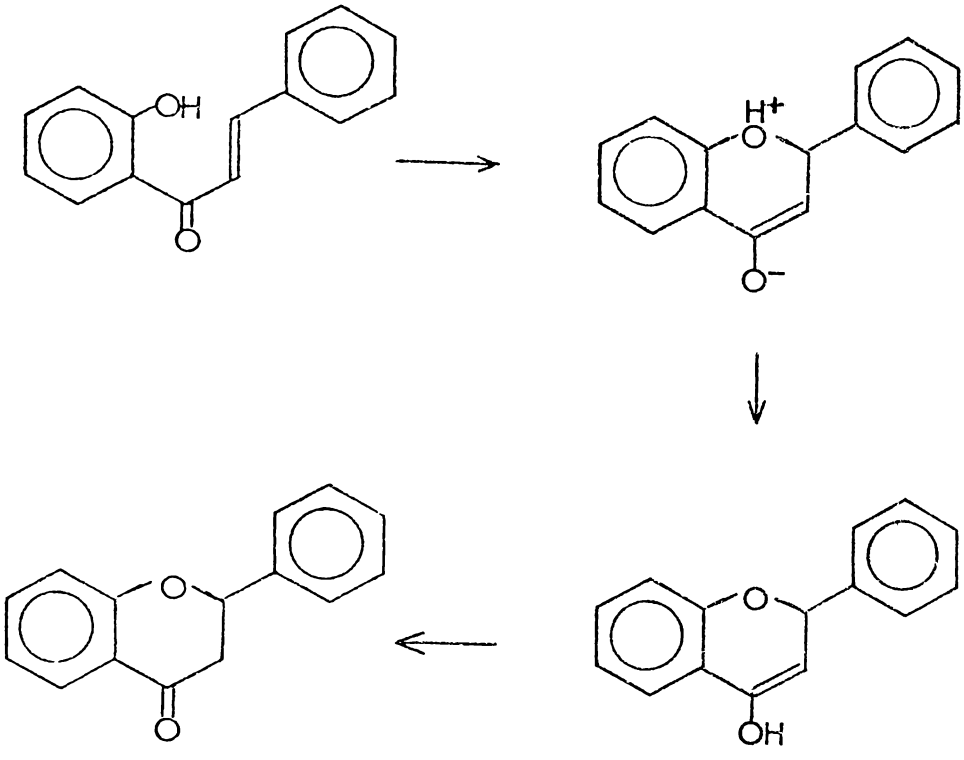


Figure 3.24. Mechanism showing cyclisation of the neutral 2'-hydroxychalcone

The mechanism for the elimination in the reverse reaction is less well defined. It may be a concerted E2 mechanism in which the proton and phenolate group depart simultaneously, with the proton being pulled off by a base as shown in Fig. 3.25, or possibly an El_{cB} mechanism involving a carbanion (enolate) intermediate as shown in Fig. 3.26.

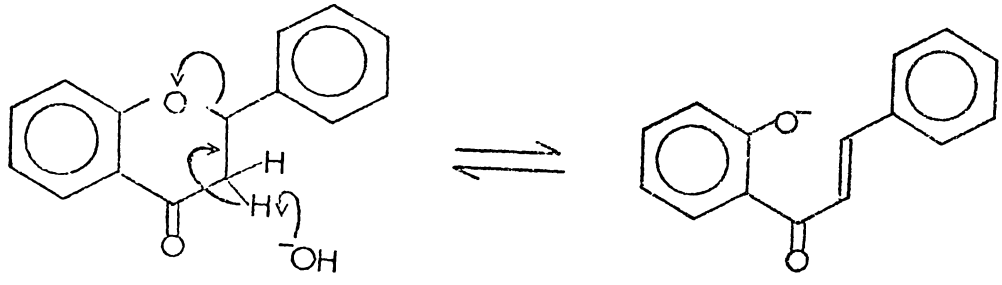
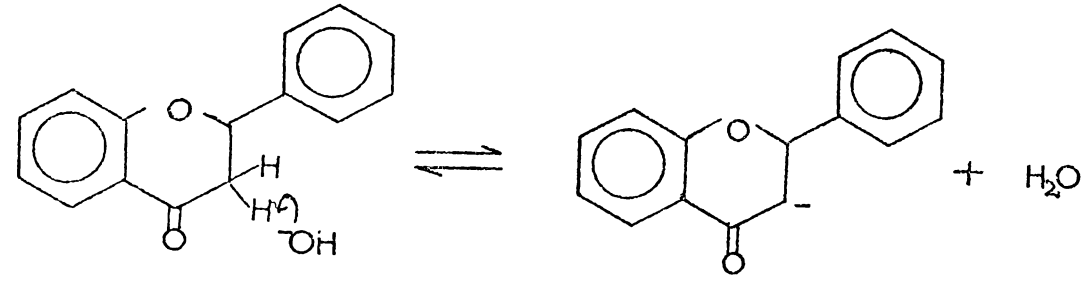


Figure 3.25. Possible E2 mechanism



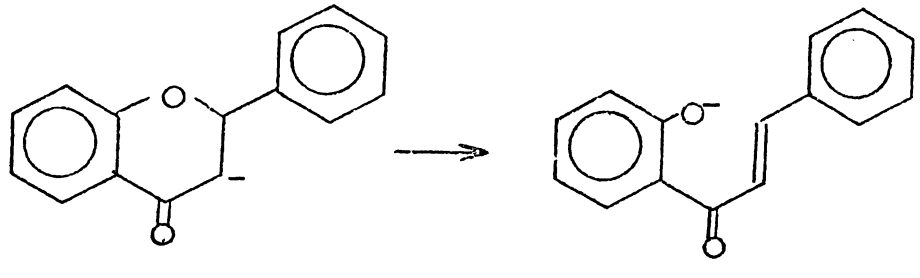


Figure 3.26. Possible El_{cB} mechanism

El_{cB} eliminations can be subdivided into irreversible El_{cB} and reversible El_{cB}. In the former case the carbanion shown in Fig. 3.26. goes on to give the chalcone more rapidly than it picks up a proton to revert to the flavanone, while in the latter case the carbanion returns to the flavanone more quickly than it decomposes to the chalcone. Using the steady state treatment (Saunders and Cockerill, 1973) it can be shown that an irreversible El_{cB} elimination is kinetically indistinguishably from an E2 elimination, and both should be dependent on buffer concentrations. In many cases a reversible El_{cB} mechanism is kinetically distinguishable from the other two mechanisms in that the rate should show an inverse dependence upon the conjugate acid of the base involved, and should be independent of buffer concentrations. In our case the conjugate acid is the reaction solvent (H_2O) and it was thus not possible to differentiate on these grounds.

There was very little proven buffer catalysis except for N-ethylmorpholine, diethanolamine and tris(hydroxymethyl)aminomethane buffers and Fedor (1967) has shown that N-alkylmorpholines, which are of similar basicity to the three amines used in the present study may be poor catalysts for eliminations resulting in the formation of a double bond α,β to a carbonyl group. This suggests that the observed catalysis in these cases is probably not as a result of their being general base catalysis in the reverse reaction, but rather catalysis of some type in the forward reaction. This indicates that the mechanism involved in the reverse reaction could be a reversible El_{cB}, but much more work on buffer catalysis studies would be needed to substantiate this premise.

For an E2 mechanism to operate it is preferable to have the five atoms involved in the same plane (Fukui & Fujimoto, 1965). The possibility exists, therefore, that the presence of certain substituents (in particular 6'-substituents) could change the mechanism by forcing the atoms involved to be out of coplanarity. Therefore, different elimination mechanisms may apply for different chalcones.

3-8.2. Attempts to Rationalise the Values of pK_a, k, k' and k"

The observed rate constants for the attainment of equilibrium of several chalcones have been found to fit the expression $k_{obs} = kfCH + k'fC^- + k''\{OH^-\}$ (equation (9), section 3-2.2) and a summary of the values of best fit derived for k, k', k" and the pK_a are shown in Table 3-11.

TABLE 3-11. Summary of the results from this section.

Chalcone	pK_a	10^6k	$10^4k'$	10k"
2'-hydroxy	9.55	10	75	22.5
2'-hydroxy-4'-methoxy	9.55	7	27	11.7
2'-hydroxy-6'-methoxy	8.95	8	5.3	6.33
2'-hydroxy-4',6'-dimethyl	9.25	4	10	1.90
2'-hydroxy-5',6'-benzo	8.5	16	5.0	1.7

The values calculated for 2'-hydroxy-5',6'-benzochalcone have been included in Table 3-11 to enable general comparisons to be made, but because of the uncertainty associated with these values no attempt will be made to rationalise them specifically. Similarly there will be no attempt to rationalise the calculated values of k as they are subject to significant experimental error in that they are very small values obtained by subtracting relatively large k' and k" $\{OH^-\}$terms from k_{obs} (they make little contribution to the total rate of the reaction

in the region of main interest). Also, as a consequence of the method used to determine them they may include contributions from processes other than the uncatalysed cyclisation of neutral chalcone in the forward direction. In any case the values for k differed very little between the chalcones which would make any attempts at rationalisation rather suspect.

3-8.2.1. Discussion of $_pK_a$ values

2'-hydroxychalcones may exist, at least partially, in a hydrogen bonded configuration in aqueous solution as shown in Fig. 3.27 and the calculated $_pK_a$ values may be partly a measure of the relative strength of this hydrogen bond as well as a measure of the inherent basicity of the conjugate base. There is no doubt about the dominance of the hydrogen bonded configuration in non-polar solvents but its extent in aqueous solution is less certain.

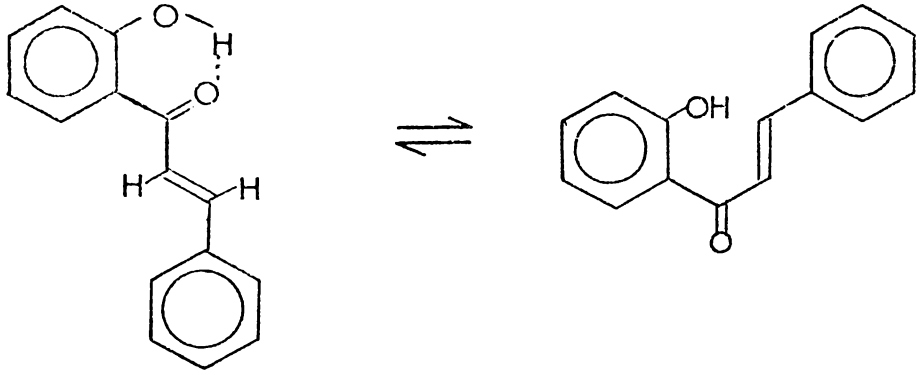


Figure 3.27. Hydrogen-bonding in 2'-hydroxychalcones

Lamola and Sharp (1966) showed that very little intramolecular hydrogen-bonding existed in *o*-hydroxybenzophenones when in hydroxylic solvents, presumably because the solvent could form intermolecular hydrogen bonds with the ortho hydroxyl group. They do not exclude the possibility that there may be a small degree of intramolecular hydrogen bonding in aqueous solution, and in our example the ability of the carbonyl oxygen to withdraw electrons from the double bond, and hence increase its charge density, may in fact make intramolecular hydrogen bonding even more favourable.

Assuming that hydrogen bonding is significant a lowering of the pK_a for chalcones with 6'-substituents could be explained if the interaction shown in Fig. 3.28. caused a weakening of the hydrogen bond. The relative pK_a values within chalcones with 6'-substituents could be explained by the extent of this interaction. If this is so, a direct correlation of basicity, as measured by the pK_a, with nucleophilicity need not necessarily follow.

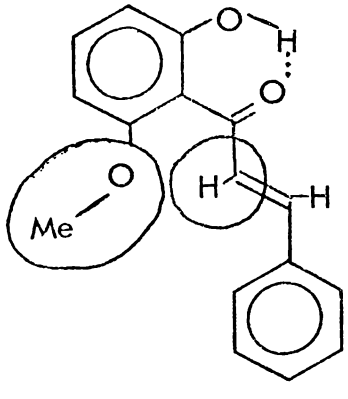


Figure 3.28. Possible interaction, in the hydrogen bonded configuration of chalcones with 6'-substituents

Had purely electronic effects been considered, quite incorrect predictions for the relative values of pK_a would have resulted. For example, the pK_a for 2'-hydroxy-6'-methoxychalcone would have been predicted to be greater than that for 2'-hydroxychalcone, and approximately the same as that for 2'-hydroxy-4'-methoxychalcone. The similarity of the pK_a values for 2'-hydroxychalcone and 2'-hydroxy-4'-methoxychalcone is difficult to rationalise as no steric effects of the type outlined above could apply, and electronic considerations would have predicted that the latter compound should have a higher pKa.

3-8.2.2. Discussion of k' values

A relationship was first looked for between k' and the calculated pK_a as shown in Fig. 3.29. It can be seen that there is a general trend, with k' increasing as the pK_a increases but the correlation is not linear. The range of pK_a values is very small, however, and a trend may have been more obvious if this range could have been extended. The lack of a linear correlation between log k' and pK_a, for the chalcones under study, coupled with the mentioned expectation that relative pK_a values may not indicate the relative nucleophilicities of the 2'-o^- ions in the chalcones studied, requires that other factors need to be considered in an exploration of the relative k' values.

Substituents which are likely to affect the rate constant for the cyclisation of the ionised chalcone (k') (see Fig. 3.23.) can be grouped as follows:

(i) Those which affect the nucleophilic power of the 2'-o^- ion.

(ii) Those which affect the electron withdrawal by the carbonyl group from the double bond (to which cyclisation occurs),

(iii) Those that cause a preference for conformations that are sterically unsuitable for cyclisation.

These factors can be influenced by steric and/or electronic effects as listed below in points A to D.

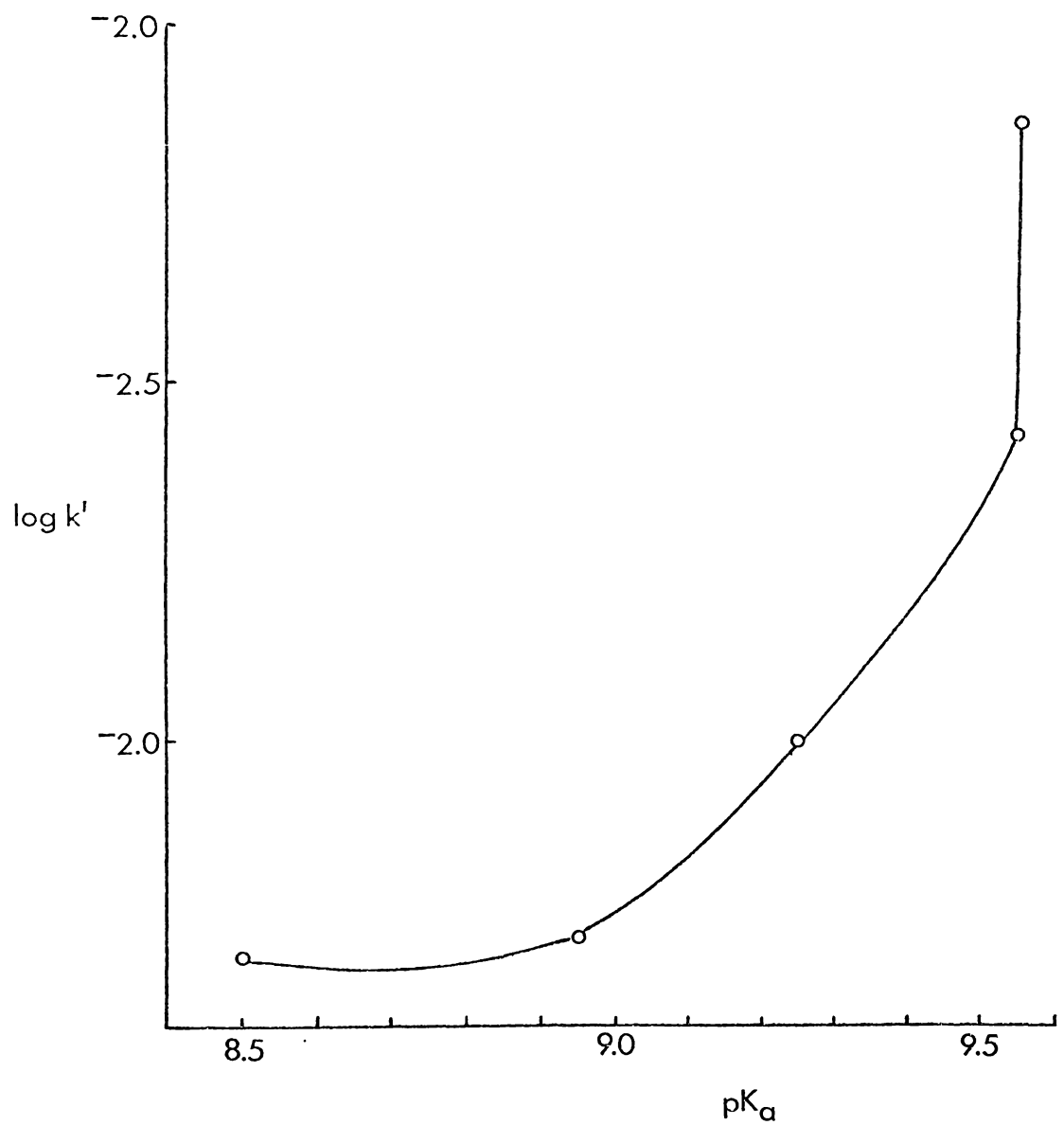


Figure 3.29. Relationship between log k' and pK_a

(A) The carbonyl group could be forced out of planarity with the A-ring either by steric interaction with any 6'-substituent or, in the case of a 6'-methoxyl group, possibly by an electrostatic repulsion between the oxygen atoms as shown in Fig. 3.30.

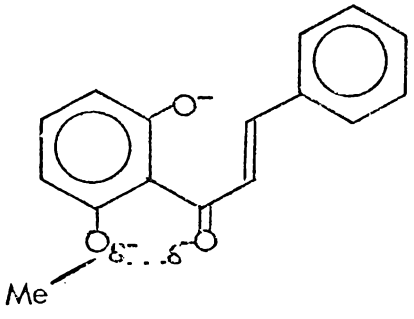


Figure 3.30. Possible charge-charge repulsion between a 6'-methoxyl group and the carbonyl oxygen

Delocalisation of π-electrons from the A-ring and ring substituents as shown in Fig. 3.31. can occur if the

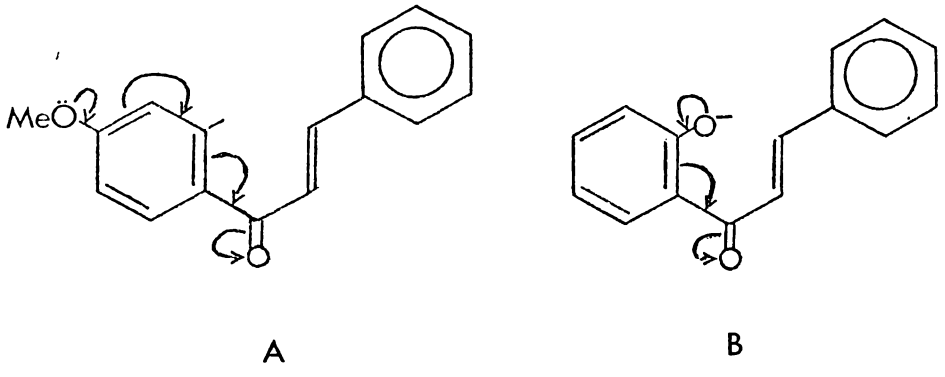


Figure 3.31. Delocalisation of electrons favourable when the carbonyl group is in the plane of the ring.

A. 4'-methoxyl group

B. 2'-o⁻ group.

carbonyl is in the plane of the ring, but if it is forced to lie out of the plane the degree of such delocalisation would be reduced. Any such reduction would allow an increase in the extent of electron withdrawal (inductive and resonance effects) by the carbonyl group from the double bond as in Fig. 3.32, and would result in an increase in k'. In addition, the hindering of the delocalisation shown in Fig. 3.31 B would increase the nucleophilic power of the 2'-o- ion and this effect would also increase the value of k'.

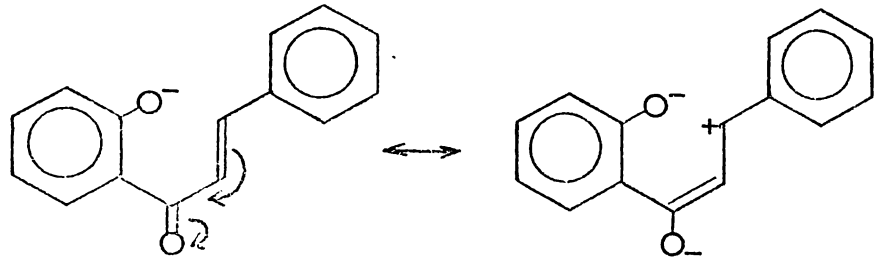


Figure 3.32. Delocalisation from the double bond which is aided by an out-of-plane carbonyl group

(B) An out of plane carbonyl group may, however, affect the availability of suitable conformations for cyclisation by forcing the 2'-o$^-$ ion away from the β-carbon atom, an effect which would decrease k'. Steric considerations would probably ensure that attack of the 2'-o$^-$ ion was from above the planar α,β-unsaturated carbonyl system and therefore, unless the distortions were very large (and model studies suggest that this is probably not the case),

this point may not be very significant.

(C) If a 6'-substituent causes the carbonyl to be out of plane in the ground state and it must come nearer to planarity in the transition state, then the repulsion between the 6'-substituent and the carbonyl oxygen must increase in the transition state, an effect that would lead to a decrease in k'.

(D) Substituents in the A-ring may also affect the value of k' by purely electronic means. Those that can donate electrons to the carbonyl group by inductive (+I) or resonance (+R) effects will prevent the carbonyl group from effecting as great a delocalisation of electrons from the double bond and therefore will make the double bond less susceptible to nucleophilic attack by the 2'-o^- ion at the β-carbon atom. This effect would lead to a decrease in k'. Under these conditions the charge on the 2'-o^- ion can be less easily delocalised onto the carbonyl group (see Fig 3.31B) leading to an increase in its nucleophilicity, and this would partially counteract the former effect. Substituents which withdraw electrons (inductive, -I; resonance, -R) will have effects which are the direct opposite to those for electron donating substituents.

Owing to the lack of any clear trends in k' values for the various chalcones, comparisons are made between pairs of compounds in the following sections.

The first comparison made is between two chalcones that lack a 6'-substituent and should therefore have similar steric requirements, the second is between chalcones that should be subject to similar electronic effects, but different steric effects (since one has a 6'-substituent and the other does not), and the third is between two chalcones that have different 6'-substituents.

(i) Possible explanations why k' for 2'-hydroxy-4'-methoxychalcone is less than k' for 2'-hydroxychalcone.

The effect of the degree of coplanarity of the carbonyl group should be unimportant in a comparison of these two compounds. The calculated $_pK_a$ values are the same and should correlate reasonably well with nucleophilicity in these cases as there are no 6'-substituents to affect the degree of hydrogen-bonding (see earlier discussion). The observation that k' for 2'-hydroxy-4'-methoxychalcone is lower than that for 2'-hydroxychalcone, therefore, is explicable in terms of the lessening of the extent of electron withdrawal by the carbonyl group from the double bond, due to the +R effect of the 4'-methoxyl group (see Fig. 3.31A) as described in (D) above.

(ii) Attempts to explain why k' for 2'-hydroxy-6'-methoxychalcone is less than k' for 2'-hydroxy-4'- methoxychalcone.

The 6'-methoxyl group can potentially push the carbonyl group out of the plane of the ring by steric and/or electrostatic interaction with the carbonyl oxygen (see Fig. 3.30), and if the effects described in (B) and (C) outweigh those in (A) this may account for the smaller k'

value in the 6'-methoxychalcone. The electronic effects described in (D) would be similar for the two compounds since the methoxyl group in the 6'-position can delocalise electrons onto the carbonyl group in a manner analogous to that shown for a 4'-methoxyl group in Fig. 3.31 A, and any -I effect of the 6'-methoxyl group would be small in comparison with the +R effects. A further point regarding possible effects in chalcones with 6'-substituents is that such substituents may affect the rate of cyclisation by solvation effects associated with their proximity to the carbonyl group.

Although there can be no certainty regarding which effect actually operates, the smaller value of k' for 2'-hydroxy-6'-methoxychalcone compared with k' for 2'-hydroxy-4'-methoxychalcone could be explained either by the intrinsic nucleophilicity of the 2'-o^- ion being lower in the former case (i.e. assuming some correlation between $_pK_a$ and nucleophilicity) or by the possible steric and/or electrostatic effects associated with the 6'-methoxyl group as outlined in (B) and (C). In particular, the effect outlined in (C) may be quite important as an explanation for the low k' value of 2'-hydroxy-6'-methoxy chalcone.

(iii) Attempts to explain why k' for 2'-hydroxy-4',6'-dimethylchalcone is larger than k' for 2'-hydroxy-6'-methoxychalcone.

The interaction of a 6'-methyl group with the carbonyl oxygen is potentially different from that of a 6'-methoxyl group as the electrostatic repulsion possible for the 6'-

methoxyl group (see Fig. 3.30.) can not apply to the methyl group. This means that the bringing together of these groups in the transition state would be a less unfavourable process than that for the 6'-methoxyl case where both steric and electrostatic repulsions could apply. Thus, if the effect described in (C) is important for chalcones with 6'-substituents this may explain why k' is greater for 2'-hydroxy-4',6'-dimethyl-chalcone than for 2'-hydroxy-6'-methoxychalcone.
The electronic effects should be similar as both methyl and methoxyl groups can donate electrons to the carbonyl group via mesomeric effects, and any +I effect of the 6'-methyl substituent would be small after transfer through the number of bonds involved.

3-8.2.3. Discussion of k" values

This part of the discussion involves the reverse reaction (Fig. 3.33.) in which the flavanone is converted to the chalcone.

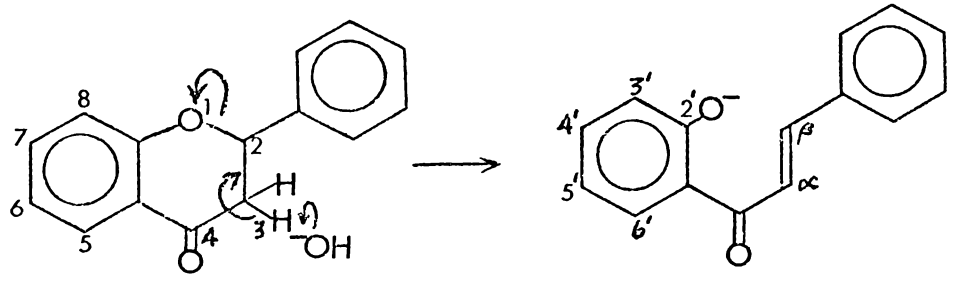


Figure 3.33. Conversion of flavanone to chalcone

It should be noted that in this section the substituent numbering will be based upon the flavanone numbering system, except for the 2'-o$^-$ leaving group for which no ambiguity arises.

The rate constant for the above reaction $k_r = k'' \{OH^-\}$ and factors which will increase k" are listed below.

(i) Any effect that can increase the acidity of the α-hydrogen atom would favour base abstraction of this proton and hence increase k".

(ii) Good leaving groups would increase k". However, just as there is uncertainty regarding the degree of correlation between pKa and the nucleophilicity of the 2'-o- ion, so must there be uncertainty about that between pKa and the leaving group ability of the 2'-o- ion in the chalcones studied.

(iii) Any release of steric strain in the transition state for ring opening would favour an increase in k".

The compounds chosen for comparison in this section are the same as those compared in section 3-8.2.2.

(i) Attempts to explain why k" for 7-methoxyflavanone is smaller than k" for flavanone. (The calculated k" values are recorded in Table 3-11, but listed under the corresponding chalcone).

The inherent leaving group abilities should be similar since the pKa values were calculated to be the same for the two compounds, but the 7-methoxyl group could delocalise electrons onto the carbonyl oxygen in competition with the delocalisation

from the departing 2'-o$^-$ ion (Fig. 3.34). This would make the carbonyl group less effective at stabilising the developing 2'-o- ion and hence lessen the leaving group ability of this ion in 7-methoxyflavanone compared with flavanone. The delocalisation from the 7-methoxy group would also lower the acidity of the α-hydrogen and either (or both) of these effects could account for k" being lower for 7-methoxyflavanone than for flavanone.

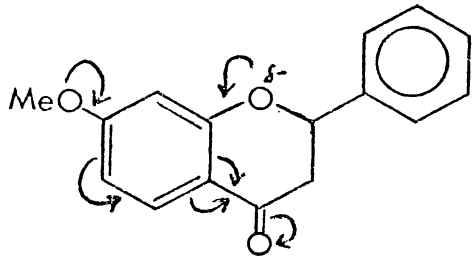


Figure 3.34. Competing delocalisation of electrons onto the carbonyl group.

(ii) Attempts to explain why k" for 5-methoxyflavanone is smaller than k" for 7-methoxyflavanone.

In 5-methoxyflavanone there is the possibility that the carbonyl group may lie out of the plane of the A-ring, but the overall effect of this is difficult to determine. If the carbonyl group was significantly out of the plane of the ring this would increase the acidity of the α-proton, but would make the departure of the 2'-o- ion more difficult,

since in this position the carbonyl oxygen can not delocalise the electrons from the 2'-o^- leaving group so effectively. The electronic effects of the methoxyl groups should be similar for the two compounds, and are therefore no help in explaining the observed results.

A comparison of the pK_a values might suggest that the leaving group ability should be better for 5-methoxyflavanone than for 7-methoxyflavanone, but this would increase k" for the former compound over that for the latter, which is opposite to the experimentally determined effect. There is the possibility of steric and electrostatic repulsion between the 5-methoxyl group and the carbonyl group in the ground state, and this may be partially relieved in the transition state, an effect which would again lead to a rate acceleration of 5-methoxyflavanone compared with 7-methoxyflavanone. It is thus extremely difficult to see why k" for 5-methoxyflavanone is in fact smaller than k" for 7-methoxyflavanone. One possible explanation could be that in 5-methoxyflavanone the carbonyl group does lie out of the plane of the ring, and that its effectiveness at limiting the departure of the 2'-o^- ion is far more important than its ability to increase the acidity of the α-proton.

(iii) Attempts to explain why k" for 5,7-dimethyflavanone is smaller than k" for 5-methoxyflavanone.

The k" value for 5,7-dimethylflavanone was the lowest determined and an explanation for this low value may be the same as that suggested above for 5-methoxyflavanone.

There may be steric repulsion in the ground state of 5,7-dimethylflavanone which could be relieved in the transition state, but the nett relief of both steric and electrostatic repulsion effects in the case of the transition state for 5-methoxyflavanone, if greater than that for 5,7-dimethylflavanone, could account for the larger k" value for the latter compound.

3-8.3. Conclusions

Probably the most obvious, and most disappointing result was that the variation in rates was very small. It had been hoped that some of the substituents would have had a considerable effect upon the rate which would have allowed a clear picture to develop as to what the major substituent effects are.

It is observed that electron releasing groups at the 4'- or 6'-positions decrease the rate. This rate retardation is particularly pronounced for 6'-substituents and it appears to be the steric interaction of this substituent with the carbonyl group that causes the major diminution in the observed rate. However, the effect is not dramatic.

From a synthetic point of view these results would suggest that the most satisfactory conditions for the conversion of 2'-hydroxychalcones to flavanones would be under weakly basic conditions where the rate of the reverse reaction is low. As long as the base strength was not too high a good degree of conversion to the flavanone would result, and at a reasonable rate. A study of the various repetitive scans for the conversion of the 2'-hydroxychalcones to flavanones shows that even beyond pH 10 the percentage of flavanone in the final product is still very high.

4. THE KINETICS AND MECHANISM OF THE CYCLISATION OF 2'-HYDROXYCHALCONE EPOXIDE TO 3-HYDROXYFLAVANONE IN WATER

Although 2'-hydroxychalcone epoxides have never been isolated or detected in natural sources they have often been postulated (see Section 1-3.4) as important intermediates in the biosynthesis of flavonoids. Consequently a study of their reactivity in aqueous solutions, particularly in the neutral region, is of interest and might explain why, if they do occur naturally, they remain undiscovered. If 2'-hydroxychalcone epoxides are intermediates in the formation of flavonoids in the AFO reaction (see section 1-4.1) and in the formation of aurones from chalcone dibromides (see section 1-4.2), as has been often suggested, such a study might also explain the failure to detect them in these systems.

Since no kinetic studies have been performed on 2'-hydroxychalcone epoxides we set out to determine the rate of cyclisation of unsubstituted 2'-hydroxychalcone epoxide to 3-hydroxyflavanone in water (Fig. 4.1.).

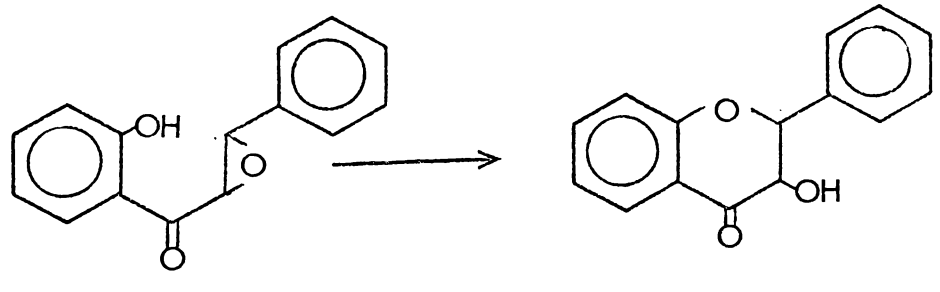


Figure 4.1. Cyclisation of 2'-hydroxychalcone epoxide to 3-hydroxyflavanone

4-1 EXPERIMENTAL

Unless indicated otherwise the experimental conditions were as described in section 3-1. The epoxide had initially been added to various buffers as a methanol solution, but it became apparent that the epoxide was cyclising to 3-hydroxyflavanone in the methanol solution. It was thought that traces of H_2O in the methanol might have been responsible for the cyclisation, but even very dry methanol still allowed slow reaction. The water soluble solvent in which the epoxide proved ultimately to be most stable was dioxan. The epoxide remained stable in dioxan for many days if light was excluded from the solution, but exposure to light appeared to photocatalyse the cyclisation to 3-hydroxyflavanone.*

The following buffers were used from pH 1.11 - 2.4, hydrochloric acid solutions; from pH 2.92 - 3.77, formate; from pH 3.91 - 5.22 acetate, and for the higher pH values, phosphate. The ionic strengths of all buffers was adjusted to 0.5 mol l^{-1} by the addition of potassium chloride.

*Bodforss (1918) found that irradiation of chalcone epoxides with light gave dibenzoylmethanes and Jain (1965) suggested that if the original chalcone epoxide had a 2'-hydroxyl group, the resulting *o*-hydroxydibenzoylmethane could cyclise, leading on to the flavone. Following the photochemical reaction of 2'-hydroxychalcone epoxide spectrophotometrically failed to reveal any evidence of *o*-hydroxydibenzoylmethane (or flavone) and the only product detected was 3-hydroxyflavanone which can be formed from cyclisation of the epoxide but not by cyclisation of *o*-hydroxydibenzoylmethane. It should be noted that our study was performed in dioxan solution and hence our results do not exclude the possibility that Jain's suggestion may still be applicable under the conditions applying in natural sources.

The reaction was started by the addition of 15μℓ of the epoxide as a solution in dioxan to the aqueous buffer solution. By following the decrease in absorbance at 262 nm (see Fig. 4.2.) as the epoxide reacted, pseudo first-order rate constants were determined. The temperature for all kinetic runs was 30°C.

Preliminary kinetic runs were performed at constant pH, using different epoxide solutions. That is, the epoxide was injected into the buffer as a methanol solution, a dimethylsulphoxide solution or a dioxan solution. The observed rate constants were found, within experimental error, to be independent of the solvent used to dissolve the epoxide.

4-2 BUFFER DILUTION STUDIES

The effect of the buffer concentration on the observed rate constant for phosphate, acetate and formate buffers was measured and the results are shown in Table 4-1.

The results in Table 4-1 show that the observed rate constant, at a particular pH, is independent of the buffer concentration, effectively establishing that within the buffer concentration range tested there is no significant general acid or general base catalysis.

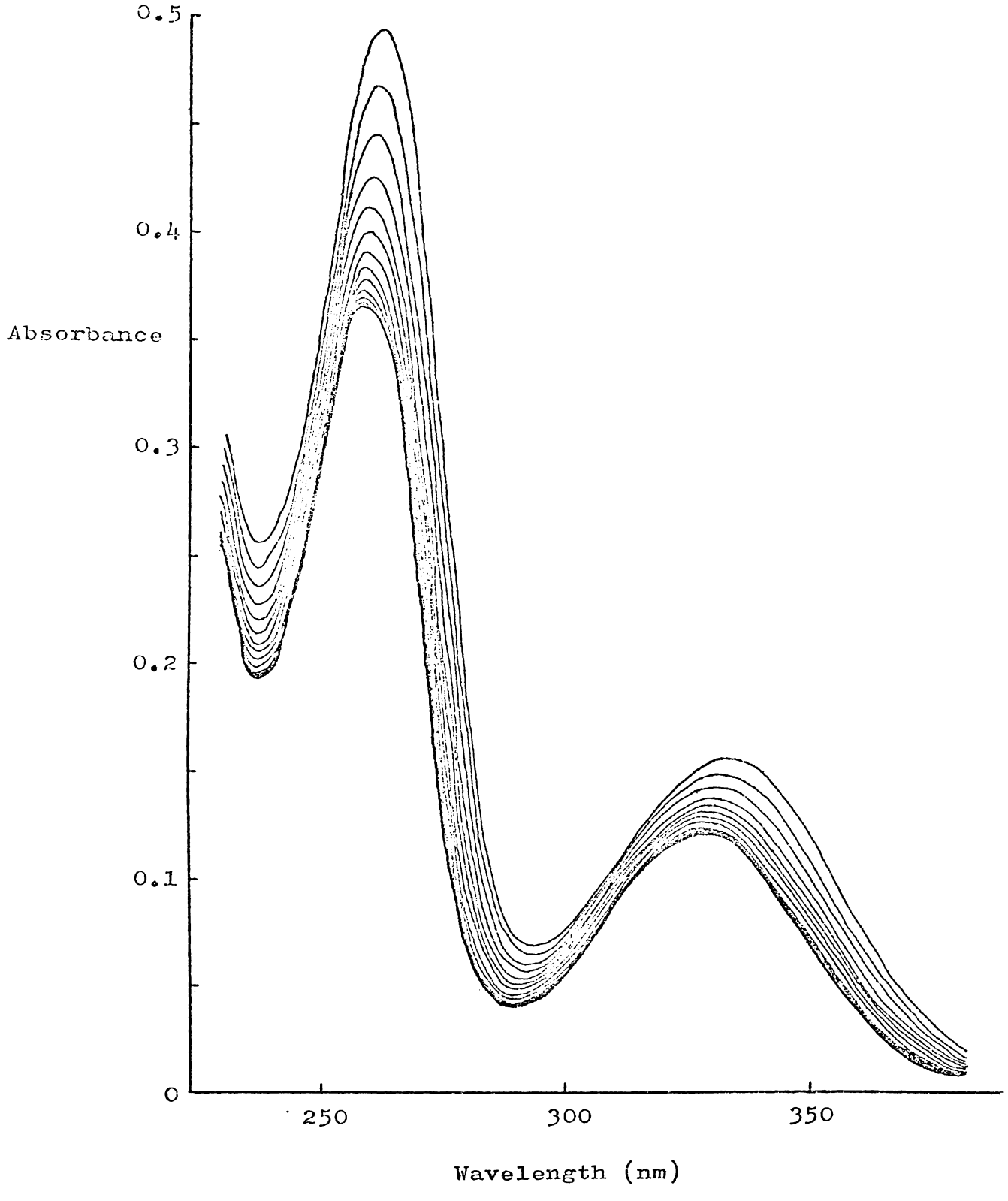


Figure 4.2. Repetitive wavelength scans at 5 min. intervals and pH 2.4. showing the absorbance decrease during the course of the cyclisation reaction of 2'-hydroxychalcone epoxide to 3-hydroxyflavanone.

Table 4-1 Effect on the observed rate constant of changing the total buffer concentration at constant pH and constant ionic strength.

Buffer	Concentration (mol l^{-1})	$10^4 k_{obs}/s^{-1}$
Phosphate (pH = 5.98)	0.1	258 ± 8
	0.02	254 ± 8
	0.01	263 ± 8
	0.005	262 ± 8
Acetate (pH = 4.57)	0.1	10.3 ± 0.3
	0.02	10.1 ± 0.3
	0.01	10.3 ± 0.3
	0.005	10.4 ± 0.3
Formate (pH = 3.67)	0.1	2.00 ± 0.06
	0.01	1.95 ± 0.06

4-3 THE EFFECT OF pH ON THE RATE OF CYCLISATION OF 2'-HYDROXYCHALCONE EPOXIDE TO 3-HYDROXYFLAVANONE.

The pH dependence of the rate of cyclisation of 2'-hydroxychalcone epoxide to 3-hydroxyflavanone was observed over the range pH 1.11-6.32 (Table 4-2). By reference to this table it can be seen that the rate constant is at a minimum around pH 3.4 but increases rapidly below pH 3 and above pH 4.5. It was thus decided to initially consider the data in these two pH ranges separately.

TABLE 4-2 Effect of pH on the observed rate constant for the cyclisation of 2'-hydroxychalcone epoxide to 3-hydroxyflavanone in water at 30°C and μ=0.5 mol l^{-1}

pH	$10^4 k_{obs}/s^{-1}$	$10^4 k_{obs}/s^{-1}$*
1.11	183	
	178	
	182	
	183	183 ± 5
1.62	57.0	
	55.0	56 ± 2
2.08	19.8	
	19.5	19.7 ± 0.6
2.40	9.87	
	9.78	9.8 ± 0.3
2.92	3.35	
	3.42	3.4 ± 0.1
3.10	2.55	2.55 ± 0.08
3.32	1.88	1.88 ± 0.06
3.42	1.78	1.78 ± 0.05
3.60	1.87	1.87 ± 0.06
3.67	2.00	2.00 ± 0.06
3.77	2.30	2.30 ± 0.07
3.91	2.85	
	2.87	2.86 ± 0.09
4.63	11.48	
	11.55	
	11.53	11.5 ± 0.3
5.22	42.63	
	44.12	43 ± 1.3
5.98	253	
	250	
	259	
	257	255 ± 8
6.32	570	
	566	
	559	565 ± 17

*Mean values taking 3% experimental error in k_{obs} into account

4-3.1 Acid-Catalysed Pathway

If we assume the mechanism shown in Fig. 4.3. to be applicable in the range pH 1-3 we can derive a relationship between k_{obs} and $\{H^+\}$.

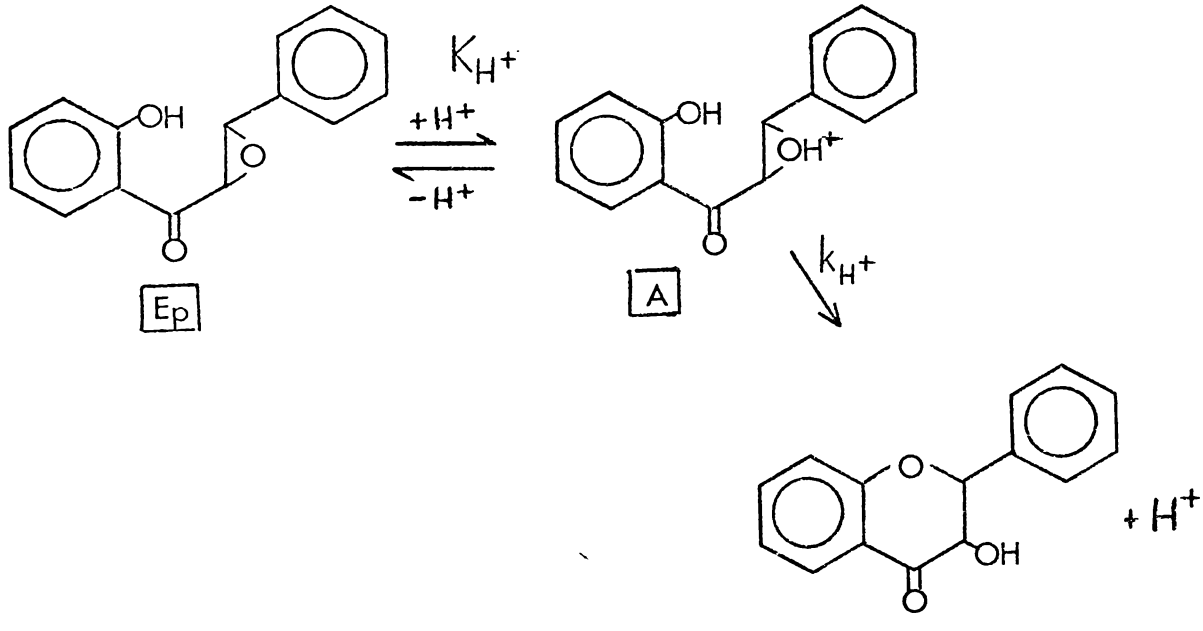


Figure 4.3. Proposed acid-catalysed mechanism

From the above mechanism

$$\text{Rate} = k_{H^+}[A] \quad \text{and} \quad K_{H^+} = \frac{[A]}{E_p\{H^+\}}$$

$$\therefore \text{Rate} = k_{H^+} K_{H^+} [E_p]\{H^+\} \quad \ldots\ldots \text{theoretically}$$

$$\text{Rate} = k_{obs}[E_p] \quad \ldots\ldots \text{experimentally at constant pH}$$

$$\therefore k_{obs} = k_{H^+} K_{H^+} \{H^+\}$$

$$\text{OR} \quad k_{obs} = k_a\{H^+\}$$

Therefore if the above mechanism is correct a plot of k_{obs} versus $\{H^+\}$ in the range pH 1-3 should be a straight line with a slope equal to the acid-catalysed rate constant ka. This plot is in fact linear as shown in Fig. 4.4., thus supporting the proposed mechanism and has a slope of 0.235 activity^{-1}s^{-1}. Therefore in the range pH 1-3 the equation

$$k_{obs} = 0.235\ \{H^+\}\ \text{activity}^{-1}\text{s}^{-1}$$

accounts fully for the observed kinetics.

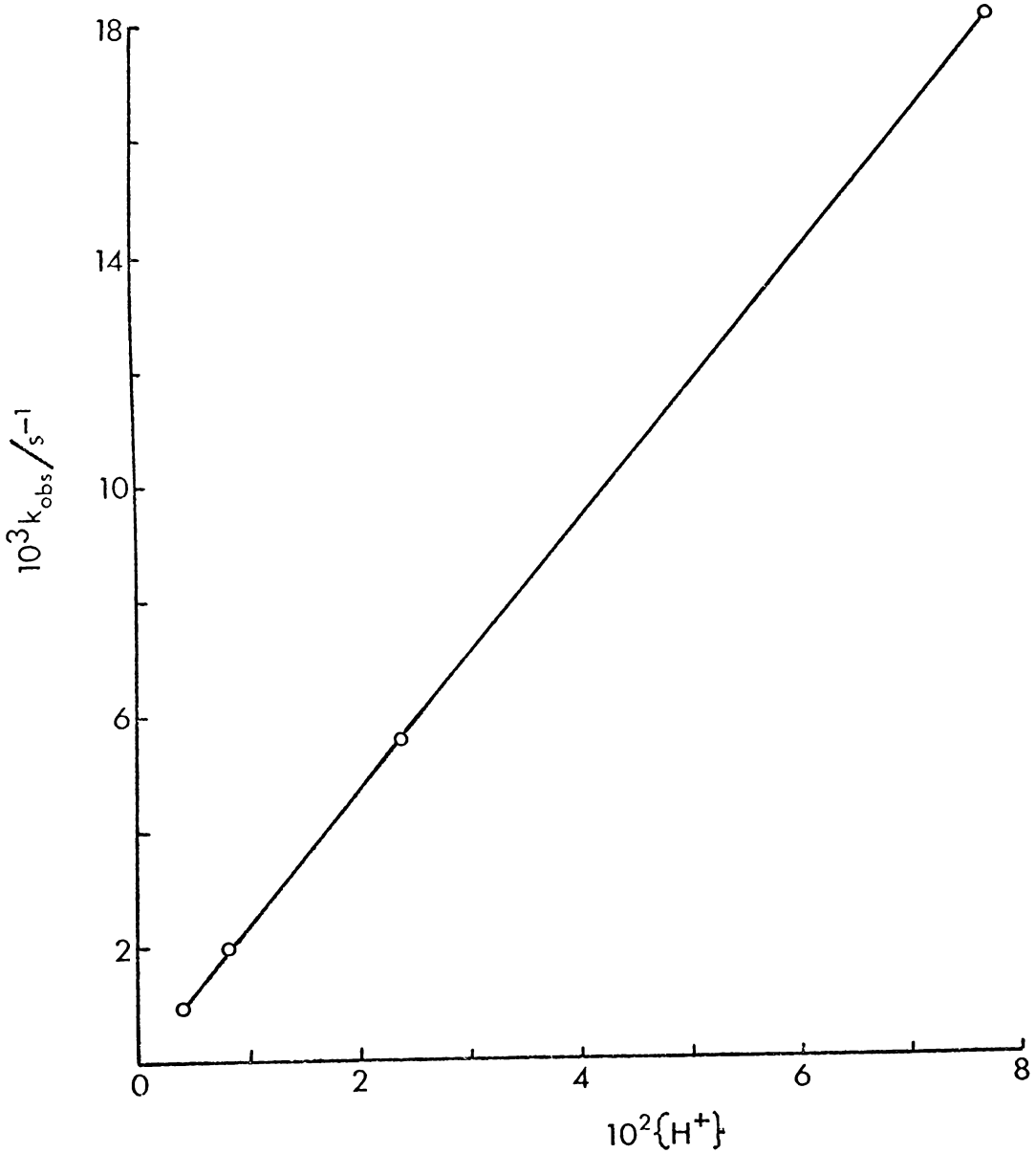


Figure 4.4. Plot of k_{obs} versus $\{H^+\}$ in the range pH 1 - 3.

4-3.2 Base-Catalysed Pathway

By analogous reasoning to that proposed for the acid-catalysed pathway we can derive a relationship between k_{obs} and OH^- in the range pH 4.5-6.5. The proposed mechanism in this range is shown in Fig. 4.5.

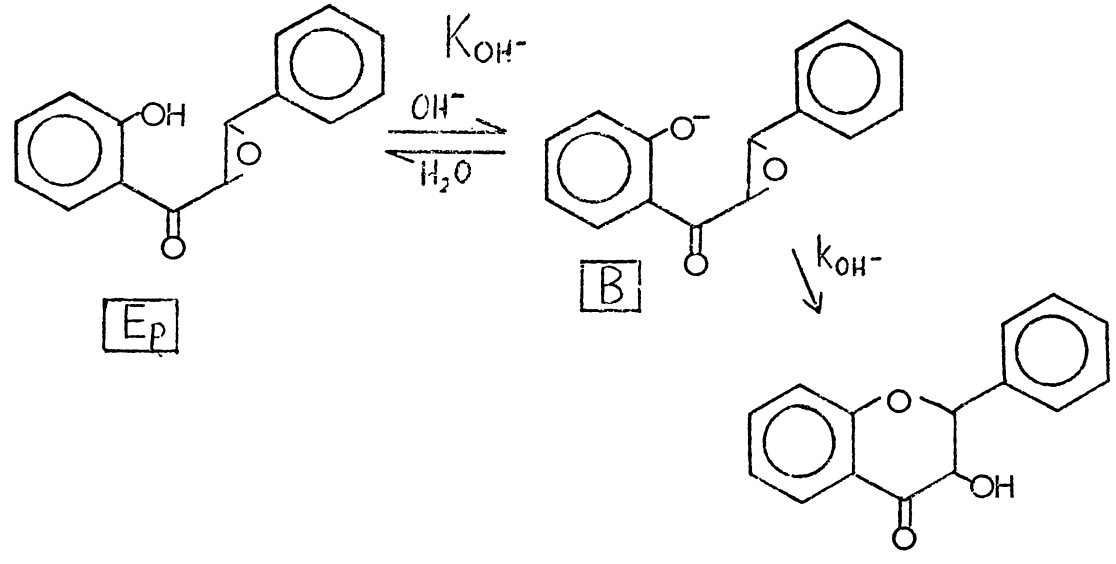


Figure 4.5. Proposed base-catalysed mechanism

From the above mechanism

$$\text{Rate} = k_{OH^-}[B] \qquad \text{and} \qquad K_{OH^-} = \frac{[B]}{[E_p]\{OH^-\}}$$

$$\therefore \quad \text{Rate} = k_{OH^-} K_{OH^-} [E_p]\{OH^-\} \quad \ldots\ldots \text{ theoretically}$$

$$\text{Rate} = k_{obs} [E_p] \quad \ldots\ldots \text{ experimentally at constant pH}$$

$$\therefore \quad k_{obs} = k_{OH^-} K_{OH^-} \{OH^-\}$$

$$\text{OR} \quad k_{obs} = k_b \{OH^-\}$$

Therefore if the above mechanism is correct a plot of k_{obs} versus $\{OH^-\}$ in the range pH 4.5-6.5 should be a straight line with a slope equal to the base-catalysed rate constant k_b. This plot is indeed linear as shown in Fig. 4.6. which supports the mechanism suggested and it has a slope of 1.85×10^6 activity^{-1}s^{-1}. Therefore in the range pH 4.5-6.5 the equation

$$k_{obs} = 1.85 \times 10^6 \{OH^-\} \text{ activity}^{-1}\text{s}^{-1}$$

accounts fully for the observed kinetcs.

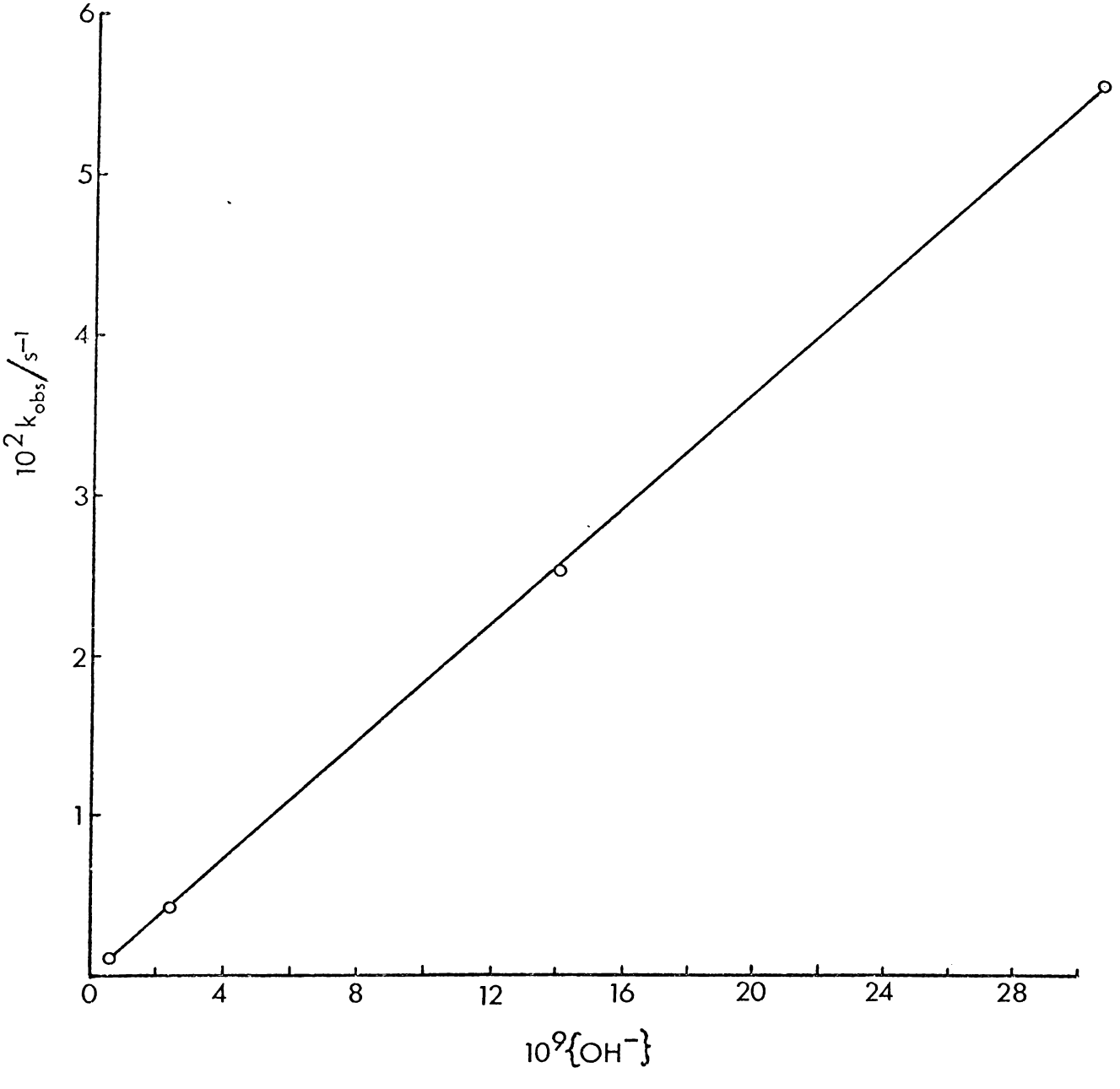
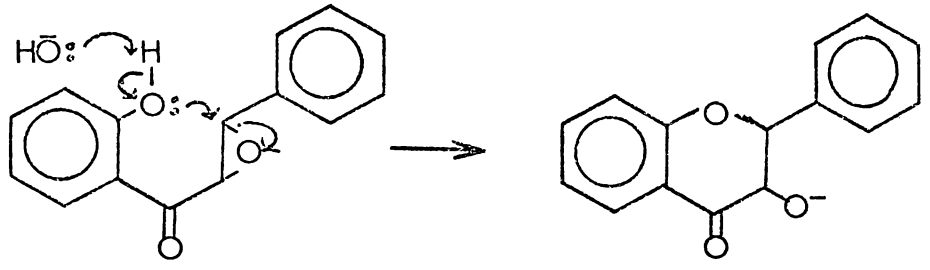


Figure 4.6. Plot of k_{obs} versus {OH-} in the range pH 4.5 - 6.5

An alternative mechanism consistent with the kinetic form is the following in which hydroxide acts as a base on the neutral epoxide to assist cyclisation.



However, if this mechanism does apply it would be expected that general bases would also catalyse the cyclisation. No buffer catalysis was observed. However, it is possible that buffer catalysis might have been detected at higher buffer base concentrations so the possibility of this general base-catalysed mechanism is not completely eliminated, even though it is less likely than the specific base-catalysed mechanism.

4-3.3 The Overall Rate Expression

The previously described acid-catalysed and base-catalysed mechanisms effectively account for the prescribed pH ranges, but as can be seen in the complete pH-rate profile (Fig. 4.7), neither is alone capable of explaining the intermediate range. Here we need to take account of a combination of the two pathways, leading to the following overall rate equation.

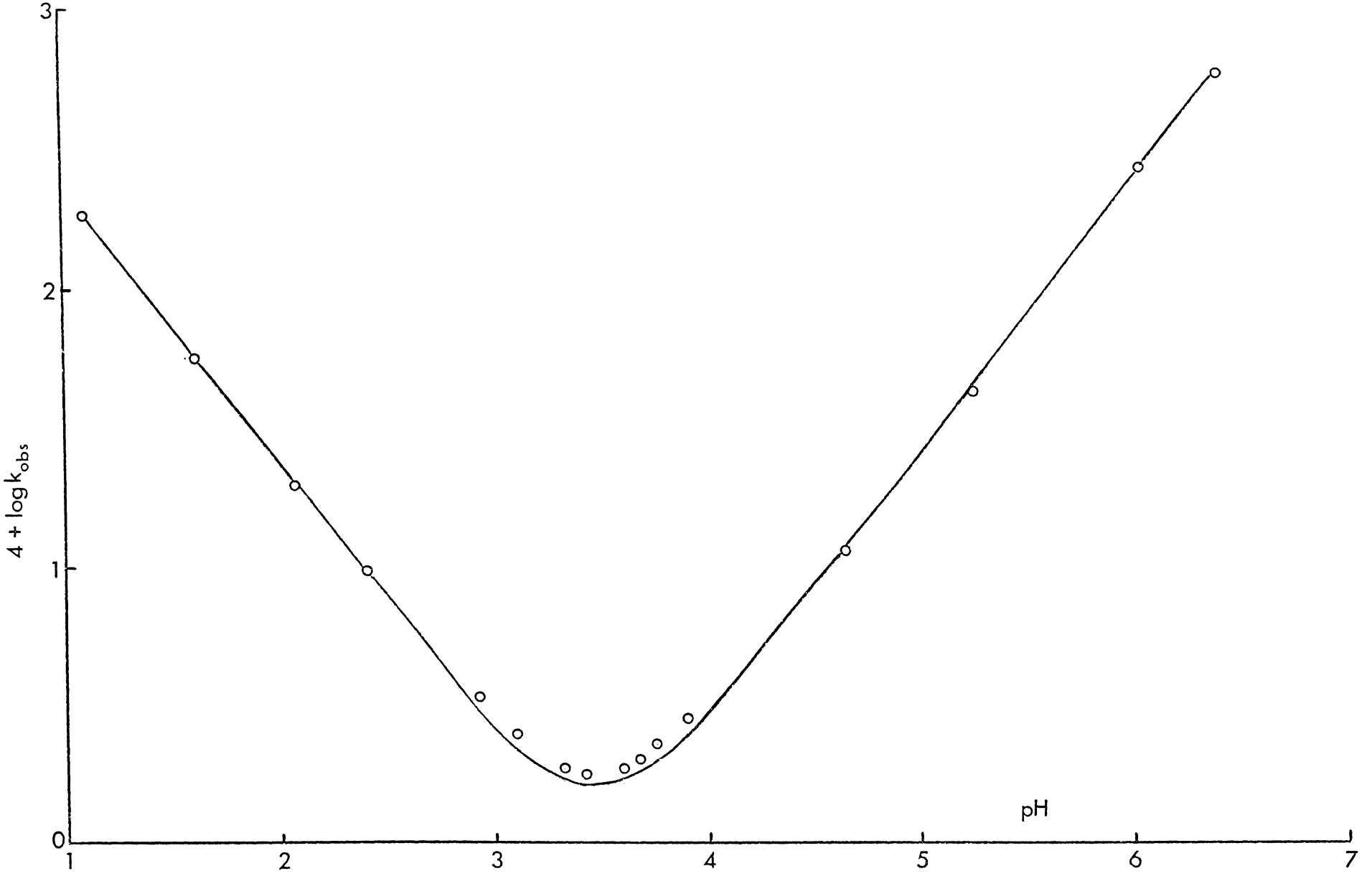


Figure 4.7. pH-log rate profile for the cyclisation of 2'-hydroxychalcone epoxide to 3-hydroxyflavanone in water at 30°C, μ= 0.5 mol l^{-1}

Rate = .235 [Epoxide] $\{H^+\}$ + 1.85 x 10^6 [Epoxide] $\{OH^-\}$ mol $^{-1}s^{-1}$

In Fig. 4.7. the experimentally determined rate constants are plotted as points, while the line is theoretical, being based on the above rate expression. It should be noted that log k_{obs}, rather than k_{obs}, has been plotted against pH resulting in each arm of the profile having unit slope. The agreement between the experimental points and the theoretical line indicates that the kinetic data are consistent with the above rate equation at all pH values employed. The overall mechanism suggested can be summarised as in Fig. 4.8. Both the pathways shown make significant contributions to the overall reaction in the intermediate pH range.

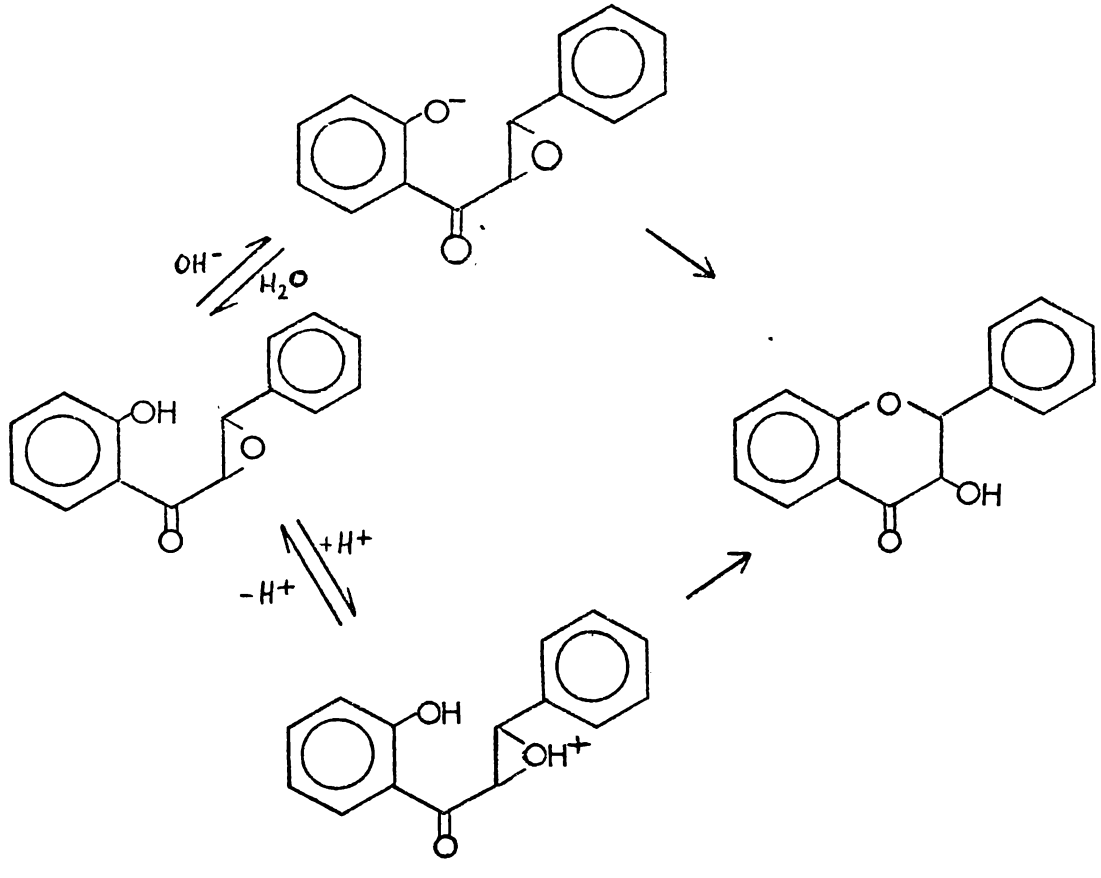


Figure 4.8. Proposed mechanism for the cyclisation of 2'-hydroxychalcone epoxide to 3-hydroxyflavanone in water.

Although all the points shown in Fig. 4.7 lie, within experimental error, on the theoretical curve there is a tendency for the experimental points in the intermediate region to lie slightly but consistently above the curve. This might imply a minor contribution to the overall rate from uncatalysed cyclisation in this pH region, where the acid- and base-catalysed reactions are slowest.

4-4 CONCLUSIONS

The results of this study show that 2'-hydroxychalcone epoxides are likely to be highly unstable near and above neutrality (by extrapolation of Fig. 4.7., $t_{\frac{1}{2}}$ for the epoxide at pH 7 can be estimated as about 2.5s) Additional substituents in the aromatic rings will of course affect both the pKa of the 2'-hydroxyl group and the rate of cyclisation of the anion but one can not conceive of groups which could stabilise the epoxide markedly. Electron donating groups in the A-ring, for instance, would decrease the extent of ionisation of the 2'-hydroxyl group but increase the nucleophilicity of the anion once formed so that compensating effects would doubtless apply. It seems probable therefore that if 2'-hydroxy-chalcone epoxides are intermediates in flavonoid biosynthesis, they may well not be present in natural sources in sufficient concentration to be isolated or even detected.

If 2'-hydroxychalcone epoxides are biosynthetic intermediates, it seems reasonable that any enzyme involved in their conversion to 3-hydroxyflavanones might employ a basic group in the region of the 2'-hydroxyl group and/or an acid group in the region of the epoxide oxygen to assist cyclisations via the types of mechanisms outlined above. In view of the high reactivity of the epoxide at neutral pH, however, a more important role of an enzyme might be to bring the

reacting groups, the 2'-phenoxide ion and the β-carbon of the epoxide into close proximity. Reactions giving aurone type products could equally be achieved by bringing the α-carbon but not the β-carbon into proximity with the 2'-phenoxide group. All these ideas are speculative however, in the absence of any evidence that epoxides really are involved in flavonoid biosynthesis.

2'-hydroxychalcone epoxides have also been postulated as intermediates in the AFO reaction (see section 1-4.1). In this reaction the chalcone is reacted with alkaline hydrogen peroxide and the first detectable product is a 3-hydroxyflavanone. This could be derived from an epoxide but if an epoxide is formed our data shows that under these strongly basic conditions they would react very rapidly, making their detection very difficult. If we assume that the pKa of the epoxide is approximately 10 (by comparison with the pKa of 2-hydroxyacetophenone (10.06) Perrin, 1958), we can make an estimate of the reaction half-life at higher pH values. Referring to the base-catalysed mechanism we can determine approximately the rate constant (k) for the conversion of the epoxide anion to 3-hydroxyflavanone.

$$E_p \overset{OH^-}{\rightleftharpoons} E_p^- \overset{k}{\rightarrow} \text{3-hydroxyflavanone}$$

$$\text{Assuming Ka} = 10^{-10} = \frac{[E_p^-][H^+]}{[E_p]}$$

$$\text{Base-catalysed rate} = k[E_p^-] = 1.85 \times 10^6 [E_p][OH^-]$$

$$\therefore \quad k = 185 s^{-1}$$

$$\therefore \quad t_{\frac{1}{2}} = \frac{0.6931}{185} s \simeq 4 \text{ milliseconds}$$

If the mechanism suggested does apply, therefore,2'-hydroxychalcone epoxides would be very short-lived indeed even in moderately basic solutions.

Donnelly & Doran (1975) found that the ratio of aurone to flavone formed by the cyclisation of 2'-hydroxy-6'-methoxychalcones in basic solution increased with increasing base concentration. To account for this effect of base concentration they proposed a mechanism for aurone formation involving a chalcone epoxide (see section 1-4.2). However, once again, these reaction conditions would ensure that any 2'-hydroxychalcone epoxides formed would be undetectable.

Chalcone epoxides with 6'-substituents might be expected to cyclise to the α-position and hence form aurones. Gormley & O'Sullivan (1972) (see also section 1-4.1) suggest that once a chalcone epoxide is formed within the AFO reaction the presence of a 6'-methoxyl group determines that attack by the 2'-O^- group will occur exclusively at the α-position, leading to aurones. Although we failed to isolate the required 2'-hydroxy-5',6'-benzochalcone epoxide from the reaction of m-chloroperoxybenzoic acid with 2'-hydroxy-5',6'-benzochalcone we did isolate the corresponding 3-hydroxyflavanone (see section 2-3.2). This had probably been formed through an epoxide, possibly during chromatography, and if so it must have come from β-cyclisation and not the expected α-cyclisation. This means that although aurones may also be formed, they are not the sole products from 2'-hydroxy-chalcone epoxides with 6'-substituents. Marathey(1955) studied the

the effect of 5',6'-benzo substitution in a 2'-hydroxychalcone and found that under AFO conditions he did not obtain 3-hydroxyflavanone (or flavonol). Most of the product from 2'-hydroxy-5',6'-benzo-4-methoxychalcone was the corresponding flavanone, which is not an oxidation product.

5. THE KINETICS AND MECHANISMS OF REACTION OF *ERYTHRO*-2'-HYDROXYCHALCONE DIBROMIDE AT pH 7.88

Combined kinetic and spectrophotometric measurements have been used to determine the routes by which *erythro*-2'-hydroxychalcone dibromide converts to 3-bromoflavanone. The ratio of the E- and Z-2'-hydroxy-α-bromochalcones formed as intermediates in the reaction has never previously been established for *erythro*-2'-hydroxychalcone dibromide or any substituted derivative. In previous synthetic studies (see section 1-4.3) such isomers have been isolated in the presence of 3-bromoflavanones which from the results of David (1979) would doubtless be formed much faster from the Z- than from the E- isomer. 3-bromoflavanone may also be formed by direct cyclisation of dibromide and to our knowledge there have been no previous attempts to establish how effectively this direct substitution reaction competes with the elimination - addition sequence via the 2'-hydroxy-α-bromochalcones as a route from the dibromide to 3-bromoflavanone (Fig. 5.1.)

In the present study, the major difficulty in determining the proportions of the three products formed from the dibromide was associated with the rapid cyclisation of the 2'-hydroxy-α-bromochalcones once formed. It is the product ratio of these compounds that is required but unfortunately before their formation is complete significant proportions have, especially in the case of the Z-isomer, already cyclised to 3-bromoflavanone. Other difficulties arise from the subsequent reaction of 3-bromoflavanone to form flavone, and the fact that the extinction coefficients for the reactive compounds involved can not be directly determined under the reaction conditions.

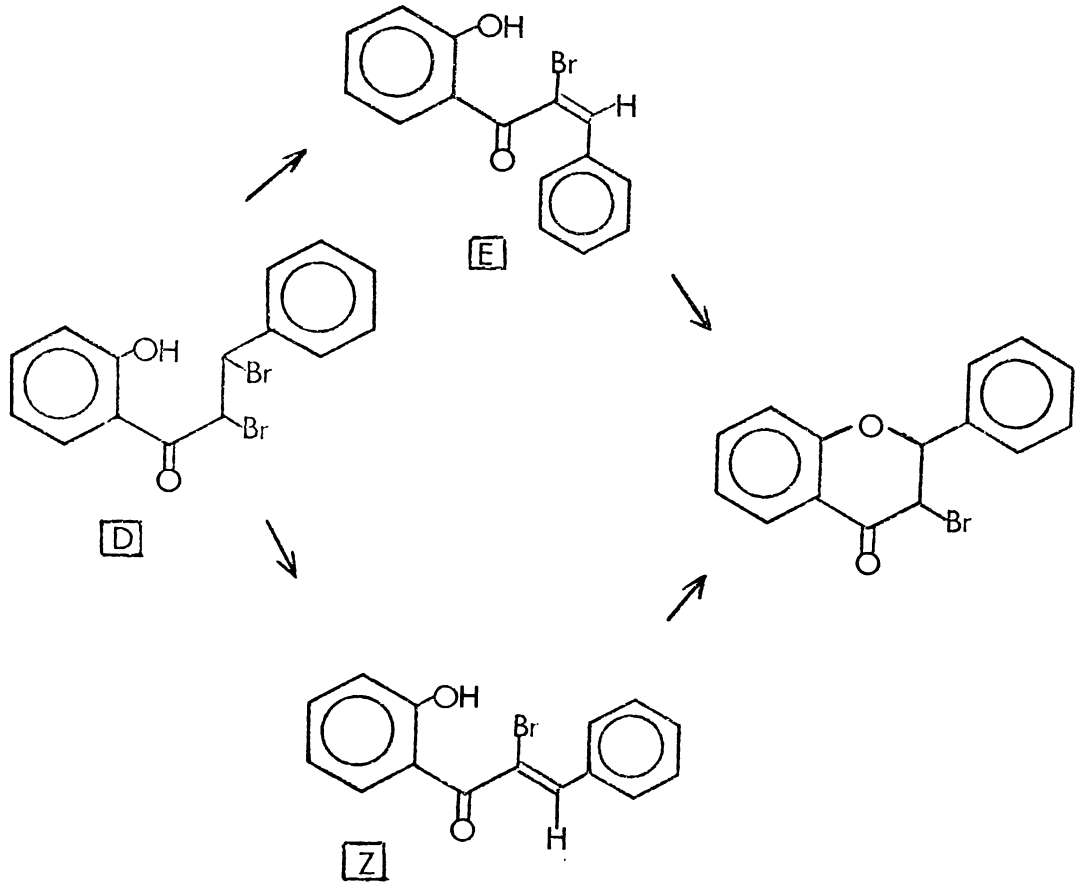


Figure 5.1. Possible routes for the conversion of *erythro*-2'-hydroxychalcone dibromide (D) to 3-bromoflavanone.

5-1 EXPERIMENTAL

The general experimental conditions were as described in section 3-1. Unless indicated otherwise the results are for N-ethylmorpholine as buffer (pH 7.88) in ethanol-water (1:4 by mixed volumes) at 30°C. In this study the ionic strength was maintained at 0.16 mol l^{-1} in order to reproduce the conditions used by David (1979) in related work.

The three starting materials, E- and Z- 2'-hydroxy-α-bromochalcone and *erythro*-2'-hydroxychalcone dibromide were all supplied in a pure form by the courtesy of Miss S.K. David and shall, in the main, be referred to by the terms E-isomer, Z-isomer and dibromide respectively. All error analyses for this section are accumulated in appendix 3. The analytical methods used for calculating the yields of the E- and Z- isomers from the reaction of the dibromide are quite different and will be treated separately.

5-2 DETERMINATION OF THE YIELD OF E-2'-HYDROXY-α-BROMOCHALCONE FROM THE REACTION OF *ERYTHRO*-2'-HYDROXYCHALCONE DIBROMIDE AT pH 7.88 IN ETHANOL-WATER (1:4)

We needed to study the reaction of the E-isomer going to 3-bromoflavanone within the overall reaction of the dibromide going to 3-bromoflavanone. In order to accomplish this it was necessary to use a wavelength to follow the reaction such that the absorbance change for the reaction of the E-isomer going to 3-bromoflavanone was large in comparison to changes for other reactions, and such that there was little or no absorbance change for the subsequent slow reaction of 3-bromoflavanone going to flavone. If these requirements are met then the contribution to the absorbance change (at a particular wavelength) made by the reaction of the E-isomer initially formed can be determined and this change can be related to the final absorbance (representing 3-bromoflavanone) and used as an estimate of the % reaction of the dibromide leading to the E-isomer. The absorbance change measures how much E-isomer is formed and the final absorbance measures the total 3-bromoflavanone formed from all sources, i.e. from all reactions of the initial total dibromide.

Repetitive scans for the reaction of 3-bromoflavanone going to flavone (Fig. 5.2.) show that isosbestic points exist at 264 and 272.5 nm. At both of these wavelengths, large absorbance changes occur for the reaction of the E-isomer going to 3-bromoflavanone(Fig. 5.3.) and, fortuitously, the reaction of the Z-isomer going to 3-bromoflavanone (Fig. 5.4.) has isosbestic points at 258 and 272 nm. Thus the wavelength 272.5 nm was selected as being very suitable for studying the reaction of the E-isomer alone, without significant interference from absorbance changes associated with the Z-isomer going to 3-bromoflavanone and on to flavone. Measurements were also made at 264 nm as a means of comparison. At this wavelength, however, there is some interference from the rapid reaction of the Z-isomer, in the early stages of the reaction which must be taken into account.

5-2.1. Method for calculating the yield of the E-isomer

(a) The reaction of the pure E-isomer going to 3-bromoflavanone was followed by recording the absorbance (A^E) at 272.5 nm (or 264 nm) against time until a constant infinity absorbance reading (A^E_∞) was obtained. The first order rate constants were determined by the conventional infinity plot method and extrapolation of this graph to zero (mixing) time gave the zero-time absorbance (A^E_O). The ratio A^E_O/A^E_∞ equals the ratio of the extinction coefficients of the E-isomer and 3-bromoflavanone at the wavelength concerned.

(b) The run was then repeated, under identical conditions, except that this time the reaction of the dibromide going to 3-bromoflavanone was followed. The absorbance (A^D) at 272.5 nm (or 264 nm) was again recorded against time until a constant infinity absorbance (A^D_∞) was obtained. The change in absorbance, which is due almost totally to the cyclisation of the E-isomer at the wavelengths used, is now smaller in relation to the infinity absorbance (A^D_∞) since

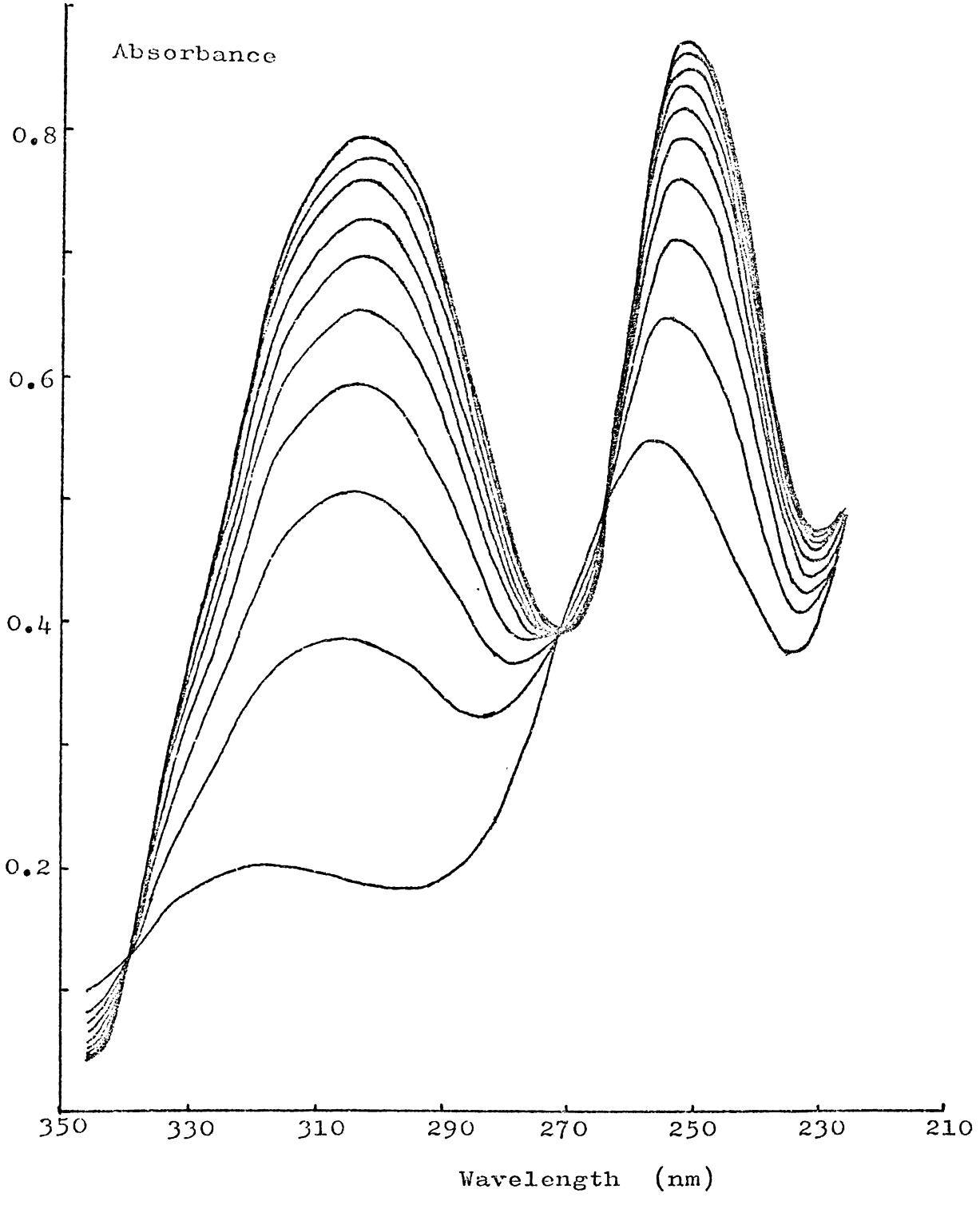


Figure 5.2. Repetitive wavelength scan for the reaction of 3-bromoflavanone going to flavone. 4 min. time intervals, pH≈11

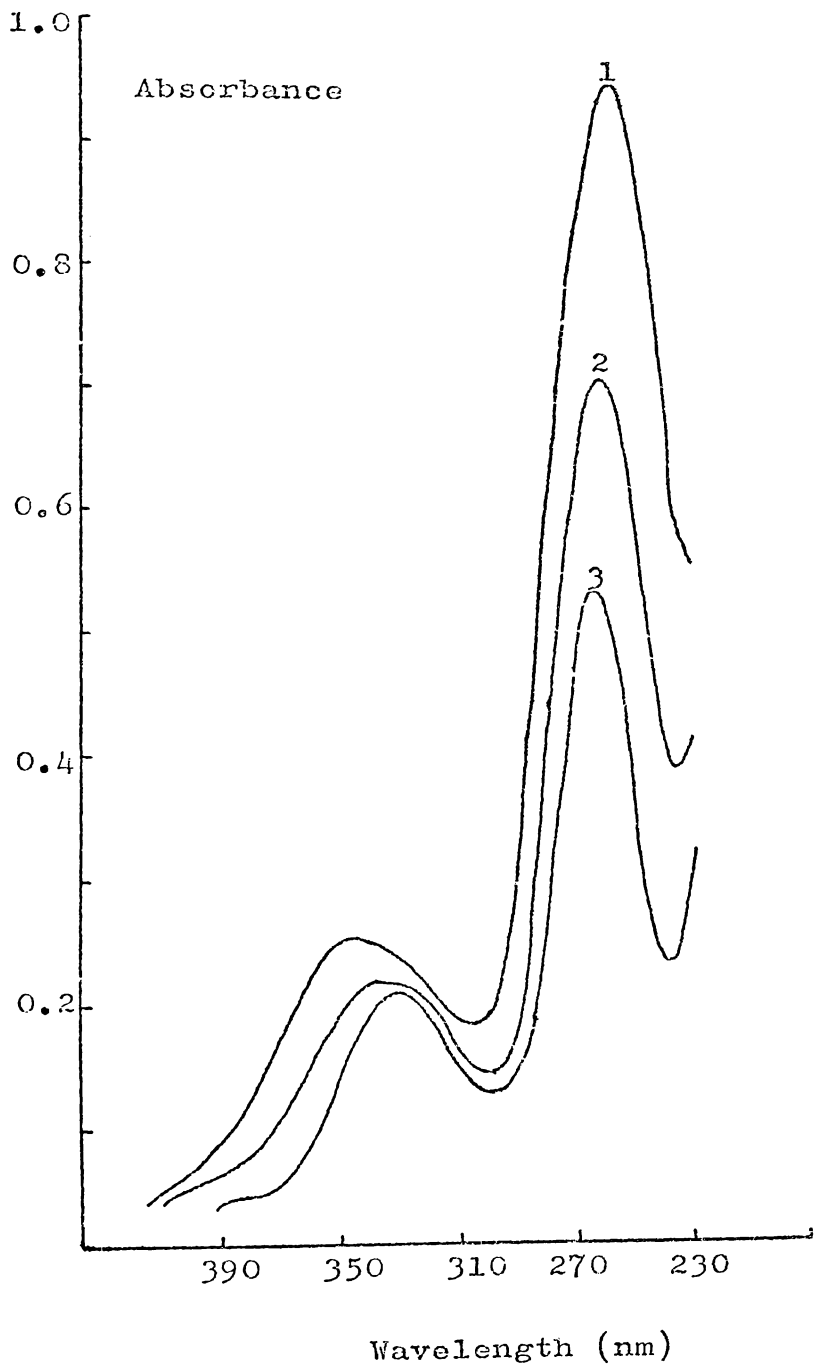


Figure 5.3. Repetitive wavelength scan for the reaction of E-2'-hydroxy -α-bromochalcone going to 3-bromoflavanone in N-ethylmorpholine, pH 7.88.

(1) initial scan, (2) 15 min, (3) 60 min.

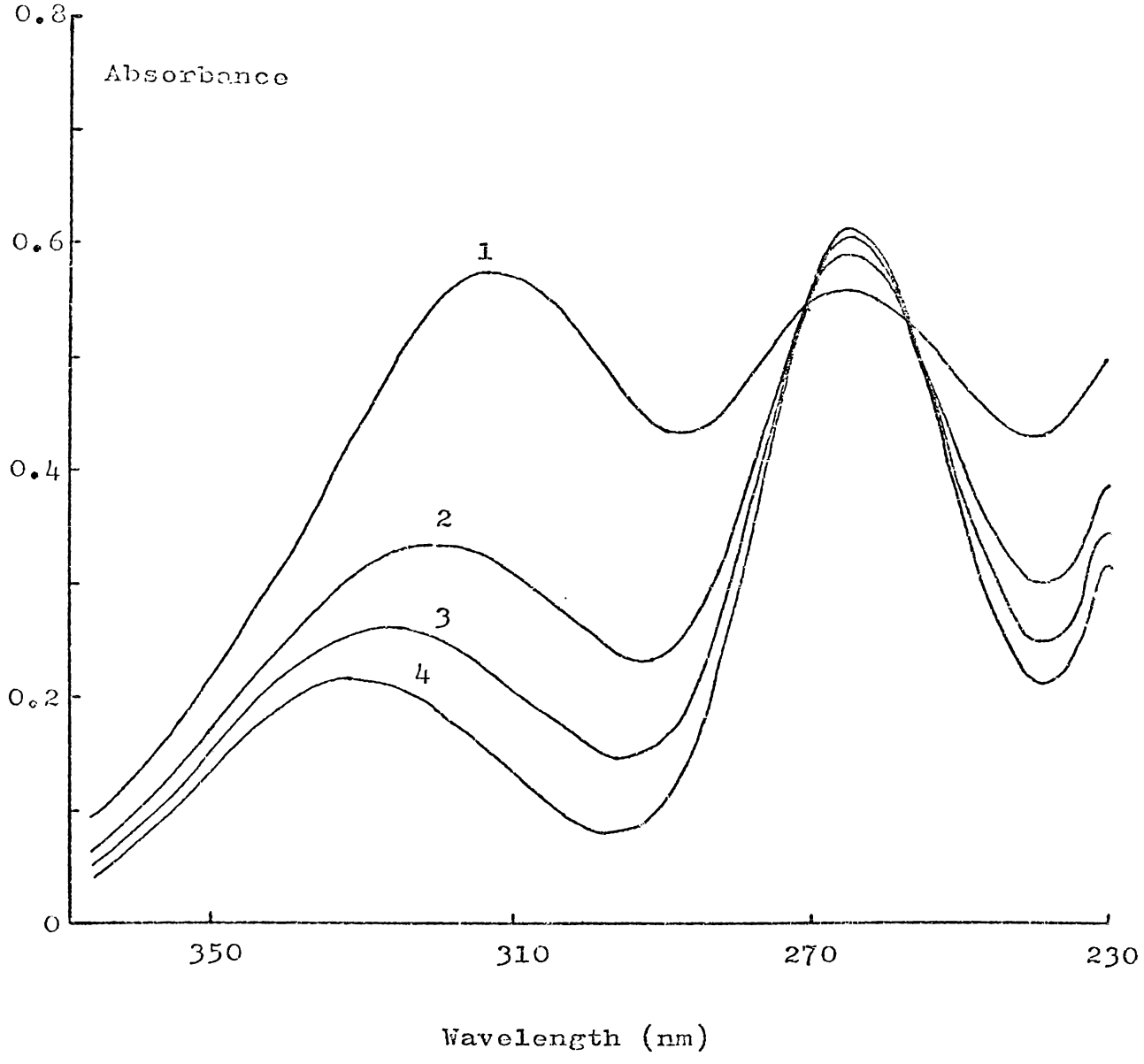


Figure 5.4. Repetitive wavelength scan for the reaction of Z-2'-hydroxy-α-bromochalcone going to 3-bromoflavanone in N-ethylmorpholine, pH 7.88 (1) ≈15s, (2) 75s, (3) 135s, (4) 315s

the latter represents 3-bromoflavanone formed not only from the E-isomer, but also from the Z-isomer and possibly by direct cyclisation of the dibromide. From this data we can calculate the theoretical zero-time absorbance which would have applied if all the dibromide had been converted to the E-isomer. This is given by the infinity absorbance from the dibromide reaction (A_∞^D) multiplied by the ratio of the extinction coefficients of the E-isomer and 3-bromoflavanone. That is, the theoretical zero-time absorbance can be represented as $A_\infty^D A_o^E / A_\infty^E$. To establish the experimental zero-time absorbance of the E-isomer in the dibromide reaction (A_o^D), the absorbance data were plotted by the first order method. These plots deviated from linearity in the initial stages because of interference from the residual dibromide, and in the case of the measurements at 264 nm because of the reactions of the Z-isomer also. The extrapolation to zero-time was accomplished from the linear portion of the plot*. In this case, zero-time for the E-isomer is not mixing time since only the dibromide is present initially. A further complication is that before all of the E-isomer has been formed some will have cyclised. Thus we have chosen an effective zero-time for the E-isomer somewhat arbitrarily as 30s, at which time 76% of total E-isomer will have been formed (the fraction of dibromide remaining after 30s has been calculated as 0.237 in section 5-3.2(b)). The choice of effective zero-time does not prove to be too critical. For instance, changing it to 60s (95% reaction) decreased the calculated yields, shown in Table 5-1, by 1%.

(c) The yield of the E-isomer from the dibromide reaction can be calculated as the ratio of the experimentally observed absorbance change to the theoretical absorbance change (i.e. the change that would have occurred had the dibromide yielded 100% of the E-isomer).

$$\text{Yield (\%) E} = 100\ (A_o^D - A_\infty^D) / [A_\infty (A_o^E / A_\infty^E) - A_\infty^D]$$

$$= 100 [(A_o^D / A_\infty^D - 1] / [(A_o^E / A_\infty^E) - 1]$$

*To ensure internal consistency, the k_{obs} value was always calculated from the extrapolated curve, and checked against the k_{obs} values obtained for the formation of 3-bromoflavanone from the pure E-isomer.

5-2.2 Results

(a) Values of A_o^E and A_∞^E were obtained from five runs starting with the E-isomer; three following the reaction at 272.5 nm and two at 264 nm. These values, together, with the observed rate constants for the formation of 3-bromoflavanone from the E-isomer, are presented in Table 5-1(a).

(b) Values of A_o^D and A_∞^D were also obtained for five runs starting with the dibromide and using identical conditions to those employed for the study of the pure E-isomer. The results are recorded in Table 5-1(b). The calculated k_{obs} values based on absorbance changes at the wavelength concerned are seen to be consistent with those determined for the formation of 3-bromoflavanone from the E-isomer.

From Table 5-1 it can be seen that the yield (%) of E-2'-hydroxy-α-bromochalcone from *erythro*-2'-hydroxychalcone dibromide is (35±2%). (For the calculation of the experimental errors in the yield of the E-isomer see appendix 3). Further it can be seen that the yield is not significantly affected by any of the following:

(i) the wavelength used in the analysis;
(ii) the concentration of N-ethylmorpholine buffer used;
(iii) the age of the solution of dibromide in ethanol added.

The yield of the E-isomer thus accounts for 35% of the reaction of the dibromide. The remaining 65% reaction represents the yield of the Z-isomer plus the yield of any 3-bromoflavanone formed by direct cyclisation.

TABLE 5-1 Absorbance data and calculated yields of E-2'-hydroxy-α-bromochalcone from the reaction of *erythro*-2'-hydroxychalcone dibromide in ethanol–water (1:4) buffered with N-ethylmorpholine μ = 0.16 mol l^{-1}, 30°C.

(a) E-isomer

	A_o^E	A_∞^E	Mean A_o^E/A_∞^E	$10^3 k_{obs}/s^{-1}$ (k_1^E)
λ = 264 nm, pH = 7.88, 0.01 mol l^{-1} NEM	0.509	0.274		1.01
	0.551	0.301	1.844	0.98
λ = 272.5 nm, pH = 7.88, 0.01 mol l^{-1} NEM	0.445	0.221		0.95
	0.425	0.210	2.019	1.00
λ = 272.5 nm, pH = 7.81, 0.05 mol l^{-1} NEM	0.458	0.234	1.957	0.90

(b) Dibromide

	A_o^D	A_∞^D	A_o^D/A_∞^D	$10^3 k_{obs}/s^{-1}$	Yield (%)[a]
λ = 264 nm, pH = 7.88 0.01 mol l^{-1} NEM	0.658	0.511	1.287	1.06	34[b]
	0.629	0.493	1.275	1.06	33[c]
λ = 272.5 nm, pH = 7.88 0.01 mol l^{-1} NEM	0.550	0.408	1.348	1.01	34[b]
	0.643	0.469	1.371	1.00	36[b]
λ = 272.5 nm, pH = 7.81, 0.05 mol l^{-1} NEM	0.695	0.520	1.337	0.94	35[b]

[a] Calculated using the mean value of A_o^E/A_∞^E for the conditions concerned and taking effective zero-time for calculated A_o^D values as 30s.

[b] Dibromide solution in ethanol freshly prepared.

[c] Dibromide solution prepared 27 hours in advance.

5-3 DETERMINATION OF THE YIELD OF Z-2'-HYDROXY-α-BROMOCHALCONE FROM THE REACTION OF *ERYTHRO*-2'-HYDROXYCHALCONE DIBROMIDE AT pH 7.88 IN ETHANOL-WATER (1:4)

A method based on the time taken for the Z-isomer to reach maximum concentration (measured at 310 nm) was used to determine its yield. The method requires recourse to kinetic theory and the necessary background is outlined in section 5-3.1.

5-3.1. Kinetic Theory

The standard treatment (Frost & Pearson, 1961) for series first order reactions relates to the following scheme in which D and Z represent the dibromide and the Z-isomer respectively.

$$D \xrightarrow{k_1} Z \xrightarrow{k_2} \text{3-bromoflavanone} \qquad \text{SCHEME 1}$$

In the special case where D yields other products in addition to Z we have also

$$D \xrightarrow{k_3} \text{all products} \qquad \text{SCHEME 2}$$

The yield (%) of Z from D under conditions of kinetic control can be calculated, if rate coefficients are established, as $100k_1/k_3$.

The differential equations are as follows:

$$dD/dt = -k_3D \qquad (1)$$

$$dZ/dt = k_1D - k_2Z \qquad (2)$$

From equation (1) $D = D_oe^{-k_3t}$ (3)

So that equation (2) becomes, multiplying through by the factor $e^{k_2 t}$,

$$e^{k_2 t} dZ/dt + k_2 Z e^{k_2 t} = k_1 D_o e^{(k_2-k_3)t} \quad (4)$$

This can be rewritten as

$$d/dt(e^{k_2 t} Z) = k_1 D_o e^{(k_2-k_3)t} \quad (5)$$

Integration of equation (5) gives

$$e^{k_2 t} Z = k_1/(k_2-k_3)\ D_o e^{(k_2-k_3)t} + Z_o$$

so that

$$Z = [k_1/(k_2-k_3)]\ D_o e^{-k_3 t} + Z_o e^{-k_2 t} \quad (6)$$

Since at $t = 0$, $Z = 0$

$$Z_o = -D_o[k_1/(k_2-k_3)]$$

and so equation (6) can be rewritten as

$$Z = [k_1/(k_2-k_3)]\ D_o(e^{-k_3 t} - e^{-k_2 t}) \quad (7)$$

If Z reaches a maximum (Z_{max}) at time t_{max}, equation (7) becomes

$$Z_{max}/D_o = [k_1/(k_2-k_3)]\ (e^{-k_3 t_{max}} - e^{-k_2 t_{max}}) \quad (8)$$

If Z_{max}/D_o and t_{max} can be measured experimentally and if k_2 is known from independent study of compound Z, k_1 and k_3 are left as the unknowns in equation (8). Further, k_3 can be determined from k_2 and t_{max} as can be seen by differentiating equation (7):

$$dZ/dt = [k_1/(k_2-k_3)]\, D_o(k_2e^{-k_2t}-k_3e^{-k_3t}) \qquad (9)$$

and noting that Z reaches a maximum when dZ/dt is zero. This leads to

$$k_2e^{-k_2t_{max}} = k_3e^{-k_3t_{max}} \qquad (10)$$

which can be rewritten as

$$\ln k_3 - k_3t_{max} = \ln k_2 - k_2t_{max} \qquad (11)$$

Given k_2 , there are two solutions for k_3, one being the trivial one $k_3 = k_2$ which implies Z is the only product from D, the other being the required solution. Once this value of k_3 is calculated, k_1 remains as the only unknown in equation (8) and can be calculated along with the yield (%) of Z, given by $100k_1/k_3$.

5-3.2. Method and Results

(a) Determination of t_{max} and hence k_3. Spectral scans for the Z-isomer (Fig. 5.4.) and the dibromide (Fig. 5.5.) show there is a strong absorbance by the Z-isomer at 310 nm, but little absorbance due to competing reactions. The subsequent formation of flavone from 3-bromoflavanone (Fig 5.2.) has a significant absorbance change at this wavelength but the extent of this reaction is negligible in the time span involved. We therefore measured t_{max} as the time after mixing in dibromide solution at which the absorbance at 310 nm reached a maximum. Care was taken to inject the dibromide solution into the reaction solution as quickly as possible to ensure a quick mixing time, and hence minimise errors in t_{max}. The volume of the added dibromide solution was increased five-fold in order to determine the effect that reaction delay due to incomplete mixing would have on t_{max}. The effect was found to be insignificant.

Values of t_{max} were determined in duplicate runs in N-ethylmorpholine buffer solutions of total concentration 0.008, 0.016, 0.04 and 0.08 mol l^{-1}. In all cases the value of t_{max} was found to be 30 ± 1s.

The value of k_2 in equation (11) is the rate constant for the conversion of the Z-isomer to 3-bromoflavanone, and has been determined by David (1979) to be 0.0220 ± 0.0005s^{-1} (c.f. our values for k_2^Z in Table 5-2) under the same reaction conditions. Since t_{max} has been determined as 30 ± 1s, the non-trivial solution for k_3 in equation (11) can be calculated as

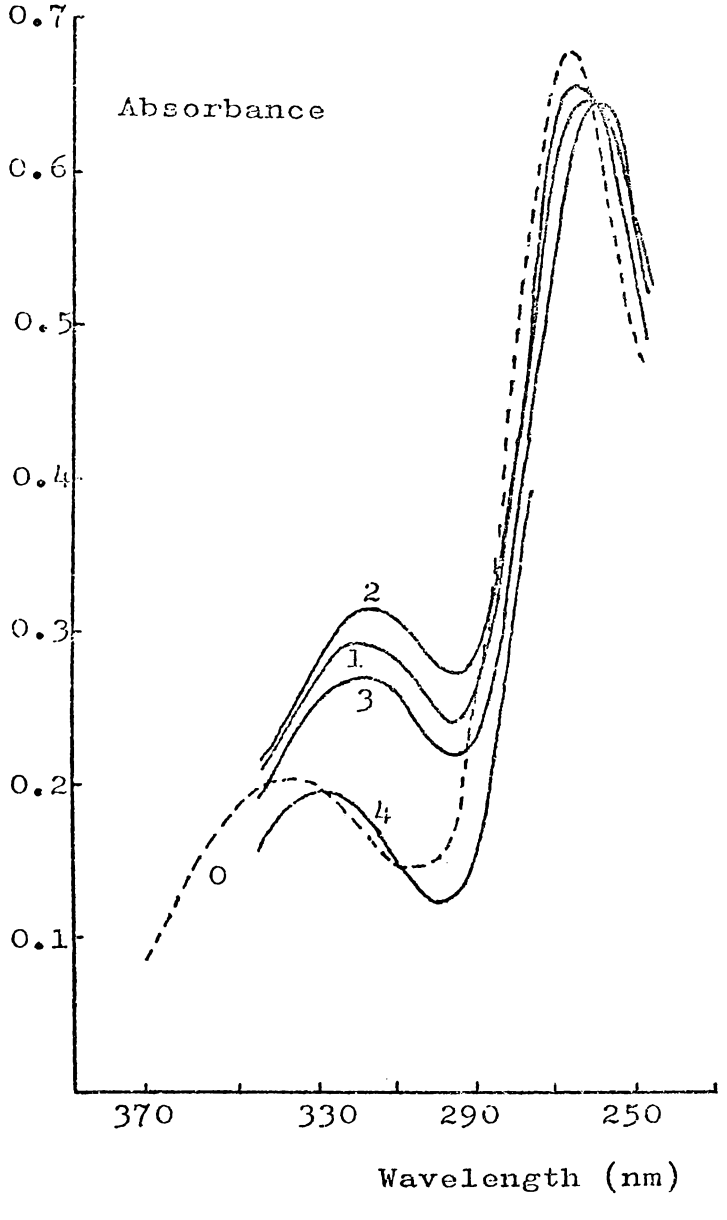


Figure 5.5. Repetitive wavelength scan for the reaction of *erthyro*-2'-hydroxychalcone dibromide in N-ethylmorpholine, pH 7.21. (1) 45s, (2) 90s, (3) 270s, (4) 700s. Scan (o) is that of an approximately equivalent amount of dibromide in 0.16 mol l^{-1} HCl. Scan (4) is incomplete for clarity.

$0.048 \pm 0.003 s^{-1}$*.

Substituting these values for k_2^Z, k_3 and t_{max} into equation (8) gives the relationship

$$Zmax/D_o = 10.77k_1^Z$$

$Zmax/D_o$ represents the fraction of total initial dibromide present as the Z-isomer at t_{max} and can be written as f^Z. Therefore we can rewrite the above equation as

$$k_1^Z = 0.093f^Z \quad (12)$$

(b) Determination of $Zmax/D_o(f^Z)$ and hence k_1^Z. The absorbance change due to the Z-isomer between t_{max} and the time ($t\infty$) at which all the dibromide is converted to 3-bromoflavanone gives a measure of the amount of Z-isomer present at t_{max} (Zmax). A measure of the total initial dibromide (D_o) is given by the absorbance at $t\infty$ ($A\infty$).

At any wavelength the measured total absorbance change between t_{max} and $t\infty$ (ΔA) will contain contributions from reactions of the Z- and E-isomers and of the residual dibromide, i.e.

$$\Delta A = \Delta A^Z + \Delta A^E + \Delta A^D \quad (13)$$

where ΔA^Z represents the contribution that the change in absorbance due to reaction of that amount of the Z-isomer present at t_{max} makes to the overall absorbance change ΔA.

*Independent measurements at pH 7.21 gave values of k_2^Z ($0.0067 s^{-1}$) and t_{max} (104s) which again were independent of the concentration of the N-ethylmorpholine buffer solution (see below). Equation (11) in this case leads to a value for k_3 of $0.0135 s^{-1}$, which, like k_2^Z is approximately three and a half times lower than the corresponding value at pH 7.88.

Buffer Concentration	$k_{obs}/s^{-1}(k_2^Z)$	t_{max}/s
0.008 mol l^{-1}	0.0066, 0.0066	104,104
0.04 mol l^{-1}	0.0069	103
0.08 mol l^{-1}	0.0068	104

Similarly for ΔA^E and ΔA^D. The values of ΔA^Z, ΔA^E and ΔA^D depend on the fractions of the respective compounds present at t_{max} and on their individual absorbance changes when they convert to 3-bromoflavanones.

The fractions of E-isomer (f^E) and dibromide (f^D) present at t_{max} can be calculated from earlier kinetic data. Substituting values for k_3 ($0.048s^{-1}$) and t(30s) into equation (3) gives

$$\underline{f^D = D/D_o = 0.237}$$

To determine f^E use is made of equation (7), replacing Z with E, i.e.

$$f^E = E/D_o = [k_1/(k_2-k_3)]\ (e^{-k_3t}-e^{-k_2t})$$

In this case k_1 is the rate constant for the reaction of the dibromide going to the E-isomer, k_2 is the rate constant for the reaction of the E-isomer going to 3-bromoflavanone which has been determined by David (1979) to be $0.0010s^{-1}$ (c.f. our value for k_2^E in Table 5-2) and k_3 is again the rate constant for the dibromide leading to all products. (see Scheme 1, but replacing Z with E). The value for k_1^E as defined above is calculated as the product of k_3 and the fraction of E-isomer formed from the dibromide (see Section 5-2.2), i.e. $k_1^E = 0.048s^{-1} \times 0.35 = 0.0168s^{-1}$ Taking t as 30s and the above values of k_1^E, k_2^E and k_3 and substituting into the above equation gives,

$$\underline{f^E = E/D_o = 0.262}$$

To determine values for the ratio of the initial to final absorbance (A_o/A_∞) for the formation of 3-bromoflavanone from each of the E- and Z-isomers and from the dibromide, a suitable wavelength needed to be chosen. The wavelength used for determining t_{max} (310 nm) was not suitable for measuring

A_∞ values because the interfering reaction of 3-bromoflavanone going to flavone became observable long before completion of the reactions being studied. To ensure the reliability of the A_∞ values the wavelength chosen needed to give a constant A_∞ value even after there had been significant flavone formation. Measurements were therefore taken at 338.5 nm, which is another isosbestic point for the reaction of 3-bromoflavanone going to flavone (see Fig. 5.2.). The correct wavelength setting was initially determined by trial and error as being that at which the infinity absorbance of a dibromide run was constant. This wavelength needed to be exactly reproduced between runs and this was found to be visually impossible, but it was achieved by recording the absorbance reading of a standard solution of flavone in ethanol at the correct wavelength. On subsequent occasions, therefore, the setting was reproduced exactly by adjusting the wavelength until the standard flavone solution gave the same absorbance reading.

The values of (A_o/A_∞) for the formation of 3-bromoflavanone from pure samples of the E-isomer and the Z-isomer were determined at 338.5 nm by the method previously described for the E-isomer at 272.5 and 264 nm (see section 5-2.1 (a)). Once again the k_{obs} values were checked for consistency as shown in Table 5-2.

The initial absorbance value at 338.5 nm for the dibromide (A_o^D) had to be determined indirectly, for at this wavelength the initial kinetic data reflects the series reactions occurring and can not be linearly plotted in any way for extrapolation to zero time. The measurement was therefore made using hydrochloric acid solution (0.01 mol l^{-1}) in ethanol-water (1:4) containing potassium chloride (0.15 mol l^{-1}); a solution in which the dibromide is quite stable. Any uncertainty in this indirect measurement of A_o^D is associated with the possibility that at pH 7.88, at which the measurement should in principle have been made, a small percentage of the dibromide could be present in ionised form whereas at pH2, at which the measurement

TABLE 5-2 Values of A_o/A_∞ at 338.5 nm for reactions in N-ethylmorpholine buffer (0.0080 mol l^{-1}) in ethanol-water (1:4) at pH 7.88 and 30°C . μ= 0.16 mol l^{-1}.

Z-isomer	A_o^Z	A_∞^Z	Mean A_o^Z/A_∞^Z	k_{obs}/s^{-1} (k_2^Z)
	0.985	0.420		0.0223
	0.684	0.293	2.34	0.0215
E-isomer	A_o^E	A_∞^E	Mean A_o^E/A_∞^E	k_{obs}/s^{-1} (k_2^E)
	0.626	0.400		0.00103
	0.584	0.376	1.56	0.00100
Dibromide	$A_o^{D*\neq}$	$A_\infty^{D\neq}$	Mean A_o^D/A_∞^D	
	0.455	0.340		
	0.457	0.338		
	0.453	0.342	1.34	

* Determined by measurement in hydrochloric acid solution (0.01 mol l^{-1} in ethanol-water (1:4)).

≠ Identical amounts of dibromide solution added.

was made, this would not be the case. Since the pKa of the dibromide is likely to be around 10 by analogy with 2'-hydroxyacetophenone (Perrin, 1958) any error should be small. The corresponding value of A_∞^D was established by adding the same amount of dibromide to N-ethylmorpholine buffer solution, as used in the other determinations, and recording the infinity absorbance. The results are recorded in Table 5-2.

Values of ΔA (equation 13) can now be obtained using the above values for f and A_o/A_∞. If after all the dibromide has been converted to 3-bromoflavanone the absorbance is A_∞^D, then the corresponding theoretical zero-time absorbance which would have applied if <u>all</u> the dibromide had been converted to the Z-isomer can be calculated as $A_\infty^D A_o^Z/A_\infty^Z$ and the theoretical absorbance change as this converted to 3-bromoflavanone would be $A_\infty^D(A_o^Z/A_\infty^Z - 1)$. The change in measured absorbance between t_{max} and t_∞ due to reaction of that amount of the Z-isomer present at t_{max} is given by the theoretical change times the fraction of the Z-isomer present at t_{max}, so that

$$\Delta A^Z = f^Z A_\infty^D[(A_o^Z/A_\infty^Z)-1] \qquad (14)$$

Similar equations apply for ΔA^E and ΔA^D and so equation (13) can be rewritten as

$$\Delta A/A_\infty^D = f^Z[(A_o^Z/A_\infty^Z)-1] + f^E[(A_o^E/A_\infty^E)-1] + f^D[(A_o^D/A_\infty^D)-1] \qquad (15)$$

Substituting known values of f and A_o/A_∞ leads to

$$\Delta A/A_\infty^D = 1.34f^Z + 0.227 \qquad (16)$$

(c) Calculation of the yield of Z-2'-hydroxy-α-bromochalcone. Values of ΔA and A_∞^D were obtained from four dibromide runs at 338.5 nm, using different concentrations of N-ethylmorpholine buffer. The results are shown in Table 5-3 together with the calculated values of f^Z, k_1^Z and the yield (%) of the Z-isomer. The experimental error in the values of yield (%) can be calculated (see Appendix 3) to be quite large (ca 8%) leading to a mean value for the yield (%) of

TABLE 5-3. Absorbance data and yield of Z-2'-hydroxy-α-bromochalcone from the reaction of *erythro*-2'-hydroxychalcone dibromide in ethanol-water (1:4) buffered at pH 7.88 with N-ethylmorpholine. μ = 0.16 mol l^{-1}, 30°C.

[a] 10^3[B]	[b] $A_{t_{max}}$	[b] A_∞	$\Delta A/A_\infty$	[c] f^Z	[d] $10^2 k_1^Z/s^{-1}$	[e] Yield (%)
8	0.411	0.245	0.678	0.336	3.12	65
16	0.416	0.250	0.652	0.317	2.94	61
40	0.416	0.250	0.664	0.326	3.03	63
80	0.379	0.228	0.662	0.325	3.01	63

[a] [B] is total N-ethylmorpholine mol l^{-1}

[b] Measured at 338.5 nm

[c] Determined from equation (16)

[d] Rate constant for the formation of the Z-isomer from the dibromide as determined from equation (12)

[e] Yield of Z-isomer calculated as $100k_1^Z/k_3$, k_3 being $0.048s^{-1}$

Z-2'-hydroxy-α-bromochalcone from *erythro*-2'-hydroxychalcone dibromide of 63 ± 8%. This amount of uncertainty would have been lower had it been possible to measure the absorbance change at 310 nm where there is a larger absorbance change due to the reaction of the Z-isomer, but the problem of the subsequent flavone-forming reaction prevented the use of this wavelength.

5-4 DISCUSSION

5-4.1 Experimental Uncertainty

The analytical method assumes that, irrespective of the starting material, there will always be the same ratio of *cis*- and *trans*-3-bromoflavanone at the infinity stage of the reaction. David (1979) has suggested that the Z-isomer may be the only precursor, but, if not, the method requires that the proportions of *cis*- and *trans*-3-bromoflavanone be the same either as directly formed from all different precursors, which is unlikely, or become the same through isomerisation before the infinity absorbance is attained. Clean first-order kinetics were obtained for the N-ethylmorpholine-catalysed elimination from a 3:1 mixture of *cis*- and *trans*-3-bromoflavanone at pH 7.87 (David, 1979) and this suggests that such an isomerisation occurs fast in comparison with the elimination of hydrogen bromide to form flavone. The isomerisation equilibrium should therefore be attained by the time infinity absorbance readings are made. However, even if this is not the case, little error would be introduced into the analysis figures if the extinction coefficients for *cis*- and *trans*-3-bromoflavanone were very similar at the analysis wavelength. That they could very nearly be identical is suggested by the report that this is indeed the case for *cis*- and *trans*-6-methyl-4'-methoxy-3-bromoflavanone (Joshi & Kulkarni, 1957; Kulkarni 1961).

5-4.2. Products

The results establish that the major reaction of *erythro*-2'-hydroxychalcone dibromide under these conditions is the elimination to form E- and Z-2'-hydroxy-α-bromochalcone. The direct cyclisation of the dibromide to 3-bromoflavanone can not be completely excluded but if it occurs it is certainly a minor reaction. 2'-Hydroxy-α-bromochalcones have often been proposed as intermediates in the cyclisation of 2'-hydroxychalcone dibromides which form aurone and flavone (Class 2) under the conditions of the Emilewicz-von-Kostanecki reaction (Auwers & Anschutz, 1921; Hutchins & Wheeler, 1937; Donnelly et al, 1973, 1973a, 1975, 1975a, 1977), but 2'-hydroxychalcone dibromides which, like the one under study, yield only flavone (Class 1) in aqueous alcoholic alkali have previously been regarded as yielding 3-bromoflavanone by direct cyclisation, as proposed by Auwers & Anschutz (1921). However, the present study suggests that the elimination - addition route via 2'-hydroxy-α-bromochalcone is the major one even for dibromides which give no aurone.

Previous reports of the synthesis of E- and Z-isomers of variously substituted 2'-hydroxy-α-bromochalcones from *erythro*-2'-hydroxychalcone dibromides (Donnelly et al, 1975a, 1977; David, 1979) show that the Z-isomer usually, but not always predominates. The precise ratio of isomers as formed has not been previously established, however, since product yields do not account for all reagent consumed, 3-bromoflavanone and flavone products, isolated in some cases, presumably accounting for the remainder, so that the isolated product ratio is dependent on the reactivities of the E- and Z-isomers.

Lutz et al (1951) found that the elimination of hydrogen bromide from *erythro*-chalcone dibromide by means of potassium acetate gave a mixture containing 66% Z-α-bromochalcone and 34% E-α-bromochalcone. Since this system lacks a 2'-hydroxyl group it is not complicated by subsequent reactions, and hence the synthetic yields do represent the ratio of isomers formed in the reaction. Although the reaction conditions are different, the similarity between our results and those of Lutz et al are worth noting. Donnelly et al (1977) have used similar conditions to those employed by Lutz et al to study the elimination of hydrogen bromide from 2'-acetoxy-4-nitrochalcone dibromide. NMR data indicated that the E-isomer/Z-isomer ratio was 25:75 in this case.

5-4.3. Mechanism

The invariance with base (N-ethylmorpholine) concentration of the yields of E- and Z-2'-hydroxy-α-bromochalcone and of their rate constants for the subsequent cyclisation to 3-bromoflavanone shows unambiguously that the rate constants for the formation of the E- and Z-isomers from *erythro*-2'-hydroxychalcone dibromide are not measurably changed with the concentration of N-ethylmorpholine buffer over the range 0.008 - 0.08 mol l^{-1} . This contrasts markedly with the results for the elimination of hydrogen bromide from 3-bromoflavanone for which the observed rate constant is changed 8-fold over a similar range of buffer concentration. In this case the mechanism for the elimination has been suggested to be E2 (David, 1979).

The lack of any detectable base catalysis in the elimination of hydrogen bromide from *erythro*-2'-hydroxychalcone dibromide shows that an E2 mechanism involving N-ethylmorpholine as base is not important. An E2 mechanism involving the lyate species HO^- and EtO^- is possible

although this implies a sharp reversal in the relative base reactivities of HO^- (and/or EtO^-) and N-ethylmorpholine between the two different eliminations. This suggests that an E2 mechanism is unlikely, but does not definitely exclude it as a possibility since the eliminations under comparison have very different characteristics. The elimination from *erythro*-2'-hydroxychalcone dibromide involves a proton which is much more acidic than the one involved in the elimination from 3-bromoflavanone and, further, the bromide ion departs from a carbon atom in the dibromide which is much less electron deficient than that involved in the 3-bromoflavanone. It is therefore possible that relative base reactivities may differ in such dissimilar eliminations. Of interest in this context is the observation (Fedor, 1967), that N-methylmorpholine is only weakly catalytic in the general base-catalysed formation of 4-methyl-3-penten-2-one from 4-methyl-4-acetoxy-2-pentanone. This reaction is similar to the elimination from the dibromide under study here in that there is also a double bond being formed α,β to the carbonyl group. This same worker has observed that N-ethylmorpholine shows no detectable catalysis in the same elimination reaction. However, this result does not definitely exclude general base catalysis by this amine as the measurements in this case were carried out at a high pH at which the hydroxide-catalysed reaction was sufficiently fast that additional catalysis by N-ethylmorpholine, could very easily pass undetected at the amine concentrations employed. Nevertheless, the results do indicate that N-alkylmorpholines might be relatively poor catalysts in this type of elimination involving formation of a double bond conjugated to a carbonyl group.

If an E2 mechanism does not apply, alternative mechanisms consistent with the lack of buffer catalysis are of the E1 or $E1_{cB}$ type. An E1 mechanism would require that k_3, the rate constant for the elimination of hydrogen bromide from the dibromide, should be invariant with pH. The value of k_3 decreases from $0.048s^{-1}$ at pH 7.88 to $0.013s^{-1}$ at pH 7.21 (see footnote p.162), whereas if ionisation of the β-bromine were the rate-limiting stage such a large decrease in the rate constant would not be expected.

The lack of buffer catalysis alternatively suggests the possibility of a mechanism in which a conjugate base is formed in equilibrium with the reactant, and then releases the leaving group (in this case the bromide ion) in the rate-limiting stage. The position of the equilibrium would be dependent on pH but not on buffer concentration and would thus account for the experimental observations in the dibromide case. Such a reaction is known for the elimination of methoxide ion from 4-methoxy-2-butanone and from 4-methoxy-4-methyl-2-pentanone., (Fedor, 1969), and is favoured in this case by having a relatively poor leaving group. Mechanisms of this type are referred to as $E1_{cB}$ if the conjugate base is a carbanion or enolate ion, but in this case owing to the proximity of the phenolic group, it would be possible that the phenoxide ion (Fig.5.6.) could be responsible for the elimination. A concerted *anti*-elimination would give E-2'-hydroxy-α-bromochalcone.

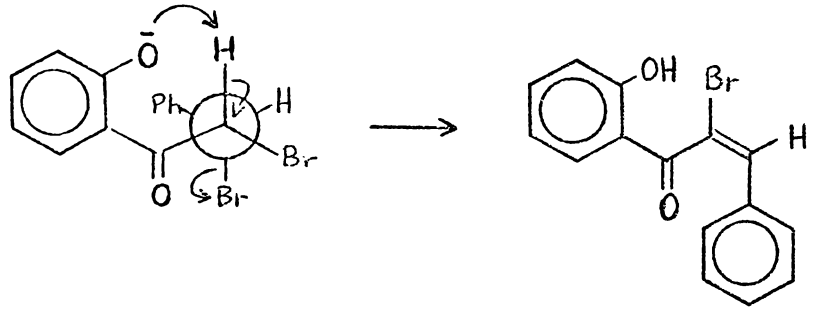


Figure 5.6. Possible concerted *anti*-elimination from *erythro*-2'-hydroxychalcone dibromide leading to E-2'-hydroxy-α-bromochalcone.

The formation of Z-2'-hydroxy-α-bromochalcone could arise by a concerted *syn*-elimination as shown in Fig. 5.7. The product proportions

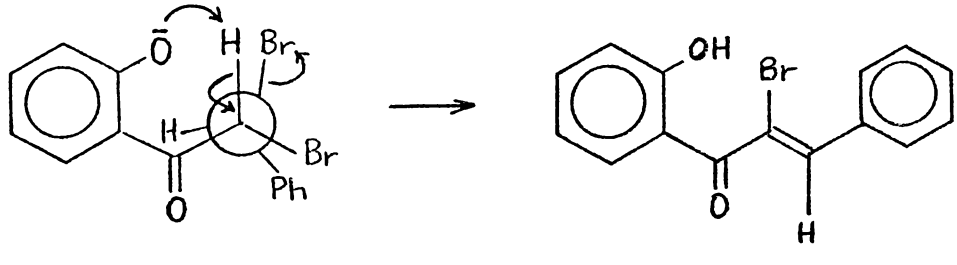


Figure 5.7. Possible concerted *syn*-elimination from *erythro*-2'-hydroxychalcone dibromide leading to Z-2'-hydroxy-α-bromochalcone.

would require that *syn* elimination should be favoured over *anti* elimination, and although this is true in certain special cases, by

analogy with other dehydrobrominations it would be unexpected if this were the situation here. By referring to figures 5.6 and 5.7 it can be seen that there is less steric interaction between the two aromatic rings in the *syn* conformation as compared to the *anti* conformation and this could possibly promote *syn*- over *anti*-elimination in this system.

A further alternative is that rapid α-proton exchange may occur in the *erythro* -2'-hydroxychalcone dibromide leading to the *threo*-2'-hydroxychalcone dibromide which could undergo a concerted *anti*-elimination to form Z-2'-hydroxy-α-bromochalcone. Any such exchange would require the formation of a carbanion at the α-position and a simpler, though not necessarily correct explanation for the product distribution is that elimination occurs directly from such a carbanion to give predominantly the more stable Z-isomer. If such a carbanion is involved, it must necessarily be formed in equilibrium with the *erythro*-2'-hydroxychalcone dibromide in order that the independence of the rate on the buffer concentration at constant pH be observed (Fig. 5.8.).

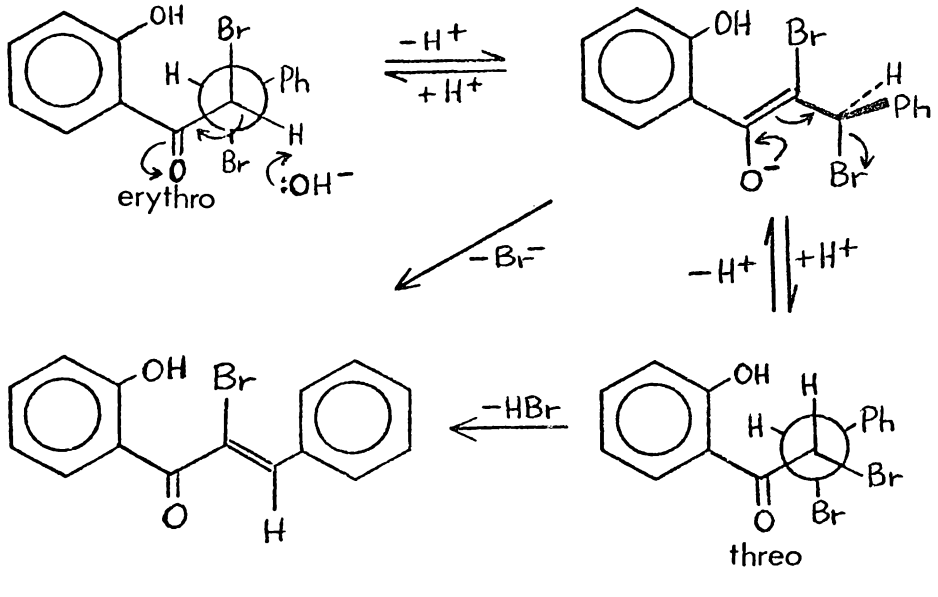


Figure 5.8. Possible routes to Z-2'-hydroxy-α- bromochalcone from *erythro*-2'-hydroxychalcone dibromide.

It is evident that the presence of the 2'-hydroxyl group may favour carbanion formation by enolate protonation (Fig. 5.9.) irrespective of whether the carbanion (enolate) is involved only in the isomerisation of *erythro* to *threo*-2'-hydroxychalcone dibromide or whether it directly expels bromide ion.

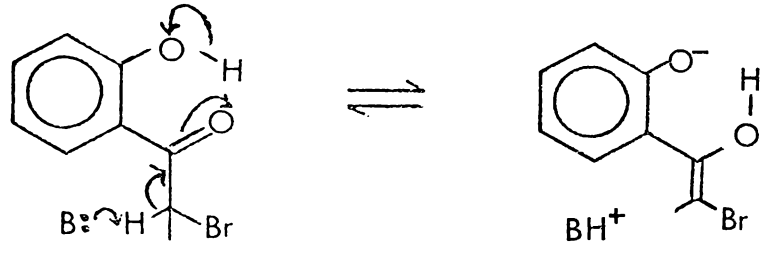


Figure 5.9. Possible assistance of carbanion (enolate) formation by protonation.

The observed reduction in k_3 with pH is consistent with both an El_{cB} type of mechanism and an E2 mechanism involving HO^- and/or EtO^-. In the former case the reduction can be accounted for by a decrease in the concentration of conjugate base in equilibrium with the dibromide and in the latter case by a decrease in the activity of HO^- and EtO^-.

In summary, an E1 mechanism has been eliminated on kinetic grounds, but E2 and El_{cB} type mechanisms remain as acceptable alternatives. The lack of any detectable dependence on buffer concentration of the rate constants or of the proportions of the E- and Z-isomers formed points to El_{cB} rather than E2, but with the particular elimination involved (i.e. a double bond being formed α,β to a carbonyl group) there is

precedence for small catalysis by N-alkylmorpholines and therefore, it is not certain that the lack of measurable buffer catalysis does in fact mean a total lack of catalysis. Catalysis could possibly be found at higher buffer concentrations. Further, if the elimination is by an El_{cB} type mechanism, a carbanion is not necessarily involved, as the elimination could proceed from the phenolate ion as conjugate base, the phenoxide group acting in place of an external base.

APPENDIX 1. DETERMINING ERROR IN k_{obs}

The conventional infinity plot method for determining first order rate constants involves calculating the slope of a plot of ln $(A-A_\infty)$ versus time, where A is the absorbance at time t and A_∞ is the infinity absorbance. The analysis of errors in k_{obs} will be treated in two parts.

(a) The error in k_{obs} using the absorbance range 0 - 0.5

The absolute error in A is taken as ±0.001

$\therefore$ absolute error in $(A - A_\infty)$ = ±0.002

The absolute error in $\ln(A - A_\infty)$ was calculated as $\frac{\Delta(A - A_\infty)}{(A - A_\infty)}$

The following example uses absorbance values obtained from the cyclisation of 2'-hydroxychalcone to flavanone at pH 9.20 (see Table 3-2).

Time interval = 50s A_∞ = 0.075

Time (50s intervals)	A	$(A - A_\infty)$	$\ln(A - A_\infty)$
1	0.373	0.298 ± 0.002	-1.211 ± 0.007
2	0.341	0.266 ± 0.002	-1.324 ± 0.008
3	0.311	0.236 ± 0.002	-1.444 ± 0.009
4	0.285	0.210 ± 0.002	-1.561 ± 0.010
5	0.261	0.186 ± 0.002	-1.682 ± 0.011
6	0.239	0.164 ± 0.002	-1.808 ± 0.012
7	0.222	0.147 ± 0.002	-1.917 ± 0.014
8	0.206	0.131 ± 0.002	-2.033 ± 0.015
9	0.191	0.116 ± 0.002	-2.154 ± 0.017
10	0.178	0.103 ± 0.002	-2.273 ± 0.019
11	0.167	0.092 ± 0.002	-2.386 ± 0.022

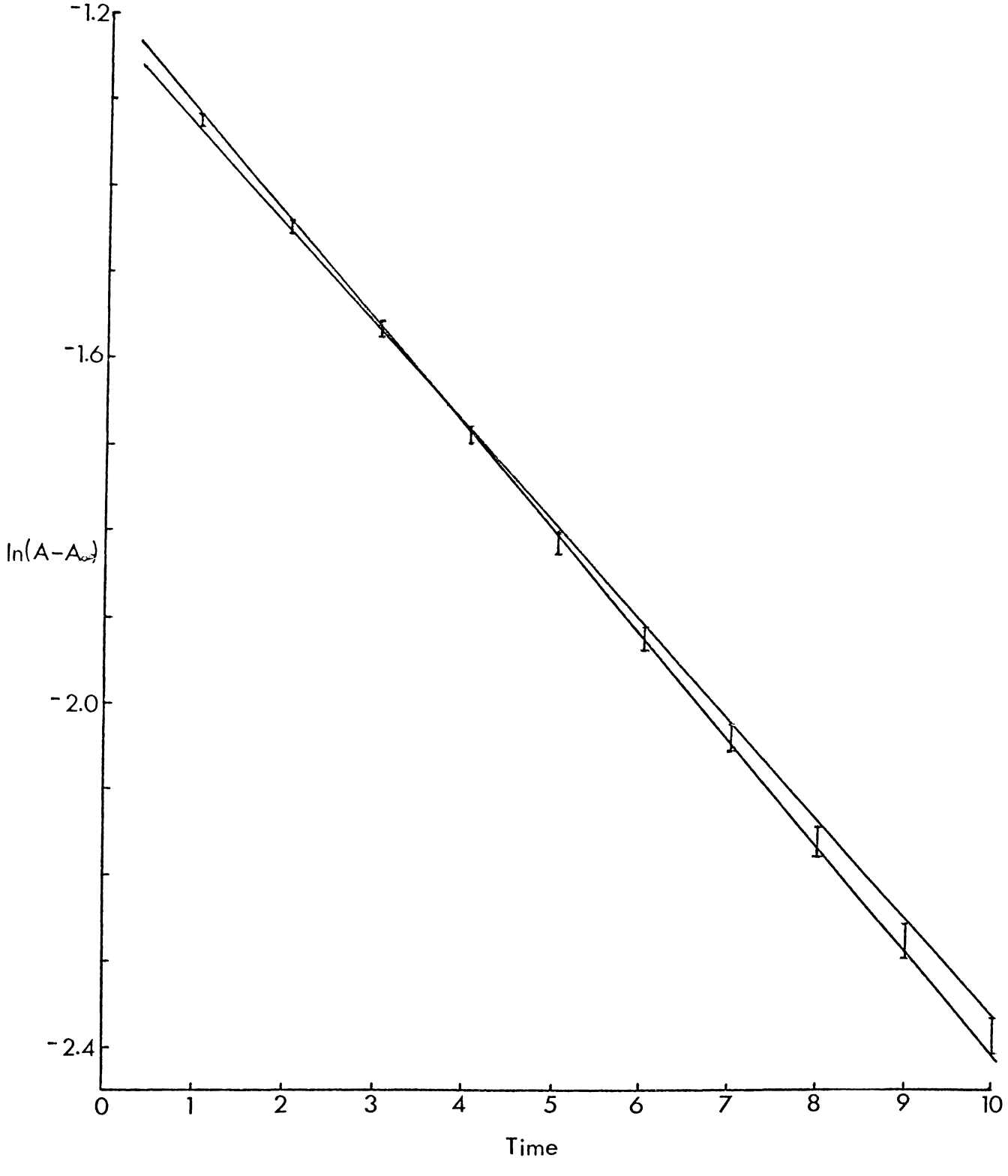


Figure A1(a) Plot of $\ln(A - A_\infty)$ versus time for kinetic runs using the absorbance range 0 - 0.5 (Time in 50s intervals).

A plot of ln $(A - A_\infty)$ versus time is shown in Fig. A1(a).

From the graph:

Maximum slope = $24.3 \times 10^{-4} s^{-1}$

Minimum slope = $23.0 \times 10^{-4} s^{-1}$

Least squares analysis = $23.7 \times 10^{-4} s^{-1}$

$\therefore$ $k_{obs} = (23.7 \pm 0.7) \times 10^{-4} s^{-1}$

The error in k_{obs} when using the absorbance range 0 - 0.5 is, therefore, taken to be $\simeq \pm 3\%$.

(b) The error in k_{obs} using the absorbance range 0 - 0.2

This example uses absorbance values obtained from the cyclisation of 2'-hydroxy-4'-methoxychalcone to 7-methoxyflavanone at pH 8.90 (see Table 3-8).

Time interval = 235s $\quad A_\infty = 0.005$

Time (235s intervals)	A	$(A - A_\infty)$	$\ln(A - A_\infty)$
1	0.139	0.134 ± 0.002	-2.010 ± 0.015
2	0.124	0.119 ± 0.002	-2.129 ± 0.017
3	0.110	0.105 ± 0.002	-2.254 ± 0.019
4	0.098	0.093 ± 0.002	-2.375 ± 0.022
5	0.088	0.083 ± 0.002	-2.489 ± 0.024
6	0.078	0.073 ± 0.002	-2.617 ± 0.027
7	0.070	0.065 ± 0.002	-2.733 ± 0.031
8	0.063	0.058 ± 0.002	-2.847 ± 0.034
9	0.057	0.052 ± 0.002	-2.957 ± 0.038
10	0.051	0.046 ± 0.002	-3.079 ± 0.043
11	0.046	0.041 ± 0.002	-3.194 ± 0.049

A plot of $\ln(A - A_\infty)$ versus time is shown in Fig. A1 (b).

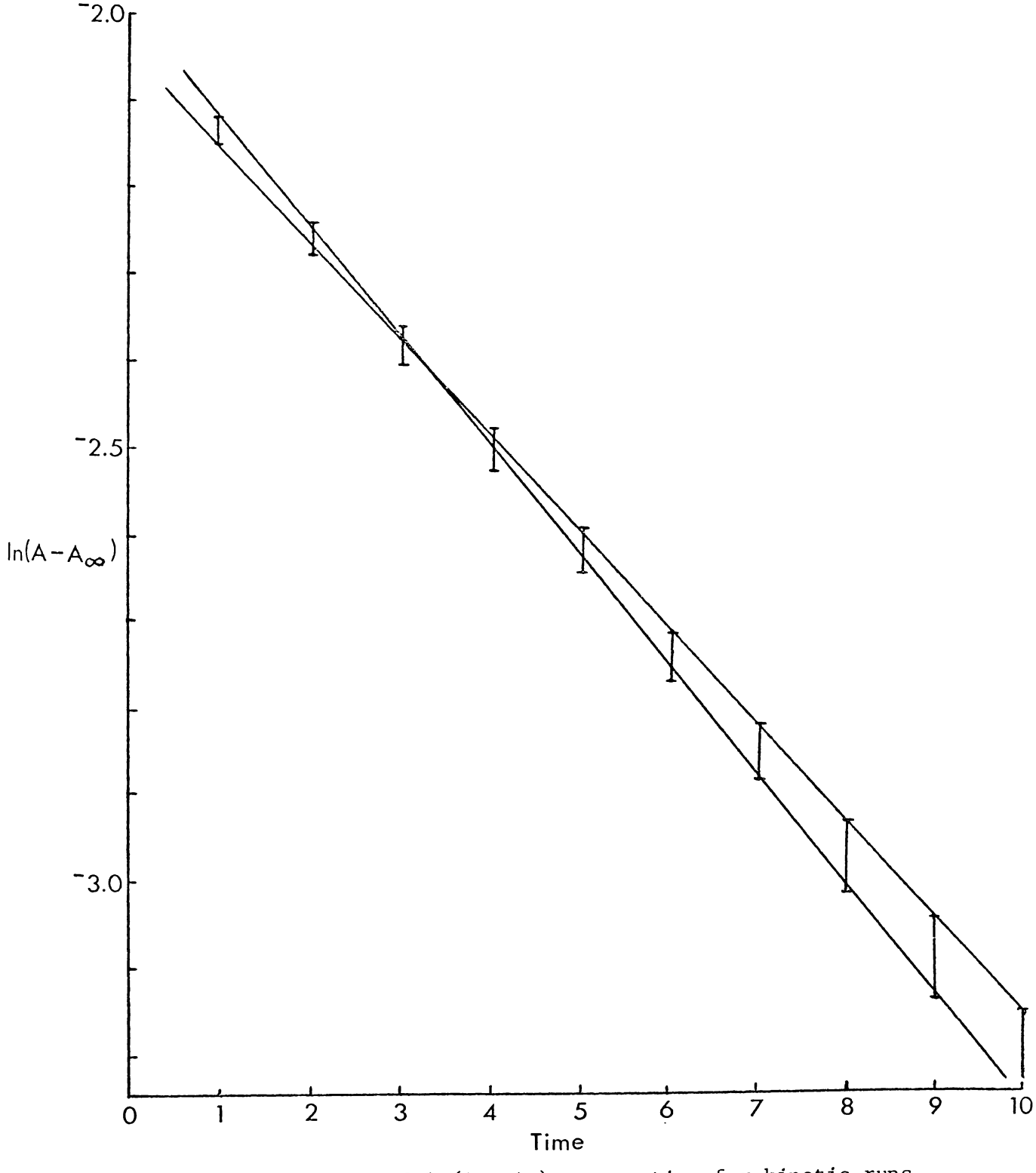


Figure A1(b). Plot of $\ln(A - A_\infty)$ versus time for kinetic runs using the absorbance range 0 - 0.2. (Time in 235s intervals).

From the graph:

Maximum slope = $5.40 \times 10^{-4} s^{-1}$

Minimum slope = $4.77 \times 10^{-4} s^{-1}$

Least squares analysis = $5.08 \times 10^{-4} s^{-1}$

$\therefore k_{obs} = (5.08 \pm 0.32) \times 10^{-4} s^{-1}$

The error in k_{obs} when using the absorbance range 0 - 0.5 is, therefore, taken to be $\approx \pm 6\%$.

APPENDIX 2. DETERMINING THE BEST FIT VALUES OF pK_a, k, k', k"

The following is a description of the method used to determine the best fit values of pK_a, k, k' and k", from the observed rate constants for the attainment of the 2'-hydroxy-6'-methoxychalcone --- 5-methoxyflavanone equilibrium (see section 3-6). The values were required to fit the expression,

$$k_{obs} = kfCH + k'fC^- + k''\{OH^-\}$$

Initially a visual estimate of the pK_a was made by reference to the pH-rate profile, and using a value for k' as one half k_{obs} at this pH a table of the form shown in tables A2-1 to A2-5 was constructed. Tables were similarly constructed for pK_a values at 0.1 pH units either side of the first estimate of pK_a.

Explanation of Tables A2-1 to A2-5

(1) Ratio of ionised chalcone to neutral chalcone.

(2) Fraction of ionised chalcone.

(3) Fraction of neutral chalcone

(4) First estimate of k' based on $k' \simeq \frac{k_{obs}}{fC^-}$, and shown as $10^4 k'/s^{-1}$.

From the above expression $k' = \frac{k_{obs}}{fC^-} - (\frac{kfCH}{fC^-} + \frac{k''\{OH^-\}}{fC^-})$

therefore this initial approximation of k' is expected to be higher than the best fit value.

(5) First approximation to k" based on $k'' = \frac{k_{obs} - k'fC^-}{\{OH^-\}}$, and shown as $k''/activity^{-1}s^{-1}$

TABLE A2-1. Assumed pKa = 8.8 and $k' = 4.6 \times 10^{-4}\ s^{-1}$

pH	$10^4 k_{obs}/s^{-1}$	$\frac{C^-}{CH}$ (1)	fC^- (2)	fCH (3)	First Estimates of:			First Correction to k' (7)	Second Estimate of k' (8)
					k' (4)	k'' (5)	k (6)		
6.94	.133	.0138	.0136	.9864	9.768		7.134	5.137	4.631
7.29	.200	.0309	.0300	.9700	6.672		6.403	2.332	4.340
7.79	.433	.0977	.0890	.9110	4.864		2.579	.788	4.076
8.14	.800	.2188	.1795	.8205	4.457		-	.399	4.058
8.43	1.34	.4266	.2990	.7010	4.481		-	.257	4.224
8.77	2.26	.9333	.4827	.5173	4.682			.200	4.482
8.90	2.60	1.2589	.5573	.4427	4.665			.202	4.463
9.25	3.58	2.8184	.7381	.2619	4.850			.273	4.577
9.39	4.16	3.8905	.7955	.2045	5.229	1.388		.335	4.894
9.85	5.35	11.2202	.9182	.0818	5.827	1.083		.799	5.028
10.12	6.13	20.8930	.9543	.0457	6.423	.988		1.424	4.999
10.23	6.58	26.9153	.9642	.0358	6.825	.860		1.814	5.011
10.50	7.89	50.1187	.9804	.0196	8.047	.728		3.318	4.729
10.83	11.51	107.1519	.9908	.0092	11.617	.700		7.017	4.600

TABLE A2-2. Assumed pKa = 8.9 and $k' = 5.1 \times 10^{-4}\ s^{-1}$

pH	$10^4 k_{obs}/\ s^{-1}$	$\frac{C^-}{CH}$ (1)	fC^- (2)	fCH (3)	First Estimates of:			First Correction to k' (7)	Second Estimate of k' (8)
					k' (4)	k'' (5)	k (6)		
6.94	.133	.0110	.0108	.9892	12.263		7.854	6.461	5.802
7.29	.200	.0245	.0240	.9760	8.348		7.972	2.929	5.419
7.79	.433	.0776	.0720	.9280	6.011		7.073	.984	5.027
8.14	.800	.1738	.1481	.8519	5.404		5.275	.492	4.912
8.43	1.34	.3388	.2531	.7469	5.295		6.595	.308	4.987
8.77	2.26	.7413	.4257	.5743	5.309			.226	5.083
8.90	2.60	1.0000	.5000	.5000	5.200			.222	4.978
9.25	3.58	2.2387	.6912	.3088	5.179			.277	4.902
9.39	4.16	3.0903	.7555	.2445	5.506	.851		.333	5.173
9.85	5.35	8.9125	.8991	.1009	5.950	.735		.760	5.190
10.12	6.13	16.5959	.9432	.0568	6.499	.682		1.339	5.160
10.23	6.58	21.3796	.9553	.0447	6.888	.685		1.701	5.187
10.50	7.89	39.8107	.9755	.0245	8.088	.628		3.097	4.991
10.83	11.51	85.1138	.9884	.0116	11.645	.651		6.532	5.113

TABLE A2-3. Assumed pKa = 8.95 and $k' = 5.5 \times 10^{-4} s^{-1}$

pH	$10^4 k_{obs}/s^{-1}$	$\frac{C^-}{CH}$ (1)	fC^- (2)	fCH (3)	First Estimates of: k' (4)	k'' (5)	k (6)	First Correction to k' (7)	Second Estimate of k' (8)
6.94	.133	.0098	.0097	.9903	13.743		8.055	8.266	5.477
7.29	.200	.0219	.0214	.9786	9.342		8.405	3.737	5.605
7.79	.433	.0692	.0647	.9353	6.692		8.245	1.240	5.452
8.14	.800	.1549	.1341	.8659	5.965		7.206	.607	5.358
8.43	1.34	.3020	.2319	.7681	5.777		8.370	.367	5.410
8.77	2.26	.6607	.3978	.6022	5.681		11.935	.252	5.429
8.90	2.60	.8913	.4712	.5288	5.517			.238	5.279
9.25	3.58	1.9953	.6661	.3339	5.374			.275	5.099
9.39	4.16	2.7542	.7336	.2664	5.670	.347		.324	5.346
9.85	5.35	7.9433	.8882	.1118	6.024	.447		.713	5.311
10.12	6.13	14.7911	.9367	.0633	6.544	.505		1.246	5.298
10.23	6.58	19.0546	.9501	.0499	6.925	.543		1.580	5.345
10.50	7.89	35.4813	.9726	.0274	8.112	.547		2.868	5.244
10.83	11.51	75.8578	.9870	.0130	11.662	.612		6.038	5.624

TABLE A2-4. Assumed pKa = 9.0 and $k' = 5.8 \times 10^{-4}\ s^{-1}$

pH	$10^4 k_{obs}/s^{-1}$	$\frac{C^-}{CH}$ (1)	fC^- (2)	fCH (3)	First Estimates of:			First Correction to k' (7)	Second Estimate of k' (8)
					k' (4)	k'' (5)	k (6)		
6.94	.133	.0087	.0086	.9914	15,403		8.364	9.271	6.132
7.29	.200	.0195	.0191	.9809	10,457		9.081	4.190	6.267
7.79	.433	.0617	.0581	.9419	7.455		10,207	1.388	6.067
8.14	.800	.1380	.1213	.8787	6.596		10.981	.677	5.919
8.43	1.34	.2692	.2121	.7879	6,319		13.958	.405	5.914
8.77	2.26	.5888	.3706	.6294	6,098		17.549	.271	5.827
8.90	2.60	.7943	.4427	.5573	5.873			.254	5.619
9.25	3.58	1,7783	.6401	.3599	5.593			.282	5.311
9.39	4.16	2.4547	.7105	.2895	5.855	.108		.327	5.528
9.85	5.35	7.0795	.8762	.1238	6.106	.258		.700	5.406
10.12	6.13	13.1826	.9295	.0705	6.595	.382		1.214	5.381
10.23	6.58	16.9824	.9444	.0556	6.968	.442		1.537	5.431
10.50	7.89	31.6228	.9693	.0307	8.140	.488		2.782	5.358
10.83	11.51	67.6083	.9854	.0146	11.680	.583		5.846	5.834

TABLE A2-5. Assumed pKa = 9.1 and $k' = 6.5 \times 10^{-4} s^{-1}$

pH	$10^4 k_{obs}/s^{-1}$	$\frac{C^-}{CH}$ (1)	fC^- (2)	fCH (3)	First Estimates of: k' (4)	k'' (5)	k (6)	First Correction to k' (7)	Second Estimate of k' (8)
6.94	.133	.0069	.0069	.9931	19.357		8.895	13.106	6.251
7.29	.200	.0155	.0153	.9847	13.113		10.242	5.909	7.204
7.79	.433	.0490	.0467	.9533	9.274		13.585	1.938	7.336
8.14	.800	.1096	.0988	.9012	8.096		17.501	.928	7.168
8.43	1.34	.2138	.1761	.8239	7.608		23.681	.538	7.070
8.77	2.26	.4677	.3187	.6813	7.092		27.680	.336	6.756
8.90	2.60	.6310	.3869	.6131	6.721			.299	6.422
9.25	3.58	1.4125	.5855	.4145	6.114			.296	5.818
9.39	4.16	1.9498	.6610	.3390	6.294	-		.330	5.964
9.85	5.35	5.6234	.8490	.1510	6.301	-		.653	5.648
10.12	6.13	10.4713	.9128	.0872	6.715	.102		1.112	5.603
10.23	6.58	13.4896	.9310	.0690	7.068	.212		1.400	5.668
10.50	7.89	25.1189	.9617	.0383	8.204	.353		2.515	5.689
10.83	11.51	53.7032	.9817	.0183	11.724	.516		5.262	6.462

(6) First approximation to k based on $k = \frac{k_{obs} - k'fC^-}{fCH}$, and shown as $10^6 k/s^{-1}$.

(7) Contribution of $(\frac{kfCH}{fC^-} + \frac{k''\{OH^-\}}{fC^-})$ to k' based on the first estimates of k and k".

(8) k' given by the value in column (4) minus the first correction in column (7).

Values of k are only shown at low pH, and values of k" at high pH, since it is only in these respective regions that their values could be expected to be constant.

From the first plotted tables we would expect the following:

(i) The first estimate of k' should be at a minimum near the pK_a and should be approximately constant in this region. The range of values for the second estimate of k' should be small.

(ii) Since the assumed k' (based on $k_{obs}/2$ at the assumed pK_a) will be too high, the first estimate for k" will be too low and should decrease with increasing pH.

(iii) As a result of the assumed k' being too high, the first estimate of k would be expected to be too low and to decrease with increasing pH.

By reference to the relevant tables we can determine which values of pK_a and k' best satisfy the above requirements.

<u>Assuming $pK_a = 8.8$ and $k' = 4.6 \times 10^{-4} s^{-1}$</u>

(i) The minimum value for k' at pH 8.14 possibly occurs a little too far below the assumed pKa. The range of values for the second estimate of k' is ≈24%.

(ii) k" decreases which is not as required.

(iii) k decreases too quickly.

Assuming pKa = 8.9 and k' = $5.1 \times 10^{-4} s^{-1}$

(i) The minimum value for k' occurs at a pH slightly above the assumed pKa, but is satisfactory in this respect. The range of values for the second estimate of k' is ≈17%.

(ii) k" still decreasing, with increasing pH, but not as fast as for pKa 8.8.

(iii) The slow decrease in k is acceptable.

Assuming pKa = 9.0 and k' = $5.8 \times 10^{-4} s^{-1}$

(i) Position of minimum value for k' acceptable. The range of values for the second estimate of k' is ≈18%.

(ii) k" is increasing as required, but too quickly.

(iii) k is increasing too quickly.

Assuming pKa = 9.1 and k' = $6.5 \times 10^{-4} s^{-1}$

(i) The range of low values for k' occurs at pH values too far above the assumed pKa. The range of values for the second estimate of k' is ≈31%.

(ii) k" increases too quickly.

(iii) k increases too quickly.

From the above it can be seen that the best fit pKa appears to lie between 8.9 and 9.0. A pKa of 8.9 appears slightly low as k" decreases with increasing pH, which means that when a better fit value for k' (lower value) is used, k" will decrease at an even faster rate. In contrast using pKa 9.0, k" increases with increasing pH. Similar comparisons for k values show that at pKa 8.9 k decreases and at pKa 9.0 k increases. Further, by comparing the range in the second estimates of k' a better fit might also be expected between

8.9 amd 9.0. From Table A2-3 it can bee seen that assuming a pK_a of 8.95 and $k' = 5.5 \times 10^{-4} s^{-1}$ a better fit is obtained. k is almost constant over the range expected, k'' increases slowly with increasing pH and the range of values for the second estimate of k' is ≈10%.

Having established the pK_a, the value of k' was decreased by small amounts until the most constant values of k'' and k were obtained. Corrections were made to k and k'' by including the minor terms that were ignored in their early estimates. That is, $\frac{-kfCH}{\{OH^-\}}$ was included in the calculation of k'', and $\frac{-k''\{OH^-\}}{fCH}$ was included in the calculation of k. Finally, a theoretical curve was plotted and compared with the experimental points. Minor adjustments to the calculated values were then made in order to obtain the best agreement between the theoretical curve and the experimental points.

APPENDIX 3. ESTIMATION OF ERROR IN CALCULATED VALUES OF YIELD (%) FOR E- AND Z-2'-HYDROXY-α-BROMOCHALCONES

In this section, data (from Chapter 5) used in one particular calculation of yield (%) for (i) the E-isomer, and (ii) the Z-isomer are treated taking experimental error into account so as to obtain an estimation of the error in the final yield (%) figures. It is assumed that a similar magnitude of error applies in the other cases as well.

(i) E-2'-hydroxy-α-bromochalcone. The data in the first line of Table 5-1(a) and the first line of Table 5-1(b) led to a value of 34% for the yield of the E-isomer for these conditions. Assuming an error of 0.001 in absorbance readings, the error in the calculated yield is as follows:

$$\text{Mean value } A_o^E/A_\infty^E = 1.844 \pm 0.010$$

$$\text{Mean value } A_o^D/A_\infty^D = 1.287 \pm 0.005$$

Therefore, from the equation in section 5-2.1,

$$\text{Yield (\%)} = 100[(A_o^D/A_\infty^D) - 1]/[(A_o^E/A_\infty^E) - 1]$$

$$= 100\ [0.287 \pm 0.005]/\ [0.844 \pm 0.010]$$

$$= 34.0 \pm 1.0$$

Adding an error of 1% in yield to cover uncertainty in effective zero time leads to a total error of 2% in the yield of the E-isomer.

(ii) Z-2'-hydroxy-α-bromochalcone. The error in this case depends on the errors in values of rate constants calculated in sections 5-3.1 and 5-3.2.

(a) Error in k_3 (section 5-3.1).

k_3 is calculated from a knowledge of the values of k_2 ($0.0220 \pm 0.0005s^{-1}$) and t_{max} ($30 \pm 1s$) as $0.048 \pm 0.003s^{-1}$. The error was calculated as follows.

Taking k_2 to be $0.0225s^{-1}$, instead of $0.0220s^{-1}$, but leaving t_{max} unchanged at 30s, gives k_3 as $0.047s^{-1}$, a change of $0.001s^{-1}$.

Likewise, keeping k_2 at $0.0220s^{-1}$ but changing t_{max} to 31s instead of 30s, gives k_3 as $0.046s^{-1}$, a change of $0.002s^{-1}$

The total error is therefore taken as $0.003s^{-1}$.

(b) Error in the value of $(Z_{max}/D_o)/k_1^Z$ (i.e. of f^Z/k_1^Z) from equation (8), section 5-3.1.

The original calculation of this value as 10.77 was based on values of k_3 of $0.048s^{-1}$ and k_2 of $0.0220s^{-1}$. To calculate the minimum possible value within error the following values were used in equation (8):

$$k_2 = 0.0215s^{-1}$$

$$k_3 = 0.051s^{-1}$$

$$t_{max} = 31s$$

The resulting value is $f^Z/k_1^Z = 10.44$.

Keeping k_2 and k_3 as stated and putting $t_{max} = 29s$ gives the same value of f_Z/k_1^Z, i.e. changing t_{max} by 2s has no effect on the value.

Therefore, the value of f^Z/k_1^Z taking errors into account is 10.77 ± 0.33 which leads to the relationship

$$k_1^Z = (0.093 \pm 0.003)\ f^Z$$

which corresponds to equation (12) in section 5-3.1.

(c) Errors in values of A_o/A_∞ required for calculation of $\Delta A/A_\infty^D$ (equation 15, section 5-3.2.).

Taking absorbance error into account, values obtained for $(A_o/A_\infty) - 1$ are as follows:

Z: 1.34 ± 0.01

E: 0.56 ± 0.009

D: 0.34 ± 0.006

Substituting these values into equation (15) gives $\Delta A/A_\infty^D$ = $(1.34 \pm 0.01)f_Z + 0.227 \pm 0.004$ which corresponds to equation (16) in the text.

(d) Errors in f^Z

Using the last line of data from Table 5-3.

$\Delta A = 0.151 \pm 0.002$

$A_\infty^D = 0.228 \pm 0.001$

So that $\Delta A/A_\infty^D = 0.662 \pm 0.012$

Substituting values into the equation (16) above leads to

$f^Z = 0.325 \pm 0.014$

(e) Errors in k_1^Z and yield (%) Z-isomer.

From the equation at the end of section (b) above

$k_1 = (0.093 \pm 0.003)(0.325 \pm 0.014)$

$= 0.030 \pm 0.002 s^{-1}$

The yield (%) Z-isomer, given by $100k_1/k_3$ can therefore be calculated to be 63 ± 8%.

It is assumed that similar errors apply to the other values of

yield (%) in the last column of Table 5-3.

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