Enthalpy–entropy compensation effect in the chalcone formation from naringin in water–ethanol mixtures †

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The isomerisation equilibrium between the flavanone and chalcone forms of the naturally occurring flavonoid naringin (7-rhamnoglucosyl-4′,5-dihydroxyflavanone) has been studied in water–ethanol mixtures in the presence of NaOH. The variation of the observed pseudo-first order rate constant for the equilibrium reaction, k_{obs}, and the equilibrium composition were determined as a function of the base concentration ($-4.0 < \log[NaOH] < -2.4$) and the water molar fraction ($0.03 \leq X_W \leq 1.00$) of the solvent mixture. The variation of the ring opening k_{op}, and the cyclisation k_{cy}, rate constants with the base concentration and solvent composition indicated that the isomerisation reaction is mediated by a carbanion intermediate. The temperature effect on k_{op} and k_{cy} showed that only the activation enthalpy and entropy changes for the ring-opening reaction, $\Delta H_{op}^{\ddagger}$ and $\Delta S_{op}^{\ddagger}$, were dependent on the solvent composition. In fact, a good linear correlation of a plot of $\Delta H_{op}^{\ddagger}$ *vs.* $\Delta S_{op}^{\ddagger}$, indicate the existence of an enthalpy–entropy compensation effect. This result was associated with changes in the balance of hydrogen-bonding interactions between the intermediate carbanion and its solvation sphere as solvent composition was modified.

Introduction

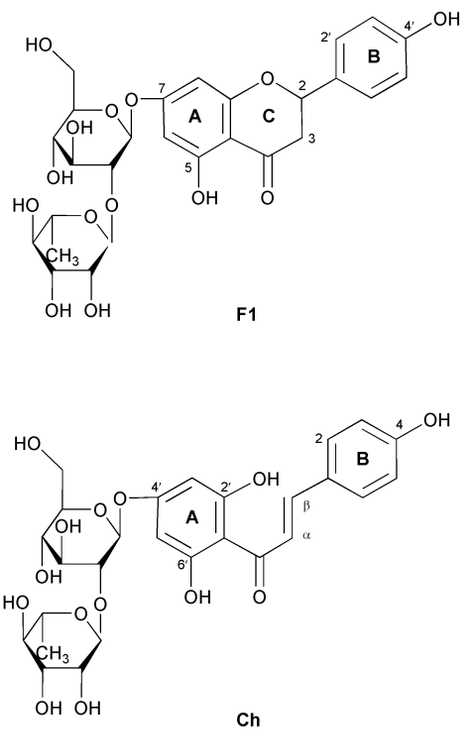
Flavonoids are bioactive polyphenolic compounds widely distributed in plants, where they play several physiological functions. In the last years, this family of natural products has received large attention due to their health-related properties,[1] mainly based in their antioxidant properties,[2] and their potential application in cancer and heart disease treatments.[3]

In particular, the flavanone naringin (**Fl**), Scheme 1, is commonly found in the peel and juice of grapefruits, and it is the responsible of the characteristic bitter taste of the fruit. In fact, this compound is commercially used as a bitter additive in some food preparations.[4]

In acid and neutral solutions of protic solvents the flavanone form of naringin is largely stable.[5] However, it is well known that in basic conditions the closed form of naringin (**Fl**) is transformed to the corresponding chalcone (**Ch**), Scheme 1.[5] It is interesting to note that catalytic hydrogenation of the α–β double bond of the naringin chalcone yields the corresponding dihydrochalcone, which is a non-nutritive semi-synthetic sweetener with considerable commercial potential.[6] However, the complete transformation of the naturally occurring precursor naringin to its chalcone is precluded by the strong tendency of the open form to cyclise back to the flavanone form.[5]

The mechanism of the flavanone–chalcone equilibrium has received large attention.[5,7–14] It has been reported that as the base concentration increases, the intermediate of the cyclisation and ring-opening reactions shift progressively from an enolate equilibrium to a carbanion intermediate mechanism.[5,8–13]

In this work, we present a kinetic study of the solvent effect on the isomerization equilibrium of naringin in water-ethanol mixtures under alkaline conditions where the carbanion mechanism takes place. The ring opening (k_{op}) and cyclisation (k_{cy}) reaction rate constants, and the isomerisation equilibrium constant K, were determined as function of the NaOH concentration and the solvent composition. A linear correlation between the activation enthalpy and entropy changes, $\Delta H_{op}^{\ddagger}$ and $\Delta S_{op}^{\ddagger}$, was only found for the ring-opening reaction. This enthalpy–entropy compensation effect is discussed as function of changes in the balance of hydrogen-bonding interactions between the intermediate carbanion and its solvation sphere as the solvent composition change.



Scheme 1 Chemical structure of naringin, **Fl** (7–rhamnoglucosyl-4′,5-dihydroxyflavanone) and its chalcone, **Ch** (4′–rhamnoglucosyl-2′,6′,4-trihydroxychalcone).

Experimental

Materials

Naringin (7-rhamnoglucosyl-4′,5-dihydroxyflavanone) 97% was from Fluka (Fluka Chemie AG, Buchs, Switzerland) and

† Dedicated to Prof. Florinda Bobbio (UNICAMP, Campinas, SP, Brazil) and in memory of Prof. Paulo Bobbio.

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used as received. Hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium acetate (NaOAc) were analytical degree from Merck Química Argentina (Buenos Aires, Argentina). Absolute ethanol (99%, HPLC degree) was from Sintorgan (Buenos Aires, Argentina). The water used in the solution preparation was triply distilled.

The naringin chalcone (4′-rhamnoglucosyl-2′,6′,4-trihydroxychalcone) was obtained by the following recommended method:[7] 1 g of naringin was added to a 4 ml of 1 : 1 ethanol–water solution containing 2 g of NaOH and heated on a steam-bath for *ca.* 5 min. The deep red solution obtained was filtered into an excess of ice-cold 2 M HCl solution saturated with NaCl. After acidification a yellow solid was formed, which was filtered and redissolved with ethanol and recrystallised from ethyl ether. The procedure was repeated twice. The yellow powder was dried under N_2 atmosphere until constant weight. The melting point was 182–186 °C, which is a narrower range compared with previous literature values, *e.g.* 185–200 °C[7] and 170–183 °C.[5] UV–visible spectra analysis in ethanol yielded the typical spectra of chalcone derivatives,[5,15] with λ_{max} = 366 nm and log ε = 4.51. HPLC analysis (see details below) yielded 98 % of purity.

Methods

UV–visible spectra were determined with a Hewlett-Packard 8453 diode array spectrophotometer operating in both spectral and kinetic modes. In order to avoid photochemical secondary reactions, the intensity of the analysing beam was abated with a neutral density filter. In all cases, the sample was placed in a 1 cm quartz cell. The cell holder was settled ±0.1 °C using a thermostat bath.

Typically, water–ethanol solutions (with water molar fractions, X_W, ranging between 0.03 and 1.00) of *ca.* 40 μM of the flavanone or chalcone forms were studied in the presence of NaOH (0.13–4.0 mM). Standard solutions (*ca.* 10^{-3} M) of both isomers in ethanol and 0.1 M NaOH in water were prepared, and the necessary amounts of each substrate were added by a microsyringe to a 2 mL of the solvent mixture, which was previously thermostated at the experimental temperature. The kinetic determinations were monitored measuring the absorbance changes at several wavelengths. The final equilibrium composition was determined by spectrophotometry, since each form has a distinct characteristic spectrum. In some cases, it was also determined by HPLC analysis. Both methods yielded similar results within ±10% of standard deviation.

HPLC experiments were performed using a Konik Liquid Chromatograph, model KNK 500 Series A, equipped with a Konik ODS-2 column (250 × 46 mm id; 5 μm) operating at 25 °C. The mobile phase was water–acetonitrile–acetic acid (79.5 : 20 : 0.5, v/v) and it was used under isocratical conditions, at a flow rate of 1.2 ml min^{-1}. Detection was performed at 284 nm and 366 nm using a Konik UV-Vis 200 detector.

All the experiments were performed twice, and their average values with their standard deviations are reported.

Results

The thick solid lines in Fig. 1 show the UV–visible spectra of the flavanone and chalcone forms of naringin in water–ethanol solutions (X_W = 0.03). In acid solutions, the spectra of both isomers were unchanged, indicating that in neutral conditions the flavonoids are in their fully protonated form. However, the presence of NaOH (higher than 0.1 mM) produces an instantaneous bathochromic shift on the flavonoid spectra (dash line spectra in Fig. 1). Similar changes were observed under all water–ethanol mixture conditions ($0.03 \leq X_W \leq 1.00$). In the flavanone spectrum new bands at 245 nm and 365 nm, together with a small shift of 4 nm of the band at 284 nm, were observed (Fig. 1A). In the case of the chalcone spectrum, a large red shift

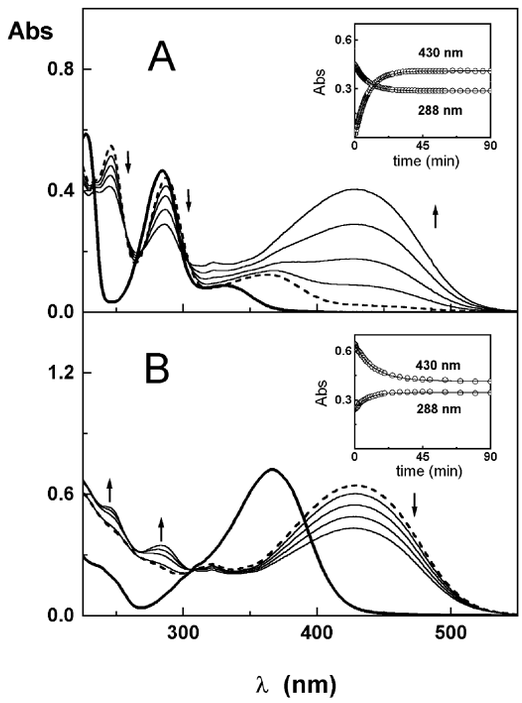


Fig. 1 UV–visible spectral changes after the addition of 1.25 mM NaOH to water–ethanol (X_W = 0.03) solutions of: (A) flavanone; and (B) chalcone forms of naringin. Spectra observed at: (——) t = 0 s, (- - -) t = 20 s, and (—) t = 5–90 min after the NaOH addition. Insets: kinetic profiles monitored at 288 and 430 nm, respectively.

(*ca.* 70 nm) is produced after the base addition, Fig. 1B. All these absorption changes are characteristic of the spectra of the ionised forms of polyphenolic compounds.[15]

In all cases, the instantaneous spectral change was followed by a slower transformation (normal solid line spectra in Fig. 1). For the ionised flavanone the bands at 245 nm and 288 nm decrease with simultaneous increment of a band at 430 nm (Fig. 1A). For the ionised chalcone the opposite changes were observed (Fig. 1B). The time-dependent absorbance change A_t, was fitted using a first-order kinetic law at the monitored wavelength, eqn. (1).[16]

$$A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obs}t) \quad (1)$$

A_0 and A_∞ are the absorbance values at the initial and final time of the reaction, and k_{obs} is the observed first-order rate constant. Under the same experimental conditions, kinetic runs using either the flavanone or chalcone forms as initial substrate yielded similar k_{obs} value, Table 1. This fact, together with the UV–visible and HPLC analysis indicating the presence of both ionised flavonoid forms at the end time of the reaction, support the occurrence of the flavanone–chalcone equilibrium of naringin. Thus, k_{obs} is the sum of the pseudo-first-order rate constants for the ring-opening (k_{op}) and cyclisation (k_{cy}) reactions (e.g. $k_{obs} = k_{op} + k_{cy}$). In order to separate k_{op} and k_{cy} from the measured k_{obs}, an independent determination of the isomerisation equilibrium constant K (= k_{op}/k_{cy}) is necessary.[16] Table 1 also shows the K values calculated taking into account the initial substrate concentration, and the initial and final absorbance values at 430 nm (log ε_{430} = 4.46) where only the ionised chalcone form absorbs. Thus, combining k_{obs} and K, the respective rate constants k_{op} [= $k_{obs}K/(1+K)$] and k_{cy} [= $k_{obs}(1+K)^{-1}$] were calculated.[16]

The variation of k_{op} and k_{cy} with the NaOH concentration follow a sigmoidal behaviour (Fig. 2A). In turns, k_{op} depends on the medium composition and comes to a maximum value at *ca.* X_W = 0.5, while the cyclisation rate k_{cy} remains unchanged (Fig. 2B).

Table 1 Observed first-order rate constant k_{obs}, and equilibrium constant K for the flavanone–chalcone isomerisation equilibrium of naringin in water–ethanol solutions at 25 °C, using either the flavanone or chalcone forms as initial substrate

Water–ethanol solutions (X_W)	[NaOH] (mM)	k_{obs} (s^{-1})/10^3		K ($= k_{op}/k_{cy}$)
		Naringin + NaOH	Chalcone + NaOH	
0.03	1.25	1.4 ± 0.5	1.3 ± 0.2	1.7 ± 0.2
0.15	1.25	1.9 ± 0.5	1.8 ± 0.3	2.4 ± 0.2
0.36	1.25	2.3 ± 0.5		3.0 ± 0.2
0.52	0.13	2.6 ± 0.5	2.5 ± 0.5	0.07 ± 0.02
0.52	0.25	2.5 ± 0.1		0.09 ± 0.02
0.52	0.40	2.5 ± 0.1		0.8 ± 0.1
0.52	1.25	2.2 ± 0.2	2.3 ± 0.1	3.4 ± 0.3
0.52	4.00	2.2 ± 0.2		3.3 ± 0.2
0.64	1.25	1.8 ± 0.3		2.7 ± 0.3
0.76	1.25	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.2
0.91	1.25	0.8 ± 0.1	0.8 ± 0.1	0.57 ± 0.05
0.97	1.25	0.5 ± 0.1		0.29 ± 0.08
1.00	0.32	1.3 ± 0.1		0.04 ± 0.01
1.00	0.89	0.56 ± 0.04		0.18 ± 0.02
1.00	1.25	0.49 ± 0.02	0.48 ± 0.01	0.23 ± 0.04
1.00	3.16	0.49 ± 0.05		0.24 ± 0.10

Table 2 Activation parameters for the ring-opening and cyclisation reactions of the flavanone–chalcone isomerisation equilibrium of naringin as a function of the water fraction X_W in water–ethanol mixtures

X_W	$\Delta H_{op}^{\ddagger}$/kJ mol^{-1}	$\Delta S_{op}^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta G_{op}^{\ddagger}$(25 °C)/kJ mol^{-1}	$\Delta H_{cy}^{\ddagger}$/kJ mol^{-1}	$\Delta S_{cy}^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta G_{cy}^{\ddagger}$ (25 °C)/kJ mol^{-1}
0.03	60 ± 3	−100 ± 7	90 ± 5	72 ± 3	−65 ± 5	91 ± 4
0.52	54 ± 3	−118 ± 5	89 ± 4	72 ± 2	−69 ± 5	93 ± 4
0.76	66 ± 3	−85 ± 5	91 ± 4	73 ± 2	−66 ± 3	93 ± 4
0.91	77 ± 2	−53 ± 5	93 ± 4	73 ± 2	−60 ± 5	91 ± 4
1.00	85 ± 2	−38 ± 5	86 ± 4	72 ± 3	−69 ± 4	93 ± 3

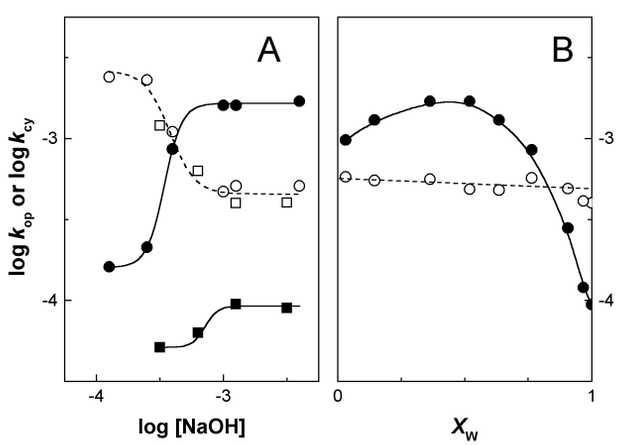


Fig. 2 Ring-opening k_{op} (solid symbols) and cyclisation k_{cy} (open symbols) pseudo-first-order constants for the isomerisation equilibrium of naringin as a function of: (A) NaOH concentration in water–ethanol solution ($X_W = 0.52$, circles) and in water (squares); and (B) water molar fraction, X_W.

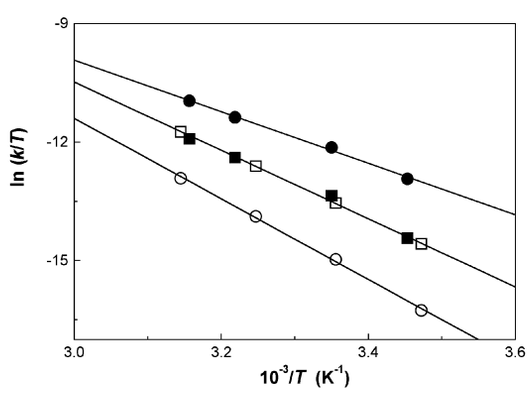


Fig. 3 Effect of the temperature on the ring-opening rate constant k_{op} (circles), and on the cyclisation rate constant k_{cy} (squares) reactions in water–ethanol solution ($X_W = 0.52$, solid symbols) and in water (open symbols).

The temperature effect on k_{op} and k_{cy} was determined in several water–ethanol mixtures using transition state theory,[16] eqn. (2).

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT} \qquad (2)$$

Here k represents either k_{op} or k_{cy}, k_B is Boltzmann's constant; h, Planck's constant; and R, the gas constant. From the intercept and slope values of the plot $\ln(k/T)$ *vs.* $1/T$, Fig. 3, the standard activation entropy and enthalpy changes, $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$, were obtained respectively. Table 2 collects the activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ (calculated at 25 °C as $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$) at several X_W values. The activation parameters for the cyclisation reaction ($\Delta H_{cy}^{\ddagger}$, $\Delta S_{cy}^{\ddagger}$ and $\Delta G_{cy}^{\ddagger}$) were independent on the composition of the solvent mixtures (Table 2), and similar to data reported for other related chalcones.[10–12] However, for the opening reaction both $\Delta H_{op}^{\ddagger}$ and $\Delta S_{op}^{\ddagger}$ change with the solvent composition, but the $\Delta G_{op}^{\ddagger}$ value remains almost constant (within the experimental error bars), Table 2.

Discussion

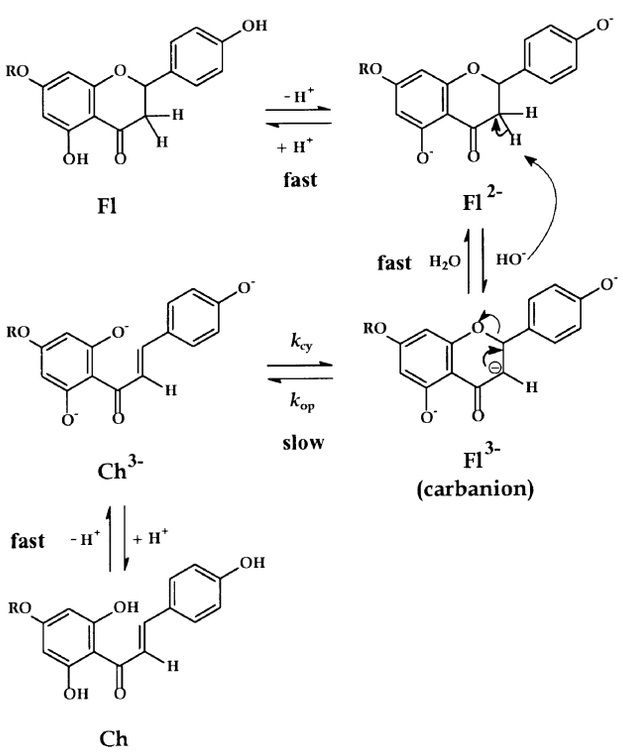
The UV–visible spectral features shown in Fig. 1 are typical of flavanone compounds that in acid and neutral media are characterised by a strong absorption band between 240–290 nm called Band II, which is due to π ⟶ π* transitions at ring A.[15] On the other hand, for chalcones the main absorption (Band I) is in the 340–390 nm range, as a result of a larger conjugated π system involving ring B.[15] The instantaneous spectral changes produced after NaOH addition are due to the ionisation of the phenolic groups of the flavonoid compounds.[15] For the chalcone form of naringin pK_a values of 6.9 (2′–OH), 8.5

(4–OH) and 12 (6′–OH) were reported in aqueous media.[5] Therefore, under our experimental conditions ($-4.0 < \log[NaOH] < -2.5$) deprotonation of the 2′–OH and 4-OH groups of the chalcone form can be expected, producing the strong absorption band at 430 nm, dashed line spectrum in Fig. 1B.

Instead, for the flavanone form a $pK_a > 10$ for the 5-OH group can be assumed, considering the reported value for 5-hydroxy-4′,7-dimethoxyflavanone.[9] It is interesting to note that the addition of the weaker base NaOAc (0.1 M) to naringin flavanone solutions only produces the appearance of the new band at 365 nm, without additional spectral changes with time, indicating that the ring opening of the flavanone does not take place (spectra not shown). Moreover, the addition of a small excess of diluted HCl solution reproduces the original spectra of the flavanone. Therefore, the red shift of Band I to 365 nm of the naringin flavanone (dashed line spectrum of Fig. 1A) can be associated with the deprotonation of the more labile 4′–OH, since the NaOAc test is commonly used for the specific detection of the more acidic hydroxyl group in flavonoids.[15]

As was mentioned in the Results section, the UV-visible spectral changes confirm that under our experimental conditions the flavanone–chalcone isomerisation equilibrium is established between the ionised forms of the flavonoids. As it was shown in Table 1, the equilibrium constant $K(=k_{op}/k_{cy})$ increases sharply with the base concentration, indicating that the equilibrium shifts to the ionised chalcone form. Moreover, the solvent dependence of k_{op}, Fig. 2B, cannot be explained in terms of general solvent effects, such as variation of the solvent permittivity.[16]

The current results can be interpreted in accordance with a carbanion intermediate mechanism *via* a pseudoacid equilibrium,[13] Scheme 2.



Scheme 2 Carbanion intermediate mechanism for the flavanone–chalcone isomerisation equilibrium of naringin in basic media.

According to this mechanism, a carbanion $\mathbf{Fl}^{3-}$ is formed by base-assisted removal of the labile α-hydrogen from the ionised flavanone $\mathbf{Fl}^{2-}$ in a fast pre-equilibrium pathway. Afterwards, this carbanion opens by intramolecular rearrangement producing the α–β double bond and the 2′-phenolate anion of the ionised chalcone $\mathbf{Ch}^{3-}$. This ring-opening reaction could be labelled *conjugate base unimolecular elimination* (E1cB).[17]

In turn, for the cyclisation reaction the deprotonated group at the 2′-position of the chalcone attacks the olefinic double bond, as a strong nucleophile, resulting in the formation of the carbanion $\mathbf{Fl}^{3-}$ which reacts with any proton donor (e.g. water solvent molecules) to form the ionic flavanone $\mathbf{Fl}^{2-}$. In this mechanism both unimolecular processes are the rate-determining steps (slow equilibrium) and therefore are independent on the base concentration, Scheme 2. The sharp sigmoidal behaviour of k_{op} and k_{cy} supports this carbanionic mechanism, since at higher NaOH concentrations both rate constants become independent on the base concentration but remain first order relative to the flavonoids.

The flavanone–chalcone equilibrium via the carbanion intermediate prevail at log [NaOH] > −3 where the fast pseudoacid equilibrium is completely shifted to the carbanion formation. At lower base concentrations the ring-opening reaction is abated, and the flavanone form prevails. It has been claimed that in weakly basic solution the cyclisation reaction from the chalcone isomer proceeds straightforwardly to the flavanone through an enolisation mechanism.[5,8–13]

The variation of the activation parameters for the ring-opening reaction can be related with the carbanion-mediated mechanism. As mentioned before, only $\Delta H_{op}^{\ddagger}$ and $\Delta S_{op}^{\ddagger}$ were affected by the solvent composition, but $\Delta G_{op}^{\ddagger}$ was almost constant, Table 2. This type of behaviour is called the *enthalpy–entropy compensation* effect.[18,19] Because $\Delta G = \Delta H - T\Delta S$, similar changes in magnitude and sign in ΔH and $T\Delta S$ cancel ΔG totally or partially.[18,19,20] The enthalpy–entropy compensation effect (for both standard and activation parameters) is most often found for liquid solutions, being particularly significant for aqueous solutions. This thermodynamic effect arises in dilute solutions when the dynamic solute–solvent and solvent–solvent equilibria are perturbed by a particular factor. The most straightforward evidence that the compensation effect is operating, is when $\Delta G^{\ddagger}$ is constant along a reaction series while $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ vary significantly, as is observed for the ring-opening reaction of naringin, Table 2 and Fig. 4. It is important to note that in this case, the variation of the activation parameters is much larger compared to the experimental error of each magnitude. This fact rules out a dominant statistical compensation pattern that should arise solely from experimental errors of $\Delta H_{op}^{\ddagger}$ and $\Delta S_{op}^{\ddagger}$ by using eqn. (2).[21]

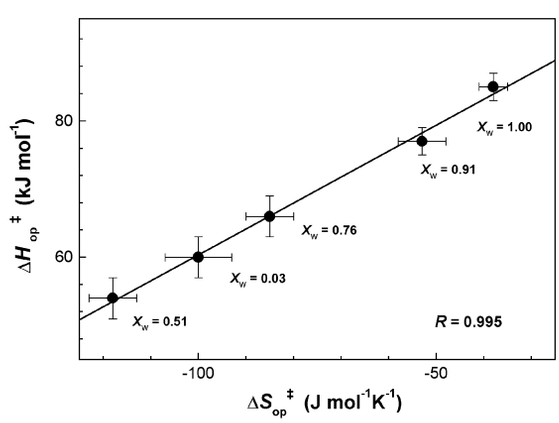


Fig. 4 Isokinetic relationship between $\Delta H_{op}^{\ddagger}$ and $\Delta S_{op}^{\ddagger}$ observed for ring-opening reaction of naringin.

The slope of the plot in Fig. 4 is called the isokinetic temperature T_{ik}, where the same k_{op} value should be observed for all composition of the solvent mixture. In the present case T_{ik} = 380 K (110 °C), which is within the range (280–450 K) obtained for several reactions in water and water–organic solvent mixtures.[22] The existence of an isokinetic relationship

supports (but does not prove) a claim that a single mechanism operates along the solvent series.

The activation parameters for the cyclisation reaction were independent on X_W, indicating that both the transition state and the ionic chalcone $\mathbf{Ch}^{3-}$ were not affected by the solvent composition. Therefore, the compensation effect should involve the carbanion $\mathbf{Fl}^{3-}$ intermediate. Since water and ethanol molecules have strong tendency to participate as both hydrogen bond donor and acceptor, the favourable formation of hydrogen bonds should produce a decrease of enthalpy. In turn, hydrogen bonds require that the participating molecules become relatively fixed, and therefore the entropy also decreases. In water–ethanol mixtures the hydrogen bonding interactions are maximal at $X_W = 0.5$, as concluded from the larger negative value of the excess molar volume V^E measured for the mixture.[23] At the same solvent composition, the lower $\Delta H_{op}^{\ddagger}$ and larger $\Delta S_{op}^{\ddagger}$ values were observed for the ring-opening reaction, indicating that the interaction between the carbanion and its solvation sphere is minimal, Fig. 5. Therefore,

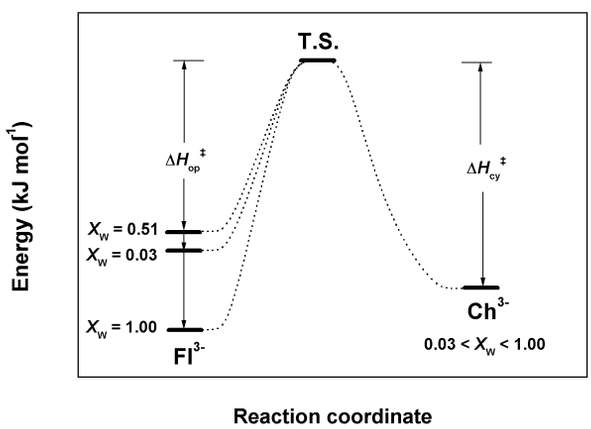


Fig. 5 Reaction coordinate scheme for the flavanone–chalcone isomerisation reaction of naringin as a function of the water molar fraction, X_W, in ethanol–water solutions.

the hydrogen-bonding ability of the solvent mixture regulates the stability of the carbanion intermediate varying the ring-opening reaction rate.

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