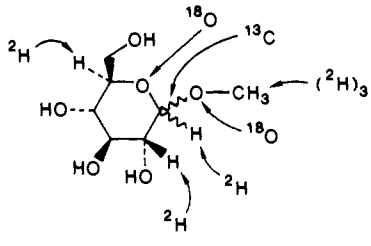
Complete Kinetic Isotope Effect Description of Transition States for Acid-Catalyzed Hydrolyses of Methyl α- and β-Glucopyranosides†

Andrew J. Bennet*‡ and Michael L. Sinnott*

Contribution from the Department of Organic Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, England. *Received February 18, 1986*

Abstract: The following kinetic isotope effects (k_{light}/k_{heavy}) have been measured by the isotopic quasi-racemate method for the hydrolyses of methyl α- and β-glucopyranosides, respectively in 2.0 M $HClO_4$ at 80 °C (α-D 1.137 ± 0.007, 1.089 ± 0.006; β-D 1.073 ± 0.003, 1.045 ± 0.004; γ-D (C5) 0.987 ± 0.002, 0.971 ± 0.003; leaving group d_3 1.006 ± 0.001, 1.015 ± 0.002; leaving group ^{18}O 1.026 ± 0.001, 1.024 ± 0.001; ring ^{18}O 0.996_5 ± 0.001, 0.991 ± 0.002; anomeric ^{13}C 1.007 ± 0.001, 1.011 ± 0.002). In conjunction with studies of the effect of added solutes on the rates of hydrolysis of various aldopyranosyl derivatives, which indicate such reactions are truely unimolecular, and model ring ^{18}O and β-deuterium effects, it is possible to locate the dihedral angles about the O5–C1 and C1–C2 bonds at the transition state using these data. If the dihedral angles so derived are used as constraints in calculations using N.L. Allinger's MM2 molecular mechanics program, transition-state structures are obtained. The geometry of these transition states stands in contradiction to the "theory of stereoelectronic control".

Glycosyl transfer is a biologically important process.[1] This being so, the physical organic chemistry of acetal and of glycoside hydrolysis has received much attention.[2,3] Although the basic features of the mechanism of acid-catalyzed hydrolysis of glycopyranosides were established over 20 years ago,[4] there remain unanswered questions with regard to the timing of bond-making and bond-breaking processes and with regard to stereochemistry, which are particularly pertinent to considerations of enzymic glycosyl transfer.[5] Since isotopic substitution does not in general alter the potential energy surface for a reaction,[6] kinetic isotope effects are the ideal method for addressing these questions. We accordingly elected to measure the kinetic isotope effects on the acid-catalyzed hydrolyses of methyl α- and β-glucopyranosides, corresponding to the isotopic substitution pattern as shown in structure I.



I

The acid-catalyzed hydrolysis of glucopyranosides is known[4] to proceed by specific acid catalysis, the exocyclic oxygen atom being reversibly protonated and then the C1–oxygen bond cleaving. Since the leaving group oxygen atom is completely protonated before glycone–aglycone fission begins, the magnitude of the leaving group ^{18}O kinetic isotope effect is a direct measure of the extent to which the glycone–aglycone bond is broken at the transition state.[7]

Any ring ^{18}O effect will arise because the endocyclic C1–O bond acquires double-bond character at the transition state as the positive charge on C1 is delocalized by one of the oxygen lone pairs of electrons. It will thus be inverse ($k_{16}/k_{18} < 1.0$), and its magnitude will reflect the degree of double-bond character of the C1–O bond and hence the charge on oxygen. The magnitude of the ^{13}C kinetic isotope effect will, in the absence of nulceophilic participation, reflect the degree to which breaking of the glycone–aglycone bond is compensated for by the tightening C1–O5 bond.[6]

There are three readily pictured phenomena which can be used in the rationalization of secondary deuterium kinetic isotope effects. The first, which applies wherever deuterium is substituted, is the inductive effect: deuterium behaves as if it were slightly electron donating with respect to hydrogen. Conventionally, this is attributed to anharmonicity of the C–H or C–D bond,[6] which results in the center of electron density of the C–D bond being slightly closer to the carbon atom than that of the C–H bond, although Williams[8] has recently been able to reproduce theoretically substantial inductive isotope effects on solvolysis reactions without invoking anharmonicity. The isotope effect arising from deuterium substitution in the leaving group of methyl glucosides will be exclusively, and that arising from deuterium substitution at C5 will be largely, due to this inductive effect; information similar to that contained in a Taft ρ^* value is contained in these effects.[9] (A small (<1%) conformational isotope effect, of uncertain origin,[10] may be superimposed on the inductve effect in the case of deuterium substitution at C5.) Inverse, inductive secondary

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‡ Present address: Smissman Laboratories, University of Kansas, Lawrence, KS 66045.

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(9) Recently it has been proposed that the apparently inductive deuterium isotope effect on the pK_a's of methylamine and methanol in fact arises because of the differing relative importance of negative hyperconjugation in the protonated, neutral, and negative species: $CH_3-{}^+OH_2 \nleftrightarrow H^-CH_2{}^{2+}OH_2$; $CH_3-OH \leftrightarrow H^-CH_2{=}{}^+OH$; $CH_3-O^- \leftrightarrow H^-CH_2{=}O$. (Williams, I. H. *J. Mol. Struct. Theochem.* **1983**, *105*, 105-117). However, "inductive" effects of deuterium of comparable magnitude are observed in systems where such a phenomenon cannot be important, e.g., the protolysis of (2,4,6-trideuteriophenyl)trimethylsilane, for which $k_{H_3}/k_{D_3} = 0.79$ (Szele, I. *Helv. Chim. Acta* **1981**, *64*, 2733-2737).

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deuterium kinetic isotope effects have been observed previously on an oxocarbonium-ion-generating reaction.[11]

The α-deuterium kinetic isotope effect is considered to arise largely from a weakening of a C–H bending vibration as the reaction center changes from sp^3 to sp^2 hybridization. The effects are traditionally used to estimate the degree of rehybridization of the reaction center at the transition state and continue to be measured for various acetal and glycoside hydrolyses,[11,12] but their use as a way of distinguishing S_N1 from S_N2 reactions of acetals has been shown to be unwarranted.[13,14]

The dominant mechanism for the appearance of a β-deuterium kinetic isotope effect is a weakening of the C–H or C–D bond consequent upon hyperconjugation into an electron-deficient p orbital on an adjacent carbon atom.[6] The magnitude of the effect, especially with freely rotating CD_3 groups, has been used as an estimate of the buildup or decrease in charge on a vicinal carbon atom.[11,15] The hyperconjugative component of the β-deuterium kinetic isotope effect is however geometry dependent, being at a maximum when the C–D bond and the electron-deficient p orbital are exactly aligned in the transition state and zero when they are orthogonal.[6] In the present case, therefore the β-deuterium kinetic isotope effect is a probe of transition-state conformation.

All the foregoing effects are anticipated to be small[6] and all except the α-deuterium effect not conveniently measurable by direct methods. We have, therefore, taken advantage of the isotopic quasi-racemate method.[16,17] This method addresses the problem of systematic errors (in temperature or concentration) inherent in any comparison of individually measured rate constants by performing the reaction of the unlabeled material and its (highly enriched) isotopically labeled antipode in the same vessel—a polarimeter cell. The optical rotation is followed as a function of time, and generally, a maximum of rotation is observed. In the ideal case, when the concentrations of the quasi-enantiomeric species are identical and there is no isotope effect on the optical rotation, any optical activity of the solution arises solely from the kinetic isotope effect as the reaction progresses. Further factors influencing the choice of the isotopic quasi-racemate method for the present study were the availability of L-glucose and extensive literature on the synthetic manipulation of D-glucose, whereby isotopes could be introduced in high enrichment at all the desired sites.

We also report the effects of added solutes on the rates of neutral hydrolysis of three aldopyranosyl derivatives as a probe of the possibility of nucleophilic involvement. We chose 2,4-dinitrophenolate and 4-bromoisoquinoline as leaving groups to avoid the uncertainties of correcting for solute effects on the preequilibrium step of an acid-catalyzed reaction. Measurement of the lifetimes of oxocarbonium ions[18,19] led to the prediction[18] that the methoxymethyl cation was "too unstable to exist" and that consequently reactions supposedly involving it were in fact bimolecular. Subsequent work[13,14] showed that the reactions of methoxymethyl derivatives in water were indeed typical preassociation reactions,[20] with a very negative β_{lg} value, very low β_{nuc} value (or low dependence on nucleophilicity, if different types of nucleophiles were compared), and α-deuterium kinetic isotope effects sometimes in the region hitherto thought typical of S_N1 reactions. Importantly however, the *order* of nucleophilic efficacy against methoxymethyl derivatives was in accord with conventional expectation. Since glycosides are much less labile than methoxymethyl compounds, it was suggested[18] that aldopyranosyl cations were "too unstable to exist": this prediction was borne out in ethanol–trifluoroethanol mixtures,[21] but the position in aqueous media was not clear.

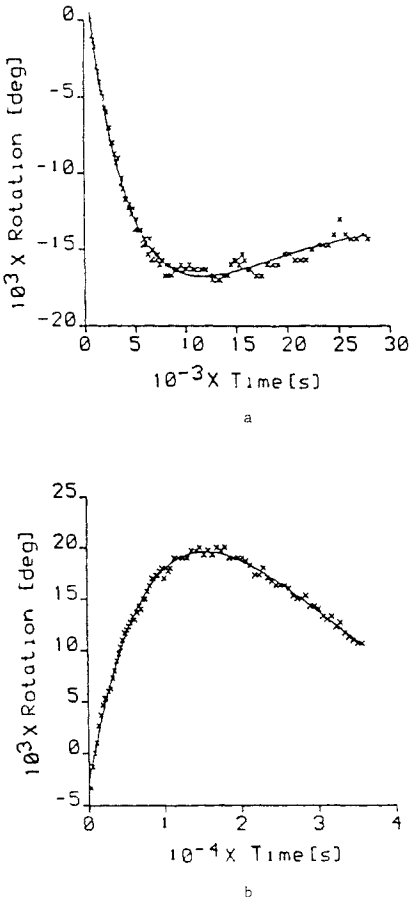

Figure 1. Time courses of optical rotation of isotopic quasi-racemates of methyl glucopyranosides (ca. 8 mg/mL each quasi-antipode) in 2.00 M $HClO_4$ at 80.0 °C. L isomer is always unlabeled. (a) Methyl [1-^{13}C]-β-glucopyranoside. (b) Methyl [1-^{18}O]-α-glucopyranoside.

Results

We found first-order rate constants for the hydrolyses of methyl α- and β-D-glucopyranosides in 2.00 M aqueous perchloric acid at 80.0 °C to be 7.33×10^{-5} and 1.41×10^{-4} s^{-1}, in good agreement with the results of previous workers.[4] The reactions were clearly first order for at least the five half-lives they followed.

A representative plot of optical rotation against time for two isotopic quasi-racemates studied is given in Figure 1. The continuous line is the computed best fit to eq 1 (for details, see

$$\alpha(t) = Ae^{-kt} + Be^{-kt/C} + \alpha(\infty) \quad (1)$$

Experimental Section). Kinetic isotope effects for each individual run are tabulated in Table I, together with calculated equilibrium effects for the protolysis of rotamers II and III of methanediol,[22]

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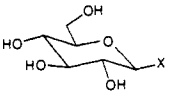
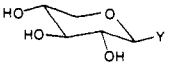
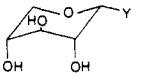
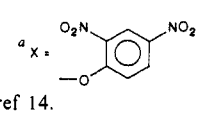
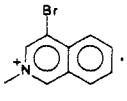
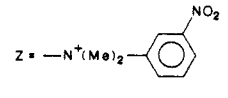
(22) Williams, I. H., unpublished results. Calculations were carried out at the 4-31G level. For estimates of the reliability of E.I.E. calculations carried out with a similar (3-21G) basis set see: Yamataka, H.; Nagase, S.; Ando, T.; Hanafusa, T. *J. Am. Chem. Soc.* **1986**, *108*, 601–606. For the method of calculation of normal-mode vibrational frequencies see: Haut, R. F.; Levi, B. A.; Hehre, W. J. *J. Comput. Chem.* **1983**, *4*, 499–505.

Table I. Kinetic Isotope Effects for the Acid-Catalyzed Hydrolyses of Methyl α- and β-Glucopyranosides in 2.0 M $HClO_4$ at 80.0 °C

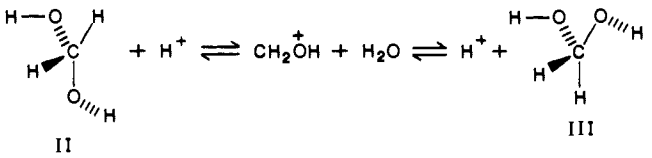
site and isotope of substitution	methyl α-glucoside KIE	methyl α-glucoside av	methyl α-glucoside calcd EIE	methyl β-glucoside KIE	methyl β-glucoside av	methyl β-glucoside calcd EIE
	1.1327	1.137 ± 0.007	1.188	1.0877	1.089 ± 0.006	1.137
	1.1460			1.0866		
α-D	1.1298			1.0897		
	1.1333			1.0959		
	1.1433			1.0927		
				1.0790		
	1.0695	1.073 ± 0.003		1.0457	1.045 ± 0.004	
	1.0746					
β-D	1.0771			1.0489		
	1.0713			1.0403		
	0.9875	0.987 ± 0.002		0.9752	0.971 ± 0.003	
γ-D	0.9884			0.9686		
	0.9830			0.9690		
	0.9878			0.9702		
	0.9884					
	1.0050	1.006		1.0153	1.015	
leaving group D_3	1.0065			1.0152		
	1.0055			1.0156		
	1.0251	1.026 ± 0.001	1.019	1.0240	1.024 ± 0.001	1.021
leaving group ^{18}O	1.0259			1.0240		
	1.0250			1.0250		
	1.0274			1.0229		
	0.9964	0.996_5 ± 0.001	0.978	0.9943	0.991 ± 0.002	0.976
	0.9983			0.9911		
ring ^{18}O	0.9968			0.9904		
	0.9954			0.9900		
	0.9959			0.9904		
	1.0064	1.007 ± 0.001	1.004	1.0099	1.011 ± 0.002	1.004
anomeric ^{13}C	1.0086			1.0078		
	1.0064			1.0122		
	1.0072			1.0131		
				1.0106		
				1.0114		

Table II. Effects of 1.0 M Added Solute on Rates of Neutral Hydrolysis of Various Derivatives

solute	 $10^5k/s^{-1}$ at 25.0 °C	CH_3OCH_2X $10^4k/s^{-1}$ at 39.0 °C[a]	 $10^5k/s^{-1}$ at 80 °C	 $10^5k/s^{-1}$ at 80 °C	CH_3OCH_2Z $10^5k/s^{-1}$ at 25.0 °C[b]
none	0.74	7.2	2.90	10.8	
$(NH_2)_2C{=}S$	0.74	15.5 (in M $NaClO_4$)	2.72	12.0	
NaF	0.77	11	4.07	13.8	38.2
NaCl	0.82	12.6	2.37	10.9	63.7
NaBr			2.09	10.6	110
NaI	0.73	23			302
$NaClO_4$	0.55	6.1	1.96	7.09	24.1
NaN_3	1.03	20	2.62	19.7	302
NaOAc	1.04	10.1	2.90	14.4	48
$NaNO_3$			2.18	9.39	

[a] X =  , Y =  , Z =  Calculated from data in ref 13. See also text. [b] Calculated from data in ref 14.

rotamer II being a model for methyl α-D-glucopyranoside in the ground-state $^4C_1$ conformation and rotamer III being a model for its β anomer in the same ground-state conformation.



II III

Since the kinetic isotope effect measured in an isotopic quasi-racemate experiment can be critically affected by the chemical purity of both quasi-enantiomers, each effect in Table I was measured with more than one batch of substrates (in fact a set of anomalous results was traced back to a single batch of methyl β-L-glucopyranoside). To confirm that the quasi-racemate method did not in some unsuspected way introduce an error proportional to the size of the effect, it was shown by comparison of separate runs that the α-deuterium kinetic isotope effect on methyl α-glucoside hydrolysis was indeed 1.13 (13%).

Table II gives first-order rate constants for neutral hydrolysis of various aldopyranosyl derivatives in the presence of various salts and of the neutral nucleophile thiourea. For comparison, reported second-order rate constants for reactions of methoxymethyl 2,4-dinitrophenolate[13] and *N*-(methoxymethyl)-*N*,*N*-dimethyl-*m*-nitroanilinium ion[14] with nucleophiles have been used to calculate

expected first-order constants at molar concentrations of solute. This will somewhat underestimate the true value in the case of the 2,4-dinitrophenolate, since the variation of rate constants with solute concentration is nonlinear.[13] (The pH at which these measurements were made (6.3) is at least 2 pH units away from the pH of incursion of base-catalyzed pathways for their hydrolysis[23,24] and 5 pH units from the pH where acid catalysis of 2,4-dinitrophenyl β-D-galactopyranoside[23] is apparent.)

Discussion

It is immediately apparent from the data in Table II that, although the nature of the added solute does have a pronounced effect on the rate of hydrolysis, this effect, if anything, varies with basicity of the anion rather than its nucleophilicity, and the neutral nucleophile thiourea has no effect. The behavior of aldopyranosyl systems is thus completely different from that of methoxymethyl systems, and we must therefore conclude that the reactions of aldopyranosyl derivatives in water are truly S_N1 reactions, with no preassociation. This conclusion accords with recent work of Kresge et al.,[25] who have estimated the lifetime of tertiary alkyl cations (e.g., 2-methyl-2-butyl) as $\sim 10^{-10}$ s, just long enough for the species to have a real existence, and work of this group some years ago,[26] which indicated that tertiary alkyl cations and glycopyranosyl cations have comparable stabilities in water.

The change in behavior, from preassociation in ethanol–trifluoroethanol mixtures,[21] to true unimolecularity in water, must arise from the greater polarity of water favoring processes with greater charge development at the transition state. Kresge et al.[11] have recently suggested a similar change in mechanism in reactions formally generating the 1-ethoxyethyl cation, consequent upon addition of dioxane to an aqueous reaction medium.

Two questions arise from the data of Table II: what is the origin of the observed, quite large, salt effects, and why are the reactions of aldopyranosyl derivatives clearly unimolecular in water when the ca. 10^2-fold faster reactions[2] of methoxymethyl derivatives are bimolecular preassociation reactions. The answer to the second question could well be that primary, unhindered methoxymethyl derivatives react faster than aldopyranosyl derivatives precisely because of the nucleophilic assistance which does not occur on the relatively hindered aldopyranosyl derivatives, in which the reaction center is in a six-membered ring and that in a nonnucleophilic medium aldopyranosyl ions could well be more stable than methoxymethyl cations.

Loss of a phenolate ion from a 2-(aryloxy)tetrahydropyran is about 10^6 times faster than loss of the same phenolate ion from an aryl β-D-glucopyranoside.[2] Salt effects on the hydrolysis of 3,4-dinitrophenoxytetrahydropyrans are small and show no marked dependence on the nature of the anion,[13] and the reactions of (aryloxy)tetrahydropyrans are cleanly unimolecular.[27] Although the equatorial substituents in the glucopyranosyl derivatives will have some kinetic effect, by anchoring the pyranose ring in the $^4C_1$ chair conformation, studies with various different aldopyranosides[23] indicate that such effects are comparatively modest. Most of the 10^6-fold reactivity difference arises from the electron-withdrawing inductive effect of the hydroxyl groups, which destabilize the oxocarbonium ion. The inductive effect of a hydroxyl group will, to some degree, depend on the group which accepts a hydrogen bond from it—the more basic the hydrogen bond acceptor, the less the electron-withdrawing inductive effect of the hydroxyl group. Stronger or weaker hydrogen bonding by the anions of the salts in Table II could therefore account for the patterns of hydrolytic reactivity observed, particularly striking amongst which is the reversal of the nucleophilic reactivity order of the halides ($F^- > Cl^- > Br^-$).

Whatever the origin of the observed salt effects, it is clear that they do not have their origin in a nucleophilic interaction and that, therefore, for the present purposes the position of an incoming nucleophilic water molecule need not be considered when considering transition states for methyl α- and β-glucoside hydrolyses which account for observed isotope effects (Table I). It is convenient to examine first the leaving group ^{18}O and leaving group secondary deuterium effects. Since the reaction is specific, rather than general acid catalyzed, the proton is known to be completely attached to the leaving oxygen atom at the transition state, and the only unknown is the degree of C–O cleavage. This cleavage must be nearly complete, since observed effects are close to equilibrium effects calculated for the model reaction by ab initio methods (Table I) and to equilibrium effects calculated from measured vibration frequencies for conversion of a nitrophenyl β-glycopyranoside to nitrophenol and a glucopyranosyl cation (1.024).[7] (The small difference in ^{18}O kinetic isotope effects for the two anomers, if real, would accord with the detectably different β_{lg} values obtained for acid-catalyzed hydrolyses of aryl α- and β-glucopyranosides (0.01 ± 0.01[28] and 0.27 ± 0.04,[29] respectively); both differences are in the sense of more bond breaking with the α-anomer). The secondary deuterium effects are small, confirming that the charge on the leaving group oxygen is roughly the same in the ground state and in the transition state and that, therefore, since in the transition state the oxygen is protonated, the C–O bond is largely cleaved. If the effects were purely inductive, the effects would be zero in the α case and slightly inverse in the β case, to accord with β_{lg} values. However, the effects are so small (0.2% and 0.5% per deuterium) that extended considerations of their magnitude are unwarranted.

The ^{13}C effects are greater than the equilibrium effects calculated for the model reaction. This means that, even though the leaving group has virtually left at the transition state, electronic and geometrical reorganizations of the glycone lag some way behind. The ^{13}C effects, although bigger than calculated effects, are nonetheless small and in agreement with the work of Goiten et al.[30] who report a value of k_{12}/k_{14} of 1.010 ± 0.006 for the α-carbon effect on the loss of pyrophosphate from 5-phosphoribosyl pyrophosphate. This value, corresponding to a ^{13}C effect of ca. 0.5%,[31] is well in-line with our results. S_N2 reactions are commonly associated with high α-carbon kinetic isotope effects[6] (e.g., $k_{12}/k_{13} = 1.08$[32] for reaction of solvated methoxide ion with a methyl sulfonium salt and k_{12}/k_{14} between 1.12 and 1.16 for reaction of *N,N*-dimethylanilines with benzyl arenesulfonates[33]), so the low value we observe confirms the conclusions from the data in Table II that there is no nucleophilic assistance.

The results with ^{18}O in the ring were quite unexpected. The effects are both very small—well under half the calculated equilibrium effects—and radically different as between the anomers. As expected, both effects are inverse, but there is much more double-bond character in the transition state for β-glucoside hydrolysis than for α-glucoside hydrolysis. This implies more buildup of charge on the ring oxygen in the case of the β-glycoside, and this picture is confirmed by the secondary effects of deuterium at C5, the inverse effect, of inductive origin, being twice as large in the β case as in the α case. Given that the exocyclic carbon–oxygen bond is largely broken at the transition state and that a nucleophile is not involved, the smallness of the ring ^{18}O effects can only have its origin in reduced orbital overlap, largely due

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(25) (a) Chiang, Y.; Chwang, W. K.; Kresge, A. J.; Powell, M. F.; Szilagyi, S. *J. Org. Chem.* **1984**, *49*, 5218–5224. (b) Chiang, Y.; Kresge, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 6363–6367.
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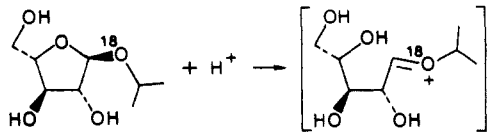
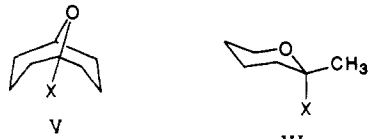
(32) Wong, O. S.-L.; Schowen, R. L. *J. Am. Chem. Soc.* **1983**, *105*, 1951–1954.
(33) Ando, T.; Tanabe, H.; Yamataka, H. *J. Am. Chem. Soc.* **1984**, *106*, 2084–2088.

to the constraints imposed by the ring. The acid-catalyzed hydrolysis of 2-propyl α-arabinofuranoside (IV), which proceeds via



IV

a ring-opening pathway,[34] exhibits a kinetic isotope effect of −1.2%[35] as a result of ^{18}O substitution in the indicated atom, well above even the result for methyl β-glucopyranoside. Since the low ring ^{18}O kinetic isotope effects are attributable to poor conjugation, they contain conformational information. It can be deduced from the work of Briggs et al.[36] that only the p-type lone pair on oxygen is effective at stabilizing a positive charge on the adjacent carbon atom by forming a carbon–oxygen double bond. These authors showed[36] that compounds of structure V, which



V VI

are constitutionally incapable of giving an oxocarbonium ion stabilized by a p–p, rather than a sp–p interaction, lost X^- some 3-fold slower than their carbocyclic analogues.[37] Therefore, overlap from an sp lone pair of electrons just fails to counterbalance the inductive effect of oxygen, which can be estimated to decelerate an oxocarbonium-ion-generating reaction by around a factor of 10^3.[38] This modest effect of sp–p overlap can be contrasted with the relative rates of departure of X^- from compounds V and VI—about 10^{13}.[37] Since the overlap of p orbitals is proportional to the square of the cosine of the dihedral angle between them,[39] and only p–p overlap will contribute significantly to the observed ring ^{18}O kinetic isotope effect, we can write eq 2 where ω is the

$$\ln (k_{16}/k_{18}) = \ln (k_{16}/k_{18})_{max} \cos^2 \omega \qquad (2)$$

dihedral angle between the empty p orbital on Cl and the p-type lone pair on the ring oxygen and $(k_{16}/k_{18})_{max}$ is the maximum effect possible in a system in which the oxocarbonium ion system carries the same degree of positive charge as the present one but which possesses optimum geometry for p–p overlap. Since the leaving group ^{18}O effects of Table I indicate that the glycone–aglycone bond is largely broken at the transition state the glycone must carry a nearly full positive charge and therefore the calculated equilibrium ^{18}O effects in Table I will be close to (though, if accurate, necessarily further from unity than) $(k_{16}/k_{18})_{max}$. If these equilibrium effects are used to calculate ω, values of 66° and 52°, respectively are calculated for the transition states for methyl α- and β-glucopyranoside hydrolyses.

The ^{18}O effect on the hydrolysis of the glycoside IV, which gives an acyclic oxocarbonium ion, can give an estimate of $(k_{16}/k_{18})_{max}$ at the other extremum so that we can locate ω as having to lie between two values for each methyl glucoside. The values of ω obtained from this second model are 54° and 24° for the α- and β-anomers, respectively. However, there is no reason for thinking the acyclic *arabino* system is geometry optimized for oxocarbonium ion formation, merely a reasonable presumption that the geometry is better than in the cyclic cases. True values of ω are thus likely to be much closer to the limits estimated from calculated equilibrium effects than from the experimental effect for hydrolysis of compound IV.

The β-deuterium kinetic isotope effects of Table I enable another geometrical parameter of the transition states to be located, this time within fairly narrow limits. The hyperconjugative component of the β-deuterium kinetic isotope effect varies as the square of the cosine of the dihedral angle (θ) between the electron-deficient p orbital and the C–H or C–D bond. One can therefore write eq 3 where $(k_H/k_D)_{max}$ is the hyperconjugative

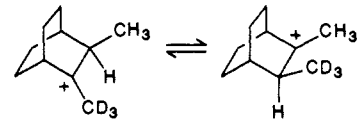
$$\ln (k_H/k_D) = \cos^2 \theta \ln (k_H/k_D)_{max} + \ln (k_H/k_D)_i \qquad (3)$$

isotope effect arising from an optimally aligned C–D bond and $(k_H/k_D)_i$ is the inductive effect of a single deuterium. The problems of the geometry of the model used to estimate the maximal isotope effect disappear in the case of β-deuterium effects since data are available on systems with a freely rotating methyl group. It has been shown[40] that a freely rotating CD_3 has a hyperconjugative isotope effect of $\exp(^3/_2 \ln (k_H/k_D)_{max})$; we can therefore write[41] eq 4. The value of $(k_H/k_D)_i$ can be taken as

$$\ln (k_H/k_D)_{max} = {}^2/_3[\ln (k_{CH_3}/k_{CD_3}) - 3 \ln (k_H/k_D)_i] \qquad (4)$$

0.96;[8] derived values of θ are in any event relatively insensitive to this small correction. They are also relatively insensitive to the model used to estimate $(k_H/k_D)_{max}$, and it transpires that θ can be located within quite narrow ranges.

Even though the glycone carries a nearly full positive charge in the transition state for methyl α- and β-glucopyranoside hydrolysis, some charge is delocalized on to the ring oxygen atom. Therefore, the charge at Cl must be less than that on a full-blown simple carbonium ion at equilibrium. Siehl and Walter[42] reported the equilibrium constant for the interconversion of VIIa and VIIb



VIIa VIIb

as a function of temperature, and their data extrapolate to an equilibrium constant of 1.206 at 80 °C. This results in an estimate of a maximum value of θ of 50° for the β transition state and 43° for the α transition state.

The charge at Cl must also be greater than that in a much faster hydrolysis of a more labile acetal which proceeds via a much more stable oxocarbonium ion. If we use the kinetic effect k_{CH_3}/k_{CD_3} for the acid-catalyzed hydrolysis of acetaldehyde diethyl acetal,[43] lower limits of θ of 41° and 31° are obtained in the β and α cases, respectively.

These limits for θ are narrower than the limits for ω obtained from the ring ^{18}O effects, but there is no strong probability that θ lies toward one limit, as there is with ω.

The α-deuterium kinetic isotope effects for both methyl α- and β-glucosides are about two-thirds of the calculated equilibrium effects (Table I). Since the ring ^{18}O and leaving group ^{18}O effects indicate that leaving group departure and C=O$^+$ bond formation are not synchronous, the simplest rationalization of this effect, that at the transition state the anomeric carbon atom is about two-thirds rehybridized, is not valid. It has recently been pointed out[8] that the inductive effect of deuterium is very commonly unjustifiably neglected in considerations of secondary isotope effects on solvolysis reactions. (The present work, the −2.9% effect of γ-deuterium in β-methyl glucoside hydrolysis, supports this contention.) The inductive reduction of an α-DKIE will of course

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(38) Estimated from the relative rates of hydrolysis of glycosides and their 2-deoxy analogues,[2] and the common assumption that inductive effects are attenuated by a factor of approximately 3 per carbon removed from the reaction center.
(39) Burkett, U.; Allinger, N. L. *Molecular Mechanics*; ACS Monograph 177; American Chemical Society: Washington, DC, 1977.
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(43) The datum in ref 11, which pertains to 25 °C, was corrected to 80 °C on the assumption that $T \ln (k_H/k_D)$ = constant.

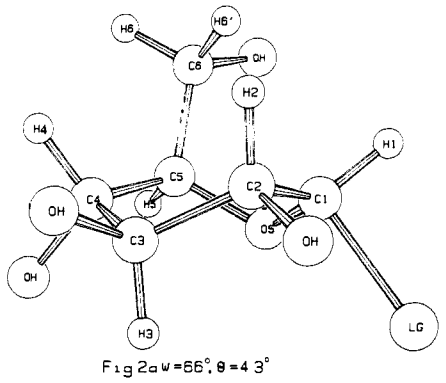


Fig 2a ω=66°, θ=43°

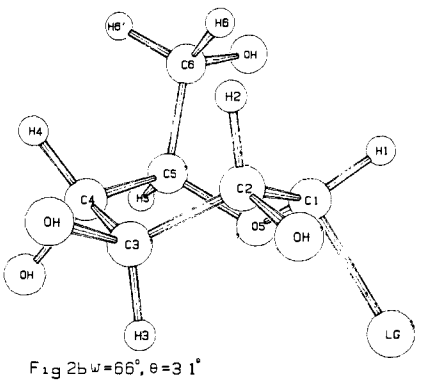


Fig 2b ω=66°, θ=31°

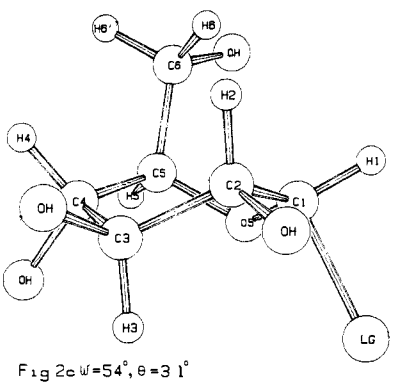


Fig 2c ω=54°, θ=31°

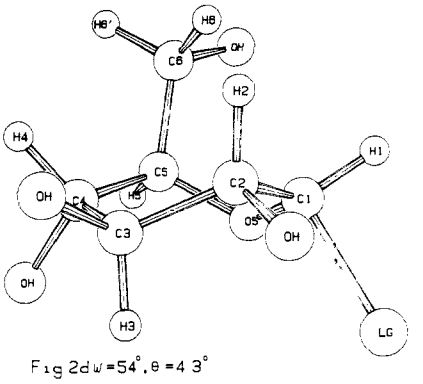


Fig 2d ω=54°, θ=43°

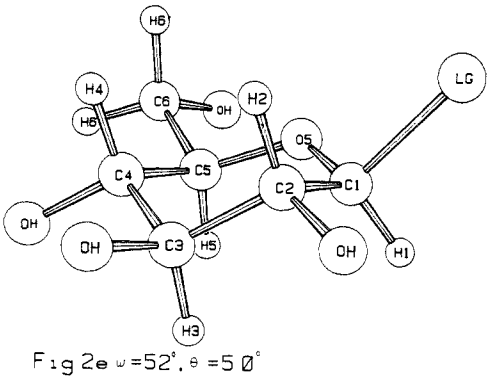


Fig 2e ω=52°, θ=50°

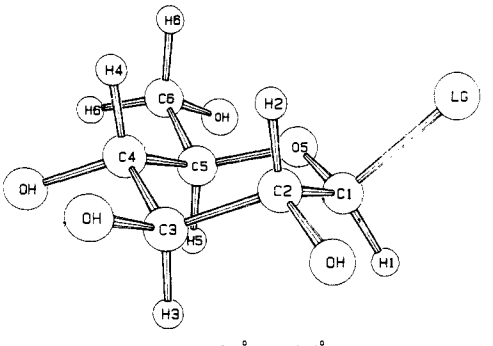


Fig 2f ω=52°, θ=41°

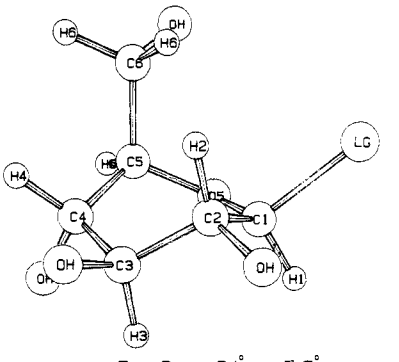


Fig 2g ω=24°, θ=50°

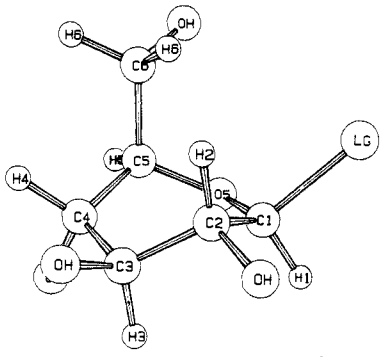


Fig 2h ω=24°, θ=41°

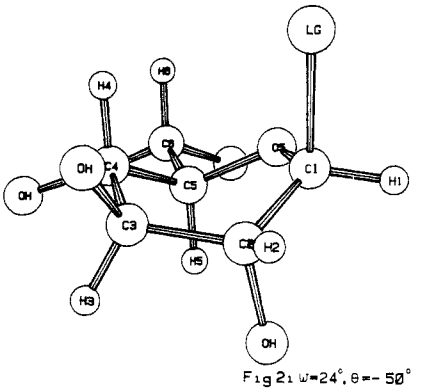


Fig 2i ω=24°, θ=-50°

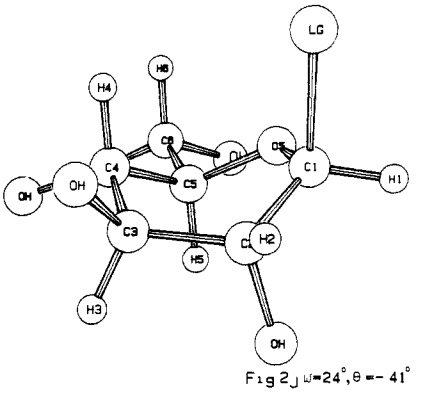


Fig 2j ω=24°, θ=-41°

Figure 2.

increase with the effective positive charge at the reaction center, and our heavy atom effects show that this is much higher than would be expected if formation of the C=O$^+$ double bond and cleavage of the glycone–aglycone bond were exactly in step.

Since this inductive reduction of the α-deuterium kinetic isotope effect will be greater in the α case than in β, we consider that the reaction center is essentially completely rehybridized in the transition state for methyl α-glucopyranoside hydrolysis, and nearly so in the case of the β-anomer.

With estimates of the dihedral angles θ and ω, and of the degree of rehydridization of the anomeric center, available from the experimental data of Table I, it is possible to calculate the preferred conformation adopted by the rest of the two transition states, using these experimental geometrical parameters as constraints. The upper and lower limits of θ and ω generate four sets of constraints per anomer; the energy-minimized structures corresponding to them were calculated by using Allinger's MM2 molecular mechanics program.[44] The 10 low-energy structures

so generated are displayed in Figure 2.

Only one structure of low energy was found for each pair of θ and ω values for the transition state for methyl α-glucoside hydrolysis. Moreover the transition states were all qualitatively similar—a $^1S_3$ skew boat flattened toward planarity (Figure 2a–d).

When the more probable values of ω are used to calculate the transition state for methyl β-glucopyranoside hydrolysis, a single structure was obtained for each value of θ (Figure 2e and 2f). These were both clearly qualitatively similar, and $^4C_1$ chairs flattened somewhat toward the $^4H_3$ half-chair. When the less plausible values of ω, derived from taking the experimental ^{18}O effect for isopropyl arabinofuranoside hydrolysis as a model for $(k_{16}/k_{18})_{max}$, are used to calculate transition-state structures, two low-energy structures are generated. One of them is half-chair-like (sofa, Figure 2i and 2j) and this is in both cases the lowest energy structure; lying slightly above it is a flattened $^{2,5}B$ boat (Figure 2g and 2h).

The transition states of Figure 2 stand in flat contradiction to the "theory of stereoelectronic control",[45] or "antiperiplanar lone pair hypothesis"[3,46] but accord with antecedent and long-held views about patterns of reactivity of Cl of sugars. In the course of their acid-catalyzed hydrolyses, the leaving group of methyl α- and β-glucoside is protonated, and since the reaction is specific acid catalyzed, the leaving group oxygen must carry a full positive charge for a meaningful length of time. It is known that the reverse anomeric effect is powerful enough in organic solvents to change the conformation of (tetra-*O*-acetyl-α-D-glucopyranosyl)imidazole to a boat when the imidazole is protonated;[47] we have also suggested that the $^1S_3$ conformation is the ground-state conformation of deprotected α-D-glucopyranosylpyridinium ions in water.[24] On the basis of these experimental precedents, therefore, it is to be expected that protonated α-methyl glucoside would adopt a boat conformation. The transition state of Figure 2a–d which represents a flattened $^1S_3$ conformation, is thus, on the basis of experimental precedent, not too surprising.

The reverse anomeric effect will freeze the conformation of protonated methyl β-glucopyranoside still more firmly in the $^4C_1$ conformation: the transition-state structure is accordingly a flattened version of this ring conformation.

We consider that the transition states depicted in Figure 2 are fatal to the idea that leaving groups from sp^3-hybridized carbon substituted with one or more heteroatoms depart from conformations in which the leaving group makes a dihedral angle of 180° with an sp^3 lone pair on the heteroatom—the antiperiplanar lone pair hypothesis (ALPH). The conformations required of hydrolyzing glycosides by ALPH have been clearly enunciated by its originator "α-glycosides must hydrolyze via their ground-state conformation, whereas β-glycosides must first assume a boat conformation in order to fulfill the stereoelectronic requirement".[48] Although it is not possible to subject a literal reading of this statement to experimental test, because of the Curtin–Hammett principle,[49] it can be understood as requiring α-glycosides to hydrolyze through transition states resembling $^4C_1$ chairs, but β-glycosides to hydrolyze through boat-like transition states. In fact precisely the opposite happens.

If ALPH were operational in this case, an angle of 180° (antiperiplanar) could be predicted for ω at the transition state for the hydrolysis of both methyl α- and β-glucosides, which clearly should give maximum ring ^{18}O effects (eq 2). Similarly, ALPH would predict an angle of 180° for θ ($^4C_1$-like transition state) for the hydrolysis of methyl α-glycoside. Using eq 3, a β-deuterium isotope effect of 1.16 can be calculated with this value of θ for an oxocarbonium-ion-type transition state. Since there are a number of boat conformations which fulfill the requirements of ALPH, a value of θ cannot be estimated with any accuracy for the methyl β-glucoside case.

We have pointed out elsewhere that the experimental results which led to the formulation of ALPH can, with greater economy, be regarded as manifestations of the principle of least nuclear motion,[24] which is not expected to apply to reactions with late transition states, such as glycoside hydrolysis. ALPH as a guide to reactivity[50] is misleading.

Experimental Section

A. Synthesis of Labeled Materials. (i) General. Since purity, and particularly anomeric purity, of the various methyl α- and β-glucopyranosides is of key importance for reliable kinetic measurements, care was taken that any mixture of isomers produced could be readily recrystallized to give the desired isomer. Thus, Fischer glycosidation with H^+ resin[51] was used to make all the methyl α-glucopyranosides except the 2-^2H and 1-^{18}O compounds, since the α-anomer (mp 167–169 °C) could readily be separated from contaminating β-anomer (mp 112.5–113.5 °C) by recrystallization from methanol or ethanol. Stereochemical purity of methyl β-glucopyranosides was ensured by careful recrystallization of the 2,3,4,6-tetra-*O*-acetyl derivative (mp 105–106 °C) or the 2,3,4,6-tetra-*O*-benzoyl derivative (mp 162.5–163.5 °C), as well as the glycoside itself, obtained by Zemplén deacetylation[52] of the pure acylated precursors.

Methyl α- β-L-glucopyranosides were made from commercially available L-glucose by Fischer glycosidation and via 2,3,4,6-tetra-*O*-benzoyl-α-L-glucopyranosyl bromide,[53] respectively.

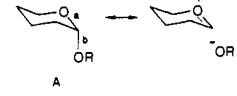
All isotopically labeled methyl glucopyranoses, except methyl 2-[^2H]-β-D-glucopyranoside (11 steps from glucose) were characterized by ^{13}C NMR and all acylated methyl glucopyranosides by ^1H NMR. All samples of methyl α-glucopyranosides used for kinetic work had melting points within the range 167–169 °C and all methyl β-glucopyranosides had melting points within the range 112.5–113.5 °C.

(ii) Deuterium at C1. D-Glucono-δ-lactone was reduced with sodium borodeuteride under the conditions of Bhattacharjee et al.;[54] crude 1-[^2H]-D-glucose was converted to methyl α-D-glucopyranoside by Fischer glycosidation and to its β-anomer via tetrabenzoylglucosyl bromide.[53,55]

(iii) Deuterium at C2. Reaction of 2,3,4,6-tetra-*O*-acetyl-2-[^2H]-α-D-glucopyranosyl bromide[56] (0.51 g) in dry methanol (10 mL) with mercuric cyanide (160 mg) and mercuric bromide (224 mg) for 6 h at 22 °C afforded, after filtration from mercuric salts, the methyl 2,3,4,6-

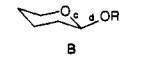
(44) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.
(45) (a) Deslongchamps, P. *Pure Appl. Chem.* **1975**, *43*, 351–378. (b) Deslongchamps, P. *Tetrahedron* **1976**, *31*, 2460–2490.
(46) We consider the term "theory of stereoelectronic control" confusing since it could apply to any electronic rationalization of any stereochemical phenomenon in any area of chemistry.
(47) Lemieux, R. U. *Pure Appl. Chem.* **1971**, *27*, 527–547.
(48) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: Oxford, 1983; p 39.
(49) Reviewed by: Seeman, J. I. *Chem. Rev.* **1983**, *83*, 83–134. In the present context, the principle means that statements can only be made about ground states and transition states, not about reactive ground-state conformations.

(50) As a guide to *ground states*, ALPH has received wide-ranging theoretical support. Lengthening of bond b and shortening of bond a in structure



A

A and stabilizing the axial orientation of electronegative substituents of C2 of tetrahydropyranyl derivatives are not unreasonably rationalized by the proposal of interaction between the p-type lone pair and the C–X σ^* orbital. However, even in respect of ground-state phenomena there are problems. ALPH (unlike the antecedent electrostatic explanation) does not explain the reverse anomeric effect, and recent work by Briggs et al. (Briggs, A. J.; Glenn, R.; Jones, P. G.; Kirby, A. J.; Ramaswamy, D. *J. Am. Chem. Soc.* **1984**, *106*, 6200–6206) has found that whereas indeed in axial tetrahydropyranyl derivatives a shortens and b lengthens according to eq i and ii, two thirds of the effect is also observed with equatorial compounds of type B, in which eq iii and iv hold.



B

$$R(a)/\text{Å} = 1.364 + (3.639 \times 10^{-3})pK_a(\text{ROH}) \quad \text{(i)}$$
$$R(b)/\text{Å} = 1.493 - (6.495 \times 10^{-3})pK_a(\text{ROH}) \quad \text{(ii)}$$
$$R(c)/\text{Å} = 1.394 + (2.14 \times 10^{-3})pK_a(\text{ROH}) \quad \text{(iii)}$$
$$R(d)/\text{Å} = 1.456 - (4.76 \times 10^{-3})pK_a(\text{ROH}) \quad \text{(iv)}$$

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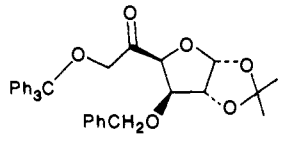
tetra-*O*-acetyl-2-[^{2}H]-β-D-glucopyranoside. Its α-anomer was obtained by reaction of methanol with dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride,[57] followed by hydrolysis of the oxime function of the methyl uloside oximes, reduction of the methyl ulosides with sodium borodeuteride, and acetylation, essentially as described by Lemieux et al.[58] In our hands the methyl 2,3,4,6-tetra-*O*-acetyl-2-[^{2}H]-α-D-glucopyranoside (15%) so produced was associated with a similar quantity of the β-manno stereoisomer, most of which crystallized on workup and the remainder of which was separated by flash chromatography on silica gel (1:1 ethyl acetate–light petroleum as solvent).

(iv) **^{18}O and Deuterium in the Leaving Group.** 1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranose (9.72 g) was reacted with [^{18}O]methanol[59] or CD_3OD (1.01 mL) with stannic chloride in dichloromethane as described by Hanessian and Banoub.[60] The methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside could be cleanly anomerized to the α-anomer by the method of Lindberg.[61]

(v) **^{13}C at the Anomeric Center.** The extensive data of Blazer and Whaley[62] on the Kiliani reaction of D-arabinose with cyanide ion led us to choose the following protocol. Sodium cyanide (1.0 g, 91% atom ^{13}C, Prochem batch No. 60 × 11) and sodium hydroxide (0.84 g) in distilled water (100 mL) were added to a solution of D-arabinose (3.02 g) and sodium hydrogen carbonate (1.7 g) in distilled water (100 mL). The solution was left open to the atmosphere (in a well-ventilated fume hood) for 92 h and then purged with nitrogen for 4 h and concentrated to 15 mL under reduced pressure. Isolation of barium gluconate, largely free from barium mannonate, and conversion of the barium 1-[^{13}C]gluconate to 1-[^{13}C]-D-glucono-δ-lactone followed standard procedures,[63] and the lactone was reduced to glucose with borane in tetrahydrofuran.[54]

(vi) **Deuterium and ^{18}O at C5.** The key intermediate was compound VIII.[64] Reduction of the ketone with lithium aluminum hydride gives D-gluco and L-ido epimers in 9:1 ratio.[64] We found that after removal



VIII

of the benzyl and triphenylmethyl groups from the alcohol with lithium in liquid ammonia,[64] the 1,2-isopropylidene-α-D-glucofuranose could be readily crystallized from its L-ido epimer in ethyl acetate. The isopropylidene group of the purified monoacetone glucose could be removed by 24-h reflux in 50% aqueous acetone in the presence of a 2-fold weight excess of Duolite C-225 (H^+ form); the crude lyophilized product was used directly. To introduce deuterium at C5, therefore, ketone VIII was reduced with lithium aluminum deuteride, to introduce ^{18}O the ketone was exchanged with $H_2{}^{18}O$ and then reduced with lithium aluminum hydride. For the exchange, ketone VIII (10 g) was dissolved in anhydrous tetrahydrofuran (35 mL), and to this was added water (0.33 mL) (70 atom % ^{18}O, Amersham batch No. PB1/12) followed by dry hydrogen chloride gas. The exchange (at ambient temperature) was followed by infrared ($\nu_{C=^{16}O}$ 1734 cm^{-1}; $\nu_{C=^{18}O}$ 1702 cm^{-1}). When equilibrium had been reached silver oxide (100 mg) was added, the solution was filtered, and the solvent was evaporated. The exchange was carried out again with 70 atom % $H_2{}^{18}O$ and then twice with 98 atom % $H_2{}^{18}O$ (Amersham, batch No. 1). After the final exchange the ^{18}O-enriched ketone was reduced immediately.

(vii) **Isotopic Enrichments.** All deuterium enrichments were estimated to be >95% from the lack of a signal in the ^{1}H NMR spectrum and also by the absence of the corresponding carbon signal in the (proton-decoupled) ^{13}C NMR spectrum. The enrichments of the anomeric carbons (88.1%) and the ring oxygen (80.0%) were estimated from the M-31 ion cluster in the mass spectrum of the methyl derivatives. The ^{18}O enrichment in the leaving group (97%) was estimated from the relative intensities of the ions at *m/z* 153 and 155 in the mass spectrum of *p*-nitroanisole made from the labeled methanol for other purposes.[35]

B. Kinetic Measurements. Reactions of methyl α- and β-glucopyranosides were followed by change in rotation at 404.6 nm in a 1-dm, 1-mL jacketed cell in a Perkin-Elmer 241 MC spectropolarimeter equipped with the manufacturer's digital printer. Water, thermostated to ±0.2 °C was passed through the jacket from a Julabo Paratherm V4 water bath.

AnalaR 60–62% aqueous perchloric acid was diluted with HPLC-grade water and standarized by titration against standard sodium hydroxide solution.

Methods used to measure the spontaneous hydrolysis of 2,4-dinitrophenyl β-D-glucopyranoside[23] and xylosyl pyridinium salts[47] have been described elsewhere, as have the sources of the substrates.

Calculation of Isotope Effects. For all the deuterium kinetic isotope effects, and for the leaving group ^{18}O kinetic isotope effect, the value of k_{light}/k_{heavy} (*C* in eq 1) was calculated by using a nonlinear least-squares minimization routine (E04HFF) from the "NAG" library on a Honeywell mainframe computer (AUCC) to fit the experimental time course of optical rotation to eq 1. *A* (eq 1), the optical rotation change for complete reaction for the light isotopomer, was measured in a separate experiment: *B*, *C*, *k*, and $\alpha(\infty)$ were treated as variables. In all cases it was checked that $B \sim -A$, that *k* was within experimental error, identical with that measured in a separate experiment, and that the experimental points were evenly distributed about the calculated "best-fit" line.

For the very small ring ^{18}O and anomeric ^{13}C effects, there was insufficient information in the time course of the optical rotation to treat *k* as a completely unlocated variable. Accordingly a constrained least-squares minimization routine (E04LAF) was used in which the value of *k* was constrained to ±10% of that measured in a separate experiment; temperature is the main source of day-to-day variation in *k*, and the 10% limit is very pessimistic (corresponding to a change of 0.8° for a reaction with an activation energy of around 30 kcal/mol).

The effects of the ring ^{18}O and anomeric ^{13}C were corrected for isotopic impurities.[16] Because of the digital output of the polarimeter, an rms deviation of 290 μdeg corresponds to a perfect fit of the data to eq 1: the calculated rms deivation for the majority of runs was 300–500 μdeg, with the worst rms deviation being 760 μdeg.

C. Molecular Mechanics Calculations. Since MM2 contains no provision for solvent interactions, and the treatment of dipolar interactions takes the molecule as being in vacuo, we considered that a more realistic model for a conformationally constrained structure in water would be obtained by complete neglect of dipolar interactions. This was particularly true of the dipoles associated with the OH groups, which in water would be H bonded. Thus, the OH group was treated as a single atom of mass 17, and the leaving group was similarly treated as a single atom of mass 32. The length of the fissile bond was taken as 2.0 Å at the transition state. The degree of rehybridization at the anomeric center was set by changing the C2–C1–O1 angle of minimum potential for bending vibrations to 90° for the α-anomer and 95° for the β-anomer.

The lone pairs on oxygen were treated as two sp^3 lone pairs perpendicular to the C5–O5–C1 plane. For each pair of values of θ and ω, it was confirmed that the same transition-state structure was obtained whatever the starting conformation ($^4C_1$, $^1S_3$, $^{2,5}B$, or $^1C_4$) or that the program failed to find a low-strain-energy structure. The calculated structures are slight refinements of those presented at the 15th Steenbock Symposium.[5]

Acknowledgment. We are deeply indebted to Dr. M. J. T. Robinson, University of Oxford, for the unstinting way he made his expertise on the isotopic quasi-racemate method available to us and to Dr. I. H. Williams for much helpful discussion and for communicating his theoretical results to us before publication.

Registry No. D_2, 7782-39-0; ^{18}O, 14797-71-8; methyl α-D-glucopyranoside, 97-30-3; methyl β-D-glucopyranoside, 709-50-2; 2,4-dinitrophenyl β-D-glucopyranoside, 25775-97-7; methoxymethyl 2,4-dinitrophenolate, 67106-75-6; β-D-xylopyranosyl-4-bromoisoquinolinium, 104213-73-2; α-D-xylopyranosyl-4-bromoisoquinolinium, 104213-74-3; *N*,*N*-dimethyl-*N*-(methoxymethyl)-*m*-nitroanilinium, 75123-86-3.

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