Generation of Glycopyranosyl Cations in the Spontaneous Hydrolyses of 2,4-Dinitrophenyl Glycopyranosides. Evidence for the General Intermediacy of Glycopyranosyl Cations in the Acid-catalysed Hydrolyses of Methyl Glycopyranosides

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The rate of liberation of 2,4-dinitrophenol from 2,4-dinitrophenyl β-D-galactopyranoside at 25.0 °C is independent of pH between pH 1.6 and 8.4: catalysis by buffer is absent. Above pH 8.4, 2,4-dinitrophenolate is liberated by a specific base-catalysed process. At 25 °C. the rates of spontaneous hydrolysis of a series of four homeomorphs each of 2,4-dinitrophenyl β-D-gluco- and -galacto-pyranoside at pH 6.5 exhibit a linear free energy relationship ($|r| = 0.92$) with the rates of hydrolysis of the corresponding methyl glycopyranosides in 2.0M-hydrochloric acid. The 2-acetamido-2-deoxy-β-D-glucopyranosyl system obeys this relationship.

ACID-CATALYSED hydrolyses of alkyl and aryl glycopyranosides are considered to take place by pre-equilibrium protonation of the exocyclic oxygen atom, followed by unimolecular heterolysis of the glycone–oxygen bond to yield a glycopyranosyl cation and the aglycone.[1] This pathway, rather than the alternative one involving an acyclic α-oxocarbocation, has been demonstrated to be predominant in the case of methyl α-D-glycopyranoside, but, as has been pointed out,[1-3] definitive evidence of its generality for other glycones has still to be obtained. Axial substituents in the pyranose ring would tend to accelerate both the ring-opening and the cyclic-ion pathways,[1] causing the predominant pathway in any given case to be *a priori* indeterminable.

If indeed the hydrolyses of a series of methyl glycopyranosides proceeded uniformly through the cyclic cations, then a linear free energy relationship should be observed

[1] B. Capon, *Chem. Rev.*, 1969, **69**, 407.
[2] C. K. De Bruyne and J. Wouters-Leysen, *Carbohydrate Res.*, 1971, **19**, 45.
[3] J. N. BeMiller, *Adv. Carbohydrate Chem.*, 1967, **22**, 25.

between the rates of acid-catalysed hydrolyses of the methyl compounds and the rates of some process which spontaneously gives rise to the same glycopyranosyl cations, provided always that changes in the glycone are insufficient to affect the position of the pre-equilibrium in the methyl glycoside hydrolyses. The major hindrance to this approach has hitherto been the difficulty of finding a leaving group whose departure from a deprotected glycoside was effected by neither acid nor base. However, work in these laboratories has demonstrated the utility of 2,4-dinitrophenolate as a leaving group for solvolysis studies,[4] and a ready synthesis of deprotected 2,4-dinitrophenyl glycopyranosides has recently become available.[5] Following the discovery of a pH-independent, spontaneous pathway for the hydrolysis of 2-(*p*-nitrophenoxy)tetrahydropyran,[6] we expected that a 2,4-dinitrophenyl glycopyranoside would undergo a similar, uncatalysed hydrolysis, and we now report its observation with the β-galactopyranoside between pH 1.5 and 8.

We also report spontaneous rates of hydrolysis of eight other 2,4-dinitrophenyl glycopyranosides and compare them with rates of acid-catalysed hydrolysis of the corresponding methyl glycopyranosides. Because of the differing steric requirements of methoxide and 2,4-dinitrophenolate, structural changes in the glycone were confined to those sites removed from C-1; this restriction would also obviate complications from effects on the pre-equilibrium in the acid-catalysed hydrolyses of the methyl compounds. The only exception, the 2-acetamido-2-deoxy-D-glucopyranosyl system, was studied to clarify the possible role of neighbouring group participation by amide in catalysis by *N*-acetyl glucosaminidases.

EXPERIMENTAL

The 2,4-dinitrophenyl glycopyranosides used have been described,[5] with the exception of the 6-chloro-6-deoxy-β-D-galactopyranosyl compound.

2,4-Dinitrophenyl 6-Chloro-6-deoxy-β-D-galactopyranoside.— 6-Chloro-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose [7] (5 g) was dissolved in a mixture of acetic anhydride (6.3 ml) and 45% (w/v) hydrogen bromide in glacial acetic acid (15 ml). After 24 h at 22 °C the solution was diluted with chloroform (75 ml) and was poured into water (100 ml). The organic layer was washed with ice–water, and then with saturated aqueous sodium hydrogen carbonate until evolution of carbon dioxide ceased. Evaporation of the dried ($MgSO_4$) organic layer yielded 2,3,4-tri-*O*-acetyl-6-chloro-6-deoxy-α-D-galactopyranosyl bromide (7.3 g) as a gum which was used without further purification to prepare *2,4-dinitrophenyl 2,3,4-tri*-O-*acetyl-6-chloro-6-deoxy-β-D-galactopyranoside* (1.15 g, 13% from the isopropylidene derivative), m.p. 167—168.5° (from methanol), $[\alpha]_D^{20}$ +80° (*c* 1 in $CHCl_3$), by the method of Latham *et al.*[8] (Found: C, 44.05; H, 4.05; Cl, 6.75; N, 5.85. $C_{18}H_{19}ClN_2O_{12}$ requires C, 44.05; H, 3.85; Cl, 7.25; N, 5.7%). De-*O*-acetylation in methanolic hydrogen chloride [5] yielded the title *compound*, m.p. 174—176°, $[\alpha]_D^{20}$ −148° (*c* 1 in $Me_2N{\cdot}CHO$) in 67% yield (Found: C, 39.5; H, 3.8; Cl, 10.1; N, 7.1. $C_{12}H_{13}ClN_2O_9$ requires C, 39.5; H, 3.55; Cl, 9.75; N, 7.7%).

Methyl Glycopyranosides.—Fully *O*-acetylated derivatives of the following methyl β-D-glycopyranosides were made by the method of Helferich and Ost,[9] except that a tenfold excess of methanol was used (yields in parentheses): methyl 6-chloro-6-deoxy-β-D-glucopyranoside (49%), m.p. 137—139°, $[\alpha]_D^{20}$ −7° (*c* 1 in $CHCl_3$) {lit.,[10] m.p. 141°, $[\alpha]_D^{19}$ −10° (pyridine)} (Found: C, 46.3; H, 5.95; Cl, 10.75. Calc. for $C_{13}H_{19}ClO_8$: C, 46.1; H, 5.6; Cl, 10.5%); *methyl 6-chloro-6-deoxy-β-D-galactopyranoside* (58%), $[\alpha]_D^{20}$ −11° (*c* 1 in $CHCl_3$) (Found: C, 45.9; H, 5.75; Cl, 10.25%); *methyl 6-deoxy-β-D-galactopyranoside* (27%), m.p. 98—99°, $[\alpha]_D^{20}$ −6° (*c* 1 in $CHCl_3$) (Found: C, 51.7; H, 6.9. $C_{13}H_{20}O_8$ requires C, 51.3; H, 6.6%), methyl 6-deoxy-β-D-glucopyranoside (29%), m.p. 102—103°, $[\alpha]_D^{20}$ −15° (*c* 1 in $CHCl_3$) {lit.,[11] m.p. 100°, $[\alpha]_D^{20}$ −20° (in EtOH)}; and *methyl 2-acetamido-2-deoxy-β-D-glucopyranoside*, m.p. 155—157°, $[\alpha]_D^{20}$ −13° (*c* 1 in $CHCl_3$) (Found: C, 49.25; H, 6.45; N, 4.05. $C_{15}H_{23}NO_9$ requires C, 49.85; H, 6.35; N, 3.9%).

De-*O*-acetylation of the above acetyl derivatives with 0.17% (w/v) sodium methoxide in methanol (for 2 h at 22 °C) gave *methyl 6-chloro-6-deoxy-β-D-galactopyranoside* (98%), m.p. 100—103°, $[\alpha]_D^{20}$ −4.5° (*c* 1 in MeOH) (Found: C, 39.1; H, 6.3; Cl, 16.9. $C_7H_{13}ClO_5$ requires C, 39.55; H, 6.1; Cl, 16.7%); methyl 6-chloro-6-deoxy-β-D-glucopyranoside, m.p. 153—155°; $[\alpha]_D^{20}$ −29° (*c* 1 in MeOH) {lit.,[11] m.p. 157—159°, $[\alpha]_D^{30}$ −49° (*c* 1 in H_2O)}; methyl 6-deoxy-β-D-glucopyranoside, m.p. 130—132°, $[\alpha]_D^{20}$ −52° (*c* 1 in MeOH) {lit.,[10] m.p. 133°, $[\alpha]_D^{20}$ −55° (H_2O)}; methyl 6-deoxy-β-D-galactopyranoside, m.p. 119—121°, $[\alpha]_D^{20}$ −17° (*c* 1 in MeOH) {lit.,[12] m.p. 117—119°, $[\alpha]_D^{20}$ + 16° (H_2O) for the enantiomer}; and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, m.p. 205—207°, $[\alpha]_D^{20}$ −46° (*c* 1 in MeOH) {lit.,[13] m.p. 199—200°, $[\alpha]_D^{20}$ −48° (*c* 0.3 in H_2O); lit.,[14] m.p. 190—191°, $[\alpha]_D^{20}$ −42° (*c* 2 in H_2O)}.

Kinetic Measurements.—Rates of acid-catalysed hydrolysis were measured polarimetrically as described previously [15] and calculated using the Guggenheim method; 2.00M-hydrochloric acid was obtained by diluting commercial (B.D.H.) concentrated acid appropriately, with standardisation (through sodium carbonate) with standard 1.0N-sulphuric acid.

The hydrolyses of 2,4-dinitrophenyl glycosides were followed in the Unicam SP 1800 system described elsewhere: [16] initial rates of absorbance increase were measured and divided by the calculated final optical density, extinction coefficients being measured from solutions of recrystallised 2,4-dinitrophenol. The variation of extinction coefficient at both 390 and 370 nm was accurately governed by a pK_a of 3.96; this figure refers to 0.8M-potassium chloride, in

[4] I. D. Page, J. R. Pritt, and M. C. Whiting, *J.C.S. Perkin II*, 1972, 906.
[5] F. Ballardie, B. Capon, J. D. G. Sutherland, D. Cocker, and M. L. Sinnott, *J.C.S. Perkin I*, 1973, 2418.
[6] T. H. Fife and L. H. Brod, *J. Amer. Chem. Soc.*, 1970, **92**, 1681.
[7] J. B. Lee and T. J. Nolan, *Canad. J. Chem.*, 1966, **44**, 1331.
[8] H. G. Latham, E. L. May, and E. Mosettig, *J. Org. Chem.*, 1950, **15**, 884.
[9] B. Helferich and W. Ost, *Chem. Ber.*, 1962, **95**, 2612.
[10] B. Helferich and A. Schneidmuller, *Ber.*, 1927, **60**, 2002.
[11] E. Fischer and K. Zach, *Ber.*, 1912, **45**, 3761.
[12] J. Minsaas, *Rec. Trav. chim.*, 1932, **51**, 475.
[13] S. Umezawa, T. Tsuchiya, and K. Tatsuta, *Bull. Chem. Soc. Japan*, 1966, **39**, 1235.
[14] W. Roth and W. W. Pigman, *J. Amer. Chem. Soc.*, 1960, **82**, 4608.
[15] D. Cocker, L. E. Jukes, and M. L. Sinnott, *J.C.S. Perkin II*, 1973, 190.
[16] M. L. Sinnott and O. M. Viratelle, *Biochem. J.*, 1973, **133**, 83.

which all hydrolysis-rate measurements of 2,4-dinitrophenyl glycosides were done. Buffer systems were hydrochloric acid (to pH 2), 0.025M-potassium hydrogen phthalate–0.025M-hydrochloric acid (pH 2.3—3.8), 0.025M-potassium hydrogen phthalate–0.025M-sodium hydroxide (pH 4.1—5.9), 0.025M-sodium dihydrogen phosphate–0.025M-sodium hydroxide (pH 6.2—7.7), 0.025M-borax–0.025M-hydrochloric acid (pH 8.0—9.8), 0.025M-borax–0.025M-sodium hydroxide (pH 10.1—10.7), 0.025M-disodium hydrogen phosphate–0.025M-sodium hydroxide (pH 11.0—11.9), and sodium hydroxide (pH 12.2—12.7). Solutions were adjusted to the required pH at room temperature on an E1L 23A pH meter standardised with standard buffer solutions of pH 4.0, 7.0, and 10.0 (B.D.H.). Double-distilled water was used throughout.

Estimation of 2,4-Dinitrophenyl β-D-Galactopyranoside Remaining after the Initial Rate Period.—A sample (100 μl) of a solution of 2,4-dinitrophenyl β-D-galactopyranoside at pH 10.1 was added to 0.1M-sodium phosphate buffer, 10^{-3}M in magnesium chloride (3.0 ml), after 8% reaction. β-Galactosidase (Boehringer, Ltd., lot no. 7423216) (1 μl; 5 mg ml^{-1}) was added and the extinction measured after 15 min.

RESULTS AND DISCUSSION

Dependence of the Rate of Hydrolysis of 2,4-Dinitrophenyl Galactopyranoside on pH.—Figure 1 shows the first-order rate constant at 25 °C for the liberation of 2,4-dinitrophenol as a function of pH, as measured by the initial rate method. This technique was adopted since O(1) ⟶ O(2) migration [17] of the dinitrophenyl group seemed a possible complication if rates were followed to completion, whereas such aryl group migration would invalidate the initial rate approach only if it were fast enough significantly to lower the total concentration of

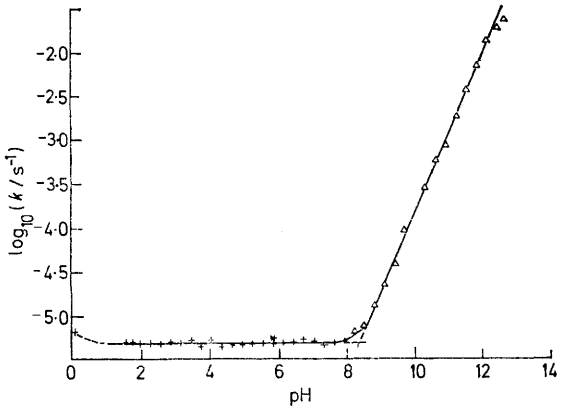
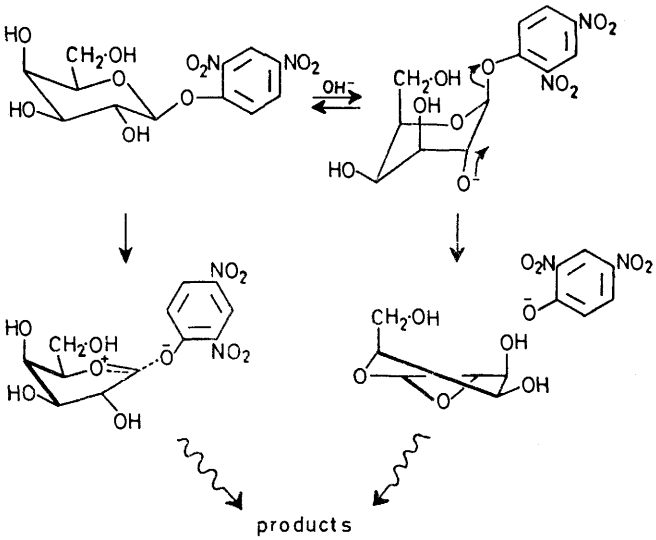


FIGURE 1 Liberation of 2,4-dinitrophenol from 2,4-dinitrophenyl β-D-galactopyranoside at 25 °C and I 0.8

glycoside during the 'initial rate' period. The near linearity of the spectrophotometer traces up to between 5 and 10% reaction would argue against such a fast rearrangement, but we independently confirmed this deduction by showing that after liberation of 8% dinitrophenolate at pH 10.1, a further 72% could be liberated at pH 7.0 by β-galactosidase: there is every reason to believe that 2-*O*-(2,4-dinitrophenyl)galactose is not a substrate for β-galactosidase.

The pH–rate profile consists of two intersecting linear portions: above pH 8.5 the rate is dependent on $[OH^-]^{0.92}$; this region probably corresponds to displacement of 2,4-dinitrophenolate by the ionised 2-hydroxy-group of the glycone (Scheme).[1] Direct S_N2_{Ar} reactions



SCHEME Pathways for the hydrolysis of 2,4-dinitrophenyl β-D-galactopyranoside

are rendered unlikely since the rate of hydrolysis of 2,4-dinitrophenolate at pH 11.9 (where the glycoside has a hydrolysis rate of *ca.* 10^{-2} s^{-1}) is only $(5.9 \pm 0.3) \times 10^{-6}$ s^{-1}; moreover direct S_N2 attack on aliphatic carbon is also possible with this substance.

The horizontal portion of the pH–rate profile represents a spontaneous S_N1 reaction of the 2,4-dinitrophenyl β-D-galactopyranoside to yield a galactopyranosyl cation and 2,4-dinitrophenolate. The kinetic insignificance of buffer catalysis under our conditions, implicit in the observation of the same spontaneous hydrolysis rate with three buffer systems, was confirmed by the absence of any rate enhancement on doubling the buffer concentration at pH 5.9. There remains the possibility that the reaction being observed is indeed bimolecular, but that water is a much more efficient general acid (or base) catalyst than its pK_a would warrant. This possibility can be excluded by consideration of the relative rates of acetolysis and spontaneous hydrolysis. This latter reaction is a mere 20 times faster at 25 °C than the acetolysis of 2,4-dinitrophenyl β-D-galactopyranoside [$k = (2.4 \pm 0.2) \times 10^{-7}$ s^{-1}] [18] corresponding, insofar as the Winstein–Grunwald equation is valid, to the low m value of 0.26. Any special catalytic role for water thus seems unlikely, unless a yet more effective catalytic role, for which there is no evidence, is also proposed for acetic acid.

Acid-catalysed Hydrolyses of Methyl Glycopyranosides.—The rates of acid-catalysed hydrolysis of the methyl glycosides (Table) show the positive entropies of activation associated with an $A1$ process (whether to yield a

[17] D. Horton and A. E. Leutzow, *Chem. Comm.*, 1971, 79.
[18] D. Cocker, Ph.D. Thesis, Bristol, 1975.

cyclic or an acyclic ion), and are as anticipated. It is noteworthy that the hydrolysis of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside shows the normal strongly positive entropy of activation. As changes in rotation, processed by the Guggenheim method (notorious for giving clean linear plots from reactions which are not first-order) were used to follow the reaction, there was some danger that the measured rate represents not rate of methoxy-group removal, but rate of de-*N*-acetylation to give the acid-inert glucosaminide. The high measured departure of methoxide (Figure 2) is only adequate (correlation coefficient 0.92) if rates of acid-catalysed methyl glycoside hydrolysis extrapolated to 25 °C are used. That this mere adequacy is a consequence of the accumulation of experimental errors in the extrapolation over 35 °C, rather than a phenomenon of mechanistic significance, is shown by an improvement in the correlation coefficient to 0.99 if *experimental* rates of methyl glycoside hydrolysis at 60 °C are plotted on the abscissa instead of the extrapolated rates.

Hydrolysis of methyl glycopyranosides in 2.0M-hydrochloric acid

Glycone	t/°C	$10^7\ k$/s^{-1}	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta S^{\ddagger}$/cal mol^{-1} K^{-1} (at 60 °C)
β-D-Glucopyranose [b]	25.0 [a]	0.276	33.8 ± 0.3	20.2 ± 0.7
β-D-Galactopyranose [b]	25.0 [a]	1.74	31.6 ± 0.3	16.6 ± 1.1
β-D-Xylopyranose [b]	25.0 [a]	1.54	33.0 ± 0.5	20.8 ± 1.5
α-L-Arabinopyranose [b]	25.0 [a]	6.39	29.8 ± 0.2	13.1 ± 0.5
6-Deoxy-β-D-glucopyranose	60.2 ± 0.1	449 ± 8.4	32.5 ± 0.6	16.9 ± 1.4
	80.1 ± 0.1	7 124 ± 130		
	25.0 [a]	1.37		
6-Deoxy-β-D-galactopyranose	60.2 ± 0.1	3 222 ± 55	30.4 ± 0.5	14.5 ± 1.3
	80.1 ± 0.1	42 790 ± 290		
	25.0 [a]	14.3		
6-Chloro-6-deoxy-β-D-glucopyranose	60.2 ± 0.1	38.7 ± 2.0	32.2 ± 0.8	11.2 ± 2.3
	80.1 ± 0.1	600 ± 18		
	25.0 [a]	0.123		
6-Chloro-6-deoxy-β-D-galactopyranose	60.2 ± 0.1	130 ± 9	33.4 ± 1.2	17.2 ± 3.5
	80.1 ± 0.1	2 234 ± 150		
	25.0 [a]	0.337		
2-Acetamido-2-deoxy-β-D-galactopyranose	60.2 ± 0.1	2 866 ± 87	29.2 ± 0.9	10.5 ± 2.5
	80.1 ± 0.1	34 220 ± 2 000		
	25.0 [a]	15.9		

[a] Extrapolated or interpolated from data at other temperatures. [b] Recalculated from W. G. Overend, C. W. Rees, and J. S. Sequeira, *J. Chem. Soc.*, 1962, 3429.

entropy of activation, however, demonstrates the insignificance of any contribution to the measured rate from such a process, since acid-catalysed amide hydrolyses show strongly negative entropies of activation (*e.g.* −20 cal mol^{-1} K^{-1} for acetamide in 2.0M-perchloric acid [19]). Piszkiewicz and Bruice [20] found only 2-acetamido-2-deoxy-glucose in the hydrolysis products of its methyl β-glyco-pyranoside.

Rates of Spontaneous Hydrolysis of 2,4-Dinitrophenyl Glycopyranosides and their Correlation with Rates of Acid-catalysed Hydrolysis of the Methyl Compounds.—On the ordinate of Figure 2 are plotted first-order rate constants for the appearance of 2,4-dinitrophenolate from the series of 2,4-dinitrophenyl glycopyranosides at 25.0 °C and pH 6.5 This pH value is 2 units away from the pH of incursion of the base-catalysed hydrolysis pathway of 2,4-dinitrophenyl β-D-galactopyranoside, and so it is unlikely that base-catalysed pathways contribute significantly to the measured rate, especially as absolute reactivities of these compounds are maximally only an order of magnitude different from that of the galactoside. Nonetheless, we confirmed that for the least and most reactive members of the series, the 6-chloro-6-deoxyglucosyl and 2-acetamido-2-deoxyglucosyl compounds, the rates obtained at pH 7.4 were identical with those obtained at pH 6.5.

The correlation obtained between the rates of unassisted departure of dinitrophenolate and proton-promoted

[19] M. M. Mhala and M. H. Jagdale, *Indian J. Chem.*, 1970, **8**, 147.

Figure 2, then, establishes the intermediacy of a common species in the reactions whose rates are plotted on ordinate and abscissa: this intermediate is the cyclic glycopyranosyl cation and so Figure 2 represents direct

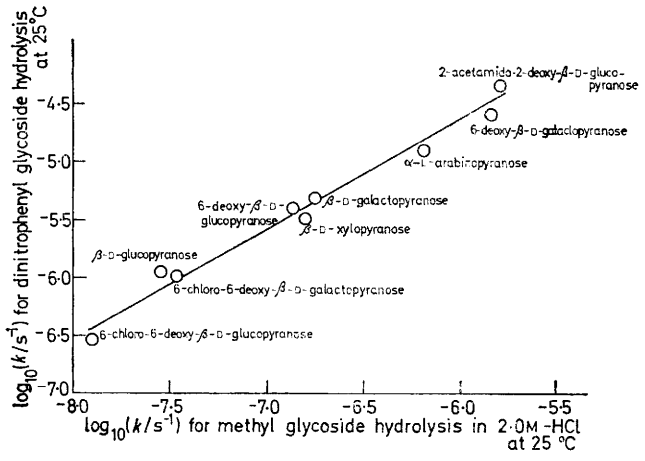


FIGURE 2 Relationship between rates of unassisted departure of dinitrophenolate and proton-assisted departure of methoxide

evidence for the general intermediacy of these cations in acid-catalysed methyl glycopyranoside hydrolysis. The gradient of the linear plot (1.01, calculated by a two-dimensional least-squares procedure) indicates that, as

[20] D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, 1968, **90**, 5844.

anticipated, the changes in glycone structure made did not effect the basicity of the methoxy-oxygen atom in this latter reaction.

The conformity of the points for the arabinosyl and 2-acetamido-2-deoxyglucopyranosyl systems to the line defined by the other glycones is noteworthy. In the α-L-arabinopyranosyl system the $^4C_1$ and $^1C_4$ chair conformers are of comparable stability,[1] and furthermore, of the glycone systems used, this is the most likely to give rise to acyclic cations. Amide group participation makes 2-acetamido-2-deoxy-β-D-glucopyranosyl the most reactive glycone used in this study, but its conformity to the relationship established by glycones without such participation means that the amide group is equally efficient in assisting the removal of methanol and 2,4-dinitrophenolate. This is in accord with the finding of Piszkiewicz and Bruice,[20] that in this system the acetamido-group was more efficient at removing methanol than bulkier neutral leaving groups. With neutral leaving groups the reverse anomeric effect dictates an equatorial or isoclinal orientation for the protonated aglycone. Amide group participation is optimised when the dihedral angle between amide and leaving group is 180°; these opposing requirements are met more readily with a small aglycone. With anionic leaving groups, of course, the reverse anomeric effect does not operate.

In acetolysis, however, the amide group is much more efficient at displacing 2,4-dinitrophenolate than its aqueous efficacy would indicate.[18] This is readily explicable by the greater importance of extensive charge-delocalisation in the transition states in the less polar solvent.

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