A Temperature-dependent Change in the Mechanism of Acid Catalysis of the Hydrolysis of *p*-Nitrophenyl β-D-Glucopyranoside indicated by Oxygen-18 and Solvent Deuterium Kinetic Isotope Effects

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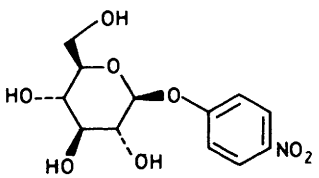
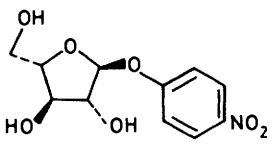
The ^{18}O kinetic isotope effect for hydrolysis of *p*-nitrophenyl [1-^{18}O]-β-glucopyranoside in 2.0M-HCl, measured by the isotopic quasi-racemate method, is 1.025, at 65.5 °C and 1.023 at 75.1 °C; there is a literature value of 1.0355 ± 0.0015 at 50.0 °C, measured mass spectrometrically. That this apparent discrepancy arises from a change in the mode of acid catalysis as the temperature is lowered is shown by the strongly temperature-dependent solvent deuterium isotope effect [log $(k_{D_2O}/k_{H_2O}) = 1.84 - 0.595 \times 10^3/T$], and by the greater effect of trifluoroacetate buffers at 45.0 than at 85.0 °C: a rough catalytic constant for general acid catalysis by trifluoroacetic acid of *ca.* $10^{-7.5}$ l mol^{-1} s^{-1} can be estimated at the former temperature.

Acetals and glycosides are generally hydrolysed in acid by a specific-acid-catalysed pathway.[1,2] Anderson and Capon,[3] arguing from the Hammond postulate, pointed out that this pathway would change to one involving undissociated acid, if the oxocarbonium ion intermediate were particularly stable, the substrate particularly unstable (*e.g.* because of steric crowding), or the leaving group were particularly weakly basic: the same conclusion in respect of the low basicity of the leaving group was reached simultaneously by Kankaanperä and Lahti.[4] After the communications of these two groups, intermolecular general acid catalysis was observed in a number of acetal systems obeying the Anderson–Capon criteria,[2] although glycosides proper remained comparatively unstudied.

This is surprising, since the first evidence for intermolecular general acid catalysis of acetal hydrolysis was obtained with sucrose in 1934, when Hammett and Long,[5] correlating rates of acid-catalysed reaction with H_0, suggested that the data obtained by Hantzsch and Weissberger[6] for inversion of sucrose by trichloroacetic acid indicated general acid catalysis. (All three Anderson–Capon criteria are met to a small degree with sucrose, since the glycosidic oxygen experiences the inductive effect of two ring oxygens, and is thus less basic than the oxygen of an alkyl glycoside, the fructofuranosyl cation, being tertiary, is more stable than an aldopyranosyl cation, and the sucrose molecule is crowded about the glycosidic link.) Intramolecular general acid catalysis of the hydrolysis of salicyl β-D-glucopyranoside was observed in 1963,[7] but until the present work the only other indication of intermolecular general acid catalysis in the hydrolysis of a glycoside came from the surprisingly large ^{18}O kinetic isotope effect measured by Rosenberg and Kirsch[8] for hydrolysis of *p*-nitrophenyl [1-^{18}O]-β-D-glucopyranoside (**I**). The value of k_{16}/k_{18} (1.0355 ± 0.0015 in 2.0M aqueous HCl at 50.0 °C) is bigger than the equilibrium



(**I**)



(**II**)

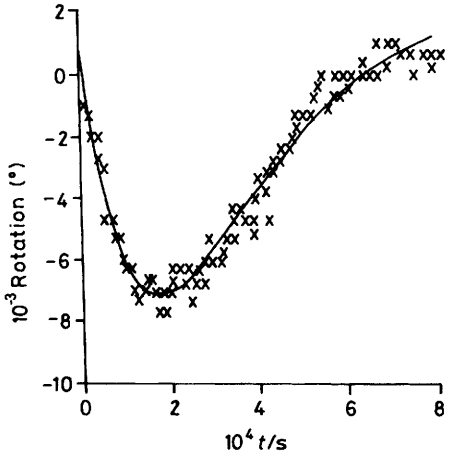


Figure 1. Optical rotation at 546 nm, as a function of time, of a solution of 9.3 mg each of *p*-nitrophenyl β-L-glucopyranoside and *p*-nitrophenyl [1-^{18}O]-β-D-glucopyranoside in 2.0M-HCl (1.0 ml), at 65.5 °C (path length 1 dm)

effect calculated from i.r. stretching frequencies for conversion of glycoside (**I**) into a glucopyranosyl cation and *p*-nitrophenol: it is in fact between this value and the equilibrium effect calculated for conversion of glycoside (**I**) into a glucopyranosyl cation and *p*-nitrophenolate ion.

We, however, observed a value of k_{16}/k_{18} of 1.023 ± 0.003 at 80.0 °C for the hydrolysis of *p*-nitrophenyl α-arabinofuranoside (**II**),[9] entirely in accord with specific acid catalysis of the hydrolysis of this glycoside, which should not be mechanistically different from that of glycoside (**I**). Rosenberg and Kirsch measured their effect by a direct mass spectrometric method,[10] whereas we had used the isotopic quasi-racemate method.[11] Given the weight of mechanistic inference the kinetic isotope effects measured by the two methods were being called upon to bear,[8,9,12,13] it was clearly imperative to investigate the cause of the apparent discrepancy. We accordingly investigated the ^{18}O kinetic isotope effect on the hydrolysis of glycoside (**I**) by the isotopic quasi-racemate method. A typical time-course of the optical rotation of an approximately equal concentration of *p*-nitrophenyl β-L-glucopyranoside and *p*-nitrophenyl [1-^{18}O]-β-D-glucopyranoside in 2.0M-HCl is given in Figure 1. The derived isotope effects, for runs at 75.1 and 65.5 °C (average 1.023 and

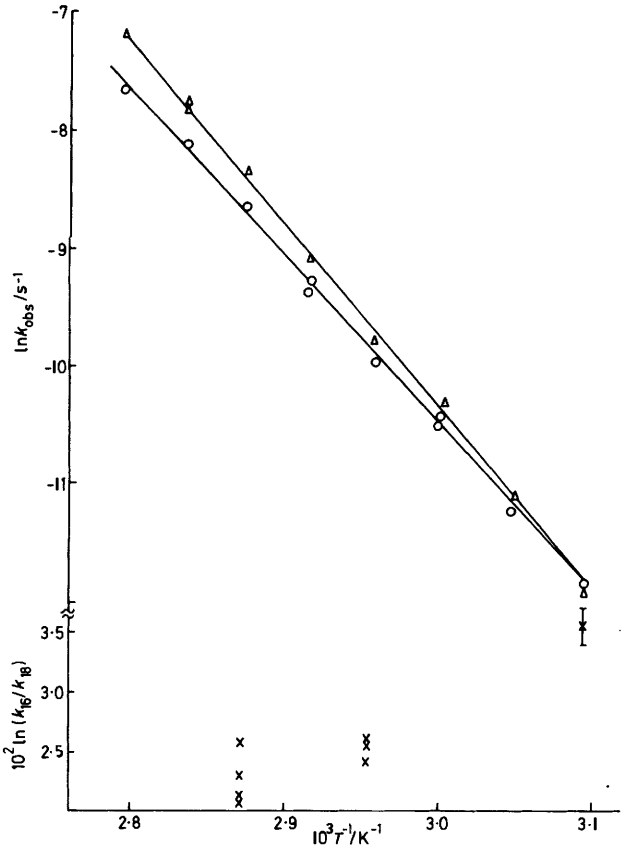


Figure 2. (Natural) logarithms of (top) observed first-order rate constants for hydrolysis of *p*-nitrophenyl β-D-glucopyranoside in (○) 2.0M-HCl in H_2O and (△) 2.0M-DCl in D_2O and (bottom) natural logarithms of ^{18}O kinetic isotope effects on the same reaction, plotted as a function of reciprocal temperature. Individual ^{18}O isotope effects are plotted in the case of the present work, but Rosenberg and Kirsch's[8] error bars are displayed

1.025_7 respectively), are plotted in Figure 2 as a function of reciprocal absolute temperature, together with Rosenberg and Kirsch's result. (It was not possible to measure the effect by the quasi-racemate method at 50.0 °C, since *p*-nitrophenyl β-glucopyranoside forms a racemic compound which is only very sparingly soluble in water at this temperature.) It is clear that either one (or both) methods of kinetic isotope effect measurement is unsound, or that there is a temperature-dependent change in mechanism, since the temperature-dependence of the ^{18}O kinetic isotope effect is much steeper than that of a classical primary kinetic isotope effect originating in zero-point-energy differences, for which $T \ln (k_{light}/k_{heavy})$ = constant. [The contribution made to observed heavy atom kinetic isotope effects by the change in molecular moments of inertia makes the temperature dependence *less* steep than that of a simple zero-point-energy effect: thus, chlorine kinetic isotope effects on displacements at n-butyl and t-butyl chlorides vary with temperature according to $\ln (k_{35}/k_{37}) = A + B/T$[14].]

That the second explanation, of a temperature-dependent change in mechanism, is the correct one is shown by the other data in Figure 2, simple Arrhenius plots for hydrolysis of *p*-nitrophenyl β-D-glucopyranoside in 2.0M-HCl in H_2O and 2.0M-DCl in D_2O. The lines are described by $\log k_{H_2O}/s^{-1} = 13.84 - 6.132 \times 10^3/T$ and $\log k_{D_2O}/s^{-1} = 15.61 - 6.705 \times 10^3/T$: the solvent deuterium isotope effect thus decreases non-classically with temperature, in accord with the change in mechanism suggested by the ^{18}O kinetic isotope effects. It in fact changes sense around 50 °C.

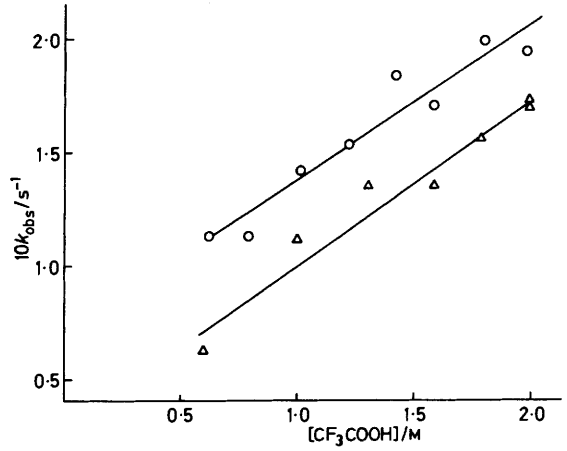


Figure 3. Rates of hydrolysis of *p*-nitrophenyl β-D-glucopyranoside in trifluoroacetic acid sodium trifluoroacetate buffers at 45.0 °C, as a function of trifluoroacetic acid concentration: (○) $[CF_3COOH]/[CF_3COONa] = 2$; (△) $[CF_3COOH]/[CF_3COONa] = 1$, ionic strength constant at 2.0M ($NaClO_4$)

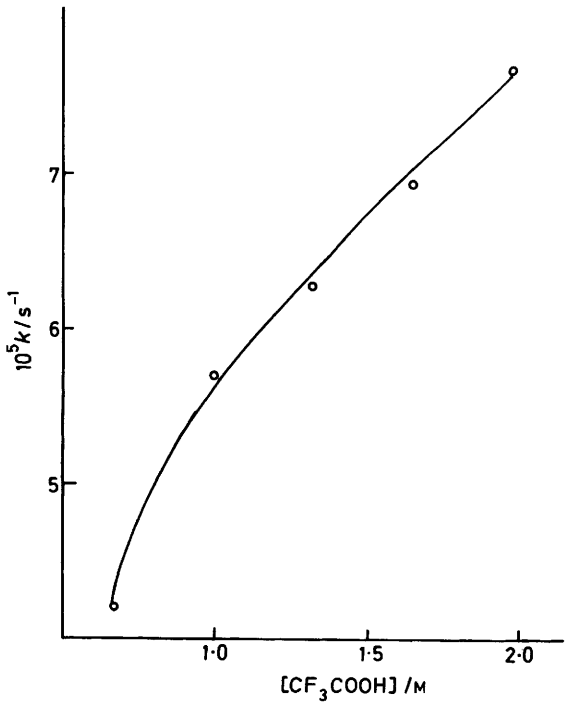


Figure 4. Rates of hydrolysis of *p*-nitrophenyl β-D-glucopyranoside in 1:1 trifluoroacetic acid–sodium trifluoroacetate buffer at 85.0 °C, as a function of trifluoroacetic acid concentration. The ionic strength was maintained at 2.0M with $NaClO_4$

Figures 3 and 4 display the results of attempting to detect general acid catalysis at 45.0 and at 85.0 °C by the traditional method of varying buffer concentration and composition at constant ionic strength. The data in Figure 3 apparently give, for both the 1:1 buffer and the 2:1 buffer, a catalytic constant of 7×10^{-8} l mol^{-1} s^{-1}. At 85.0 °C, however, a qualitatively similar, but quantitatively less pronounced effect is observed (linear least-squares treatment of the data in Figure 4 and for the 1:1 buffer in Figure 3 gives a ratio of slope to intercept of 0.84 and 2.6 l mol^{-1}, respectively). A dependence of the type shown in Figure 4 is expected in the absence of general acid catalysis for two reasons.

(i) The pK_a of trifluoroacetic acid is 0.50 (and is almost invariant with temperature),[15] therefore, even at a concentration of trifluoroacetate and trifluoroacetic acid of 1.0M each, the concentration of $[H_3O^+]$ is only 0.66 K_a. However, the literature[15] pK_a value for trifluoroacetic acid refers to infinite dilution and zero ionic strength, so it is not possible to correct for the changing concentration of H_3O^+ in the present experiments using this figure.

(ii) There are specific salt effects, averaging 20—30% for 1.0M-salt, on glycoside hydrolysis.[13]

Therefore, whereas the difference in the size of the buffer effects at 85.0 and at 45.0 °C supports our interpretation of the variation with temperature of the ^{18}O and solvent deuterium effects, as being due to a change from specific to general acid catalysis the catalytic constant derived at the low temperature is only an order-of-magnitude estimate.

At low temperature, then, the reaction is general acid-catalysed, with a proton partially transferred at the transition state, as is shown by the ^{18}O and solvent deuterium kinetic isotope effects (solvent isotope effects on reactions involving L_3O^+ are commonly low because of inverse secondary effects on the non-transferred protons[16]). The transition state for this process involves immobilisation of an acid species: as the temperature is raised this becomes relatively less favourable compared with processes involving unimolecular steps for entropic reasons, and hence the reaction mechanism changes to specific acid catalysis.

Over the past decade, it has proved illuminating to consider mechanistic dichotomies like the present one from the standpoint of the lifetime of the putative intermediate.[17] In this case the relevant intermediate is protonated *p*-nitrophenyl glucopyranoside. The lifetime of protonated tetrahydropyranyl *p*-nitrophenolate, the parent system of glycoside (**I**), has been estimated, using a number of long extrapolations and rough approximations, as *ca.* 5×10^{-17} s.[2] This is much shorter than the period of a molecular vibration (10^{-12}—10^{-13} s) and so in this case the intermediate in specific acid catalysis is too unstable to exist, and general acid catalysis is observed.[18,*] Hydrolyses of β-glucopyranosyl derivatives are *ca.* 10^6-times slower than those of tetrahydropyranyl derivatives,[2] but any Hammond effect with very good leaving groups will reduce this factor; this type of analysis therefore suggests that the lifetime of protonated glycoside (**I**) will be at least less than that of an encounter complex (10^{-10} s). In fact our experiments show that at low temperature the intermediate has a lifetime less than the period of a bond vibration, since there is a proton in flight.

It is not however clear how, from this standpoint, the high-temperature reaction should be described: when the C–O bond breaks the proton will have been transferred to the oxygen atom from acid HA (resulting in a Brønsted α near unity) but A^- will still be in the encounter complex.† Under these conditions catalysis by acids other than H_3O^+ will be experimentally very difficult to observe, but when the conjugate base of the catalysing acid is the same as the solvent, its persistence in the solvent shell is conceptually very difficult to distinguish from the situation in specific acid catalysis, where the protonated intermediate is solvent-equilibrated. Perhaps the solvent isotope effect at high temperature, which is lower than the value of k_{D_2O}/k_{H_2O} of 2—3 normally associated with acetal hydrolysis,[1] provides some distinction, although this could equally well arise from the persistence of a contribution from the general acid-catalysed pathway.

* All three of the Anderson–Capon criteria for general acid catalysis will of course tend to reduce the lifetime of the protonated acetal.

† In the reverse sense, reaction of the glucosyl cation with *p*-nitrophenol, the reaction is a preassociation reaction with A^- as a pre-association base.

Experimental

^{18}O *Kinetic Isotope Effects.*—Equipment and procedures used for these measurements, which were made by following the optical rotation of a solution of the labelled substrate and an equal quantity of its unlabelled antipode as a function of time, are described elsewhere.[9,13] Experimental time courses were fitted to equation (1) where α_t is the optical rotation at time t, A

$$\alpha_t = Ae^{-k_L t} + Be^{-k_L t/C} + \alpha' \qquad (1)$$

the optical rotation change associated with reaction of the light isotopomer, with a rate constant k_L, and C is the kinetic isotope effect. C, α', and B were taken as unlocated variables, but because of the low solubility of the racemic compound, and consequent low attainable concentrations of substrate, k_L was not treated as a completely unlocated variable, but constrained to within 10% of the value measured in a separate experiment. Because of the absorbance of the glycoside, optical rotation was followed at 546 nm.

p-Nitrophenyl β-L-glucopyranoside and *p*-nitrophenyl [1-^{18}O]-β-D-glucopyranoside were made from the acetobromosugars by standard procedures,[19] and had m.p. 166.5—168 and 166—167 °C, respectively (lit.,[20] 164 °C). The [1-^{18}O]-*p*-nitrophenol used, and the method of estimating isotropic enrichment, have been described.[9]

Hydrolysis Rates of p-*Nitrophenyl* β-D-*Glucopyranoside in Aqueous* HCl *and* DCl.—These were measured polarimetrically with a Perkin-Elmer 241MC spectropolarimeter under conditions identical with those for kinetic isotope effect measurements. DCl was prepared from benzoyl chloride by the method of Jolly.[21] Concentrations of aqueous acid were standardised by titration with anhydrous sodium carbonate.

Hydrolysis Rates of p-*Nitrophenyl* β-D-*Glucopyranoside in Trifluoroacetate Buffers.*—Initial rates of change of optical density at 350 nm of a 0.567mM solution of substrate were followed in 1 cm cells in a Unicam SP 1800 spectrometer fitted with a cell block thermostatted at 45.0 °C with a Tecam TV-14 Tempunit: $\Delta\varepsilon$ was taken as 3 390 l mol^{-1} cm^{-1} and the reaction was followed for $< 2\%$ of the total change. Hydrolysis rates at 85.0 °C were followed polarimetrically.

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