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CONVERSION OF FLAVANONES INTO CHALCONES IN ALKALINE MEDIUM. KINETIC AND SPECTROSCOPIC STUDIES

N. S. NUDELMAN

Depto. Química Orgánica, Facultad de Ciencias Exactas, Universidad de Buenos Aires, Pab. II, P. 3, 1428 Buenos Aires, Argentina

AND

J. J. P. FURLONG

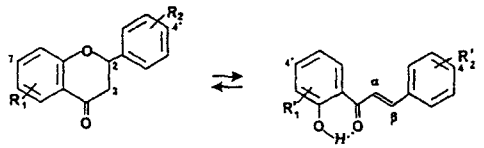
Cátedra de Química Física II, Facultad de Química, Bioquímica y Farmacia, UNSL, 5700 San Luis, Argentina

The conversion of flavanone and 7-hydroxyflavanone in alkaline water and heavy water and of the same compounds and of 4′-nitroflavanone in alkali–methanolic media into the corresponding substituted chalcones was studied kinetically and spectroscopically. Treatment of kinetic data in this work and data for the reverse reaction determined previously allowed the estimation of the partial rate coefficients for each step and of the free-energy changes for the three systems studied. To disentangle isotope effects, the conversion of [3-D_2]-flavanone was also studied. The present results confirm a previously suggested mechanism for the spontaneous reaction and afford essential information that may contribute to a more detailed understanding of the mechanism of the enzyme-catalysed reaction.

INTRODUCTION

Interest in the chalcone–flavanone system (Scheme 1) has motivated considerable research activity on their isolation from plants,[1] biological activity,[2] pharmacological properties[3] and the use in synthesis[4] of these types of natural products. The chemistry of chalcones and related compounds has been reviewed.[5]

The mechanism of chalcone–flavanone interconversion is a subject of recent interest, not only for the chemistry involved,[6–8] but also for its contribution to the understanding of the mechanism of action of chalcone isomerase. Chalcone isomerase catalyses the interconversion and is part of the flavonoid biosynthetic pathway.[9,10] This enzyme, which is ubiquitous in all plants studied,[11] has recently attracted much interest owing to its possible role in the expression of disease resistance.[3,12]



	R_1	R_2		R'_1	R'_2
Ia	H	H	IIa	H	H
b	H	4′-OH	b	H	4′-OH
c	H	4′-NO_2	c	H	4′-NO_2

Scheme 1

Two mechanisms have been postulated for the enzyme-catalysed reaction, one involving covalent catalysis[13] and the other general acid–base catalysis.[14] Recent efforts have been devoted to the purification and characterization of chalcone isomerase from soybean[15] with the aim of conducting studies to determine its mechanism of action and the factors which are responsible for catalysis.

Previous studies have been carried out in our laboratory on the mechanisms of non-enzyme-catalysed isomerizations of 2′-hydroxychalcones[7,8] as a basis for understanding the role that the enzyme plays. This paper is devoted to a kinetic study of the reverse reaction. The influence of base, solvent and substituents and isotope effects have been studied in order to gain some insight that would allow a distinction between the two proposed mechanisms[13,14] and to determine the steric course of the reaction, a point that is controversial at present.[16–18]

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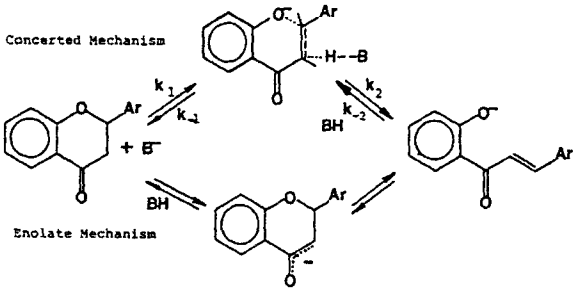
RESULTS AND DISCUSSION

The mechanisms proposed for chalcone formation are shown in Scheme 2. For the enzyme-catalysed reaction, the recent proposal by Boland and Wong[13] assumes an anti-addition of an enzyme imidazole side-chain to the double bond of the chalcone (on C-β), and simultaneous attack of a general acid on C-α to form a covalent enzyme intermediate, which then undergoes intramolecular nucleophilic displacement by the ionized 2'-OH and cyclization. We shall call this the 'doubly concerted mechanism' by analogy with the mechanism we have found for the non-enzyme-catalysed reaction. Our studies suggest that an intermediate, resulting from general acid attack on C-α which assists rotation about the CO—C-α bond, is formed in the first step. Conformation allows stabilization of this intermediate by weak interaction of the phenoxy nucleophile with the electron-deficient C-β. In the second step, nucleophilic attack occurs concertedly with release of the conjugate base (B—H bond breaking).[7,8] The previously proposed mechanism[14] assumes that the enzyme contains a general base which removes a proton from the phenolic oxygen promoting cyclization and a general acid which then protonates the enolate; this forms the flavanone by stereospecific proton transfer. We call this the 'enolate mechanism.'

Boland and Wong's strong argument against the catalysis by a general acid–base catalyst, is that 'it would cause opposite pH dependence of the forward and reverse reaction'.[13] This argument is not completely correct since general base catalysis or nucleophilic catalysis would give identical rate laws[19] and could not be distinguished by pH rate profiles. Nevertheless, in the light of that argument, it was of interest to confirm the 'doubly concerted mechanism' by the study of the reverse reaction and application of the principle of microscopic reversibility.



Scheme 2

Basicity, solvent and substituent effects

The pH conditions selected for the kinetic determinations compromised a measurable reaction rate and a reasonably high conversion. The same considerations applied to the temperature: as a wide enough range could not be used for experimental reasons, determinations at each temperature were repeated several times and the data are accurate to within $\pm 0{\cdot}1\,^{\circ}C$. The reaction shows first-order behaviour for the substrate (flavanones) and for the base (OH^-) in water and in methanol. The data are given in Table 1.

Kinetic expressions for the two alternative mechanisms were derived by applying the steady-state treatment. Assuming that the intermediate does not accumulate, equation (1) shows the kinetic law derived for the doubly concerted mechanism:

$$\frac{d[II]}{dt} = \frac{k_1 k_2 [I]_0 [B^-]}{k_{-1} + k_2} - [II]\left(\frac{k_1 k_2 [B^-]}{k_{-1} + k_2} + \frac{k_{-1} k_{-2} [BH]}{k_{-1} + k_2}\right) \quad (1)$$

and the kinetic expression derived for the enolate mechanism is

$$\frac{d[II]}{dt} = \frac{k_1 k_2 [I]_0 [B^-]}{k_{-1}[BH] + k_2} - [II]\left(\frac{k_1 k_2 [B^-]}{k_{-1}[BH] + k_2} + \frac{k_{-1} k_{-2} [BH]}{k_{-1}[BH] + k_2}\right) \quad (2)$$

In both equations $[I]_0$ represents the initial flavanone concentration, BH is the protic solvent and the factor of [II] in the second term is the sum of the forward (k_f) and reverse (k_r) reaction rate coefficients. In both cases the equilibrium constant is given by

$$K = \frac{k_{-1} k_{-2}}{k_1 k_2} = \frac{k_r [B^-]}{k_f [BH]} \quad (3)$$

Table 1 gives the kinetic data for the conversion of flavanone (**Ia**) and 4'-hydroxyflavanone (**Ib**) to the corresponding chalcones in water and in methanol at several hydroxide concentrations; it also includes data for the conversion of 4'-nitroflavanone (**Ic**) in methanol. The reactions were followed spectrophotometrically and the sums of the forward and reverse reaction coefficients were determined experimentally by regression analysis (see Experimental). Since the rates of the reverse reaction had been determined previously,[7] the second-order rate coefficients for the flavanone decomposition could be calculated and they are also presented in Table 1. The solvent isotope effect on the reactions of **Ia** and **Ib** were also studied and the results are given in Table 1.

The reaction of **Ia** in water shows only a feeble sensitivity to the substitution (**Ia** vs **Ib**), whereas a substantial decrease in rate is observed in the reaction of **Ia** when the solvent is changed. Bearing in mind the results discussed for cyclization of **IIa**,[7,8] this would also indicate similar rates of both steps for the reaction in water and change in rate control to the second step in

Table 1. Kinetic data for the flavanone–chalcone conversion[a]

System	Solvent	T (°C)	10^3[B] (M)	$10^4(k_f + k_r)$[b] (s^{-1})	$10^4 k_r$[c] (s^{-1})	$10^2\ k_f/$[B][d] (s^{-1} l mol^{-1})
Ia ⇌ **IIa**	H_2O	14·5	26·6	285	17·4	101
		14·5	26·6	266	17·4	94
		14·5	13·3	151	17·4	100
		14·5	13·3	176	17·4	109
		19·4	8·35	155	27·6	152
		20·5	8·35	166	30·65	162
		20·5	16·7	317	30·5	171
	CH_3OH	14·7	13·3	12·4	4·76	2·63
		14·7	26·6	39·7	4·76	2·88
		14·7	6·65	6·8	4·76	3·10
	D_2O	20·2	8·35	322	5·6	379
		20·0	8·35	314	5·6	369
		20·0	16·7	551	5·5	327
Ib ⇌ **IIb**	H_2O	14·6	26·6	155	1·09	58
		14·6	26·6	148	1·09	55
		14·6	16·0	91	1·09	56
		22·2	26·2	237	2·46	89
		22·2	25·0	318	2·46	86
		14·6	6·65	39	1·09	57
	CH_3OH	14·5	66·5	102	2·0[e]	15
		14·5	39·9	65	2·0[e]	16
		14·5	26·6	45	2·0[e]	16
	D_2O	22·2	25·8	383	0·22	149
Ic ⇌ **IIc**	CH_3OH	14·5	29·0	107	4·13	2·26
		14·5	43·0	138	4·13	2·24

[a]Measurement of chalcone appearance at 420 nm.
[b]B_3 parameter in equation (8).
[c]First-order rate constant for flavanone formation.[7]
[d]Second-order rate constant for flavanone decomposition.
[e]Calculated taking into account cyclization rate ratios for the 2',4-dihydroxychalcone from Ref. 8.

methanol, [$k_2 \ll k_{-1}$ in equation (1) and $k_2 \ll k_{-1}$[BH] in equation (2)], since a factor of *ca* 70 (see table 2) cannot be explained only in terms of the lower basicity of the conjugate base of methanol. This is confirmed by the absence of a primary isotope effect in the reverse reaction in methanol.[8] Reaction **Ib** → **IIb** is rate controlled by the bimolecular elimination in both solvents:[8] the rate ratio is consistent with the nucleophilic character of the base when the solvent is changed (a factor of 4). The small decrease in rate for the reaction in water observed on going from **Ia** to **Ib** is important in connection with the enzyme-catalysed reaction. In fact, it has been found that the presence of a hydroxyl group at the 4-position results in a higher rate of enzymic reaction.[15] According to Dixon *et al.*,[20] this group would interact by a hydrogen bond with a basic amino acid residue in the active site. The present results show that interpretation would be correct.

Reaction of **Ic** in methanol is rate controlled by the second step (as **Ia**), as indicated by the negligible kinetic effect when introducing the strong electron-withdrawing substituent.

To abbreviate discussion and comparison with the reverse reaction, the different factors affecting the forward and reverse reactions are shown in free-energy plots (Figure 1). ΔG and $\Delta G^{\neq}$ values were calculated according to the known equations (4) and (5), where k is the rate constant for the forward or reverse reaction, independent of the solvent and base concentration.

$$\Delta G = -RT \ln K \tag{4}$$

$$\Delta G^{\neq} = -(\ln k - \ln kT/h)RT \tag{5}$$

Table 2 gives the results for K and ΔG. Experimental rate coefficients calculated with the expressions for the concerted [equation (1)] and the enolate [equation (2)] mechanisms are also given. Taking into account the present experimental results and the influence of factors such as base concentration, substituent,[7] isotope and solvent effects[8] observed in the reverse reaction, the following comments can be made for each mechanism.

If the concerted mechanism operates for all reacting systems, reaction **Ia** ⇌ **IIa** occurs with similar contributions of both reaction steps to the rate in water, whereas in methanol the second step is rate determining (confirmed by the disappearance of the isotope effect for the reverse reaction and the almost 70-fold decrease in rate). By application of equations (1) and (3) to the

Table 2. Free-energy changes for the 2′-hydroxychalcone–flavanone interconversion at 14·5 °C[a]

Solvent	Reaction	10^6K[b]	ΔG	Concerted mechanism		Enolate mechanism	
				k[c] (s^{-1} l mol^{-1})	$\Delta G^{\neq}$	k[c] (s^{-1})	$\delta G^{\neq}$
H_2O	**IIa** → **Ia**			$6{\cdot}27 \times 10^{-5}$[d]	93·6	$3{\cdot}48 \times 10^{-3}$ d	83·9
		30·6	24·9				
	Ia → **IIa**			2·07[e]	68·7	2·07[e] (s^{-1} l mol^{-1})	68·7
	IIb → **Ib**			$1{\cdot}96 \times 10^{-6}$[f]	101·8		
		3·47	3·01				
	Ib → **IIb**			0·565[g]	71·7		
CH_3OH	**IIa** → **Ia**	669	17·5	$1{\cdot}91 \times 10^{-5}$[h]	96·4	$4{\cdot}76 \times 10^{-4}$[h]	88·7
	Ia → **IIa**			$2{\cdot}87 \times 10^{-2}$[i]	78·9	0·715[i]	71·2
	IIb → **Ib**			$8{\cdot}0 \times 10^{-6}$[f]	98·5		
		51·4	23·6				
	Ib → **IIb**			0·156[g]	74·8		
	IIc → **Ic**			$1{\cdot}66 \times 10^{-5}$[h]	96·7	$4{\cdot}13 \times 10^{-4}$[h]	89·0
		738	17·2				
	Ic → **IIc**			$2{\cdot}25 \times 10^{-2}$[i]	79·5	0·56[i]	71·8

[a]The data for the cyclization of chalcones were obtained from Ref 7. Free energy changes are in kJ mol^{-1} Error ±0·1 kJ mol^{-1}. When k is a rate ratio $\Delta G^{\neq}$ is the algebraic addition of the corresponding free energies of activation.
[b]K from equation (3) (see text).
[c]Partial rate coefficients calculated by application of equations (1) and (3) (concerted mechanism) or (2) and (3) (enolate mechanism). Actual coefficients are as follows: [d,h]k_{-2}; [e,g]k_1; [f]$k_{-1}k_{-2}/k_2$; [i]k_1k_2/k_{-1}, according to the following simplifications: [d,e]$k_2 \approx k_{-1}$ [in equation (1)] or $k_2 \approx k_{-1}$[BH] [in equation (2)]; [f,g]$k_2 \gg k_{-1}$ [in equation (1)]; [h,i]$k_2 \ll k_{-1}$ [in equation (1)] or $k_2 \ll k_{-1}$[BH] [in equation (2)].

experimental data in water, k_1 and k_{-2} could be calculated for the forward and reverse reactions, respectively, for the concerted mechanism. The same rate coefficients were calculated for the enolate mechanism, by applying equations (2) and (3). The results are given in Table 2. The corresponding free energies of activation were also calculated, which together with the free-energy changes for the equilibrium allows the plots in Fig. 1 (data for the reverse reactions were taken from Ref. 7). A change of rate control to the second step is observed for the reaction in methanol: the calculated k_1k_2/k_{-1} values for the forward and k_{-2} values for the reverse reaction are given in Table 2 for both the concerted and the enolate mechanisms. The change in rate-controlling step is shown in Fig. 1.

Reaction **Ib** ⇌ **IIb** is rate controlled by the bimolecular elimination in both solvents. The electron-releasing effect of the oxyanion in the *para* position of ring B (compare k_r values for **Ia** ⇌ **IIa** and **Ib** ⇌ **IIb** in Table 1) together with the huge isotope effects observed for the reverse reaction ($k_H/k_D = 11$ and 9·3 in water and in methanol, respectively)[8] precludes consideration of the enolate mechanism and the k_1 and $k_{-1}k_{-2}/k_2$ coefficients for the forward and reverse reactions were calculated by application of equations (1) and (3) only; the same applies to the plot for this compound in Fig. 1. The change in the rate-controlling step due to the substituent effect is more noticeable on comparing the enthalpies of activation, which are 41·0 ± 3 kJ mol^{-1} for **Ib** and 55·6 ± 3 kJ mol^{-1} for **Ia** in water. The decrease in rate in spite of the smaller energy of activation is due to the entropy factor, since the bimolecular elimination in **Ib** implies a reaction between two ions of the same charge. This finding is also relevant to our understanding of the entropic contribution of the enzyme catalysis. Although it has been claimed that freezing out favourable conformations can lead to important increases in rate,[21] recent force-field calculations[22] suggest that the rate increase is not a consequence of simply freezing but rather follows from differences in repulsive interactions between non-bonded groups.

The introduction of a strong electron-withdrawing substituent on the C-4 of ring B (compound **Ic**) exerts only a small decrease in rate; this indicates that in this case the second step is rate determining. Calculated k_1k_2/k_{-1} and k_{-2} values for the forward and reverse reactions, respectively, are given in the Table 2 for the concerted mechanism [equations (1) and (3)] and the enolate mechanism [equations (2) and (3)].

The contribution of the solvent as a proton donor, increasing the reaction rate of the proton transfer in the enolate mechanism, is indicated by the dashed lines in the energy plots (Fig. 1).

As can be seen in Table 2, in the reaction **Ia** → **IIa** the rate constant for the carbanion formation in the enolate mechanism is 2·07 s^{-1} l mol^{-1} (at 14·5 °C); it should be rate controlling, otherwise no isotope effects would have been observed for chalcone conversion.[8] The rate of carbanion formation of acetone at 25 °C in water is

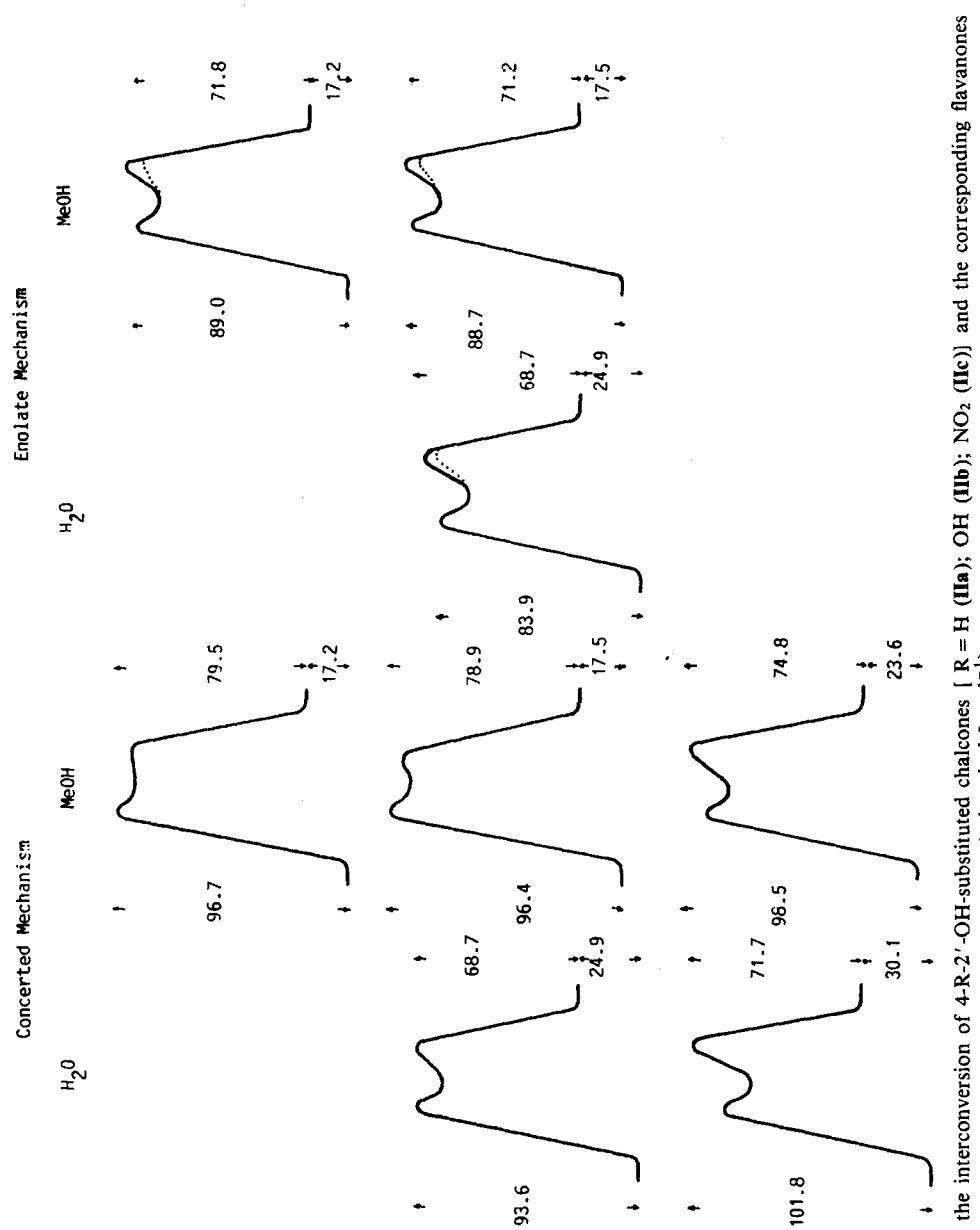


Figure 1. Energy plots for the interconversion of 4-R-2′-OH-substituted chalcones [R = H (**IIa**); OH (**IIb**); NO_2 (**IIc**)] and the corresponding flavanones (values in kJ mol^{-1})

$0{\cdot}25\ s^{-1}\,l\,mol^{-1}$.[23] This indicates that flavanone does not react via the carbanion-enolate. In addition, this intermediate should be less stable than acetone owing to its rigid structure. Further, the enolate mechanism is not able to explain either the nitro group effect on both the forward and reverse reactions or the solvent effect on the reaction **IIa** → **Ia**.

Isotope effects

The use of isotope effects to elucidate enzyme mechanisms has been reviewed.[24,25] A primary kinetic deuterium effect would indicate that the proton transfer is involved in the rate-limiting step. Two types of experiments were carried out: (a) alkaline conversion of flavanones in D_2O (Table 1) and (b) alkaline conversion of [3-D_2]flavanone in methanol (Table 3).

From the isotope solvent effect it was observed that the reaction rates in heavy water are increased ($k_H/k_D = 0{\cdot}45$ for **Ia** and 0·60 for **Ib**), owing to the higher basicity of OD^-.

For the conversion of [3-D_2]flavanone, $k_H/k_D = 5$ was observed, which is consistent with the C—D bond cleavage in the rate-controlling step. If the enolate mechanism were operating, since the carbanion reconverts to flavanone faster than it reacts to give chalcone (see Fig. 1), a considerable fraction of deuterium nuclei should be exchanged before chalcone formation and such a large isotope effect should not be observed.

Nevertheless, to explain the isotope effects on the k_1k_2/k_{-1} in terms of the concerted mechanism, a change in rate control when introducing the deuterium nuclei in **Ia** should be assumed. To check this assumpton 2′-(O^-)-α-D-chalcone was synthesized and its cyclization was studied in deuterated methanol. A k_H/k_D value of 4·7 at 25 °C was observed; this value indicates the contribution of the bimolecular elimination step in the rate control

^{1}H NMR measurements

In order to decide between *E*2 and *E*1*cB* mechanisms for the elimination process, an excess of base was added to a solution of **Ia** in deuterated methanol so that the equilibrium lays largely on the side of chalcone formation. An *E*1*cB* mechanism would imply deuteration of flavanone (on C-3) and formation of an α-D-chalcone. Conversely, an *E*2 mechanism should give non-deuterated chalcone since the reaction **IIa** → **Ia** is markedly slower (it does not depend on base concentration) and so deuteration via this mechanism is also slow. The results of two sequenced spectra are shown below.

First spectrum: **Ia** δ(methanol-d_4) 7·9 (dd, $J_{5,6} = 8$, $J_{5,7} = 2$, $J_{5,8} = 0$, 1H)(H-5); 5·5 (m, 1H)(H-2); 3·4–2·5 (m, 2H)(H-3); 7·75–6·85 (m, 8H)(H-6, H-7, H-8 and ring B protons).

Second spectrum: δ(methanol-d_4–D_2O–NaOD): 8.25 (d, $J = 15$, 1H)(H-α); 6·9–6·15 (m, 2H)(H-3′ and H-5′); 7·9–6·9 (m, 8H)(H-4′, H-6′, H-β and ring B protons).

It can be observed that the last spectrum corresponds to 2′-(O^-) chalcone and exhibits the H-α doublet consistent with an *E*2 elimination. In previous ^{1}H NMR studies on chalcone cyclizations in water,[7] we reported that flavanone formation was clearly indicated by the appearance of the H-2 singlet (5·3–5·5 ppm), confirming deuteration on C-3. Carbanion formation occurs afterwards, and it is affected by an excess of base (cyclization pH-independent zone).

Stereochemical course of elimination

In general, enzyme-catalysed reactions are observed to be highly stereoselective[26] and a lack of stereospecificity should have mechanistic implications.[27] The stereochemistry of the proton introduced at the C-3 position of the flavanone product (in the reaction catalysed by the mung bean enzyme) appears to be only partially stereoselective; the reasons for this behaviour are unknown at present. On the other hand, *E*2 reactions are highly stereoselective; the elimination in flavanone should be 'anti'[28,29] as depicted in Fig. 2. Anti-elimination requires equatorial H-3 abstraction. To confirm this assumption, NaOD was added to the flavanone in deuterated methanol in a 7 : 10 ratio. The ^{1}H NMR spectrum exhibits the H-2 quartet centred at 5·35 ppm (as expected for an ABX system). The spectra

Table 3. Conversion of flavanone to 2′-hydroxychalcone in methanol: effect of deuterium on C-3[a]

Flavanone isomer	T (°C)	10^3[Base] (M)	$10^4(k_f + k_r)$[b] (s^{-1})	10^4k_r[c] (s^{-1})	$10^4k_f/$[B][d] (s^{-1} l mol^{-1})
[3-H_2]-	15·0	25·6	11·2	4·94	2·46
	15·3	25·8	13·5	5·13	3·24
	15·2	24·3	12·3	5·07	2·98
[3-D_2]-	15·5	25·4	6·76	5·26	0·591
	15·5	25·1	6·56	5·26	0·518
	15·2	24·8	6·63	5·07	0·629

[a]Absorbance at 420 nm.
[b–d]As in Table 1.

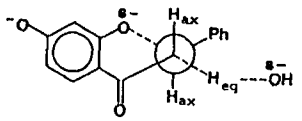


Figure 2. Stereochemical course of the *E*2 elimination in 7-hydroxyflavanone

registered several hours later show a sharp doublet ($J = 11$ Hz). This *trans*-diaxial coupling indicates that only the equatorial H-3 has been exchanged, confirming the stereochemistry of the *E*2 process. Presumably, the observation of partial stereoselectivity in the enzyme reaction may result from random protonation of the free carbanion, a process that occurs simultaneously and may also be catalysed by the enzyme. Recent studies with purified chalcone isomerase from soybeans indicate that 88% of the chalcone is converted into the (*S*)-flavanone in less than 15 s, and is followed by a slow equilibration of the chalcone with the *R* isomer.[15]

CONCLUSIONS

This and previous studies on chalcone–flavanone interconversion afford sufficient evidence to establish that the concerted mechanism is the best alternative to describe the kinetic behaviour of the spontaneous reaction. They also afford important clues toward the understanding of the enzyme-catalysed reaction, which can be summarized as follows. (a) The actual substrate is the 2′-hydroxychalconate. Chalcone isomerase from soybeans[15] has a blocked *N*-terminus, and it contains 18 lysine residues and 7 arginine residues, among other amino acids. At pH ≈ 7 the enzyme would play the role of a general base (probably through the lysine residues) which partially ionizes the substrate. (b) Modification studies of the mentioned chalcone isomerase[15] indicated that it contains a single cysteine residue and suggested that it is located in the active site. The single cysteine sulphydryl group of the enzyme is the prime candidate to function as general acid (BH) to protonate chalcone on C-α. (c) The stereochemistry of the spontaneous reaction affords a good explanation for the partial stereospecificity of the enzyme-catalysed reaction. (d) The isotope effects observed confirm the proposed mechanism. In the presence of chalcone isomerase, the enzyme-bound chalconate would undergo general acid attack by the sulphydryl moiety, concertedly with the nucleophilic attack on C-β, releasing flavanone into solution. Additional binding of the substrate to the enzyme may also assist the approach of the 2′-phenolate nucleophile to C-β (adopting the *trans-s-trans* configuration), which would be an additional factor for rate acceleration. Semi-empirical self-consistent molecular orbital calculations show the barrier for this conformational change.[30]

EXPERIMENTAL

UV–visible spectra were recorded with a Beckman DK-2A spectrophotometer. ^{1}H NMR spectra were run on a Varian EM 360 A spectrometer; shifts are referred to tetramethylsilane.

Reagents. Flavanones (**I**) were prepared as described previously.[7] High-purity [3D$_2$]flavanone and 2′-hydroxy-α-D-chalcone were available through the courtesy of the Laboratory of Applied Spectroscopy, University of San Luis, Argentina.

Kinetic measurements. Kinetic determinations were performed spectrophotometrically using 1·0-cm thermostated silica cells. The temperature was accurate to within ±0·1 °C. In all cases pseudo-first-order kinetics were observed. Standard solutions of flavanone or chalcone (*ca* 10^{-2} M) in methanol and of sodium hydroxide (0·1–5 M) in water were prepared. A typical reaction was carried out by microsyringing the necessary amount of sodium hydroxide solution into the solvent in the thermostated cell. The flavanone or chalcone solution was syringed into the cell and the absorbance recorded as a function of time. The pseudo-first-order rate constants for cyclization were calculated as previously described.[7]

Rate constants for flavanone conversion were calculated using a non-linear regression treatment based on the Newton–Raphson method.[31] Equation (6) measures chalcone formation when the flavanone does not absorb at the selected wavelength:

$$A_t = A_\infty - A_\infty \exp[-(k_f + k_r)t] \quad (6)$$

Where A_∞ is the absorbance at equilibrium. When the flavanone does absorb (e.g. **Ic**), equation (7) applies:

$$A_t = A_\infty - (A_\infty - A_0)\exp[-(k_f + k_r)t] \quad (7)$$

In every case the regression analysis was carried out on a convenient three-parameter function [equation (8)]:

$$A_t = B_1 - B_2 \exp(-B_3 t) \quad (8)$$

In all cases high correlation factors were observed ($r > 0{\cdot}97$).

Cyclization of 2′-hydroxy-α-D-chalcone in methanol. Two determinations at different base concentrations ([OCH_3^-] = 0·0183 and 0·011 M) were performed, measuring the absorbance decrease at *420 nm* and 25 °C. The average first-order rate constant is $k_D = 3{\cdot}42 \times 10^{-4}$ s^{-1}. The rate constant for the hydrogenated chalcone was analogously determined as $k_H = 1{\cdot}62 \times 10^{-3}$ s^{-1}.

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