Kinetic Isotope Effect Study of Transition States for the Hydrolyses of α- and β-Glucopyranosyl Fluorides

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Abstract: The hydrolyses of α- and β-glucopyranosyl fluorides at near-neutral pH have been studied. The water (buffer-independent) reactions were characterized by values of $\Delta H^{\ddagger}$ of 96 ± 4 and 88 ± 5 kJ mol^{-1} and of $\Delta S^{\ddagger}$ of −37 ± 12 and −38 ± 17 J mol^{-1} K^{-1}, respectively, and by multiple kinetic isotope effects measured by the isotopic quasi-racemate method. These effects, expressed as k_{light}/k_{heavy} for the α-fluoride at 80 °C and the β-fluoride at 50 °C, were, respectively, for αD (C1), 1.14_2 and 1.08_6, for βD (C2), 1.06_5 and 1.03_0, for ring ^{18}O, 0.98_4 and 0.98_5, and for anomeric ^{13}C, 1.03_2 and 1.01_7; a γD (C5) effect of 0.98_0 was also measured for the α-compound. A transition-state structure for the hydrolysis of the α fluoride, involving the ring in a flattened $^4C_1$ chair conformation, and an "exploded" S_N2 disposition of water and fluoride ion about the anomeric center were located from BEBOVIB-IV and the initial structure, parameter settings, and procedure used by Tanaka *et al.* (Tanaka, Y.; Tao, A.; Blanchard, J. S.; Hehre, E. J. *J. Biol. Chem.* under review) in their study of the glucoamylase-catalyzed hydrolysis of this compound. The effects for the β-fluoride are consistent with a more S_N1-like transition state in which the sugar ring is again in a flattened $^4C_1$ conformation. In the presence of 2.0 M sodium azide, when the reaction of the α-fluoride becomes a classic S_N2 reaction (Banait, N. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1991**, *113*, 7951), the ^{13}C effect increases to 1.08_5 but the αD effect also increases modestly (to 1.16_9). The reaction of the β-fluoride in 0.3 M succinate buffer is largely (formally) a bimolecular reaction of the acidic form of the buffer; effects are as follows: αD, 1.11_0; βD, 1.05_9; γD, 0.98_1; ring ^{18}O, 0.98_8; anomeric ^{13}C, 1.06_4. The apparent promotion of A_ND_N reactions of the β-fluoride by succinate monoanion rather than dianion suggests that the microscopic reaction is the catalysis by neutral succinic acid of the formation of 1,2-anhydroglucose from the monoanion of the glucosyl fluoride.

Introduction

The mechanisms of the hydrolysis and solvolysis of glycosyl derivatives are of interest as the simplest nonenzymic counterpart of the glycosyl transfers important in biology,[1] where the formation and cleavage of glycosidic linkages is important metabolically, structurally, and in terms of information transfer.[2] The involvement of species with many of the properties of glycopyranosyl cations in the hydrolysis and solvolysis of glycopyranosyl derivatives has long been recognized,[3] but a full understanding of the conformation of these species and the timing of the making and breaking of bonds to the reaction center has yet to be achieved.

For many years discrete glucopyranosyl cations were considered to be intermediates in hydrolysis and solvolysis of glucuopyranosyl derivatives, but measurements of the lifetimes of other oxocarbonium ions[4] cast doubt on the real existence of the less stable glucosyl cation as a solvent-equilibrated intermediate even in water. The discovery that the apparently S_N1 reactions of methoxymethyl derivatives were in fact preassociation reactions,[5] coupled with the lesser reactivity of glucosyl derivatives, reinforced the probability that the reactions of glucosyl derivatives necessarily involved the participation of the incoming nucleophile. This is certainly the case in mixtures of ethanol and trifluoroethanol,[6] but it now appears that the lifetimes of oxocarbonium ions are relatively insensitive to polar effects of the type responsible for the lower reactivity of glucosyl derivatives compared to tetrahydropyranyl derivatives,[7] so that the estimated lifetime of the glucopyranosyl cation *in water* is longer than that of the methoxymethyl cation. In accord with this, although there are salt effects on the hydrolysis of glycosyl derivatives with leaving groups which are equatorial in the preferred ground-state conformation, these effects parallel the basicity, rather than the nucleophilicity, of the anion and the neutral nucleophile thiourea has no effect.[8] Recently, however, Banait and Jencks[9] have shown that α-glucopyranosyl fluoride, in which the leaving group is axial, can undergo bimolecular nucleophilic displacements in water, but only with anionic nucleophiles. General base catalysis of the attack of a neutral nucleophile by an anionic base can also be observed. Taken together, these data suggest that the glucosyl cation has a real existence but is not in contact with an anionic nucleophile or leaving group.

The question of the conformation of the pyranose ring at the transition state for hydrolysis of glucosyl derivatives can therefore be addressed, in some cases, without having to consider the position of the incoming nucleophile. A multiple kinetic isotope effect study of the acid-catalyzed hydrolyses of methyl α- and β-glucopyranosides revealed that the likely conformation of the pyranose ring in the transition state for the hydrolysis of the β-compound was a flattened $^4C_1$ chair conformation, whereas that for the hydrolysis of the α-compound was a flattened $^1S_3$ skew conformation.[8] These results stood in flat contradiction to the predictions of the antiperiplanar lone pair hypothesis (ALPH), that the α-anomer should react through the ground-state $^4C_1$ conformation but the β-anomer would be forced to adopt a boat conformation, in order to fulfill a supposed "stereoelectronic" requirement that one of the oxygen sp^3 lone pairs of electrons

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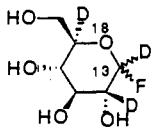
should be antiperiplanar to the leaving group.[10,11] They were however readily understood on the basis of the reverse anomeric effect of the protonated methoxy leaving group, which reinforced the normal preference of the equatorial anomer to adopt the $^4C_1$ conformation but forced the initially axial anomer to adopt the same skew conformation as α-D-glucopyranosyl pyridinium ion in solution.[12]

On this basis it would be predicted that the hydrolyses of both anomers of glucosyl derivatives with an anionic leaving group, which would experience a direct, rather than a reverse, anomeric effect, would go through transition states in which the pyranose ring approximates to the $^4C_1$ conformation of the substrate in its ground state. Such processes are known with fluoride,[14] 2,4-dinitrophenolate,[15] and dihydrogen phosphate[16] as leaving groups. We therefore now report a multiple kinetic isotope effect study of the hydrolyses of α- and β-glucopyranosyl fluorides, analogous to that previously reported for the *O*-glycosides, except that a leaving group kinetic isotope effect requires special techniques:[17] sites of isotopic substitution are shown in structure I. The kinetic

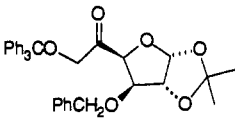


(I)

isotope effects are measured by the isotopic quasi-racemate method, in which the optical rotation of a solution containing equal concentrations of the D-enantiomer highly enriched in the isotope of interest, and of the L-enantiomer, is followed during the course of the reaction.[18] (This method therefore cannot be used with glucosyl 2,4-dinitrophenolates, which yield the intensely colored 2,4-dinitrophenolate anion; glucosyl phosphates, though they could yield a leaving group kinetic isotope effect, are unsuitable because of competitive phosphorus–oxygen fission.)

Experimental Section

Materials. D-Glucose, L-glucose, [2-^{2}H]-D-glucose, [1-^{13}C]-D-glucose (99 atom % ^{13}C), and 97 atom % $H_2{}^{18}O$ were purchased from Aldrich Chemical Co., Milwaukee, WI. 1,2,3,4,6-Penta-*O*-acetyl-[1-^{12}H]-D-glucose was made by the method of Berven and Withers.[19] Deuterium and ^{18}O at C5 were introduce as described previously:[8] the key intermediate II was reduced with $LiAlD_4$ to introduce deuterium. To introduce ^{18}O, the ketone was exchanged with $H_2{}^{18}O$ (97 atom % ^{18}O) four times, and the exchange was followed by infrared spectrometry ($\nu_{C=^{16}O}$ 1736 cm^{-1}, $\nu_{C=^{18}O}$ 1711 cm^{-1}). After the final exchange, the ^{18}O-labeled ketone, which we estimate to be >95% C=^{18}O from the complete absence of any



(II)

νC=^{16}O and FAB mass spectrometry of the derived fluorides, was reduced immediately. Glucose and pentaacetylglucose were converted to the crystalline tetraacetyl α- and β-glucopyranosyl fluorides (mp 122–124 and 79–81 °C, respectively) by literature procedures.[20] Deuterium incorporation was confirmed by [^{1}H] NMR (at 400 MHz) in $CDCl_3$, the anomeric proton at δ 5.74 (q, J_{F-H1} 53 Hz, J_{H1-H2} 3 Hz) in the α-fluoride and 5.40 (q, J_{F-H1} 52 Hz, J_{H1-H2} 6 Hz) in the β-fluoride disappearing in the 1-deuteriated compounds and becoming a doublet in the 2-deuteriated compounds, the 2-proton at δ 4.94 (sextet, J_{H2-F} 24 Hz, J_{H2-H3} 10 Hz) in the α-fluoride and δ 5.12 (sextet, J_{F-H2} 12 Hz, J_{H2-H3} 9 Hz) in the β-fluoride disappearing in the 2-deuteriated compounds and becoming a quartet in the 1-deuteriated compounds, and the multiplet at δ 4.18 in the α-fluoride and δ 3.92 in the β-fluoride disappearing in the deuterated compounds.

The tetra-*O*-acetylglucosyl fluorides were deacetylated with potassium cyanide in methanol.[21] All labeled α-glucosyl fluorides were crystalline materials (mp 118–120 °C). Due to the difficulties encountered in crystallizing the labile β-fluorides, rather than attempting to crystallize them, immediately after deacetylation, they were dried under vacuo, and after testing by NMR, they were used immediately for kinetic purposes. With small isotope effects for the β-compound, it was virtually impossible to get the system balanced and so equal quantities of the crystalline tetraacetates were weighed out and deacetylated as a mixture.

Kinetic Measurements. First-order rate constants for hydrolyses of α- and β-glucosyl fluoride, or for mutarotation of glucose, were obtained from the change in rotation at 405 nm of buffered solutions in a 1-dm, 1-mL jacketed cell in a JASCO DIP 370 polarimeter equipped with a printer. Water thermostated to ±0.1 °C was passed through the cell jacket from a Fisher Scientific temperature controller. Some measurements were made by measurement of fluoride ion release: it was confirmed in representative cases that rate constants measured by the two monitoring systems were identical. Rate constants were obtained by nonlinear least squares fitting of time courses to eq 1 using the nonlinear least squares fitting program *Kaleidagraph* (version 2:0) (Abelbeck software, bought from Synergy (PCS Inc.)) run on a Macintosh SE30 personal computer.

$$\alpha = Ke^{-kt} + L \tag{1}$$

Isotope Effects. Experimental time courses of optical rotation of an isotopic quasi-racemate in the course of a reaction were fitted to eq 2,

$$\alpha = Ae^{-k_L t} + Be^{-k_H t} + C \tag{2}$$

The optical rotation change for complete reaction for the light isotopomer (*A*) was measured in a separate experiment. For the α-fluoride in 1.0 mL (3.0 mg) in a 1-dm pathlength cell, it was 0.310° on hydrolysis and 0.600° for the reaction with 2.0 M sodium azide, and for β-fluoride (3.0 mg) on a hydrolysis, it was 0.100°. Despite the buffer acceleration of these reactions, *A* remained unaltered, indicating that any nucleophilic substitution product decomposed on a time scale fast compared to hydrolysis. For small effects, particularly with the β-fluoride, up to 6 mg of each enantiomer was used. In these cases it was confirmed that the pH after reaction remained in the pH-independent region (pH 4.0–7.0[22]). The final pH values were in the region 5.7–6.0.

The isotopic effect was then calculated as k_L/k_H. Fits of experimental data to eq 2 are very ill-conditioned, and data were accepted only when a maximum or a minimum was observed and when the size of the effect calculated by more sophisticated procedures corresponded approximately to that calculated from $\Pi = A\delta k/2.7k$, where Π is the amplitude of the maximum or minimum and δk is the difference in rate constants between light and heavy isotopomers. The procedure depends critically on *A* and *B* not being completely indeterminate: the initial estimates of *A* and *B* input into *Kaleidagraph* were those calculated from the amount of each

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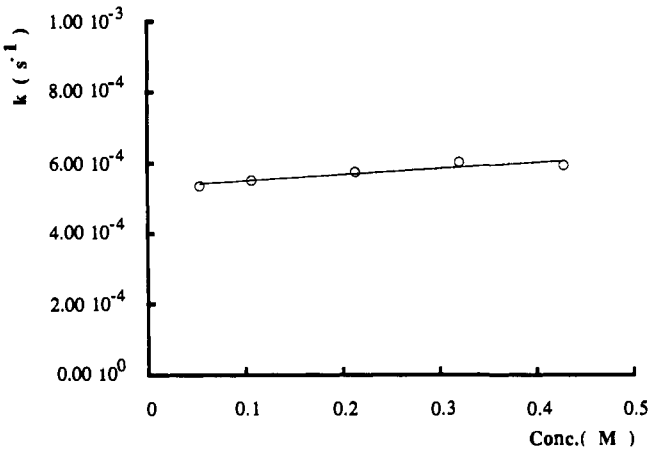

Figure 1. Effect of succinate buffer concentration on the hydrolysis of α-glucosyl fluoride at 80 °C, pH 6.0, I = 1.0 M ($NaClO_4$).

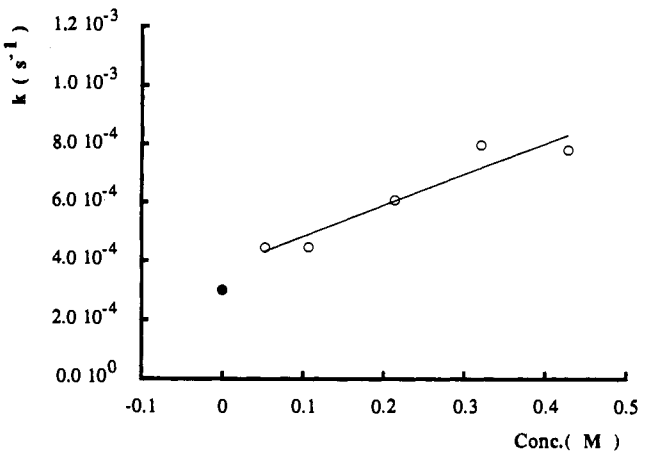

Figure 2. Effect of succinate concentration on the hydrolysis of β-glucosyl fluoride at 50 °C, pH 6.0, I = 1.0 M ($NaClO_4$). (●) In Bis-Tris buffer at 50 °C, pH 6.0, I = 1.0 M ($NaClO_4$).

antipode in the reaction mixture, and refined values were within 10% of initial estimates.

The precision within each individual run was characterized by the standard deviation of optical rotation σ_α and the effect of fluctuation of optical rotation α on the kinetic isotope effect, obtained from fitting data with eq 2. For a series of measurements of α_i (i = 1, 2, ..., N), standard deviation of optical rotation α is

$$(\sigma_\alpha)^2 = [\sum_{i=1}^{N} (\delta\alpha)_i^2]/(N-1) \qquad (3)$$

Where $(\delta\alpha)_i = \alpha_i - \alpha_i^{\text{real}}$. α_i^{real} is the point on best fitting curve at time $t = i\delta t$.

The effect of fluctuation of optical rotation α on the kinetic isotope effect, obtained from fitting data to eq 2, was estimated from eq 4:

$$(\sigma_s)^2 \le \{\sum_t (\delta\alpha)_t^2 + 2k_H \sum_t |(\delta\alpha)_t (SAte^{-k_L t} + Bt\, e^{-k_H t})| + (k_H)^2 \sum_t (SAte^{-k_L t} + Bte^{-k_H t})^2\}/\{(N-1)(\sum_t [Ak_H{}^t]^2 e^{-2k_L t})\} \quad (4)$$

where σ_s is the standard deviation of the kinetic isotope effect. S is the kinetic isotope effect (k_L/k_H), and $1\Delta t \le t \le N\Delta t$. The assumption of $\delta k_H \le k_H$ was used in the derivation of eq 3. The second and third terms contribute most of $(\sigma_s)^2$ for a nonbalanced run.

Results and Discussion

Nature of the Hydrolysis Reactions. Much of this work was initiated before we were aware of the the work of Banait and Jencks, which indicated that nucleophilic[9a] and general acid- and base-catalyzed[9b] processes contributed to the rate of liberation of fluoride ion from α-glucopyranosyl fluoride, but only with anionic reagents. On the basis of previous findings that the nature of the salt did not effect hydrolyses of various glycopyranosyl derivatives,[8] we used high concentrations of succinate buffer to neutralize the HF liberated in the hydrolysis. In the event, as is seen from Figure 1, this unfortunate choice did not affect the validity of the results with the α-fluoride: even at 0.3 M succinate buffer, only ~10% of the reaction goes through the buffer-catalyzed process.

With the β-fluoride, however, the dependence on buffer concentration was much more marked (Figure 2) and it is clear that most of the reaction at 0.3 M succinate buffer proceeded through buffer-catalyzed processes. We therefore performed an additional set of measurements on the β-fluoride using neutral Bis-Tris as a buffer.

Table 1. First-Order Rate Constants for Hydrolyses of Glucopyranosyl Fluorides in 50 mM Sodium Acetate Buffer, I = 1.0 M ($NaClO_4$)

T (°C)	k_α (s^{-1})	k_β (s^{-1})	
			$\Delta H^{\ddagger}_\beta$ = 88 ± 5 kJ mol^{-1}
31.0		5.83×10^{-5}	$\Delta S^{\ddagger}_\beta$ = −38 ± 17 J mol^{-1} K^{-1}
37.0		1.17×10^{-4}	
40.0	8.42×10^{-6}		
44.0		2.75×10^{-4}	
50.0	2.19×10^{-5}	3.98×10^{-4}	
55.0		8.79×10^{-4}	
60.0	8.91×10^{-5}		
70.0	2.31×10^{-4}		$\Delta H^{\ddagger}_\alpha$ = 96 ± 4 kJ mol^{-1}
80.0	5.46×10^{-4}		$\Delta S^{\ddagger}_\alpha$ = −37 ± 12 J mol^{-1} K^{-1}

Table 2. Comparison of the Rates of Hydrolysis of the Glucosyl Fluorides and Mutarotation of Glucose

buffer[a]	fluoride	T (°C)	k_{mut} (s^{-1})	k_{hydro} (s^{-1})	k_{hydro}/k_{mut}
0.2 M succinate	α	80	0.088	0.000 55	0.006
0.2 M succinate	α	60	0.033	0.000 23	0.007
+2 M NaN_3	α	50	0.014	0.000 083	0.006
0.3 M succinate	β	50	0.015	0.000 69	0.047
0.3 M Bis-Tris	β	50	0.0031	0.000 31	0.1

[a] At pH 6.0, I = 1.0 M ($NaClO_4$).

In Table 1 are gathered together first-order rate constants for the water reaction of both fluorides determined in low concentrations of acetate buffer. The much higher energy of activation for the α-fluoride means that the α/β ratio is very temperature dependent. The slightly negative entropies of activation classically suggest bimolecular processes, although the validty of this criterion in reactions in water in which strongly solvated ions are generated is questionable: the entropy for the β-fluoride is similar to that for the *galacto* compound reported previously.[14]

In Tables 3 and 5–7 are reported measured isotope effects for four systems—the water reaction of the α-fluoride, the bimolecular reaction of the α-fluoride with azide ion, the water reaction of the β-fluoride, and then the succinate-buffer-catalyzed reaction of the β-fluoride. Two aspects of the validity of these data, measured by the isotopic quasi-racemate method, need to be addressed, however. The first concerns mutarotation of the sugar. The method in its simple form assumes that the equilibrium composition of the anomers is reached with a time constant fast compared to hydrolysis. The data in Table 2 indicate that this is so for all reactions except that of the water reaction of the β-fluoride, where hydrolysis is only 10-fold slower than mutaro-

tation. On the worst-case assumption, that the initial hydrolysis yields α-glucopyranose exclusively, this will introduce an error of about 10% of the effect on observed effects.

The very large difference in rate between α- and β-glucosyl fluorides (see Table 1, ref 24) made it impracticable to measure kinetic isotope effects at the same temperature for both anomers.[23] We therefore measured isotope effects on the hydrolysis of the β-anomer at 50 °C and of the α-anomer at 80 °C. The effects for the α-anomer can thus be compared directly with those previously reported for methyl α- and β-glucosides.[8] If the effects are classical zero-point energy effects, then $T\ln(k_L/k_H)$ = constant and the effects for the β-anomer should be reduced by 10% of the effect for proper comparison; if there is a contribution to the heavy atom effects from the isotope effect on molecular moments of inertia, then the temperature dependence will be less steep.

Leaving group ^{18}O effects on the acid-catalyzed hydrolyses of oxygen glycosides,[8,24-26] as well as β_{lg} values of approximately 0 for the acid-catalyzed hydrolyses of glycosides and of below −1 for the spontaneous hydrolyses of 2-(aryloxy)tetrahydropyrans[27] and 2-(aryloxy)tetrahydrofurans[28] establish that the transition state for hydrolysis of oxygen glycosides is very late, whether the leaving group is protonated in a preequilibrium step or not. β_{lg} values for the spontaneous hydrolyses of glycosyl pyridinium ions[12,29] of below −1 and large leaving group ^{15}N kinetic isotope effects for nucleoside hydrolysis[30] likewise establish that at the transition state for hydrolyses of these *N*-glycosides the C–N bond is largely broken. Since there is such ample experimental evidence for the lateness of the transition states for glycosyl–oxygen and glycosyl–nitrogen bond cleavage, we assumed similar, almost complete, carbon–fluorine cleavage at the transition state for glycosyl fluoride hydrolysis.[17]

Water and Azide Reactions of α-Glucosyl Fluoride. Isotope effects in the water reaction of α-glucosyl fluoride are set out in Table 3, where they are compared with those for the acid-catalyzed reaction of methyl α-glucoside. Whereas the secondary deuterium effects for the two systems are broadly similar, the ^{13}C and ^{18}O effects are radically different. The ^{13}C effect is indeed totally incompatible with a unimolecular reaction. S_N1 reactions are commonly associated with low values of reaction center carbon isotope effects, because the loss of zero-point energy occasioned by the cleavage of the carbon leaving group bond is largely offset by the increase in zero-point energy associated with double bond character in the bonds connecting the reaction center to groups which stabilize positive charge, whereas S_N2 reactions are associated with high effects. Thus, for example, as the nucleophilicity of the solvent is decreased in the solvolysis of isopropyl 2-naphthalenesulfonate from ethanol through to hexafluoro-2-propanol, the reaction center ^{14}C effect falls from 1.095 to 1.044.[31] In respect of glycoside hydrolysis, in addition to our effects with the acid-catalyzed hydrolyses of methyl glucosides,[8] Goiten *et al.*[32] 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 corresponds to a ^{13}C effect of around 0.5%. Schramm's group has reported a somewhat higher effect for the cleavage of a glycosyl–nitrogen bond, k_{12}/k_{14} of 1.049 ± 0.010 for acid-catalyzed hydrolysis of AMP.[33] The much higher ^{13}C effect for the water reaction of α-glucosyl fluoride than for the acid-catalyzed reaction of methyl α-glucoside thus provides telling support for the conclusion of Banait and Jencks,[9] that the glucopyranosyl cation is too unstable to exist in contact with an anionic leaving group (such as fluoride) but in the presence of a neutral one (such as methanol) it has a real existence.

Table 3. Kinetic Isotope Effects for the Neutral Hydrolysis of α-Glucosyl Fluoride in 0.2 M Sodium Succinate Buffer, pH 6.0, I = 1.0 M ($NaClO_4$) at 80.0 °C

site and isotope	KIE (σ_α, σ_{KIE})	average	OMe[a]	EIE[b]
α-D	1.135 (0.0004, 0.020)	1.142	1.137	1.188
	1.138 (0.0003, 0.026)	±0.0075		
	1.144 (0.0002, 0.017)			
	1.152 (0.0002, 0.020)			
β-D	1.072 (0.0002, 0.010)	1.067	1.073	
	1.065 (0.0003, 0.014)	±0.0077		
	1.069 (0.0002, 0.009)			
	1.055 (0.0002, 0.014)			
γ(C5)-D	0.983 (0.0002, 0.0017)	0.979	0.977	
	0.976 (0.0002, 0.0006)	±0.0032		
	0.978 (0.0003, 0.0070)			
	0.971 (0.0002, 0.0050)			
1-^{13}C	1.030 (0.0007, 0.0039)	1.032	1.007	1.004
	1.033 (0.0009, 0.0042)	±0.0032		
	1.031 (0.0007, 0.0020)			
	1.037 (0.0013, 0.0062)			
5-^{18}O	0.981 (0.0005, 0.0020)	0.984	0.996_5	0.978
	0.982 (0.0010, 0.0054)	±0.0049		
	0.990 (0.0006, 0.0026)			

[a] For the acid-catalyzed hydrolysis of methyl α-glucoside, taken from ref 8. [b] Calculated for the appropriate rotamer of dihydroxymethane by I. H. Williams and quoted in ref 8.

Table 4. Kinetic Isotope Effects on the Reaction of α-Glucosyl Fluoride with Azide Ion (0.2 M Sodium Succinate Buffer, 2.0 M Sodium Azide, pH 6.0 at 50.0 °C)

site and isotope	KIE (σ_α, σ_{KIE}) 0.2 M SU/2 M NaN_3, 50 °C	average
α-D	1.178 (0.0006, 0.014)	1.169
	1.162 (0.0005, 0.017)	±0.0082
	1.167 (0.0005, 0.014)	
1-^{13}C	1.087 (0.0003, 0.026)	1.085
	1.092 (0.0004, 0.017)	±0.0082
	1.076 (0.0008, 0.014)	

The question of bimolecularity of the reactions of α-glucosyl fluoride was probed further by running the reaction in 2 M sodium azide (Table 4), conditions which are known to result in direct displacements to give β-glucosyl azide:[9] the lower temperature (50 °C) was an inescapable instrumental consequence of the faster reaction under these circumstances. The reaction center carbon effect is very high indeed (1.085 ± 0.008): we present an example of the primary data in Figure 3. This effect is not however unprecedented: Ando *et al.*[34] report ^{14}C effects of 1.12–1.16 on the reaction of *N,N*-dimethylanilines with benzyl arenesulfonates, and Wong and Schowen,[35] a ^{13}C effect of 1.08 on the reaction of methoxide with *S*-methyldibenzothiophenium ion. The azide reaction is associated with a marginally higher α-deuterium effect than the water reaction, which could be a temperature effect. Azide, though, is a "softer" nucleophile than water, and it was found in the nucleophilic reactions of *N,N*-dimethyl-*N*-(meth-

(23) For maximum accuracy of the individual readings, the polarimeter requires a 10-min integration time and the minimal requirement for a data set is around 40 points extending over four half-lives. A minimum run time of 5–6 h is thus required for the faster reacting isomer.

(24) (a) Banks, B. E. C.; Meinwald, Y.; Rhind-Tutt, A. J.; Sheff, I.; Vernon, C. A. *J. Chem. Soc.* **1961**, 3240. (b) Rosenberg, S.; Kirsch, J. F. *Biochemistry* **1981**, *20*, 3196.
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(28) Lönnberg, H.; Pohjola, V. *Acta Chem. Scand.* **1976**, *30A*, 669.
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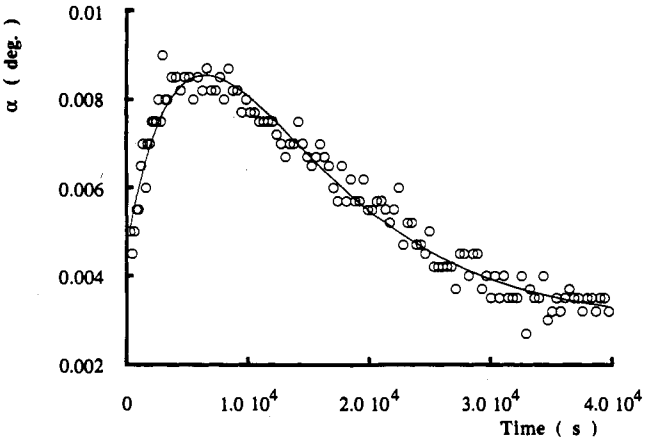

Figure 3. Time course of optical rotation of a solution containing α-L-glucosyl fluoride and [1-^{13}C]-α-D-glucosyl fluoride in 0.2 M sodium succinate, pH 6.0,2.0 M sodium azide at 50 °C (σ_α = 0.0004, σ_{KIE} = 0.0173).

oxymethyl)anilinium ions[7b] that "softer" nucleophiles gave bigger α-deuterium kinetic isotope effects.

The very different effects arising from ^{18}O substitution in the case of the fluoride (0.984 ± 0.005) compared to the methyl glycoside (0.996_5) suggest immediately that double bond character between O5 and C1 is much more pronounced at the transition state for fluoride hydrolysis than for methyl glucoside hydrolysis. The low value of the ring ^{18}O effect in the case of the methyl α-glucoside was the main determinant of our finding that the transition state for this compound was derived from the $^1S_3$ skew conformation: qualitatively, therefore, a different reactive conformation is anticipated for the fluoride.

Conformational information can be obtained from the inverse ring ^{18}O isotope effect by the following argument. Effective delocalization of charge and, hence, development of O5–C1 double bond character is possible only with the use of the p-type lone pair on oxygen: the sp-type lone pair is largely ineffective.[36] Since the strength of p–p orbital overlap depends on the square of the cosine of the dihedral angle between the two p orbitals, we can write eq 5, $(k_{16}/k_{18})_{max}$ is the isotope effect when the p-type lone

$$\ln(k_{16}/k_{18})_{obs} = \cos^2 \omega \ln(k_{16}/k_{18})_{max} \quad (5)$$

pair and the electron-deficient p orbital on C1 are exactly aligned, and ω is the dihedral angle between the p-type lone pair on oxygen and the electron-deficient p orbital on C1. In the work on the acid-catalyzed hydrolyses of the methyl glycosides,[8] two extreme estimates of $(k_{16}/k_{18})_{max}$ were used. We argued that any kinetic effect would be less than the equilibrium effect for oxocarbonium ion generation and based one estimate on Williams' theoretical calculations for the dehydration of dihydroxymethane, shown in Table 1. At the other extreme, we took the kinetic effect of 0.988 for the acid-catalyzed hydrolysis of isopropyl [1-^{18}O]-α-L-arabinofuranoside,[25] which takes place by a ring opening mechanism, in the rate-determining transition state of which the ring oxygen is protonated and the C1–O1 bond has double bond character. However, the measured inverse effects for the hydrolyses of glucosyl fluorides are bigger than those for this system, so that here 0.988 is a poor measure of $(k_{16}/k_{18})_{max}$. Despite the complication caused by the bimolecularity of the reaction, therefore, the theoretical value for the equilibrium isotope effect calculated for the conversion of the appropriate rotamer of dihydroxymethane to the protonated formaldehyde cation is an attractive zeroth approximation to $(k_{16}/k_{18})_{max}$. On this basis ω is estimated to be 32°.

(36) Briggs, A. J.; Evans, C. M.; Glenn, R.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1637.

The dihedral angle H2–C2-C1-F can likewise be approximately located from the β-deuterium kinetic isotope effect. β-Deuterium kinetic isotope effects have their origin in two phenomena, hyperconjugation and the inductive effect of deuterium, and can be considered in terms of eq 6.[37]

$$\ln(k_H k_D) = \cos^2 \theta \ln(k_H/k_D)_{max} + \ln(k_H/k_D)_i \quad (6)$$

The first term of eq 6 represents hyperconjugation of the C–L σ orbital with an electron-deficient p orbital on an adjacent carbon atom: θ is the dihedral angle between the C–L bond and this orbital. $\ln(k_H/k_D)_{max}$ is the maximal hyperconjugative effect obtained when the C–L bond and the p orbital are exactly eclipsed and increases as the positive charge on the adjacent carbon atom increases, with the associated weakening of the C–L bond. The second term in eq 6 represents the small, electron-releasing inductive effect of deuterium.

The data of Kresge and Weeks[38] on secondary deuterium isotope effects on the hydrolyses of acetaldehyde diethyl acetal and of ethyl vinyl ether at 25 °C provide a basis for estimating the values of both $\ln(k_H/k_D)_{max}$ and $\ln(k_H/k_D)_{max}$ and $\ln(k_H/k_D)_i$ for reactions which generate an oxocarbonium ion in water. In water, the hydrolysis of the acetal is specific acid catalyzed, whereas the rate-determining step in the hydrolysis of ethyl vinyl ether is protonation of the methylene carbon. Both hydrolyses involve the ethoxyethyl cation as a real intermediate. The inductive effect of α-deuterium substitution can be estimated from the isotope effect on the hydrolysis of CH_2=CL—OEt, since there is no change of hybridization at the carbocationic carbon and the effect of (0.965) is wholly inductive. Inductive effects are attenuated by a factor of approximately 2.5 per additional carbon atom between the reaction center and the site of substitution,[39] so we take $\ln(k_H/k_D)_i$ as –0.014. The hyperconjugative β-deuterium kinetic isotope effect of a freely rotating CL_3 group is $^3/_2(k_H/k_D)_{max}$,[40] and the experimental β-deuterium kinetic isotope effect for the hydrolysis of $CL_3CH(OEt)_2$ is 1.134 ± 0.011.[38] By using a value of –0.014 for $\ln(k_H/k_D)_i$, we can estimate $\ln(k_H/k_D)_{max}$ as 0.112 at 25 °C. Correction of this estimate of $\ln(k_H/k_D)_{max}$ to 80 °C assuming $T\ln(k_L/k_H)$ = constant and taking $\ln(k_H/k_D)_i$ as –0.014 allows θ for the hydrolysis of α-glucosyl fluoride to be estimated from eq 6. The resulting value of θ of 25° (i.e., a dihedral angle H2–C2–C1–F of 155°) is obtained. These estimates of θ and ω and ordinary qualitative conformational reasoning indicate that the transition state for α-glucosyl fluoride is $^4C_1$ chair flattened toward $^4H_3$.

The two other secondary deuterium effects are also consistent with this picture. The simplest possible interpretation of the α-deuterium effect, that the degree of rehybridization at C1 is merely $\ln(\alpha_{KIE})/\ln(EIE)$, leads to the conclusion that the anomeric carbon is 74% rehybrized. Whatever the corrections required for the bimolecularity of the reaction, estimation of the bond angles defined by the three nondeparting substituents and the anomeric center as 117° at the transition state will introduce errors of only a few degrees. Likewise, the magnitude of the γ(C5) effect qualitatively supports the contention that there is no large conformational change between the ground state and transition state. The C5 effect arises in principle from two phenomena, the inductive effect of deuterium and the deuterium conformational isotope effect. Whereas the latter is modest in the case of cyclohexane derivatives (the deuterium prefers the equatorial orientation by about 6 cal mol^{-1},[41] the presence of an α-heteroatom can cause it to be quite substantial.[42] Existing

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(41) (a) Anet, F. A. L.; Kopelevich, M. *J. Am. Chem. Soc.* **1986**, *108*, 1355. (b) Anet, F. A. L.; O'Leary, D. J. *Tetrahedron Lett.* **1989**, *30*, 1059.

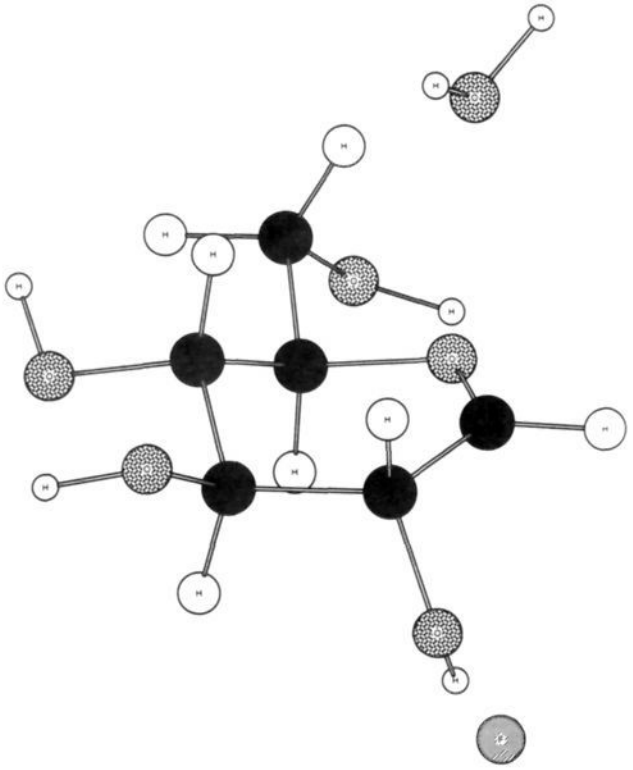

Figure 4. BEBOVIB-IV transition-state structure for α-glucosyl fluoride hydrolysis.

data (on 1,3-dioxolans)[42] suggest that the equatorial orientation of deuterium α- to a single ring oxygen should be favored by around 24 cal mol^{-1}. This by itself could account for an inverse γ-deuterium effect of 3–4%. If there were a large change in conformation, this effect would be superimposed on an inductive effect in the same sense, arising from charge buildup on the ring oxygen, which is known to be significant from the ring ^{18}O effects.

Qualitatively, therefore, the effects are all consistent with a bimolecular attack of water on α-glucosyl fluoride, through an exploded transition state immediately derived from the ground-state $^4C_1$ conformation of the substrate. This is in stark contrast *to the transition state with the neutral leaving group methanol*,[8] which does not involve a nucleophilic water and in which the conformation of the sugar ring is derived from the $^1S_3$ conformation.

Estimation of the Transition-State Structure for the Water Reaction of α-Glucosyl Fluoride Using BEBOVIB-IV. Tanaka et al. (Tanaka, Y.; Tao, W.; Blanchard, J. S.; Hehre, E. J. *J. Biol. Chem.*, under review) have recently described isotope effects for enzymic hydrolyses of α-glucosyl fluoride and have derived transition-state structures using BEBOVIB-IV. By starting with a theoretically-refined structure, bond orders were varied systematically and the resulting calculated isotope effects compared with experiment. Using their input parameters and procedure, we were able to locate the transition state for α-glucosyl fluoride hydrolysis shown in Figure 4, which reproduced the experimental effects reasonably well (Table 5). Particularly noteworthy is the H2–C2–C1–F dihedral angle, which in Figure 4 is 156°: the approximate calculation based on the β-deuterium effect using reactions which generated the ethoxyethyl cation as a model (vide supra) gave 155°. However, while the inverse ring ^{18}O effect was successfully modeled in this system, we found that systematic variation of the O5–C1 bond order gave inverse ring ^{18}O effects only over a very narrow range (1.8 < bond order < 2.0). The value of ω derived from the transition state of Figure 4 is 12°, as compared with 32° estimated empirically. The (comparatively minor) disagreement may be a consequence of the ~30% experimental errors on the ring ^{18}O effect and its cosine squared dependence on ω. A transition state for the water reaction of β-glucosyl fluoride could not be located using the parameterization of Tanaka *et al.* for the α-fluoride.

Table 5. Comparison of Experimental Kinetic Isotope Effects with Those Calculated from the Transition State of Figure 4 by BEBOVIB-IV

site and isotope of substitution	KIE(exptl)[b]	KIE(theor)[c]	bond order (bond)
α-D	1.142	1.142	0.961 (C1–H1)
β-D	1.067	1.070	0.844 (C2–H2)
5-D	0.979	0.979	0.918 (C5–H5)
1-^{13}C	1.032	1.023	
5-^{18}O	0.986	0.989	1.920 (C1–O5)
			0.001 (C1–F1)
			0.001 (C1–OH_2)

Table 6. Kinetic Isotope Effects for the Hydrolysis of β-Glucosyl Fluoride in 0.3 M Bis-Tris Buffer, pH 6.0, at 50.0 °C, I = 1.0 M ($NaClO_4$)

site and isotope	KIE (σ_α, σ_{KIE})	average	–OMe[a]	calcd EIE[b]
α-D	1.085 (0.0003, 0.0047)	1.086	1.08_9	1.137
	1.085 (0.0004, 0.0055)	±0.0012		
	1.087 (0.0006, 0.014)			
β-D	1.021 (0.0005, 0.0027)	1.030	1.04_5	
	1.033 (0.0010, 0.0078)	±0.0083		
	1.037 (0.0008, 0.0037)			
1-^{13}C	1.020 (0.0004, 0.0014)	1.017	1.01_1	1.00
	1.017 (0.0005, 0.0026)	±0.0022		
	1.016 (0.0004, 0.0012)			
5-^{18}O	0.982 (0.0006, 0.0019)	0.985	0.99_1	0.97_6
	0.991 (0.0006, 0.0015)	±0.0049		
	0.983 (0.0007, 0.0018)			

[a] For the acid-catalyzed hydrolysis of methyl α-glucoside, taken from ref 8. [b] Calculated for the appropriate rotamer of dihydroxymethane by I. H. Williams and quoted in ref 8.

Water Reaction of β-Glucosyl Fluoride. In Table 6 are set out isotope effects for the water reaction of this compound at 50.0 °C. Comparison with the effects for the acid-catalyzed hydrolysis of the methyl glucoside reveals no major differences. Particularly noteworthy is the small reaction center carbon effect: change of the leaving group from neutral MeOH to anionic F^- causes no great change in this effect, unlike the situation in the α-series. This lower reaction center carbon effect for the β-fluoride as compared to the α suggests a lower degree of nucleophilic assistance by water to the departure of fluoride. It is tempting to speculate that this may be related to the long-recognized greater susceptibility of axial leaving groups to bimolecular nucleophilic displacement than equatorial leaving groups.[43] The glucopyranosyl cation may be so finely on the border of a real existence that it is too unstable to exist with an anionic nucleophile on the α-face, but not on the β-face.

Estimation of the dihedral angles ω and θ, from the ring ^{18}O effect and β-deuterium effect in the same way as for the α-fluoride, with a temperature correction based on the assumption that $T\ln(k_l/k_h)$ = constant, gives ω = 41° and θ = 50°. Qualitatively, these angles are consistent with reaction through a flattened $^4C_1$ chair, as observed for methyl β-glucoside.[8] Although the possibility of reaction through nonchair conformers cannot readily be dismissed on the basis of these angles alone, if the β-fluoride

(42) (a) Anet, F. A. L.; Kopelevich, M. *J. Am. Chem. Soc.* **1986**, *108*, 2109. (b) Anet, F. A. L.; Kopelevich, M. *J. Chem. Soc., Chem. Commun.* **1987**, 595. (c) Carr, C. A.; Ellison, S. L. R.; Robinson, M. J. T. *Tetrahedron Lett.* **1989**, *30*, 4585.

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Table 7. Kinetic Isotope Effects on the Hydrolysis of β-Glucosyl Fluoride in 0.3 M Sodium Succinate Buffer, pH 6.0, I = 1.0 M ($NaClO_4$), at 50.0 °C

site and isotope	KIE (σ_a, σ_{KIE})	average
α-D	1.101 (0.0005, 0.0067)	1.105
	1.100 (0.0004, 0.024)	±0.0053
	1.109 (0.0006, 0.017)	
β-D	1.058 (0.0003, 0.0071)	1.059
	1.059 (0.0004, 0.0090)	±0.0010
	1.060 (0.0003, 0.0070)	
5-D	0.973 (0.0005, 0.0014)	0.981
	0.977 (0.0005, 0.0019)	±0.0074
	0.987 (0.0005, 0.0014)	
	0.988 (0.0006, 0.0013)	
1-^{13}C	1.073 (0.0009, 0.0070)	1.064
	1.056 (0.0009, 0.0049)	±0.0070
	1.063 (0.0012, 0.0049)	
	1.064 (0.0009, 0.0089)	
5-^{18}O	0.985 (0.0003, 0.0013)	0.988
	0.992 (0.0003, 0.0014)	±0.0036
	0.987 (0.0003, 0.0013)	

reacted through a boat conformation and the α-fluoride through a chair, then access of an nucleophilic water molecule to the reaction center for both substrates would be comparably ready and the interpretation of the reaction center ^{13}C effects would become problematic.

The α-deuterium effect is lower than for the α-fluoride but not as a fraction of the calculated equilibrium isotope effect: the approximation that the isotope effect is a linear function of the degree of rehybridization indicates that the reaction center is 66% rehybridized.

Succinate Reaction of β-Glucosyl Fluoride. In Table 7 are given isotope effects for the succinate reaction of β-glucosyl fluoride. The obvious interpretation of the results, supported at first sight by the very high reaction center ^{13}C effect, that one is observing the action of succinate as a nucleophile, on closer inspection fails to rationalize the facts. Most noticeably, it appears as succinate monoanion rather than succinate dianion, which is the more effective catalyst of the reaction (Figure 5). Additionally, ascription of buffer catalysis to a nucleophilic reaction encounters the problem why the catalysis should be much more marked for the β-fluoride than for the α, when the backside approach of a nucleophile is so much easier in the α-case. A further, circumstantial piece of evidence is that the rotation change on reaction with succinate is the same as that for the water reaction, so the product is glucose, not the acylal.

Bifunctional catalysis by succinate monoanion, in which –COOH acts as a general acid and $-COO^-$ as a nucleophile is sterically disfavored (the reaction would correspond to a 9-*endo-tet* cyclization).

A transition state which explains the facts is one in which most of the reaction occurs by *neutral* succinic acid acting upon the C2 oxyanion of the substrate (Figure 6). Such a transition state explains the high reaction center ^{13}C effects (since the key step is an intramolecular nucleophilic displacement by the C2

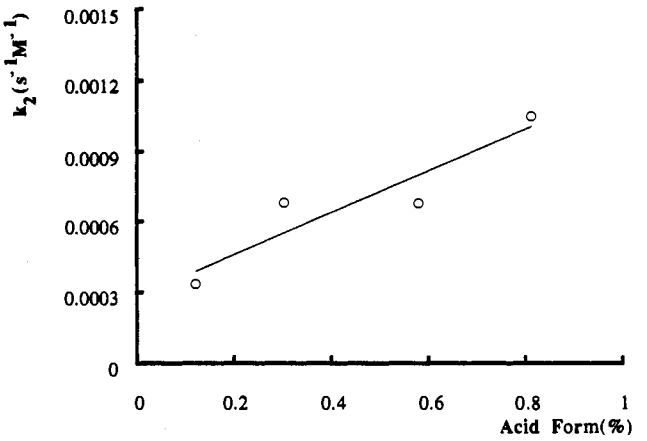

Figure 5. Dependence of the buffer catalytic constant for the hydrolysis of β-glucosyl fluoride in 0.3 M succinate at 50 °C, I = 1.0 M by $NaClO_4$ upon composition of the buffer. The buffering ionization is the second ionization of succinic acid, so that the acid form is succinate monoanion and the basic form succinate dianion.

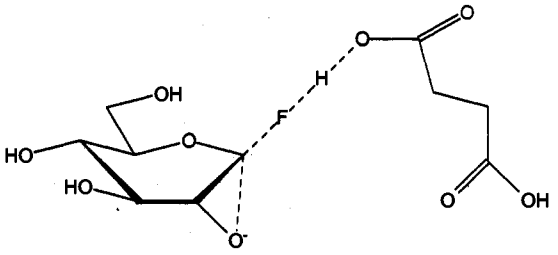

Figure 6. Qualitative transition-state structure for the succinate-catalyzed hydrolysis of β-glucosyl fluoride.

oxyanion) and also the greater susceptibility of the equatorial β-fluoride to this form of catalysis. It permits a small amount of catalysis, formally by succinate dianion, occurring through a similar transition state involving general acid catalysis by succinate monoanion of the ring closure of the substrate conjugate base. The existence of a preequilibrium, moreover, rationalizes the higher β-deuterium effect than for the anion. Formation of the substrate anion will be disfavored inductively by the β-deuterium: this phenomenon could indeed account for the whole of the observed effect.[44]

Enhanced reactivity upon ionization of a 2-hydroxy group in the *ribofurano* series has been shown to be associated with the persistence of high α-deuterium kinetic isotope effects:[45] these effects in the present system are therefore consistent with the transition state of Figure 6.

Acknowledgment. We thank the NIH for Grant GM 42469, Dr. John Blanchard and Dr. Wen Tao for assistance with the BEBOVIB calculations, and Professor W. P. Jencks for preprints of ref 9 and helpful comments on the manuscript.

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(45) Johnson, R. W.; Marschner, T. M.; Oppenheimer, N. J. *J. Am. Chem. Soc.* **1988**, *110*, 2257.