Food Chemistry **49** (1994) 73–75

Kinetic effect of sulphite ion on ester hydrolysis

B. L. Wedzicha & C. L. Allen

Procter Department of Food Science, University of Leeds, Leeds, UK, LS2 9JT

(Received 10 January 1993; accepted 25 March 1993)

The hydrolysis of *p*-nitrophenyl acetate at pH 6–8 is strongly catalysed by SO_3^{2-} (rate/mol l^{-1} s^{-1} = 1.6[ester][SO_3^{2-}] at 28 °C; the pseudo-first-order rate constant for the uncatalysed reaction is of the order 10^{-5} s^{-1} at pH 8). The corresponding second-order rate constant for the reaction catalysed by HPO_4^{2-} is 16 000 times smaller, despite both the conjugate acids having similar pK values. It is suggested that the large effect of sulphite ion is due to its high nucleophilicity; esters and lactones in food could become less stable in the presence of sulphite species.

INTRODUCTION

It has recently been suggested that sulphite species, S(IV) (e.g. HSO_3^- or SO_3^{2-}), exert a marked destabilising effect on ascorbic acid, ASA (Davies & Wedzicha, 1992). A possible mechanism is the involvement of sulphur(IV) oxospecies as acid-base catalysts of ester hydrolysis, thereby facilitating the irreversible opening of the lactone ring of ASA. This is regarded as one of the early steps in ASA degradation (Kurata & Sakurai, 1967). The idea that S(IV) may be involved at this stage stems from the observation that the hydrolysis of D-glucono-δ-lactone is also facilitated by S(IV) (Davies & Wedzicha, 1992). At pH 4·7, S(IV) is more effective than acetate buffer at the same concentration; acetic acid and acetate ions are, in fact, regarded as moderate acid-base catalysts of gluconolactone hydrolysis (Pocker & Green, 1973). It is possible that the reactive form of S(IV) is sulphite ion (i.e. it acts by nucleophilic attack on the carboxyl-carbon of the lactone, or as a base catalyst) because no evidence of a kinetic effect of S(IV) had previously been reported (Wedzicha & McWeeny, 1974) when the ASA–S(IV) reaction had been investigated at c. pH 2; at this pH, the concentration of SO_3^{2-} is 500 times smaller than at pH 4·7. There is, however, no information regarding the effect of S(IV) on the hydrolysis of simple esters; this information would form the basis of further investigations into more complex systems such as those involving lactones.

The aim of the present investigation is therefore to study the hydrolysis of a model ester in the presence of S(IV) to confirm the suspected mechanism of catalysis and to establish the nature of the reactive species.

Food Chemistry 0308-8146/93/$06.00 © 1993 Elsevier Science Publishers Ltd, England. Printed in Great Britain

MATERIALS AND METHODS

Reaction mixtures contained *p*-nitrophenyl acetate (60–600 μM) (Sigma Chemicals, Poole) and were made up in phosphate buffer (pH 6–8). For reactions with half lives $t_{1/2} < 5$ min, the ester was dissolved in absolute ethanol to give a concentration 100 times as great as that desired in the final reaction mixture. The reaction was started by adding an aliquot (5 ml) of the ethanolic solution to buffer (495 ml) containing NaCl or $NaNO_3$ (0·05–0·5M), trisodium citrate (0·01–0·3M) or sodium acetate (0·05–0·7M). The reaction pH was that measured after the reactants had been mixed. Reaction mixtures were placed in 1-cm silica cells held in the thermostatted (28 ± 0·2 °C) cell block of a Pye Unicam SP 402 spectrophotometer. The progress of the reaction was followed by measuring the rate of increase of absorbance due to *p*-nitrophenol at 398 nm. The rate of reaction was obtained by using the extinction coefficient of *p*-nitrophenol at this wavelength, measured at the pH of the reaction.

The hydrolysis of the ester in the presence of S(IV) occurred much more rapidly and was followed by means of a stop-flow kinetic apparatus (Hi-Tech Scientific SF51 Stop Flow Spectrometer). In this case, the ester solution (5 ml) was added to phosphate buffer (250 ml, pH 6·0, 10 μM NaH_2PO_4 + 1mM Na_2HPO_4) to give a concentration of 60μM. Solutions of S(IV) (0·2–1·0M) were prepared in pH 7·0 ([NaH_2PO_4] = [Na_2HPO_4] = 0·20M) and pH 8·0 ([NaH_2PO_4] = 0·02M; [Na_2HPO_4] = 0·20M) phosphate buffers. Reactant solutions were allowed to come to thermal equilibrium at 28°C and equal volumes mixed to begin the reaction. The progress of the reaction was followed spectrophotometrically as before but by using the high-speed-data-acquisition system of the stop-flow apparatus.

RESULTS AND DISCUSSION

To facilitate the measurement of the rate of reaction, it was decided to use an ester whose hydrolysis could be followed spectrophotometrically. A widely used substrate for esterase enzymes is *p*-nitrophenyl acetate; preliminary experiments showed that its rate of hydrolysis proceeds at a conveniently measurable rate in phosphate buffer in the pH range 6–8. The high sensitivity of the spectrophotometric analysis of the products of hydrolysis enables kinetic experiments to be carried out at ester concentrations that are negligible in comparison with those of other reactants, and pseudo-first-order kinetics should be observed.

The extinction coefficient of *p*-nitrophenol was found to vary with pH as predicted for an acid with pK 7·00, and values of 340 and 17 500 mol^{-1} litre cm^{-1} for the *p*-nitrophenol and *p*-nitrophenolate ion, respectively, were obtained. For reactions with $t_{1/2} > 5$ min, initial rates were obtained from concentration-time data over the first 5–10 % reaction; these gave essentially linear plots. The initial reaction was confirmed to be of first-order with respect to the initial ester concentration (60–600μM). Subsequently, all kinetic runs were carried out with *c*. 300μM ester.

The addition of up to 0·5M NaCl, $NaNO_3$, or sodium acetate had no effect on the rate of hydrolysis of the ester at pH 8·0 (50μM Na_2HPO_4 + 5mM NaH_2PO_4). Citrate ion (0·01–0·3M) at pH 8·0 caused the rate of reaction to increase slightly ($10^5k/s^{-1} = 4{\cdot}9 + 6{\cdot}9$[citrate]); the magnitude of this effect was too small to establish unequivocally the nature of the catalytic species. Phosphate buffer was seen to have a greater effect; e.g. at pH 8, the pseudo-first-order rate constant, k/s^{-1}, increased as 10^{-4}[phosphate] where the concentration term represents the sum of concentrations of all the phosphate species (11–55mM at pH 8).

If the rate of ester hydrolysis is subject to both acid and base catalysis, the pseudo-first-order rate constant, *k*, will be given by:

$$k = k_1 + k_2[HA] + k_3[A^-]$$

where k_1 is a rate constant for the uncatalysed reaction, HA and A^- represent the unionised and ionised forms of the acid, and k_2 and k_3 are the rate constants for the catalysed reactions.

Rewriting this rate equation in terms of the total concentration c (= [HA] + [A^-]) gives:

$$k = k_1 + \{k_2[H^+]/(K + [H^+]) + k_3K/(K + [H^+])\}c$$

where *K* is the dissociation constant of the acid and $[H^+]$ the hydrogen-ion concentration of the medium. In practice, the pseudo-first-order rate constant was found to increase linearly with *c*, i.e.:

$$k = k_1 + k_4c$$

Hence, the slope, k_4, of a graph of *k* against *c* is related to k_2 and k_3 by:

$$k_4 = (k_2[H^+] + k_3K)/(K + [H^+])$$

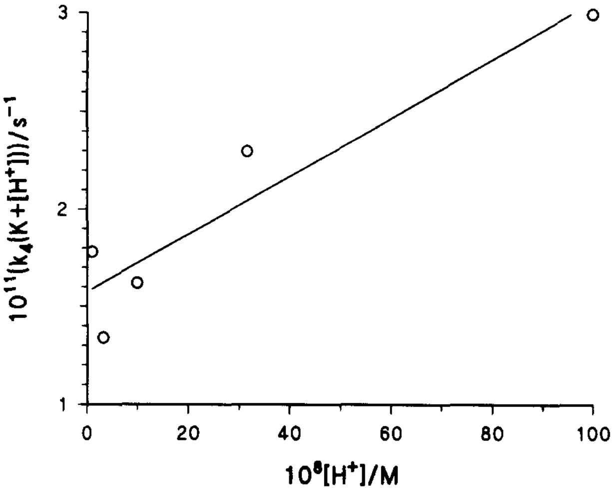


Fig. 1. Graph of $k_4(K + [H^+])$ against $[H^+]$ to elucidate the relative contributions of $H_2PO_4^-$ and HPO_4^{2-} ions to the rate-enhancing effect of phosphate buffer on the hydrolysis of *p*-nitrophenyl acetate, where *K* is the dissociation constant of $H_2PO_4^-$ and k_4 the slope of a graph of the pseudo-first-order rate constant for ester hydrolysis against ($[H_2PO_4^-] + [HPO_4^{2-}]$) as buffer concentration was varied at constant pH. Reaction conditions: [ester] = 300μM; [phosphate] = 0·1–0·6 M (pH 6), 0·07–0·33M (pH 6·5), 0·02–0·1M (pH 7), 0·016–0·08M (pH 7·5), 0·01–0·06M (pH 8); 28 °C.

Figure 1 shows a graph of $k_4(K+[H^+])$ plotted against $[H^+]$ with $K = 1{\cdot}58 \times 10^{-7}$ mol litre^{-1} (pK 6·80) for the phosphate buffer. The values of intercept and slope, k_2, are $1{\cdot}57 \times 10^{-11}$ s^{-1} and 1.45×10^{-5} mol^{-1} litre s^{-1}, respectively, which give the ratio $k_3/k_2 \approx 7$; i.e. the reaction is preferentially base-catalysed by HPO_4^{2-} with $k_3 = 1 \times 10^{-4}$ mol^{-1} litre s^{-1}.

The rate of ester hydrolysis in the presence of S(IV) (0·1–0·5M) at pH 6–8, was too great for the reaction to be followed by conventional spectrophotometry, and a stop-flow kinetic approach was required.

The effect of S(IV) concentration on the first-order rate constant for the hydrolysis of *p*-nitrophenyl acetate

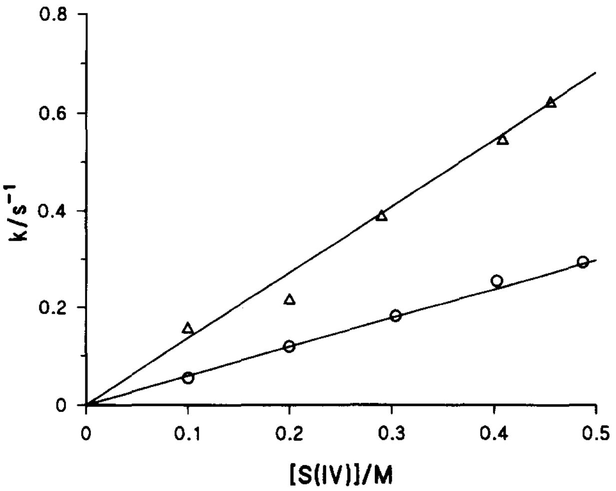


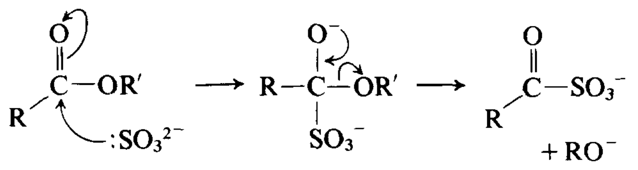
Fig. 2. Graph of pseudo-first-order rate constant, *k*, for the hydrolysis of *p*-nitrophenyl acetate against S(IV) concentration at pH 7. ○, ($[H_2PO_4^-] = [HPO_4^{2-}]$ = 0·2M) and pH 8. Δ, ($[H_2PO_4^-]$ = 0·02M, $[HPO_4^{2-}]$ = 0·2M). Reaction conditions: [ester] = 30 μM; 28 °C.

at pH 7·0 and 8·0 is shown in Fig. 2. In separate experiments, S(IV) was found not to have any measurable effect on the extinction coefficient of *p*-nitrophenol in the pH range investigated. Unlike the behaviour of phosphate buffer, the kinetic effect of S(IV) is considerable, with values of rate constants some four orders of magnitude greater than those at comparable concentrations of phosphate buffer.

The intercept of the lines in Fig. 2 is close to the origin, which confirms that, in the presence of S(IV) at the concentrations investigated, the major reaction is the S(IV)-catalysed process. By using a pK value of 7·18 (Wedzicha, 1984) for the dissociation of HSO_3^- ($K = 6{\cdot}61 \times 10^{-8}$ mol litre^{-1}) and the slopes of the two lines at pH 7 and 8 of 0·63 and 1·4 mol^{-1} litre s^{-1}, it is found that $k_3 \gg k_2$ and $k_3 = 1{\cdot}6$ mol^{-1} litre s^{-1}, which suggests that the sulphite ion is by far the most effective catalyst of ester hydrolysis.

Despite the similar pK values of $H_2PO_4^-$ and HSO_3^-, there is a considerable difference between the ability of the acids, and their conjugate bases, to catalyse the hydrolysis of *p*-nitrophenyl acetate; the value of k_3 is 16 000 times as great for SO_3^{2-} as for HPO_4^{2-}. It is suggested that this difference is due to the high nucleophilicity of the sulphite ion.

A simple mechanism to explain the behaviour of sulphite ion as a catalyst of ester hydrolysis is therefore nucleophilic attack on the ester linkage, as follows:



The intermediate obtained after elimination of RO$^-$ would be hydrolysed to the carboxylic acid and S(IV).

The low reactivity of HSO_3^- is consistent with its low nucleophilicity (Wedzicha & Goddard, 1991).

CONCLUSION

Sulphite ion is an effective enhancer of ester hydrolysis in comparison with the mono- and dihydrogen orthophosphates. Acetate ion has no significant effect at a similar concentration. It is possible that sulphite ion acts by nucleophilic attack on the ester moiety, and this mechanism could also be responsible for the destabilisation of ascorbic acid or D-glucono-δ-lactone by S(IV).

ACKNOWLEDGEMENT

We are grateful to École Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, Dijon, France, for an appointment as Professeur Visité to B.L.W., and to Paul Kajda for help during the experimental work.

REFERENCES

Davies, C. G. A. & Wedzicha, B.L. (1992). Kinetics of the inhibition of ascorbic acid browning by sulphite, *Food Add. Cont.*, **9**, 471–7.

Kurata, T. & Sakurai, Y. (1967). Degradation of L-ascorbic acid and mechanism of non-enzymic browning reaction. Part II: Non-oxidative degradation of L-ascorbic acid including the formation of 3-deoxy-L-pentosone. *Agric. Biol. Chem.*, **31**, 170–6.

Pocker, Y. & Green, E. (1973). Hydrolysis of D-glucono-δ-lactone. I: General acid-base catalysis, solvent deuterium isotope effects and transition state characterisation. *J. Amer. Chem. Soc.*, **95**, 113–19.

Wedzicha, B. L. (1984). *Chemistry of Sulphur Dioxide in Foods*. Elsevier Applied Science Publishers, London.

Wedzicha, B.L & Goddard, S. J. (1991). The state of sulphur dioxide at high concentration and low water activity. *Food Chem.*, **40**, 119–36.

Wedzicha, B. L. & McWeeny, D. J. (1974). Nonenzymic browning reactions of ascorbic acid and their inhibition. The production of 3-deoxy-4-sulphopentosulose in mixtures of ascorbic acid, glycine and bisulphite ion. *J. Sci. Food Agric.*, **25**, 577–87.