Acetolysis of 2,4-Dinitrophenyl Glycopyranosides

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A linear free energy relationship exists between rates of acetolysis of 2,4-dinitrophenyl glycopyranosides and rates of acid-catalysed hydrolysis of the methyl glycosides. For the 2-acetamido-2-deoxy-β-D-glucopyranosyl glycone, however, amide group participation is more important in acetic acid. Products from the other glycones are 1-*O*-acetylglycopyranoses, 60 ± 3% inverted. The α-deuterium kinetic isotope effect k_H/k_D for the acetolysis of 2,4-dinitrophenyl β-D-galactopyranoside is 1.099 ± 0.016.

We have shown that glycopyranosyl cations are generated during the spontaneous hydrolyses of 2,4-dinitrophenyl glycopyranosides.[1] To relate this process to S_N1 reactions in more conventional systems, it is necessary to study the solvolysis in a solvent which does not give rise to mutarotating products, and in which data on 2,4-dinitrophenolate as a leaving group is available. We therefore now report the behaviour of these glycosides in anhydrous acetic acid.[2]

EXPERIMENTAL

2,4-Dinitrophenyl glycopyranosides have been described.[1,3]

Acetic acid was dried by the method of Page *et al.*,[2b] and contained 0.01—0.02% water, as estimated by Karl Fischer titration.

Products from the glycone were determined by carrying out the acetolysis in [*U*-^{2}H]acetic acid (99.5% deuteriated; Diaprep Inc.) and examining the n.m.r. spectra of reactants and products, distinction between α- and β-acetates being made by splittings and chemical shifts of the anomeric proton. Maskill [4] has found a maximal 9% isotope effect on the ratio of retained to inverted substitution products in the butyrolysis and [*O*-^{2}H]-butyrolysis of *trans*-4-t-butylcyclohexyl *N*-nitrosoacetamide, so any isotope effect on the product composition consequent upon the change to a deuteriated solvent is likely to be less than the errors in n.m.r. integration. N.m.r. analyses were carried out on a Varian HA 100, or in cases of low substrate solubility, a JEOL Fourier transform instrument.

2,4-Dinitrophenol from the acetolysis of its β-D-galactopyranoside was estimated from the absorbance at 390 nm of a 0.1M-sodium phosphate buffer, pH 7.0 (20 ml) to which the acetolysis solution (20 μl) had been added.

Rates were measured spectrophotometrically [2] using a Unicam SP 800, SP 1800, or SP 1700 spectrophotometer with a thermostatted cell block, through which water was pumped from a Tecam or Paratherm thermostatic pump. A thermocouple was used to measure the temperature difference between the cell-block and the bath. Contrary to a recent report,[5] however, in our hands this system proved of insufficient accuracy to allow the confident determination of Arrhenius parameters, as it could give rise to large systematic errors in temperature above *ca.* 60°. We therefore only report rates at 60°. Some extrapolation using the derived parameters was however necessary to obtain these, although with the exception of the 6-chloro-6-deoxyglucoside (13° extrapolation) this was over less than 5°. The crude data are contained in Supplementary Publication No. SUP 21673 (4 pp.).†

For the measurement of the α-deuterium kinetic isotope effect we solvolysed protiated and deuteriated material in adjacent cell positions in the SP 1700, and between each run reversed the positions. Intermittent blockage of the cell-block channels was then the only factor which could systematically affect these measurements, and this was detectable by results around 10 standard deviations outside the normal error range.

RESULTS AND DISCUSSION

The glycosides are polyhydroxylic alcohols and heating them in anhydrous acetic acid could in principle acetylate them. As 2′,4′-dinitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside acetolyses *ca.* 10 times more slowly than the deprotected derivative,[6] our processing the data by the Guggenheim method would, if acetylation were

† For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1975, Index issue. Items less than 10 pp. are supplied as full-size copies.

[1] D. Cocker and M. L. Sinnott, *J.C.S. Perkin II*, 1975, 1391.

[2] (*a*) M. L. Sinnott and M. C. Whiting, *J. Chem. Soc.* (*B*), 1971, 965; (*b*) I. D. Page, J. R. Pritt, and M. C. Whiting, *J.C.S. Perkin II*, 1972, 906.

[3] (*a*) F. Ballardie, B. Capon, J. D. G. Sutherland, D. Cocker, and M. L. Sinnott, *J.C.S. Perkin I*, 1973, 2418; (*b*) M. L. Sinnott and I. J. L. Souchard, *Biochem. J.*, 1973, **133**, 89.

[4] H. Maskill, Ph.D. Thesis, Bristol, 1967.

[5] A. W. L. Dudeney and R. J. Irving, *J.C.S. Faraday I*, 1975, 1215.

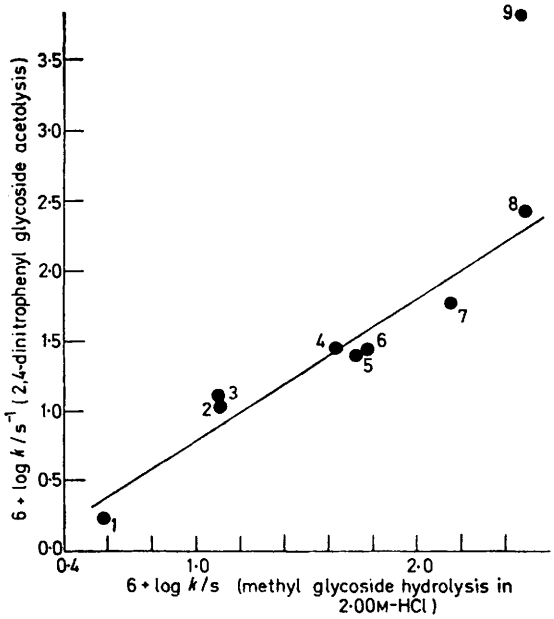
[6] L. E. Jukes, B.Sc. (Part II) Thesis, Bristol, 1972.

much faster than acetolysis, result in our obtaining the rate of the former rather than of the latter process. We therefore confirmed that the yield of 2,4-dinitrophenol from 2,4-dinitrophenyl β-D-galactopyranoside after 12 half-lives was indeed quantitative (98%). Acetylation would have greatest importance relative to acetolysis for 2,4-dinitrophenyl β-D-glucopyranoside, as the derived cation would be the least stable one which still possessed a readily acetylated primary hydroxy-group. However, although examination of the n.m.r. spectrum of this compound in [*U*-^{2}H]acetic acid immediately after solution and after 150 half-lives at 100° revealed extensive acetylation, as manifested by an alteration in shape and a considerable downfield shift of the envelope of glycone protons, after only 10—12 half-lives this envelope was unaltered in shape, although the maximum had shifted downfield by τ *ca.* 0.1. We conclude that acetylation, whilst taking place, had not affected our kinetic measurements.

The products of the acetolyses, then, are the 1-*O*-acetylglycopyranoses, and, with the exception of the 2-acetamido-2-deoxyglucopyranosyl system, these are formed with the configuration at C-1 predominantly inverted (60 ± 3% for the 6 'non-participatory' glycones). Comparison with the data of Bonner [7] for the anomerisation of the fully acetylated aldopyranoses in acetic acid–acetic anhydride–sulphuric acid indicates that this proportion of inversion is slightly less than would be observed were the products thermodynamically controlled: because of the anomeric effect, more than 60% of axial acetate would be anticipated at equilibrium. Slight assistance from the *trans*-2-hydroxy-group could be the cause, but the acetolyses of tertiary 2,4-dinitrophenolates show little preference for inversion,[8] and tertiary alkyl cations are of comparable stability to glycopyranosyl cations.[9] Further, a high preference for inversion is manifested in the methanolyses of both *o*-carboxyphenyl D-glucopyranosides.[10] 60% Inversion seems sufficiently close to the sort of product distribution expected for capture of the cation on encounter from either side of the pyranose ring that an explanation based on reaction through solvent-separated ion-pairs or free ions seems more plausible. In the more nucleophilic methanol products could arise by attack on intimate ion-pairs, but the more feebly nucleophilic acetic acid might attack only the solvent-separated ion-pairs or free ions.

The product of the acetolysis of 2′,4′-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside is exclusively the retained (β) 1-*O*-acetyl derivative, indicating that the enhanced rate observed with this compound is indeed attributable to acetamido-group participation.

In the Figure are plotted logarithmically the rates at 60° of the acetolyses of the 2,4-dinitrophenyl glycosides against those of the hydrolyses, in 2M-HCl, of the corresponding methyl compounds.[1] Rates of acetolysis at 60.0° of the 2,4-dinitrophenyl glycopyranosides of the following aglycones were as follows (in s^{-1}) β-D-glucose 1.1×10^{-5}, β-D-galactose 2.6×10^{-5}, β-D-xylose 2.8×10^{-5}, α-L-arabinose 6.0×10^{-5}, 6-deoxy-β-D-galactose 2.7×10^{-4}, 6-deoxy-β-D-glucose 2.8×10^{-5}, 6-chloro-6-deoxy-β-D-glucose 1.7×10^{-6}, 6-chloro-6-deoxy-β-D-galactose 1.3×10^{-5}, 2-acetamido-2-deoxy-β-D-glucose 6.2×10^{-3}. With the exception of the point for the 2-acetamido-2-deoxy-β-D-glucopyranosyl system, a linear free energy relationship is observed (r 0.96), confirming the intermediacy of the same glycosyl species in the acetolyses of the 2,4-dinitrophenyl glycosides, the acid-catalysed hydrolyses of the methyl glycosides, and hence also [1] the pH-independent hydrolyses of the 2,4-dinitrophenyl glycosides. The slope of the correlation line (1.0)



Relationship between rates (at 60 °C) of 2,4-dinitrophenyl glycoside acetolysis and methyl glycoside hydrolysis: 1, 6-chloro-6-deoxy-β-D-glycopyranose; 2, β-D-glucopyranose; 3, 6-chloro-6-deoxy-β-D-galactopyranose; 4, 6-deoxy-β-D-glucopyranose; 5, β-D-galactopyranose; 6, β-D-xylopyranose; 7, α,L-arabinopyranose; 8, 6-deoxy-β-D-galactopyranose; 9, 2-acetamido-2-deoxy-β-D-glucopyranose

is fairly surprising, as it indicates that at least those substituents in the pyranose ring that have been examined affect the generation of a cationic centre at C-1 to the same extent in water and in anhydrous acetic acid. As many of the structural variations examined have their effect largely through their conformational rather than their electronic effect, this implies that the conformational demands of these substituents are the same in water and acetic acid. This would apply, for instance, to the hydrogen-bonding hydroxy-group, as the relative ease of generation of the glucopyranosyl and galactopyranosyl cations is the same in acetic acid and water.

The effect of change of solvent from water to anhydrous acetic acid on the efficacy of the acetamido-group as an

[7] W. A. Bonner, *J. Amer. Chem. Soc.*, 1959, **81**, 1448.
[8] J. R. Pritt, Ph.D. Thesis, Bristol, 1972.
[9] D. Cocker, L. E. Jukes, and M. L. Sinnott, *J.C.S. Perkin II*, 1973, 190.
[10] B. Capon, *Biochimie*, 1971, **53**, 145.

intramolecular nucleophile is however marked. The acetolysis of 2′,4′-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside is 1.5 orders of magnitude faster than would be anticipated from the rate of acid-catalysed hydrolysis of the methyl compound, or indeed its own rate of spontaneous hydrolysis. This enhanced efficiency of neighbouring-group participation in the less polar acetic acid is readily understood, since the transition state for amide group participation involves dispersion of positive charge over the whole NHC=O system as well as over C-1 and O-5. Such a more efficient dispersal of charge would be of greater relative importance in the less polar solvent. This assists the resurrection of the possibility that amide group participation may after all play a role in lysozyme catalysis. Recent *X*-ray work has shown that if a saccharide unit is bound in the D subsite, it adopts a conformation suitable for Asp-52 general base catalysed participation by the 2-acetamido-group.[11]

At 75°, the α-deuterium kinetic isotope effect k_H/k_D for the acetolysis of 2,4-dinitrophenyl β-D-galactopyranoside is 1.099 ± 0.016. This is comparable with the effects observed for other reactions in which glycopyranosyl cations are generated such as the acid-catalysed hydrolysis of phenyl β-D-glucopyranoside[12] (k_H/k_D 1.13 ± 0.01) and the hydrolysis of α-D-glucopyranosyl phosphate (k_H/k_T 1.21, corresponding to k_H/k_D 1.11).[13] These isotope effects are less than the theoretical maximum, but this is often observed in acetal hydrolysis, and is attributed to reactant-like transition states rather than nucleophilic participation.[14]

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[14] H. G. Bull, K. Koehler, T. C. Pletcher, J. J. Ortiz, and E. H. Cordes, *J. Amer. Chem. Soc.*, 1971, **93**, 3002.