The Role of Sugar Substituents in Glycoside Hydrolysis

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Received June 16, 1999

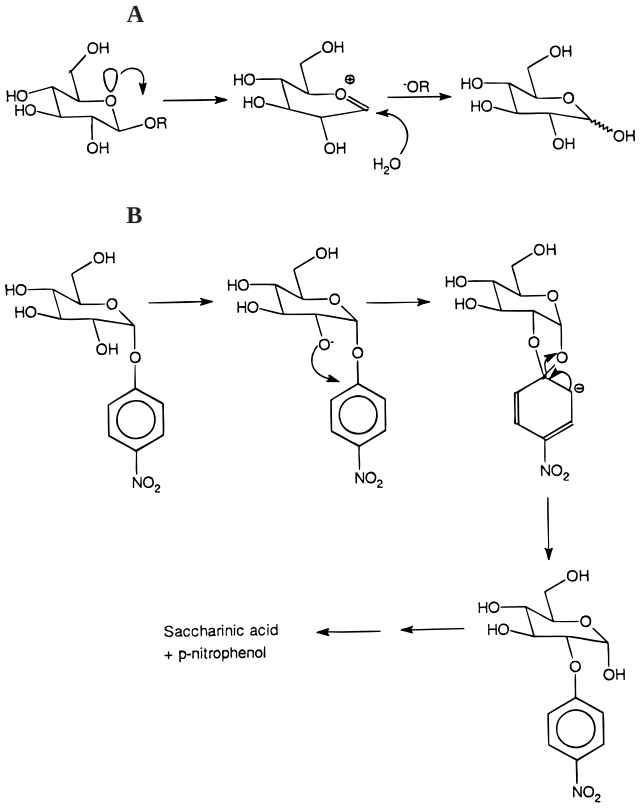
Abstract: A series of monosubstituted deoxy and deoxyfluoro 2,4-dinitrophenyl (DNP) β-D-glycopyranosides was synthesized and used to probe the mechanism of spontaneous β-glycoside hydrolysis. Their relative rates of hydrolysis followed the order 2-deoxy > 4-deoxy > 3-deoxy ≈ 6-deoxy > parent > 6-deoxy-6-fluoro > 3-deoxy-3-fluoro > 4-deoxy-4-fluoro > 2-deoxy-2-fluoro. Hammett correlations of the pH-independent hydrolysis rates of each of the 6-, 4-, 3-, and 2-position substituted glycosides with the σ_I value for the sugar ring substituent were linear ($r = 0.95$ to 0.999, $\rho_I = -2.2$ to -10.7), consistent with hydrolysis rates being largely dictated by field effects on an electron-deficient transition state. The relative rates of hydrolysis of the DNP glucosides can be rationalized on the basis of the stabilities of the oxocarbenium ion-like transition states, as predicted by the Kirkwood−Westheimer model. The primary determinant of the rate of hydrolysis within a series appears to be the field effect of the ring substituent on O5, the principal center of charge development at the transition state. Differences in the rates of hydrolysis between different *series* of hexopyranosides may not arise solely from field effects and likely also reflect differences in steric factors or solvation.

Introduction

Carbohydrate oligomers are ubiquitous in nature and, in addition to having structural and storage roles, are involved in a number of biologically important processes such as cell−cell recognition and energy metabolism. Thus the mechanism of their hydrolysis has been the focus of a great deal of research.[1−9] Hydrolysis of glycopyranosides is believed to occur by an essentially $D_N + A_N$ mechanism, leading to the generation of a cyclic oxocarbenium ion as shown in Scheme 1A. Modeling and experimental studies suggest that the stability of the resultant cation in solution is variable and dependent on the identities and locations of the ring substituents.[1,2,5−7,9−13] Studies carried out with fully hydroxylated glycosides indicate that the transition state for glycoside hydrolysis in these systems is highly preassociative, consistent with the relative instability of these species in solution.[1,2,6,10,11]

Within this framework, little is known about the role of substituents on the glycopyranose ring in determining the relative rates of glycoside hydrolysis. In general, it has been observed that the rate of hydrolysis of glycosides increases as the number of axial hydroxyl groups on the glycone increases. Edward[14] rationalized these observations on steric grounds, wherein steric

Scheme 1



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10.1021/ja992044h CCC: $19.00
Published on Web 02/02/2000

compression due to 1,3 diaxial interactions in the ground state $^4C_1$ conformation is released as the transition state half-chair is formed. Such ground-state interactions will be larger with axial substituents, thus according to this rationale the hydrolysis rates of galactosides, for example, are greater than those of glucosides.

Several studies have indicated that polar effects[15] contribute strongly to the relative rates of glycoside hydrolysis. Generally, the rate of hydrolysis of a glycoside increases as a consequence of deoxygenation,[16−19] presumably because replacement of an electron-withdrawing hydroxyl group with a hydrogen stabilizes the electron-deficient transition state. However, the increase in the rate of hydrolysis of the deoxy glycosides is not simply a function of the distance of the substitution from the anomeric center. In the studies carried out to date, the relative rates of hydrolysis of monodeoxygenated glycosides and α-glucosyl phosphates is 2-deoxy > 4-deoxy > 3-deoxy > 6-deoxy > parent.[17,19] Conversely, when a hydroxyl is replaced by a more electronegative fluorine atom, the rate of glycoside hydrolysis decreases. Indeed, a study of the rates of hydrolysis of a series of deoxyfluoro α-glucosyl phosphates showed the exact inverse order, parent > 6-deoxyfluoro > 3-deoxyfluoro > 4-deoxyfluoro > 2-deoxyfluoro.[20] Unfortunately, detailed interpretation of these rates was not possible because the observed rate constants contain contributions from at least two hydrolytic pathways, that via the neutral species and that via the conjugate acid. Further, the substitution can affect not only the rate constant for bond cleavage via both pathways but also, through effects on the basicity of the glycosidic oxygen, the concentration of the conjugate acid species.

The intent of this study is to further investigate the contribution of the ring substituents to the relative rates of glycoside hydrolysis. To this end, a series of monosubstituted deoxy and deoxyfluoro 2,4-dinitrophenyl β-D-glycopyranosides was synthesized. (Abbreviations: DNP, 2,4-dinitrophenyl; α-DKIE, α-deuterium kinetic isotope effect; all, allopyranoside; gal, galactopyranoside; glc, glucopyranoside; man, mannopyranoside; d, deoxy; F, deoxyfluoro) Both the equatorial and the axial epimers of the parent and the fluorinated glycosides were synthesized to explore the dependence of hydrolysis rate on the configuration at each of the glycone ring positions. Deoxy and deoxyfluoro substitutions are excellent probes of field effects since both are smaller than a hydroxyl group,[19] thereby minimizing steric contributions to the observed rates, while the electronic effects differ substantially. DNP glycosides were chosen for these studies because the rate of glycoside hydrolysis with this aglycone has been shown[8] to be pH-independent from pH 2 to 8. Thus rate constants determined in the pH-independent region will be unaffected by protonation equilibria, providing true first-order rate constants for the heterolysis of the glycosidic linkage, free from any complications associated with parallel pathways through neutral and conjugate acid species.

Results

Synthesis of Glycosides. Synthesis of fluorinated sugars was achieved either by deoxyfluorination with diethylaminosulfuryl trifluoride or by electrophilic fluorination using acetyl hypofluorite according to literature procedures, as described in the Supplementary Information. Deoxygenation was generally achieved by radical reduction of the bromo sugar substituted at the site of interest. Per-O-acylated sugars were converted to their hemiacetals by selective anomeric deprotection and then converted to the dinitrophenyl glycoside by reaction with fluoro 2,4-dinitrobenzene and deprotected using methanolic HCl.

Kinetic Studies. General. Rates of hydrolysis of the DNP glycosides were determined under the conditions used by Cocker and Sinnott.[8] However, reactions were followed for long periods (>3 half-lives), and rate constants were extracted by direct fit to a first-order expression rather than via initial rates analysis. To minimize possible solute−solute interactions, hydrolyses were carried out with dilute solutions of the glycosides ($\sim 10^{-4}$ M) in buffered 0.4 M KCl. Hydrolyses were followed at three different glycoside concentrations, and rate constants were found, within experimental error, to be independent of the starting concentration of substrate.

Eliminating Alternative Mechanisms. Although it is likely that hydrolysis of the DNP glycosides proceeds via the heterolytic mechanism shown in Scheme 1A, control experiments were performed to demonstrate that other modes of hydrolysis did not contribute significantly to the observed release of DNP. One possibility was that glycoside cleavage occurred by attack of water on C1′ of the aryl ring[21] rather than at the anomeric center of the sugar. However, electron impact mass spectral analysis of the products of hydrolysis of DNPglc at 60 °C in [^{18}O]H_2O, under conditions otherwise identical to those used in the kinetic studies, showed no significant incorporation of ^{18}O into the 2,4-dinitrophenol released on hydrolysis of the substrate. This is consistent with hydrolysis of the DNP glycosides proceeding only via attack of water on the sugar ring.

Horton[22] has demonstrated that under basic conditions (0.25 M KOH) the hydrolysis of *p*-nitrophenyl α-D-glucoside proceeds by a series of migrations initiated by the attack of the sugar C2 hydroxyl on the ipso position of the aryl ring to produce 2-*O*-*p*-nitrophenyl-D-glucose (Scheme 1B), which rearranges further and subsequently eliminates the phenolate. This pathway seems unlikely for hydrolysis of DNP β-D-glucosides since these are 1,2-trans glycosides, and because these experiments were carried out at near neutral pH (6.5). Further, it has been demonstrated[8] that this migration/elimination process did *not* contribute significantly to the release of phenol in the first 10% of the reaction for the hydrolysis of DNPgal. Additional evidence against the migration/elimination pathway was obtained by HPLC analysis of aliquots taken at several times during the hydrolysis of DNPglc. No new UV-absorbing, sugar-containing peaks, which would be produced by migration of the DNP group to the C2 or C3 hydroxyl, were observed.

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(15) The term polar effect refers to the observed influence of an unconjugated, sterically remote substituent on the rate of a reaction. Depending on the mechanism of transmission, polar effects in aliphatic systems may be described as inductive or field effects. Inductive effects are caused by through-bond polarization of electron density, whereas field effects are due to through-space interactions.[54] Several studies have convincingly demonstrated that polar effects in aliphatic systems in solution are best described as field effects[31,55−58] and that the inductive component is relatively minor.
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Table 1. Kinetic Data for the Spontaneous Hydrolysis of the DNP Glycosides[a]

DNP glycoside[b]	no. of temps	$\Delta H^{\ddagger}$ (37 °C) (kJ/mol)	$\Delta S^{\ddagger}$ (J/mol/K)	rate constant[c] at 37 °C (sec^{-1})
DNPglc (**2**)	13	117.8 (2.1)	33.8 (0.9)	5.58×10^{-6}
2FDNPglc (**10**)	4	120.8 (1.5)	13.3 (0.3)	1.45×10^{-7}
3FDNPglc (**8**)	8	121.4 (6.2)	29.6 (2.4)	8.23×10^{-7}
4FDNPglc (**6**)	3	123 (8.2)	27.9 (3.0)	3.74×10^{-7}
6FDNPglc (**4**)	4	126 (16)	53 (11)	1.99×10^{-6}
3dDNPglc (**15**)	3	116.7 (1.1)	42.0 (0.6)	2.23×10^{-5}
4dDNPglc (**13**)	3	109.5 (0.3)	33.0 (0.2)	1.25×10^{-4}
6dDNPglc (**11**)	4	108.3 (0.7)	16.3 (0.1)	2.60×10^{-5}
DNPgal (**17**)	4	107.0 (1.8)	12.0 (0.3)	2.61×10^{-5}
2FDNPgal (**25**)	4	104.5 (1.7)	−21.2 (0.6)	1.25×10^{-6}
3FDNPgal (**23**)	9	90.7 (4.9)	−48.1 (4.8)	1.04×10^{-5}
4FDNPgal (**21**)	8	107.1 (0.8)	−4.74 (0.06)	3.36×10^{-6}
6FDNPgal (**19**)	4	101.5 (2.3)	−14.1 (0.5)	9.40×10^{-6}
6dDNPgal (**27**)	4	104.3 (3.9)	18.4 (1.1)	1.61×10^{-4}
DNPall (**33**)	4	111.3 (0.2)	19.4 (0.06)	1.18×10^{-5}
3FDNPall (**35**)	4	119.1 (1.4)	23.4 (0.5)	9.51×10^{-7}
DNPman (**37**)	4	105.9 (0.5)	−18.0 (0.2)	1.09×10^{-6}
2FDNPman (**39**)	4	126.3 (0.7)	18.1 (0.2)	3.05×10^{-8}

[a] Standard errors for the activation parameters are given in parentheses. These, however, do not accurately reflect the precision of these parameters, as noted in the Results section. Consequently, the activation parameters are not interpreted to any significant extent. [b] Numbers in brackets are compound numbers used in the Supporting Information. [c] Rates were calculated by extrapolation or interpolation of Eyring plots.

Determination of Rate Constants. Rate constants and activation parameters determined for the hydrolyses of the DNP glycosides are presented in Table 1.[23] Reassuringly, the rate constants (4.6×10^{-6} s^{-1} for DNPgal and 1.2×10^{-6} s^{-1} for DNPglc) determined previously by initial rates analysis[8] were in close agreement with the rate constants (4.7×10^{-6} s^{-1} for DNPgal and 8.7×10^{-7} s^{-1} for DNPglc) determined in this study by following the reaction for several half-lives and fitting the time course of phenol release to a first-order equation. The wide range of hydrolysis rates observed with these glycosides made it impossible to obtain reliable data for all compounds at a single temperature. Thus the rate constants presented in Table 1 at 37 °C were determined by extrapolation or interpolation of Eyring plots derived from hydrolyses at various temperatures. As a check of the precision of the rate constants determined in this way, this complete analysis at a series of temperatures (total temperature range of 55 °C) was repeated three times for DNPglc, and calculated rate constants of 5.4×10^{-6}, 5.6×10^{-6}, and 5.4×10^{-6} sec^{-1} were obtained (Figure 1). Even though these calculated rate constants agree well and the plots are as good as most published plots of this type, values of activation entropy and enthalpy determined from these same plots were not as reproducible, the activation enthalpies ranging from 115 to 122 kJ/mol and the activation entropies from 25.8 to 46.6 J/mol·K. This indicates that the precision of the activation parameters is not well represented by the statistically calculated errors, as noted previously.[24] For this reason, only rate constants (hence, $\Delta G^{\ddagger}$) will be interpreted in any detail. The 2dDNPglc proved too labile for purification and characterization. Rate constants for its hydrolysis were therefore estimated with the aid of the excellent correlation ($r = 0.997$, $\rho = 1.1$) discovered between the rate constants for hydrolysis of the DNP glucosides and those previously determined for the analogous series of α-glucosyl phosphates (Figure 2).

(23) Some compounds are referred to by trivial designations for clarity, e.g., 3dDNPglc for 2,4-dinitrophenyl 3-deoxy glucopyranoside rather than 2,4-dinitrophenyl 3-deoxy-D-*xylo*-hexopyranoside. Note that 2dDNPglc, 3dDNPglc, and 4dDNPglc may with equal justification be referred to, respectively, as 2dDNPman, 3dDNPall, and 4dDNPgal.

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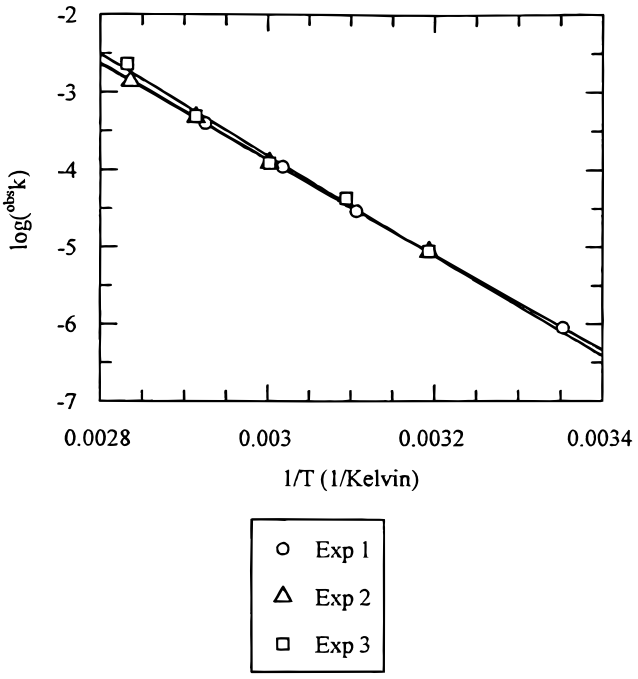

Figure 1. Eyring plots for the hydrolysis of DNPglc from three separate experiments. Line correlations for each plot are excellent ($r = 0.997-1.00$). Slight variations in the slope and intercepts of such plots result in much larger variation in the activation entropies and enthalpies than that observed for the rate constants (see text).

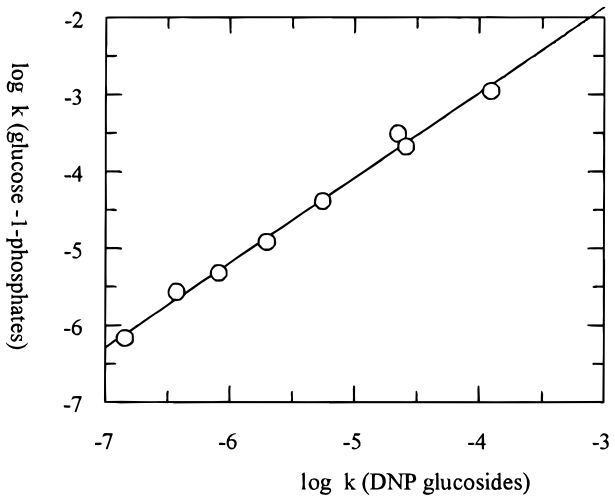

Figure 2. Comparison of rate constants for hydrolysis of DNP glucosides (37 °C) and the analogous α-glucosyl phosphates (25 °C).

Discussion

Field Effects Dominate. Data obtained in this study are consistent with previous results from other systems. A plot of the logarithm of the rate constants for hydrolysis of the DNP glucosides versus the logarithm of the rate constants for the analogously substituted α-glucosyl phosphates showed a correlation of $r = 0.997$ (Figure 2). The presence of such a correlation indicates that the two reactions proceed through similar oxocarbenium ion-like transition states. The slope of the plot was positive ($\rho = 1.1$) indicating a slightly greater sensitivity to ring substitutions for the α-glucosyl phosphates. Assuming field effects are important, this result indicates a somewhat greater degree of charge development at the transition state for hydrolysis of the glycosyl phosphates. While this difference may be due to the different leaving groups, it is also consistent with differences in the stereochemistry at the anomeric

Table 2. Correlation Coefficients (r) and Reaction Constants (ρ_I) for Hammett Plots from Each Set of DNP Glycosides

substitution position	r	ρ_I
6-glucosides	0.99	−2.2
6-galactosides	0.99	−2.5
4-glucosides	0.99	−5.1
4-galactosides	0.99	−3.1
3-glucosides	0.99	−2.9
3-galactosides	0.95	−3.5
3-allosides	0.95	−2.7
2-glucosides	0.96	−8.3
2-galactosides	0.98	−8.7
2-mannosides	0.97	−10.7

center. The heterolysis of α-glycosides proceeds via a transition state more dissociative than that of the analogous β-linked compounds, presumably for stereoelectronic reasons. This is seen in comparison of secondary deuterium kinetic isotope effects for the acid-catalyzed hydrolysis of α-methyl glucoside ($k_H/k_D = 1.188$) and β-methyl glucoside ($k_H/k_D = 1.089$).[3] It is also seen in the greater percentage of product of inverted anomeric configuration formed upon ethanolysis of β-glycosides compared to that of α-glycosides.[6]

The relative rates of hydrolysis determined in this study for the deoxy and deoxyfluoro DNP glucosides were 2-deoxy > 4-deoxy > 6-deoxy ≈ 3-deoxy > parent > 6-deoxyfluoro > 3-deoxyfluoro > 4-deoxyfluoro > 2-deoxyfluoro (Table 1), which is essentially the same order seen for the substituted α-glucosyl phosphates. This order does not reflect the distance of the modified substituent from C-1 and thus cannot be rationalized simply by the field effect felt at C-1. However, field effects likely play a major role in determining the rates of glycoside hydrolysis, because in all cases deoxygenation of the glucoside increases the rate of hydrolysis with respect to the parent glycoside, while fluorination has the inverse effect. Indeed, studies by Oppenheimer of the hydrolysis rates of a series of 2-position substituted nicotinamide β-D-ribofuranosides[25] revealed a strong correlation of rate constant with substituent σ_I ($r = 0.99$, $\rho_I = -6.7$) consistent with the importance of field effects at the 2-position. Further, such effects are also seen at the 6-position of glucosides, as is seen if the previously published rate constants for the acid-catalyzed hydrolysis of a series of 6-substituted methyl glucosides[26] are plotted against σ_I according to the equation $\log k = \rho_I\sigma_I + \log k_o$, when a correlation coefficient of $r = 0.97$ ($\rho_I = -3.0$) is observed.

To gain insight into the consequences of substitution at each position and the sources of the rate differences, Hammett plots of log k versus σ_I were constructed for each group of glycosides substituted at a particular position (Figure 3). Although these plots contain only three data points in most cases, such plots are very informative, yielding the correlation coefficients (r) and sensitivities (ρ_I) shown in Table 2. These plots provide reasonable evidence that the major factor determining the rates of hydrolysis of the substituted DNP glycosides at a given ring position is the field effect of the substituent on the oxocarbenium ion-like transition state. The sensitivities of these systems to the substituent, as reflected in ρ_I values, are consistent with their general proximity to the reaction center, with the greatest values being at the 2-position ($\rho_I = -8.3$ to -10.7) and the smallest being at the 6-position ($\rho_I = -2.2$ to -2.5). These sensitivities are quite consistent with those noted earlier from work by Oppenheimer on the 2-substituted ribosides and that by Timell on the 6-substituted glucosides.[25,26]

Transition State Structure. While the solvolysis of DNP glycosides clearly proceeds via an oxocarbenium ion-like transition state,[6,8] a species conventionally represented with partial double bond character and partial positive charge between C1 and O5, the distribution and extent of charge development between these centers is unclear and likely variable. Chemical intuition regarding the predominance of the formal charge at O-5 to maintain a stable octet of electrons on each atom is supported by ab initio calculations (6-31G*) ,which were used[27] to assess the extent of oxocarbenium ion formation in the acid-catalyzed hydrolysis of 2-methoxy tetrahydropyran. These studies indicated that lone pair donation from O5 into the nσ^* orbital at C1 was important to attainment of the transition state and that cleavage of the C1−O1 bond takes place concurrently with formation of a C1−O5 double bond. Thus the transition state for this system more closely resembles an oxocarbenium than a C1 carbonium ion. A semiempirical modeling study (PM3)[28] calculated the partial charge distributions for the ring carbons and oxygen in α-D-glucose and in a glucopyranosyl oxocarbenium ion. This study, along with AMPAC modeling of the mannopyranosyl oxocarbenium ion,[29] indicated that the greatest increases in partial positive charge between the ground state and the transition state were at O5 and C1, with the greatest increase in positive charge development at O5. Though these model studies are suggestive, none considers the contribution of the solvent, the nucleophile, or the leaving group to the stability of the oxocarbenium ion at the transition state. However, these model studies, together with experimental results of ^{2}H, ^{13}C, and ring ^{18}O kinetic isotope effects of the acid-catalyzed hydrolyses of methyl glucosides,[3] do confirm that charge development at the transition state of glycoside hydrolysis is delocalized between C1 and O5.

Kirkwood−Westheimer Analysis. Glucosides. Extending the previous σ_I analysis to examine the relative rates of hydrolysis of the DNP glucosides substituted at *different* positions, the observed relative order of rates appears to be determined by field effects according to the Kirkwood−Westheimer model.[30] Our analysis was similar to that applied to examination of the effect of ring substituents on the pK_a of carboxylic acids in 2,2,2-bicyclo systems.[31] This model predicts that the relative values of k (the equilibrium constant in this case) for two structures differing by a single substituent (x and y) can be calculated using eq 1 , where e is the charge generated

$$\log(k_x/k_y) = (e/2.3kT)[(\mu \cos\theta/R^2D_E)_x - (\mu \cos\theta/R^2D_E)_y] \quad (1)$$

and θ is the angle between a line of length R joining the point where charge is generated and the midpoint of the dipole associated with the substitution. D_E is the dielectric constant of the space through which the field effect is transmitted, and μ is the magnitude of the dipole associated with the substitution.

Extension of this analysis to comparison of the relative rates of reaction examined in the current study is afforded by the use of transition state theory. Both modeling studies[28,29] and

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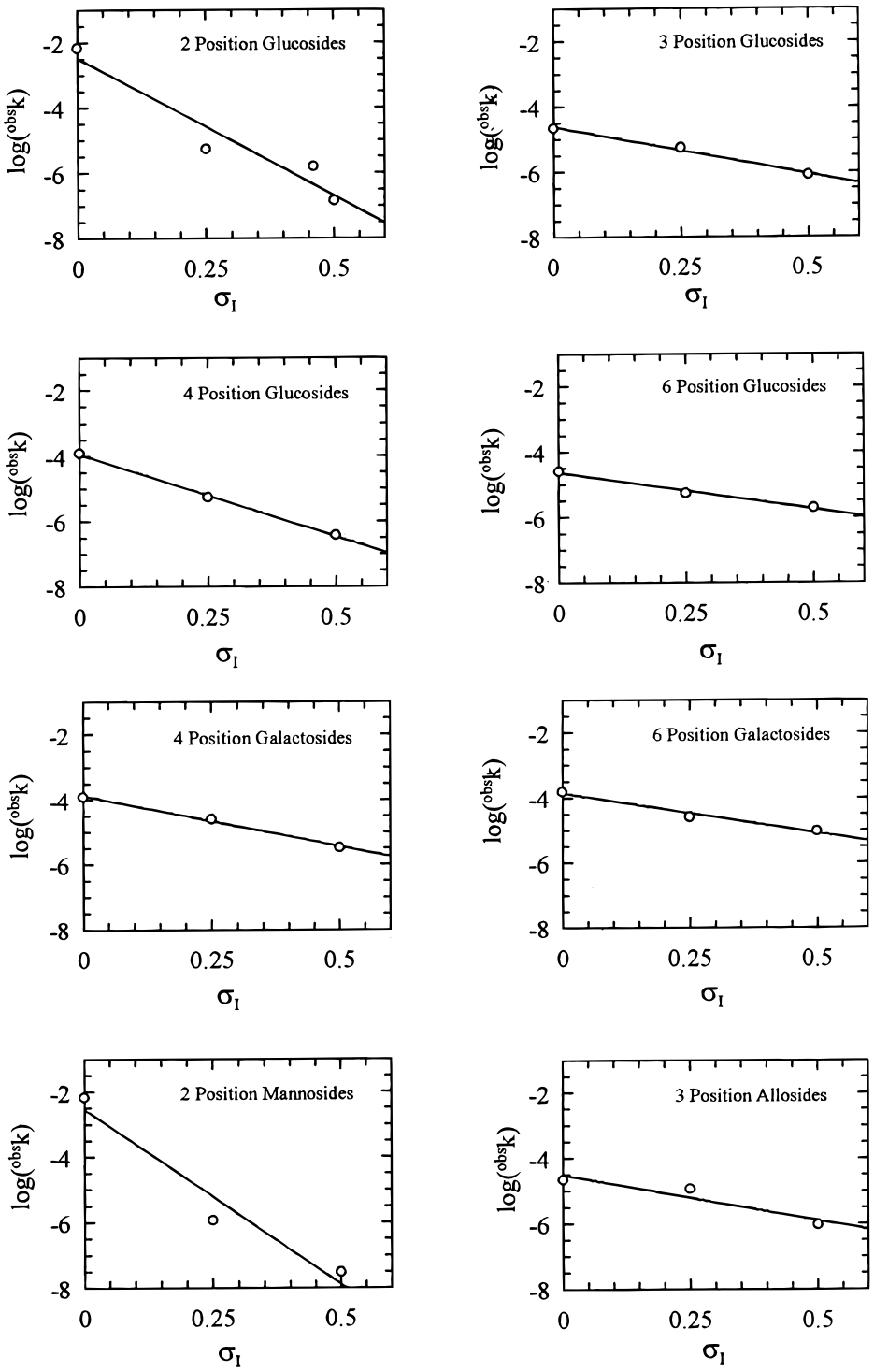

Figure 3. Hammett plots correlating rate constants for hydrolysis of the DNP glycosides at 37 °C with σ_I. Each plot represents the effects of changing the substituent at a single position with a single stereochemistry. The plot of 2-position substituted glucosides includes an additional point measured for 2,4-dinitrophenyl 2-chloro-2-deoxy β-D-glucopyranoside ($k = 1.61 \times 10^{-6}$ sec^{-1}). Details of parameters extracted from these plots are provided in Table 2.

experiment[3] indicate that the transition state of a glucosyl oxocarbenium ion is flattened toward a ^{4}H half-chair with partial charge development at *both* C1 and O5. The majority of the charge generated resides at O5.[3,28,29] Through space distances between O5 or C1 and the dipole midpoints of the substituted centers, along with values for θ, were calculated for such a glucosyl oxocarbenium ion using InsightII (Molecular Simulations Inc., San Diego, CA.). Minimization using either steepest descents or conjugate gradient algorithms converged on essentially identical conformations. Because substitutions are made on the same system at one temperature, *e*, *k*, and *T* are constant. Further, given the conservative substitutions examined in the current study, and the relative insensitivity of field effects to the ring system examined[31] it is reasonable to assume that D_E is constant. Values of σ_I were substituted for μ as measures of the substituent dipole.[32]

Values of relative rates for each of the substituted DNPglycosides $\{\log(^{calcd}k_{rel})\}$ can therefore be calculated by the summing of all of the relative field effects due to the substituents on the ring felt at *both* C1 and O5 using different charge

distributions between these two centers. Field effects, when uncomplicated by steric factors as in this case, have been shown previously to be strictly additive in a closely analogous system involving comparison of rates of hydrolysis for a series of deoxy- and deoxyfluoro α-glucosyl phosphates.[19] Therefore, determination of $\log(^{calcd}k_{rel})$ for DNPglc requires calculation of relative field effects for hydroxyl groups at C2, C3, C4, and C6, while that for 2FDNPglc requires calculation of the relative field effects for a fluorine at C2 and hydroxyl groups at the 3-, 4- and 6-positions, and so on. Since the Kirkwood−Westheimer model best describes rigid ring systems,[31] it is difficult to estimate the field effect for the 6-position substituents because they are able to rotate around the C5−C6 bond. The weighted populations for the three staggered rotamers of methyl α-D-glucoside in D_2O were therefore used to estimate values of R and θ and thus the relative field effect for this position.[33]

Thus the logarithm of the calculated *relative* rate of a substituted DNP glucoside, $\log(^{calcd}k_{rel})$, is given by eq 2, where

$$\log(^{calcd}k_{rel}) = \sum_{i=C2}^{C6} e_{C1}[\sigma_{Ii} \cos \theta_i/(R_{C1i})^2] + e_{O5}[\sigma_{Ii} \cos \theta_i/(R_{O5i})^2] \quad (2)$$

e_{C1} is the fractional charge on C1 and e_{O5} is the fractional charge on O5.

Values of $\log(^{calcd}k_{rel})$ were calculated for a range of possible charge distributions, where the total charge at the transition state was shared between O5 and C1. Figure 4 shows a series of plots of $^{calcd}k_{rel}$ values (calculated for various charge distributions between O5 and C1) versus the observed rate constants ^{obs}k, of the DNPglycosides. The calculated relative rates based on the magnitude of the field effect at O5 and C1 as predicted by the Kirkwood−Westheimer model correlate with the observed rates of hydrolysis (r = 0.92−0.98, slopes = 1.21−1.33). The best agreement of the calculated and empirical data was obtained when the majority of the charge generated at the transition state was localized at O5, a finding in agreement with previous studies of the transition state structure for glycoside hydrolysis (vide supra). The optimal fit of the data was obtained when 97% of the charge was localized on O5 (r^2 = 0.967). Further, the observed relative rates of hydrolysis for compounds substituted at C3 and C4 (i.e., 4-deoxy > 3-deoxy, 4-fluoro < 3-fluoro) are predicted by our model at all points when >60% of the charge generated was localized at O5. These data, taken together with the Hammett plots shown in Figure 3, strongly suggest that the relative rates of hydrolysis of these glucosides are dictated largely by the stabilities of the oxocarbenium ion-like transition states and that these stabilities are heavily dependent on field effects associated with the various substituents.

Galactosides and Mannosides. The calculations of relative rates based upon Kirkwood−Westheimer theory used in this analysis thus far have only dealt with cases in which the substituent has the same configuration as in the parent glucoside. Because the theory takes into account the orientation of the dipole associated with the substituent with respect to the center

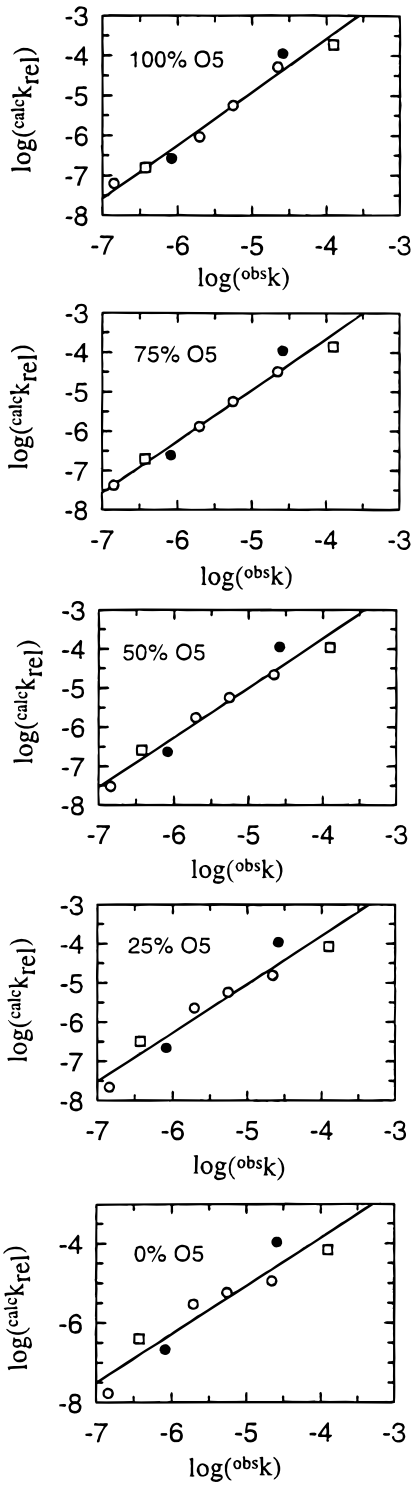

Figure 4. Comparison of the calculated and experimentally determined rate constants for hydrolysis of DNP glycosides. Relative rate constants for substituted glycosides were calculated using eq 2 and the measured rate constant for DNPglc assuming the charge distributions (O5 versus C1) shown and plotted versus observed rate constants. The quality of the fit to the data improved as the amount of charge on O5 increased (r = 0.92−0.98 using σ_I for the calculations, r = 0.91−0.97 using μ; best fit obtained at 97% of charge at O5, r^2 = 0.967 using σ_I, r^2 = 0.942 using μ). Glycosides substituted at the 3-position (filled circles) and 4-position (open squares) are highlighted to demonstrate that the observed relative rates of hydrolysis are best predicted by the model when the majority of the positive charge obtained at the transition state resides on O5. Data points on the graph represent, from the left in order of increasing ^{obs}k, 2-fluoro-, 4-fluoro-, 3-fluoro-, 6-fluoro-, parent, 3-deoxy-, 6-deoxy, and 4-deoxy.

(32) To validate the use of σ_I as a substitute for μ, the calculations used to obtain Figure 4 were carried out using both σ_I (H = 0.0, OH = 0.25, F = 0.5) (Charton, M. *J. Org. Chem.* **1964**, *29*, 1222−1227) and bond dipole moment μ (H = 0.4 D, OH = 0.7 D, F = 1.39 D) (Minkin, V. I.; Osipov, O. A.; Zhdanov, Y. A. *Dipole Moments in Organic Chemistry*; Plenum Press: New York, 1970; p 88). The data obtained with the two parameters were very similar (see Figure 4 and its legend) though slightly better correlations were obtained with σ_I.

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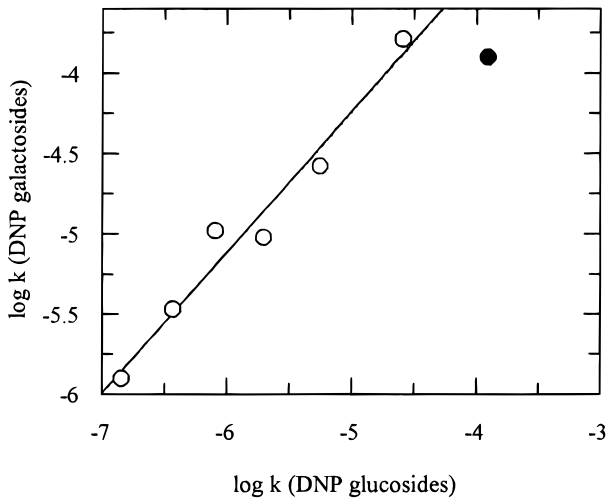

Figure 5. Comparison of rate constants for hydrolysis of DNP glucosides and DNP galactosides. A correlation coefficient of $r = 0.98$ was observed if the point for the 4-deoxyglucoside (filled circle) was omitted, as represented by the line shown.

of charge it should be possible to extend this analysis to the epimeric galactosides, allosides, and mannosides, as long as the transition state structures are similar in all cases. Considerable similarity of transition states for the hydrolysis of glucosides and galactosides is indeed indicated by the excellent linear free energy relationship ($r = 0.98$ if the 4-position is excluded) observed between the rates of hydrolysis of the two series of compounds (Figure 5), as well as by the nearly identical α-DKIE values obtained for the DNP galactoside ($k_H/k_D = 1.11 \pm 0.01$) and the DNP glucoside ($k_H/k_D = 1.09 \pm 0.02$). Predictions of rates for allosides and galactosides should therefore be feasible. Indeed, calculations predict rates 2- to 28-fold greater for galactosides than for the corresponding glucosides, factors quite consistent with the 5- to 13-fold greater rate constants actually observed. The observed rate constant for hydrolysis of the 4-deoxy "galactoside" appears to be lower than that predicted by the correlation shown in Figure 5. This may be due to the fact that, unlike any other galactosides (including 4FDNPgal), the 4-deoxy "galactoside" lacks an axial C4 substituent and thus lacks the release of steric compression proposed by Edward[14] as important to lowering of activation barriers when axial substituents are present.

There is reason to believe that this steric compression argument may not apply to mannosides, other factors possibly taking precedence given the proximity of the 2-hydroxyl to the incoming nucleophile and the departing aglycone and especially given previous suggestions of a role for the 2-hydroxyl in orienting the incoming nucleophile.[3,6] In addition, modeling studies[29] have suggested that the mannopyranosyl oxocarbenium ion adopts one of two ^{3}H half-chair conformations rather than the ^{4}H conformation. Indeed, the data in this study indicate that the relief of steric strain at the transition state is not a key factor for sugars with an axial 2-substituent, since rate constants for hydrolysis of DNPman and 2FDNPman are 5-fold *lower* than those for their corresponding glucosides. Interestingly, calculations by Woods and co-workers[34] had indeed predicted that lyxopyranosides (axial 2-hydroxyl) would generate a less stable oxocarbenium ion than xylopyranosides (equatorial 2-hydroxyl). Our results do, however, contradict the recent claim that axial electronegative substituents at C4 *accelerate* glycopyranoside solvolysis via electrostatic stabilization of the oxocarbenium ion.[35] In our case, with *sterically conservative* substituents, rate reductions are observed with electronegative substituents. Possibly release of ground-state strain was more important in their case than they had realized.

Solvent Involvement. Although the analysis of activation entropies and enthalpies has generally been avoided in this study, it is noteworthy that activation enthalpies for the galactosides are significantly lower than those for the corresponding glucosides, balanced somewhat by more negative $\Delta S^{\ddagger}$ values, with the net result of a slight decrease in $\Delta G^{\ddagger}$. Such entropy/enthalpy compensations have been noted in a variety of systems such as the ionization of carboxylic acids[36] and binding of carbohydrates to lectins[37] and have been commented on recently in relation to solvation and ligand binding.[38] A rationale proposed for these observations (see, for example, ref 39) invokes a reorganization of the solvent structure as the reaction proceeds, the extent of this being different for different substrates, thereby influencing the reaction rate in different ways. In other systems, more negative $\Delta S^{\ddagger}$ values have been interpreted as reflecting increased solvent organization at the transition state. Indeed, differences in the hydration structure of glucose and galactose and their related pentopyranose congeners[16,40−45] have been observed in a number of studies, galactose apparently being more strongly solvated than glucose in aqueous solution.[46,47] Thus if the transition states are not solvated to exactly equivalent extents, effects upon rates, which would show up in the activation enthalpies, would be expected. Such effects have been noted by others.[46,47]

Conclusions

Hammett correlations of the pH-independent hydrolysis rates of the 6-, 4-, 3-, and 2-position substituted glycosides with the σ_I value for the substituent were linear ($r = 0.95$ to 0.999, $\rho_I = -2.2$ to -10.7), consistent with hydrolysis rates being dictated largely by field effects. The slopes of the plots were similar in magnitude and sign to those seen in other systems involving electron-deficient transition states. The relative rates of hydrolysis of the DNP glucosides, i.e., 2-deoxy > 4-deoxy > 3-deoxy ≈ 6-deoxy > parent > 6-deoxy-6-fluoro > 3-deoxy-3-fluoro > 4-deoxy-4-fluoro > 2-deoxy-2-fluoro, can be rationalized on the basis of the relative stabilities of the oxocarbenium ion-like transition states (predicted by the Kirkwood−Westheimer model), which appear to be principally a function of field effects exerted by the ring substituents on O5, the principal center of charge development at the transition state. In addition the results indicated that differences in the rates of

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hydrolysis between different *series* of hexopyranosides may not arise solely from field effects but may also reflect differences in steric factors or solvation.

Experimental Section

Syntheses of DNP Glycosides. Full experimental details for the synthesis of the compounds described and full characterization data are provided as Supplementary Information. The acetylated deoxy- and deoxyfluoro-glycopyranoses were selectively deacetylated at the 1-position, either by treatment with ethanolamine[48] or hydrazine acetate[49] or by conversion of the per-O-acetylated carbohydrate to the α-glycosyl bromide, followed by silver carbonate-catalyzed hydrolysis.[50] Protected DNP glycosides were synthesized by reaction of the protected hemiacetal with fluoro-2,4-dinitrobenzene.[51] These compounds were then deacetylated by use of methanolic HCl.[52]

Kinetic Studies. General. Conditions used for measuring the rates of spontaneous hydrolysis of the 2,4-dinitrophenyl glycosides are essentially identical to those used by Sinnott for the determination of the rates of hydrolysis of a series of DNP galactosides; however, rate constants were determined by fits to full hydrolysis profiles rather than via initial rates.[8] All rates were determined at pH 6.50 in 25 mM sodium phosphate buffer and 0.40 M KCl. Glycoside concentrations were approximately 0.15 mM. All solutions were prepared with Nano-pure quality water. Hydrolysis rates were determined at three or more temperatures with each glycoside. The temperature was regulated by a Neslab RTE−210 circulating bath. Temperatures were recorded from the available digital readout and were checked using an external thermometer and found to be accurate within ±0.2 °C. The temperatures during the experiment did not fluctuate more than ±0.2 °C. Each rate was determined by fitting the A_{390} of the solution at specific times to a first-order equation using the work station on an Applied Photophysics MV 17 stopped flow spectrophotometer and employing the available fitting routine. Typically seven points were used to determine the rate, and the reactions were allowed to proceed to at least 3 half-lives. The rates at each temperature were measured three times and averaged. As a check of the first-order fits obtained, several rate constants were also determined by plotting $\ln((A_\infty - A_t)/A_\infty)$ vs time. The rates obtained were essentially identical to those found using the fitting routine.

Activation parameters for the substrates were obtained from Eyring plots of the logarithm of the averaged value for the rate constant determined at each temperature divided by temperature versus the reciprocal of the absolute temperature. These data were analyzed by linear regression using GraFit,[53] which provided the slope and intercept for the line obtained and errors from these values. Values for $\Delta H^\ddagger$ were calculated from the slope of the line and values for $\Delta S^\ddagger$ from the *y* intercept. Rate constants shown were calculated from the equations defining the lines obtained for Eyring plots of the data.

High-Temperature Hydrolysis Rates (>50 °C). Approximately 1 mL aliquots of buffered DNP glycoside solutions (approx 0.2, 0.1, and 0.05 mM) were pipetted into 1 mL Wheaton vials, flushed with nitrogen, sealed using a Bunsen burner, and then immersed in a water bath at the required temperature. After equilibrating for 5 min, samples were removed at time intervals and immediately frozen. These solutions were later thawed, and the A_{390} for each of the vials was measured at room temperature using a Pye-Unicam PU 8000 UV−visible spectrophotometer equipped with a sipper cell. Data were analyzed as described previously.

Low-Temperature Hydrolysis Rates. Samples were prepared as described above, placed in quartz cuvettes fitted with Teflon plugs, and sealed with Parafilm. These cuvettes were then placed in a Perkin-Elmer λ II spectrophotometer equipped with a thermostated cell block and temperature probe, and absorbance readings at 390 nm were recorded as a function of time. These absorbances were automatically recorded and then fit to a first-order equation as previously described.

Secondary αDKIE Measurements. The isotope effects for spontaneous hydrolysis of glycosides were determined at 45 ± 0.1 °C using the procedure employed for the low-temperature hydrolysis rates. Rate constants were determined at least six times for each of the deuterated and protiated compounds. Isotope effects were determined from the ratios of consecutive measurements of the rates for the protiated and deuterated compounds, and errors are provided as the standard deviation in the isotope effects determined.

Acknowledgment. We thank Dr. Ross Stewart for his help, encouragement, and advice throughout this project. Financial support from the Natural Sciences and Engineering Research Council of Canada and a GREAT Scholarship from the Science Council of British Columbia (for M.N.N.) are gratefully acknowledged. We also wish to thank Drs. M. L. Sinnott, N. J. Oppenheimer, S. Brown, and P. Charifson for reading early versions of this manuscript and providing helpful comments.

Supporting Information Available: Full experimental details for the synthesis of all compounds described, including their characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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