those for counting the product methyl acetate. In this way the constants α of eq 8 for the catalyst and for the product were ensured equal.

Tracer Experiments. Sodium acetate-t_1 (NaO_2CCH_2T) (New England Nuclear Corp.) (61.5 mg, 75 mCi) was diluted 150-fold with sodium acetate (Fisher Certified) by dissolving both in water distilled in glass from $KMnO_4$ and then freeze-drying the mixture. This was accomplished by freezing the solution with Dry Ice–acetone in a thin layer on the sides of a 150-ml, round-bottomed flask, and then allowing the solution to warm slowly to room temperature while a receiving flask was cooled with Dry Ice–acetone and the system continuously pumped.

A methanol solution (25 ml) 0.1 *M* in the diluted sodium acetate-t_1, 0.4 *M* in acetic acid (Fisher Certified), and 0.001 *M* in *p*-nitrophenyl acetate was thermostated in a glass-stoppered flask at 27.4 ± 0.1°. Samples of 5 ml were withdrawn periodically. To the sample was added 1.0 ml of 0.434 *M* hydrochloric acid in methanol to quench the reaction. The sample was frozen using liquid nitrogen as coolant in a thin layer on the sides of a 15 ml, round-bottomed flask attached to an evacuable, two-armed, short-path still. The still was evacuated to a pressure of 0.1 mm and closed off. The sample was allowed to distill to the receiver which contained 0.1800 g (3.34 mmol) of sodium methoxide to neutralize the acetic acid. The distillation was then repeated to separate methyl acetate-t_1 and methanol as the distillate from the sodium acetate just formed by neutralization of the acetic acid. An aliquot of this distillate was counted. The weight of the distillate and the weight of the aliquot of distillate added to the scintillation solution were determined so that the fraction of the distillate counted and the effective activity of the whole distillate could be determined.

Control experiments showed the procedure reproducibly to yield the correct activity of the starting methyl acetate within 4.5%. In the absence of labeled methyl acetate, no radioactivity appeared in the final distillate.

Acknowledgment. It is a pleasure to thank Dr. Norman S. Behn and Professors R. G. Carlson and J. K. Lee for their advice and assistance.

Glycoside Hydrolysis. III. Intramolecular Acetamido Group Participation in the Specific Acid Catalyzed Hydrolysis of Methyl 2-Acetamido-2-deoxy-β-D-glucopyranoside[1]

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Abstract: The hydrolyses of methyl and pyranosyl glycosides of glucose (Gl) and N-acetylglucosamine (NAG) have been studied in the low pH range at 78.2°, μ = 0.3. The rates of hydrolysis of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Me-β-NAG), di-N-acetylchitobiose (NAG_2), methyl β-D-glucopyranoside (Me-β-Gl), cellobiose (Gl_2), and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (Me-α-NAG) are proportional to hydrogen ion activity (a_H), indicating a specific acid catalyzed mechanism. Me-β-NAG and NAG_2 yield product solutions with $[\alpha]^{30}D$ values equal to that of NAG; Me-β-Gl and Gl_2 yield product solutions with $[\alpha]^{30}D$ values equal to that of Gl. Me-α-NAG hydrolyses with a second-order specific acid catalyzed rate (k_H) one-fourth that of its β anomer, and yields a product solution with an $[\alpha]^{30}D$ value which suggests the formation of a product mixture of methyl 2-amino-2-deoxy-α-D-glucopyranoside and NAG. When log k_H values for β-D-glycosides of NAG are plotted *vs.* the log k_H values for the corresponding β-D-glucopyranosides, a linear relationship is seen to exist for five aryl and pyranosyl glucosides. The point corresponding to the methyl glycosides deviates significantly from the line, and can be accounted for as a 50-fold rate enhancement in the hydrolysis of Me-β-NAG over that anticipated. To account for this rate enhancement two kinetically equivalent mechanisms of hydrolysis of Me-β-NAG are considered: (a) intramolecular general acid catalysis by the protonated 2-acetamido oxygen, and (b) nucleophilic displacement by the 2-acetamido oxygen of the protonated aglycone. The latter mechanism is preferred on the basis of the lower rate of hydrolysis of Me-α-NAG which sterically precludes the possibility of b but not a. Intramolecular acetamido participation in the specific acid catalyzed hydrolysis of Me-β-NAG is concluded to compete favorably with the normal path through an oxocarbonium ion intermediate because the small methyl aglycone does not inhibit the formation of the *trans*-diaxial conformation most favorable to acetamido participation. The significance of this result and its possible relation to the mechanism of lysozyme is discussed.

Lysozyme is the first enzyme to have its tertiary structure determined by X-ray crystallographic methods.[4] From chemical studies[5] and X-ray diffraction studies of complexes of lysozyme with several inhibitors,[6] it is possible to infer that carboxyl groups are the only side-chain functional groups of the enzyme which are both present at the active site and likely to be involved in the bond-breaking steps. Lysozyme specifically hydrolyzes β-linked glycosides of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM).[7] This fact led to the previous model studies in this series, which demonstrated that the acetamido group of 2-acetamido-2-deoxy-β-D-glucopyranosides assists the spontaneous hydrolysis of the glycoside bond in a stereospecific fashion,[1a] and assists in concert

(1) (a) Part I: D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, **89**, 6237 (1967); (b) part II: D. Piszkiewicz and T. C. Bruice, *ibid.*, **90**, 2156 (1968).

(2) Predoctoral Fellow, National Institutes of Health. A portion of the material to be submitted by D. P. for the Ph.D. in Chemistry, University of California at Santa Barbara.

(3) To whom inquiries should be directed.

(4) C. C. F. Blake, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 365 (1967).

(5) J. A. Rupley and V. Gates, *Proc. Natl. Acad. Sci. U. S.*, **57**, 496 (1967).

(6) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967).

(7) For a summary of known lysozyme substrates, see ref 1b.

Table I. Polarimetric Rates of Reaction and $[\alpha]^{30}D$ Infinity Values

Compd	pH	k_{obsd}, min^{-1}	k_H, M^{-1} min^{-1}	k_H (av)	$[\alpha]^{30}D$ (∞), deg	$[\alpha]D$ of product, deg
Me-β-NAG	0.75	1.36×10^{-2}	7.64×10^{-2}	6.20×10^{-2}	53.6	52.1[c]
	1.08	4.92×10^{-3}	5.91×10^{-2}			
	1.54	1.67×10^{-3}	5.80×10^{-2}			
	2.08	4.52×10^{-4}	5.43×10^{-2}			
NAG_2	0.75	2.56×10^{-3}	1.43×10^{-2}	1.15×10^{-2}	57.7	52.1[c]
	1.08	1.00×10^{-3}	1.20×10^{-2}		52.9	
	1.54	2.80×10^{-4}	0.97×10^{-2}			
	2.08	8.12×10^{-5}	0.98×10^{-2}			
Me-β-Gl	0.75	6.87×10^{-4}	3.86×10^{-3}	2.96×10^{-3}	50.6	53.3[d]
	1.08	2.22×10^{-4}	2.67×10^{-3}			
	1.54	6.77×10^{-5}	2.35×10^{-3}			
Gl_2	0.75	9.19×10^{-4}	5.16×10^{-3}	4.01×10^{-3}	51.3	53.3[d]
	1.08	2.82×10^{-4}	3.39×10^{-3}			
	1.54	1.00×10^{-4}	3.47×10^{-3}			
Me-α-NAG	0.75	3.47×10^{-3}	1.95×10^{-2}	1.64×10^{-2}	87.3[a] to 93.8[b]	52.1[c]
	1.08	1.60×10^{-3}	1.92×10^{-2}		82.4[a] to 88.6[b]	or
	1.54	3.06×10^{-4}	1.06×10^{-2}			127[e]

[a] Calculated value of $[\alpha]^{30}D(\infty)$ based on the assumption that methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride is the only optically active product. [b] Calculated value of $[\alpha]^{30}D$ (∞) based on the assumption that NAG is the only optically active product. [c] Value of $[\alpha]^{30}D$ for mutarotated NAG from ref 1a. [d] Value of $[\alpha]^{30}D$ for mutarotated glucopyranose determined in pH 0.75 HCl solution in this study. [e] Value of $[\alpha]D$ for methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride from A. Neuberger and R. P. Rivers, *J. Chem. Soc.*, 122 (1939).

with intramolecular general acid catalysis in the hydrolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[1b] In the previous studies of aryl glycosides[1] no evidence was found for intramolecular acetamido participation in the specific acid catalyzed hydrolyses of 2-acetamido-2-deoxy-β-D-glucopyranosides. The present study considers the specific acid catalyzed hydrolyses of methyl and pyranosyl glycosides. Since these compounds are chemically and structurally similar to the natural lysozyme substrates[7] such a study should help us understand the physical chemistry of these substrates, and possibly the enzyme-catalyzed reaction itself.

Experimental Section

Materials. Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Me-β-NAG) was prepared according to the method of Hough and Theobald,[8] yield 20%, mp 197–198°, $[\alpha]^{30}D$ −42° (water) [lit. mp 196–197°, $[\alpha]^{20}D$ −43° (water)[8]]. Di-N-acetylchitobiose (NAG_2) was a gift from Professor John A. Rupley. The remaining glycosides were purchased from Pierce Chemical Co.

Kinetics. All kinetics measurements were done at 78.2 ± 0.3° in aqueous HCl at μ = 0.3 with KCl. Polarimetrically determined rate constants and pH's at 78.2° were determined as described previously.[1a] The pseudo-first-order rate constants (k_{obsd}) were obtained by calculating the slope of plots of log $[(\alpha_\infty - \alpha_0)/(\alpha_\infty - \alpha_t)]$ *vs.* time (t) or by the method of Guggenheim.[9]

Results

The polarimetrically determined pseudo-first-order rate constants (k_{obsd}) of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Me-β-NAG), di-N-acetylchitobiose (NAG_2), methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (Me-α-NAG), methyl β-D-glucopyranoside (Me-β-Gl), and cellobiose (Gl_2) are presented in Table I. Values for the second-order rate constant for the specific acid catalyzed reaction (k_H) were calculated from the equation $k_H = k_{obsd}/a_H$, where a_H is the activity of hydrogen ion as determined by the glass electrode. For each compound of Table I there is general agreement in the values of k_H determined at various pH's; the mechanism of reaction therefore appears to be specific acid catalysis. For the four β-glycosides examined, Me-β-NAG, NAG_2, Me-β-Gl, and Gl_2, the α infinity values agree well with the α value of the expected products, NAG or glucose.

Me-α-NAG was found to undergo a specific acid catalyzed reaction, but the calculated α infinity value was not in agreement with the value of the expected product, NAG. In a previous study,[10] Me-α-NAG has been observed to yield methyl 2-amino-2-deoxy-α-D-glucopyranoside as well as NAG when allowed to react in an acidic medium. If these two compounds were produced in approximately equal quantities, an α infinity value would be observed similar to that found in this study. Thus, it is reasonable to conclude that in the acid-catalyzed reaction of Me-α-NAG, two reactions take place: (1) the hydrolysis of the glycosidic bond to yield NAG, and (2) the hydrolysis of the 2-acetamido group to yield the relatively stable methyl 2-amino-2-deoxy-α-D-glucopyranoside.[10,11]

Discussion

In Figure 1 the log k_H values for the β-D-glycosides of NAG are plotted *vs.* the log k_H values for the β-D-glucopyranosides. A linear relationship is seen to exist for the following aglycones: *o*-nitrophenyl (*o*-NP), phenyl (P), 1-naphthyl (1-N), *p*-nitrophenyl (*p*-NP), N-acetylglucosaminyl (NAG), or glucosyl (Gl); the rates for the glycosides of NAG are 2.1 to 2.9 times greater than those for glucose. The order of decreasing reactivity (*i.e.*, *o*-NP ≥ P > 1-N > *p*-NP > 4-NAG or 4-Gl) indicates that the rate constants for both series of glycosides are similarly dependent on the electronic and steric nature of the aglycone; neither factor alone, however, is capable of explaining the

(8) L. Hough and R. S. Theobald in "Methods in Carbohydrate Chemistry," Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press Inc., New York, N. Y., 1963, p 162.

(9) E. A. Guggenheim, *Phil. Mag.*, 2, 538 (1926).

(10) A. B. Foster, D. Horton, and M. Stacey, *J. Chem. Soc.*, 81 (1957).

(11) We might note that in the study described in ref 10, Me-β-NAG was reported to yield both NAG and methyl 2-amino-2-deoxy-β-D-glucopyranoside. Our experiments, performed under somewhat different conditions, indicate that only NAG is formed in the specific acid catalyzed hydrolysis of Me-β-NAG.

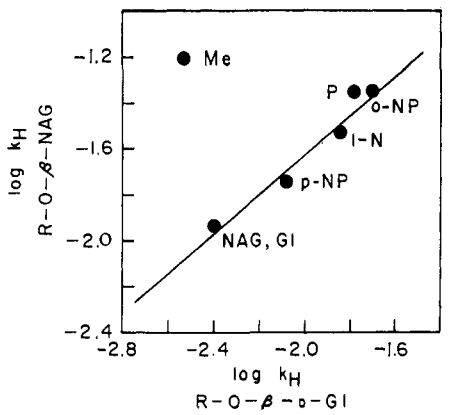


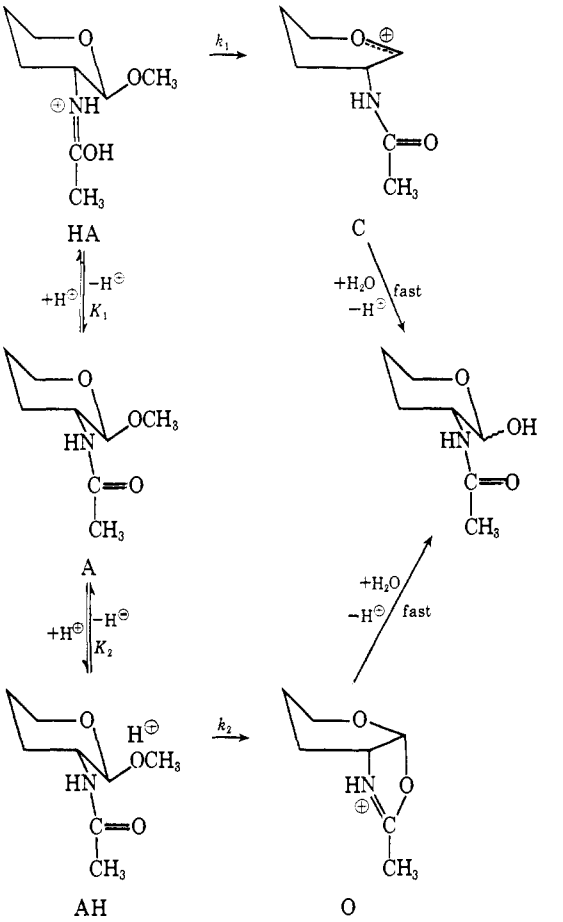
Figure 1. Plot of log k_H for β-glycosides of NAG *vs.* log k_H for β-glucopyranosides. Values of k_H for Me and NAG or Gl glycosides are from Table I. All other values are from ref 1a and 1b.

order of reactivity. Thus, the pK_a's of the conjugate acids of the leaving groups are: *o*-NP, 7.23;[12a] P, 9.98;[12a] 1-N, 9.85;[12a] *p*-NP, 7.15;[12a] 4-(pyranosyl), *ca.* 14.0.[12b] The greater reactivity of *o*-NP compared to P and *p*-NP glycosides is anticipated on the basis of the known greater reactivity of *ortho*-substituted glycosides compared to *para*-substituted glycosides.[13] The two- to threefold ratio of rate constants for the β-D-glycosides of NAG compared to those of glucose is in the opposite direction to that anticipated from steric considerations. Glycoside hydrolysis has been postulated to proceed through a planar carbonium–oxonium ion intermediate[14] formation of which shifts the bulky amide from a favorable equatorial to a less favorable quasi-axial position, thereby hindering the hydrolysis. In the ground state of the β-D-glycosides of NAG, the bulky groups attached to C-1 and C-2 are placed in close proximity, even though both are equatorial. This steric repulsion tends to push the aglycone out of the equatorial plane, twisting the chair conformation closer to the planar intermediate. Thus, greater steric demand by the aglycone has been shown to increase the hydrolysis rates of β-D-glucosides,[15] presumably because it creates a conformational change in the ground state.

Only one point in Figure 1 deviates significantly from the best line drawn for the other glycosides: that corresponding to the methyl glycosides. This deviation may be explained as either a 50-fold rate acceleration in the hydrolysis of Me-β-NAG over that anticipated, or a rate inhibition in the hydrolysis of Me-β-Gl. Our relative values for the specific acid catalyzed rate constants (k_H) for methyl (Me), phenyl (P), and *p*-nitrophenyl (*p*-NP) β-D-glucopyranosides (1:2.82:5.6) agree qualitatively with those tabulated by BeMiller[16] (1:2.16:9.46). The rationale for this order of susceptibility to acid-catalyzed hydrolysis has been treated previously.[15] If this rationale is accepted the rate of hydrolysis (k_H) of Me-β-Gl is reasonable; therefore, the value of k_H for the hydrolysis of Me-β-NAG is *ca.* 50-fold greater than would be anticipated on the basis of the linear relationship of Figure 1.

To explain this enhancement in rate one may consider two kinetically equivalent mechanisms differing only in the position of the proton (Chart I). The pK_a's of amides are distinctly greater than those of acetals[17] so that the amide oxygen should be protonated

Chart I



prior to the C-1 oxygen. The protonated amide oxygen might then act as an intramolecular general acid catalyst in the hydrolysis of the methyl glycoside bond *via* k_1 of Chart I. In this mechanism, the intermediate pyranosyl carbonium ion (C) would rapidly react with water yielding the product NAG. If this mechanism accounts for the hydrolysis of Me-β-NAG, the over-all rate expression may be written as $v = k_1HA$. On the basis of a material balance derivation, we may write that at any value of a_H

$$k_{obsd} = \frac{a_H k_1 K_2/(K_1 + K_2)}{K_1K_2/(K_1 + K_2) + a_H} \qquad (1)$$

If the value of $K_1K_2/(K_1 + K_2)$ is much larger than a_H, then k_{obsd} equals a_H times a complex constant which is equal to the determined value of k_H.

Alternatively, the oxygen of the 2-acetamido group might give intramolecular nucleophilic assistance in

(12) (a) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962, p 130. (b) The pyranosyl 4-hydroxyl group was considered to have a pK_a similar to that of pentaeryithritol; P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, 82, 795 (1960).

(13) R. L. Nath and H. N. Rydon, *Biochem. J.*, 57, 1 (1954).

(14) C. A. Bunton, T. A. Lewis, D. R. Llewellyn, and C. A. Vernon, *J. Chem. Soc.*, 4419 (1955).

(15) M. S. Feather and J. F. Harris, *J. Org. Chem.*, **30**, 153 (1965).

(16) J. N. BeMiller, *Advan. Carbohydrate Chem.*, **22**, 25 (1967), see Table VII of this review.

(17) (a) A. R. Goldfarb, A. Mele, and N. Gutstein, *J. Amer. Chem. Soc.*, 77, 6194 (1955); (b) R. B. Homer and R. B. Moodie, *J. Chem. Soc.*, 4377 (1963); (c) J. Koskikallio and S. Syrjäpalo, *Suomen Kemistilehti, B*, 37, 120 (1964).

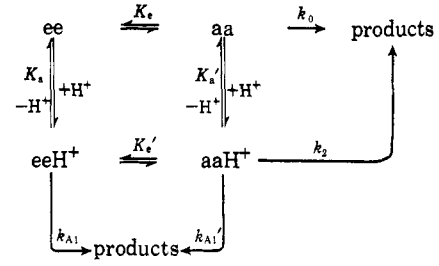
displacing the protonated glycoside bond, *via* k_2 of Chart I. Precedents for an intramolecular reaction of this type exists in the great spontaneous rates of solvolysis of *o*- and *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosides.[1a] This mechanism leads to an unstable protonated oxazoline intermediate. The structurally analogous compound 2-methyloxazoline has been shown to hydrolyze rapidly in the acid region at 25°.[18] Therefore, any oxazoline intermediate, if formed, should hydrolyze rapidly to NAG, which is the observed product of the hydrolysis. If this mechanism accounts for the hydrolysis of Me-β-NAG, the over-all rate expression may be written as $v = k_2AH$, which leads to eq 2. Here also, if $K_1K_2/(K_1 + K_2)$ is

$$k_{obsd} = \frac{a_H k_2 K_1/(K_1 + K_2)}{K_1K_2/(K_1 + K_2) + a_H} \qquad (2)$$

much larger than a_H, then k_{obsd} equals a_H times a complex constant which is equal to the determined value of k_H. Thus, either of the proposed mechanisms could explain the observed rate enhancement in the hydrolysis of Me-β-NAG, and, since they yield kinetically equivalent over-all rate expressions for k_{obsd} (eq 1 and 2), they cannot be differentiated on a kinetic basis.

Me-α-NAG was found to hydrolyze with a polarimetrically determined, specific acid catalyzed rate (k_H) one-fourth that of its β anomer (Table I), and to give an α infinity value suggesting a mixture of products. Examination of molecular models demonstrates that for Me-α-NAG, a protonated acetamido is sterically permitted to act as an intramolecular general acid catalyst (similar to the k_1 mechanism for Me-β-NAG). However, the *cis*-1,2 conformation of substituents in Me-α-NAG precludes intramolecular nucleophilic atack by the 2-acetamido group on the protonated glycoside linkage (similar to the k_2 mechanism for Me-β-NAG). It seems reasonable to conclude, therefore, that Me-β-NAG hydrolyzes *via* the k_2 mechanism, with intramolecular acetamido group participation. Me-α-NAG, which is not allowed this mechanism, must undergo hydrolysis of its glycoside linkage *via* the normal pyranosyl carbonium ion intermediate mechanism.[14] A competing specific acid catalyzed hydrolysis of the amide bond yielding methyl 2-amino-2-deoxy-α-D-glucopyranoside[10] would explain the observed α infinity values (Table I).

The results of this and previous studies on glycoside hydrolysis[1] may be explained in part on the basis of glycoside conformation. A simplified scheme for the proposed mechanism of glycoside hydrolysis—considering reactive conformations—is presented in eq 3. In eq 3, the equilibrium constant for the diequatorial

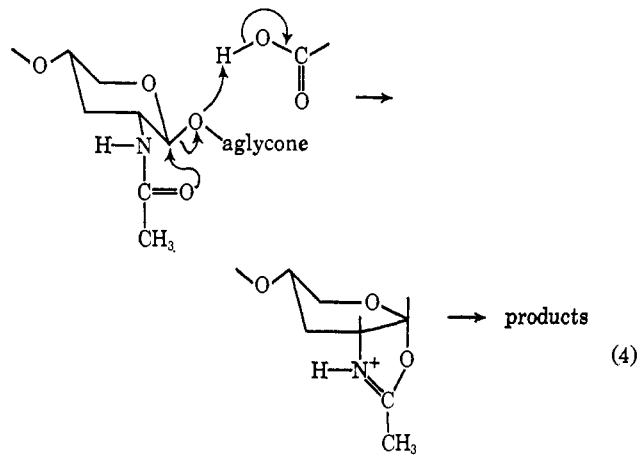


(3)

(ee) and diaxial (aa) conformations of aglycone and acetamido groups of R-O-β-D-NAG is K_e = ee/aa. The rate constants k_{A1} and k_{A1}' are for the specific acid catalyzed hydrolysis of glycosides in the ee and aa conformations, respectively. The dissociation of their conjugate acids are provided by K_a and K_a', respectively. Rate constants k_0 and k_2 pertain to hydrolytic paths involving participation of the neighboring acetamido group. An increase in size of the aglycone obviously results in an increase in K_e and K_e'. For the aryl glycosides of β-NAG in the region of low acidity (pH 2–11) the species aaH^+ and eeH^+ comprise an insignificant mole fraction of glycoside, so that the mechanisms associated with k_{A1} and k_{A1}' are of no kinetic significance. Even though aa is not as favorable a conformation as ee, the hydrolysis of the aryl glycosides occurs *via* k_0 because the aryl groups ($\rho^- = +2.6$)[1b] are good leaving groups. Thus, the spontaneous hydrolysis of the aryl glycosides of β-NAG occurs with anchimeric participation of the acetamido group even though the aa conformation represents a very small fraction of the ground state due to a lack of competition by other mechanisms. For the poor leaving groups, Me and NAG, spontaneous hydrolysis through participation of the weakly basic acetamido (k_0) is not anticipated.

In the acid range the paths of hydrolysis are expected to be through k_2, k_{A1}, and k_{A1}'. For the larger leaving groups (4-NAG, *o*-NP, *p*-NP, P, 1-N) the mole fraction of eeH^+ will far exceed aaH^+ due to severe axial–axial steric interactions. Consequently, the favored pathway is k_{A1}. For Me-β-NAG, however, aaH^+ should be more stable than in the case of the aryl glycosides and the pathways to product through k_2 and k_{A1}' are preferred. Apparently the pathway through k_2 is favored over that through k_{A1}'.

A mechanism of lysozyme-catalyzed glycoside hydrolysis has been considered which employs simultaneous general acid catalysis by glutamic acid-35 of the enzyme and intramolecular nucleophilic attack by the acetamido oxygen of the substrate[1a] (eq 4). Since the



(4)

amount of acetamido participation has been shown to be dependent on leaving-group tendencies,[1b] the significance of acetamido group participation in the enzyme-catalyzed hydrolysis of a polysaccharide where leaving-group tendencies are poor, could justifiably be questioned. This study has demonstrated that a mechanism involving acetamido group participation (eq 4) may

(18) R. B. Martin and A. Parcell, *J. Amer. Chem. Soc.*, **83**, 4835 (1961).

compete with one involving an oxocarbonium ion intermediate if a suitable acid catalyst is present and the pyranoside ring undergoes a suitable conformational change. Glutamic acid-35 of lysozyme has been proposed to act as a general acid catalyst[6] and model studies[1b] have demonstrated that a carboxyl group may act as an effective intramolecular general acid catalyst.

In attempting to elucidate the mechanism of catalysis by lysozyme we must answer the question of primary importance, is the 2-acetamido group an absolute requirement for catalysis? A suitable method of answering this question is to examine the interaction of lysozyme with β-glucose oligomers. Since cellobiose is known to bind to lysozyme,[19] larger oligomers should bind more effectively. A determination of K_m and k_{cat} for such oligomers should then demonstrate the significance of the 2-acetamido group in the enzyme mechanism. These experiments are now in progress.

Acknowledgments. This work was supported by a grant from the National Institutes of Health. We wish to thank Professor John A. Rupley for his gift of di-N-acetylchitobiose.

(19) J. A. Rupley, L. Butler, M. Gerring, F. J. Hartdegen, and R. Pecoraro, *Proc. Natl. Acad. Sci. U. S.*, 57, 1088 (1967).

Absence of Carbonyl Oxygen Exchange Concurrent with the Alkaline Hydrolysis of Substituted Methyl Benzoates[1]

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Abstract: The carbonyl oxygen-18 content of labeled methyl benzoate, methyl *p*-nitrobenzoate, methyl *p*-aminobenzoate, and ethyl benzoate was monitored during the course of alkaline hydrolysis. No oxygen-18 depletion was observed for any of the three methyl benzoates for up to 85% completion of reaction in 33.3% dioxane–water. In addition, the incorporation of solvent oxygen-18 into methyl *p*-nitrobenzoate during alkaline hydrolysis in 33.3% dioxane–water employing water enriched in oxygen-18 was measured. Although the latter results are complicated by possible exchange of oxygen-18 into the *p*-nitro substituent, a minimum value of 36.4 could be assigned to the ratio k_h/k_{ex}. These results are not in agreement with a previously published report of extensive oxygen exchange concurrent with the alkaline hydrolysis of these compounds under these conditions. Both methyl and ethyl benzoate were observed to exchange carbonyl oxygen with solvent oxygen during the course of alkaline hydrolysis in water in the absence of added dioxane. The values of k_h/k_{ex} were 27.7 and 12.6, respectively. These observations are consistent with the interpretation that the principal determinant of the k_h/k_{ex} ratio is the stability of the departing anion; but other factors including the affinity of the anion for acyl carbon as opposed to the proton and rates of proton transfer within the intermediate must be considered in a quantitative evaluation.

Four principal lines of evidence have been advanced in support of the existence of tetrahedral addition intermediates in certain acyl transfer reactions: (1) negative deviations from pH–rate profiles which cannot be attributed to reactant ionization,[3] (2) changes in product distribution after the rate-determining step in the over-all reaction,[4] (3) decreasing slopes in plots of observed rates *vs.* catalyst concentrations,[5] and (4) the existence of concurrent carbonyl oxygen exchange and hydrolysis.[6-8] While the interpretation of the first three of these four criteria in terms of a tetracovalent intermediate has not been challenged, it has been suggested that oxygen-18 exchange might proceed by a mechanism not involving an intermediate.[9] The recent demonstration that oxygen-18 exchange measurements give results entirely consonant with the pH–rate profile for the hydrolysis of ethyl trifluorothiolacetate militates against this latter interpretation at least for this one case.[6]

It has been reported that the ratios of the alkaline hydrolysis rate constants to those for carbonyl oxygen exchange for substituted methyl benzoates are sensitive to the nature of the substituent on the benzene ring. The ratio, measured in 33% dioxane–water, of k_h/k_{ex} ranged from 30 for the *p*-amino compound to 2.8 for the *p*-nitro, and the results were interpreted in terms of the lifetime of the intermediate being of the same order of magnitude as the rate of proton transfer within it.[8] Related work in this laboratory required an ester which would exchange its carbonyl oxygen atom with water in alkaline medium at a rate comparable to the hydrolytic rate. Methyl *p*-nitrobenzoate-carbonyl-

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