The Kinetics and Mechanism, and the Equilibrium Position as a Function of pH, of the Isomerisation of Naringin and the 4′-Rhamnoglucoside of 2′,4,4′,6′-Tetrahydroxychalcone

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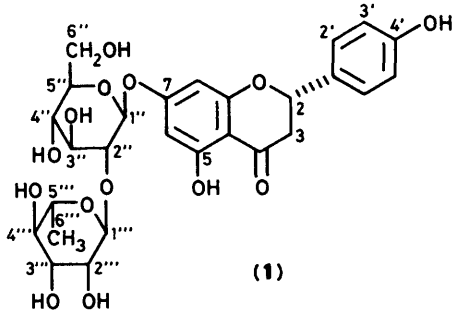
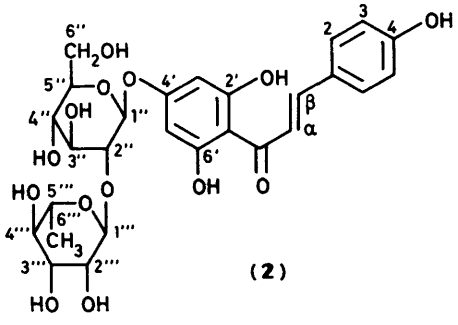
The pH–rate profile for the title reaction has been determined and accounted for in terms of contributions from the various ionised species. The effect of ionisation of a chalcone 4-OH group on rate of cyclisation is quantitatively accommodated for the first time. The rate data have been used to calculate the position of the equilibrium and its rate of attainment as a function of pH, and this information should be of general value for optimising conditions for synthesis of 2′,4,6′-trihydroxychalcones by ring-opening 4′,5-dihydroxyflavanones.

The flavanone naringin (**1**), the 7-rhamnoglucoside of 4′,5,7-trihydroxyflavanone, is readily accessible[1,2] from the fruit of the grapefruit tree, *Citrus decumana*. It can be ring-opened under basic conditions to the corresponding chalcone (**2**),[1] the 4′-rhamnoglucoside of 2′,4,4′,6′-tetrahydroxychalcone, catalytic hydrogenation across the α,β-double bond of which gives[2] the corresponding *dihydro*chalcone (not shown), a non-nutritive artificial sweetener of considerable commercial potential.[1,3] The precursor chalcone (**2**) is, however, one of the class of 2′,6′-*dihydroxy*-substituted chalcones which are unstable, being characterised[4] by a strong tendency to cyclise rapidly back to flavanone [(**1**) in this case] at neutrality and over a wide range of higher pH. Data on the pH-dependence of the position of equilibrium between and rates of interconversion of (**2**) and (**1**) are therefore of interest from a synthetic viewpoint as well as for their inherent mechanistic significance.



(**1**)



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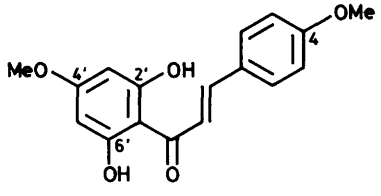

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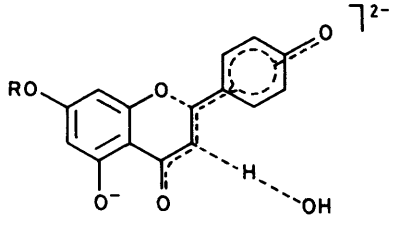


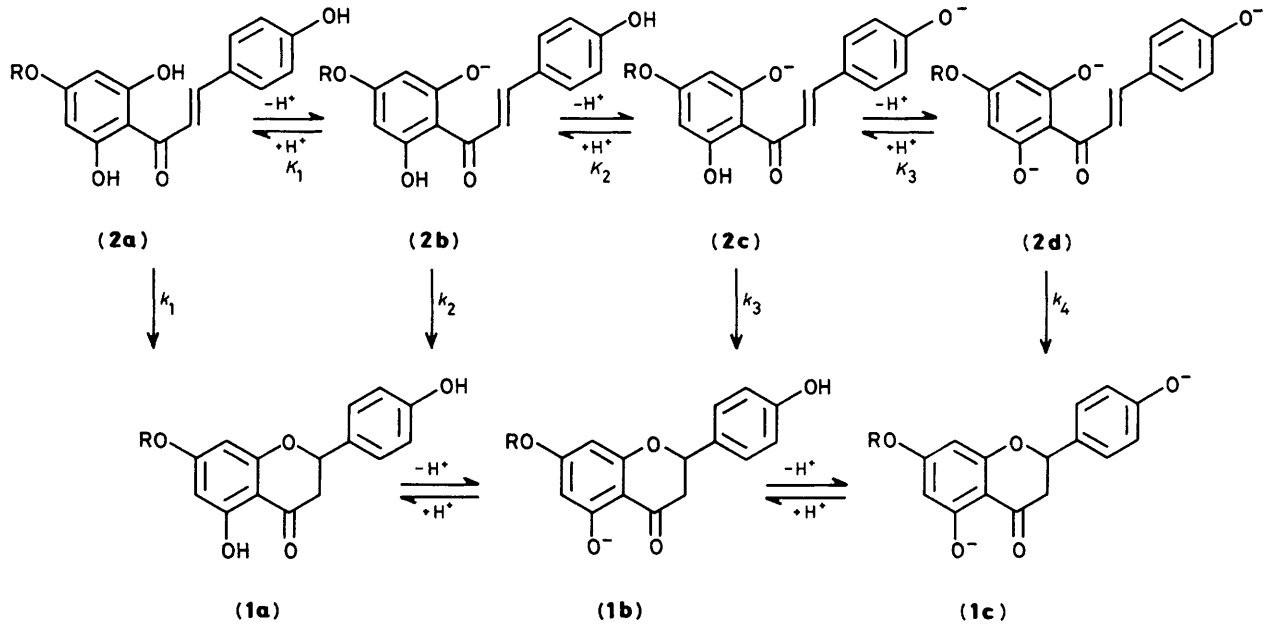
(**4**)

We recently reported[4] the first such data for a 2′,6′-dihydroxychalcone, that of 2′,6′-dihydroxy-4,4′-dimethoxychalcone (**3**), which established factors contributing to the specially high reactivity towards cyclisation to flavanone. The present study on the naringin chalcone–flavanone equilibrium (**2**) ⇌ (**1**) reveals many similarities to the earlier results[4] but there is one major difference: the naringin chalcone (**2**) has a free phenolic group (4-OH) in place of the methoxy group (4-OMe) of the chalcone in the previous study, and the ionisation of the 4-OH group between pH 8 and 9 introduces a new contributing kinetic term, reduces the rate, and results in marked changes to the pH–rate profile.

This report is the first in which the kinetics and mechanism of cyclisation of any chalcone in which the contribution of the ionisation of the 4-OH group, common to many naturally occurring chalcones, has been quantitatively accommodated.

Experimental

Naringin (**1**) (1 g) (Sigma) was added to a solution of potassium hydroxide (2 g) in 1:1 ethanol–water (4 ml) and heated on a steam-bath for *ca.* 3 min. The deep red solution obtained was filtered immediately into an excess of ice-cold hydrochloric acid (2 mol l^{-1}) saturated with sodium chloride. The precipitated yellow solid was extracted with diethyl ether. After drying ($MgSO_4$) and filtering, the ether solution was evaporated. The solid residue was recrystallised from diethyl ether–chloroform–light petroleum (b.p. 40—60 °C) (*ca.* 1:1:1) to give 4′-rhamnoglucosyloxy-2′,4,6′-trihydroxychalcone (**2**), an orange powder, m.p. 170—183 °C (lit.,[5] 185—200 °C); $\lambda_{max.}$ (acidic methanol) 367 nm (log ε 4.46) [lit.,[5] (MeOH) 370 nm]; δ_H [90 MHz; solvent $(CD_3)_2SO$; standard CD_3SOCHD_2, δ_H 2.60] 1.30 (3 H, d, *J* 6 Hz, 6‴-H_3), 3.4—3.8 (m, br, other sugar protons), 5.20 (2 H, br, anomeric 1″- and 1‴-H), 6.16 (2 H, s, 3′-, 5′-H), 6.95 (2 H, d, *J* 8 Hz, 3-, 5-H), 7.65 (2 H, d, *J* 8 Hz, 2-, 6-H), 7.70 (1 H, d, *J* 16 Hz, α-H), 8.05 (1 H, d, *J* 16 Hz, β-H), 10.20 (1 H, s, br, 4-OH), and 12.60 (2 H, s, br, 2′-, 6′-



Scheme. R = rhamnoglucosyl

OH); δ_C [22.53 MHz; solvent $(CD_3)_2SO$; standard $(CD_3)_2SO$, δ_C 43.5] 19.0 (6‴-C), 61.3 (6″-C), 69.3 (5‴-C), 70.4 and 71.3 (2‴-, 3‴-, 4″-C), 72.8 (4‴-C), 77.6 and 78.1 (2″-, 3″-, 5″-C), 95.9 (3′-, 5′-C), 98.2 (1″-C), 101.5 (1‴-C), 106.9 (1-C), 117.0 (3-, 5-C), 124.7 (α-C), 126.9 (1-C), 131.5 (2-, 6-C), 144.0 (β-C), 160.9 (4-C), 163.8 (4′-C), 164.6 (2′-, 6′-C), and 193.4 p.p.m. (C=O); assignments of the sugar ^{13}C signals are based on those of Markham and Chari[6] for naringin (Found: C, 53.3; H, 5.7. Calc. for $C_{27}H_{32}O_{14}$: C, 52.9; H, 5.3%).

Kinetic Measurements and Analysis.—Methods employed were those of the earlier study,[4] to which reference may be made for details. Reactions were carried out at 30 °C in aqueous solutions containing 4% v/v ethanol (μ 1.0 mol dm^{-3} with KCl) buffered as previously described.[4] Changing chalcone absorbance was monitored spectrophotometrically. Calculation of observed first-order rate coefficients (k_{obs}), and their analysis in terms of pH-variant contributions from reactions of neutral and ionised chalcone and flavanone species (Scheme) in order to produce a theoretical pH–rate profile, followed the methods described in detail in the previous study.[4] The analysis is complicated in the present case, however, by the additional ionisable phenolic group of the chalcone (4-OH). Acid catalysis has not been studied.

Results

The experimental rate data are given by the points in the pH–rate profile (Figure 1). For comparison, the theoretical profile (the line in Figure 1) was derived from equation (1). In this

$$k_{obs} = k_1 f^A + k_2 f^B + k_3 f^C + k_4 f^D + k_5[OH^-] \quad (1)$$

equation which corresponds to equation (4) in the previous report,[4] the first four terms represent the unimolecular cyclisations of neutral chalcone [(**2a**); first-order rate coefficient k_1; Scheme], of chalcone monoanion [(**2b**); k_2], of chalcone dianion [(**2c**); k_3], and of chalcone trianion [(**2d**); k_4]. The terms f represent the fraction of total chalcone present in the ionised form indicated by the superscript (Scheme). Values of f were calculated in the lower pH region taking into account overlapping of the first two ionisations (K_1 and K_2), f^A being given by $(a_{H^+})^2/[(a_{H^+})^2 + K_1 a_{H^+} + K_1K_2]$, f^B by $K_1 a_{H^+}/[(a_{H^+})^2 + K_1 a_{H^+} + K_1K_2]$, and f^C by $K_1K_2/[(a_{H^+})^2 + K_1 a_{H^+} + K_1K_2]$. The value of f^D associated with the well separated third ionisation (pK_3 12) at high pH is given by $K_3/(K_3 + a_{H^+})$. The fifth term in equation (1) becomes important only above pH 12 and represents, as described in detail in the previous report,[4] a contribution to k_{obs} resulting from the reverse ring-opening to chalcone of the fully ionised flavanone species [dianion (**1c**)] by reaction with hydroxide ion (second-order rate coefficient k_5).

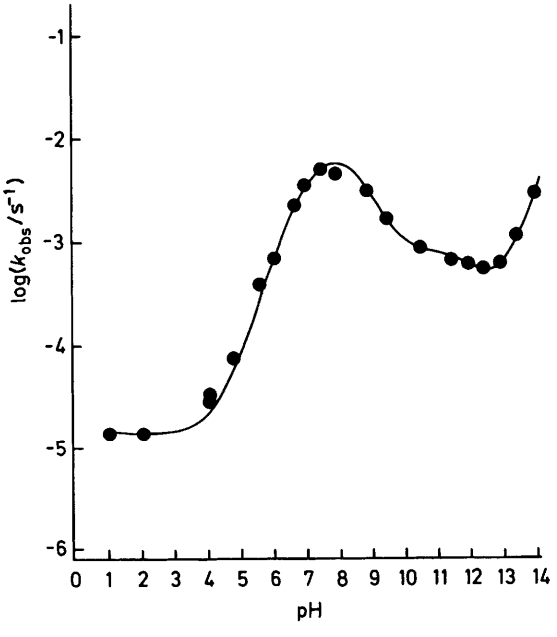


Figure 1. Semilogarithmic plot of k_{obs} in s^{-1} *versus* pH. Points are experimental. The curve is theoretical being based on equation (1) and the following rate and equilibrium constant values: k_1 1.4 × 10^{-5} s^{-1}; k_2 7.5 × 10^{-3} s^{-1}; k_3 8.0 × 10^{-4} s^{-1}; k_4 3.0 × 10^{-4} s^{-1}; k_5 2.6 × 10^{-3} dm^3 mol^{-1} s^{-1}; pK_1 6.90; pK_2 8.50; pK_3 12.0

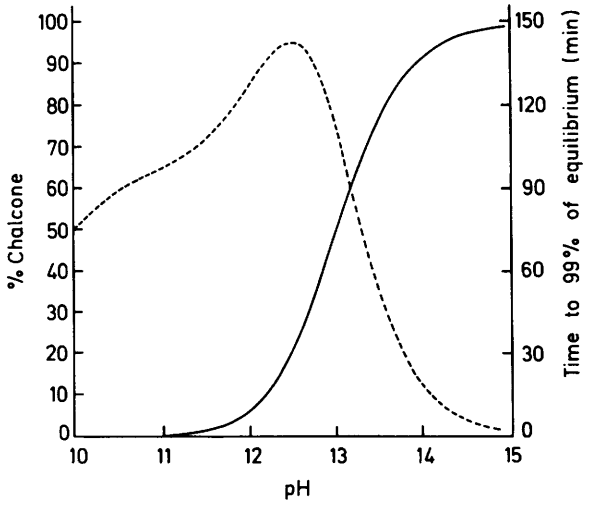


Figure 2. % Chalcone (**2**) at equilibrium (full curve) and time required for 99% reaction (dashed curve) as a function of pH. (% Chalcone at equilibrium is given by $100k_5[OH^-]/k_{obs}$ as based on the equations[7] for reversible first-order reactions, and time for 99% reaction is given by $4.6/k_{obs}$)

Values of rate and equilibrium constants found by variation to best fit equation (1) to the experimental data are given in the legend to Figure 1.

Figure 2 shows, as a function of pH, the time required for flavanone and chalcone to come nearly to equilibrium, and the percentage chalcone at equilibrium. Such data should be a useful guide when choosing conditions to synthesize chalcone (**2**) or other 2′,4,6′-trihydroxychalcones by ring-opening 4′,5-dihydroxyflavanones: unnecessarily prolonged contact with strong base may further decompose chalcones by cleavage across the enone function and is to be avoided. It should be noted that when working on a synthetic scale, calculation of the amount of NaOH required to simulate pH conditions indicated in Figure 2 would require allowance to be made for consumption of base in ionisation of phenolic groups.

Discussion

Form of the Rate Profile.—The profile (Figure 1) has much in common with that of the closely related 2′,6′-dihydroxy-4,4′-dimethoxychalcone (**3**) and the effects which contribute to make it so have been covered in detail in the report[4] on (**3**). The major difference is that the plateau between pH 8 and 11 for (**3**), which is associated with cyclisation of the monoanion, disappears for the naringin chalcone (**2**). The explanation lies in the ionisation of the ring B 4-OH group [pK_2 8.5; *cf.* un-ionisable 4-OMe in (**3**)] to form the dianion (**2c**): the dianion is much less reactive (*ca.* 10-fold) in cyclisation as shown by the decrease in k_{obs} with such ionisation between pH 8 and 9.

A full plateau in the profile representing cyclisation of (**1c**) is not attained, however, because at increasingly high pH there is a further decrease in k_{obs} as the second ring A phenol function [6′-OH in (**2c**)] starts to ionise. The rate reduction parallels that previously observed[4] with 6′-OH ionisation in the monoanion of (**3**). Another parallel with (**3**) exists at still higher pH where k_{obs} increases again as hydroxide ion at high concentration promotes the reverse ring-opening reaction of flavanone and this contributes to the measured k_{obs} which, as previously discussed, is the sum of the forward and reverse rate coefficients.

Rates of Unimolecular Cyclisation of Chalcone Species.—The chalcone cyclises only *ca.* 2-fold faster than (**3**) in both neutral [(**2a**); k_1] and monoanionic [(**2b**); k_2] forms. The large increase in reactivity ($k_2 \sim 500k_1$) with the first ionisation [(**2a**) ⟶ (**2b**)] is similar in magnitude to the increase for (**3**) which was accounted for in the previous report[4].

Ionisation of the 4-OH group to 4-O^- [(**2b**) ⟶ (**2c**)], for which there is no equivalent in the case of (**3**), leads to a reduction in rate of cyclisation by a factor of only *ca.* 10. This is apparently small (from an electrostatic viewpoint) given that the degree of delocalisation of negative charge into the enone system, where 2′-O^- is required to attack, would be expected to be much greater for (**2c**) [4′-O^-] than for (**2b**) [4′-OH]. It must be recognised, however, that such delocalisation would increase charge density on the α-carbon much more so than on the β-carbon where 2′-O^- is required to attack as a nucleophile, and also that delocalisation through to the carbonyl oxygen may inhibit competing delocalisation to the same oxygen from 2′-O^- thus enhancing the nucleophilic power of the latter in cyclisation.* The final chalcone ionisation [6′-OH; (**2c**) ⟶ (**2d**)] leads to a reduction in rate of cyclisation by a factor of *ca.* 3, almost the same as that associated[4] with 6′-OH ionisation of the monoanion of (**3**). Explanations for this unexpected reduction in reactivity have been considered in detail[4] for (**3**) and are equally applicable here.

Reverse Ring-opening Reaction of Naringin.—It is surprising that in the ring-opening reaction [(**1**) ⟶ (**2**)] with hydroxide ($k_5[OH^-]$) the flavanone dianion [(**1c**); 5-O^-, 4′-O^-] is more reactive (*ca.* 2-fold) than the monoanion of the previously studied chalcone [(**3**); 5-O^-, 4′-OMe]. Charge repulsion would be expected to slow the reaction of OH^- with a dianion more so than a monoanion. It is the case that the 4-O^- group in (**1c**) is remote from the centre (α-C) at which OH^- reacts and that an electrostatic effect over such a distance may be swamped by that of the 5′-O^- group which is much closer to the α-C and immediately adjacent to the activating carbonyl oxygen: the 5′-O^- group is common to both the dianion (**1c**) and the monoanion of (**3**). Nevertheless the higher reactivity of the dianion (**1c**) with OH^- is sufficiently unusual to raise the question of whether electron donation from 4′-O^- can sufficiently assist expulsion of the departing phenolate (2′-O^-) in a transition state of the type indicated by (**4**) to more than offset any destablising electrostatic repulsion effect between OH^- and 4′-O^-. A similar explanation is applicable to a stepwise mechanism involving enolate formation prior to rate-limiting ring-opening. A general study of the effect of 4′- as opposed to 3′-substituents may therefore provide a lead to understanding of the mechanism of ring-opening of flavanones.

Acknowledgements

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* Such delocalisation into the enone system is not possible if the ring B hydroxy group is in the 3- rather than the 4-position, as applies for a number of other naturally occurring chalcones, such as that from hesperidin: significantly different pH–rate profiles may be expected in their case.

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