# ADVANCES IN ENZYMOLOGY

## AND RELATED AREAS OF MOLECULAR BIOLOGY

Founded by F. F. NORD

Edited by ALTON MEISTER

CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK

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# PHYSICAL ORGANIC MODELS FOR THE MECHANISM OF LYSOZYME ACTION

By BEN M. DUNN, *Bethesda, Maryland* and THOMAS C. BRUICE, *Santa Barbara, California*

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## I. Introduction

Given the ubiquitous array of polysaccharides as components of connective tissues (hyaluronic acid), bacterial cell walls (poly-NAG–NAM), plant cell walls (cellulose), crustacean exoskeleton (poly-NAG or chitin), and as energy stores (glycogen), an understanding of the enzymatic mechanisms by which these materials are synthesized and degraded would be of great general interest. At present the most thoroughly studied glycosidase is hen's egg white lysozyme (E.C.3.2.1.17).

Interest in the mechanism of lysozyme action was greatly stimulated by the elegant work of Phillips and collaborators which has revealed the three-dimensional structure of the crystalline protein. The reader is referred to references 1–9 for a complete discussion of the

1

structure. For the purposes of this chapter it will suffice to state that the binding site accommodates six sugar residues (A,B,C,D,E,F) and that the substrate binding site is predominately hydrophobic and contains only two side chain groups that are considered as candidates for involvement in the catalytic mechanism—the carboxyl groups of the side chains of Glu 35 and Asp 52 located at the junction of sites D and E.

The generally employed substrate for lysozyme, the cell walls of *Micrococcus luteus*, consists of an alternating copolymer of 2-acetamido-2-deoxy-D-glucose (NAG) and N-acetylmuramic acid (NAM) units, crosslinked by polypeptide chains through amide bonds to the lactyl side group of the muramic acid residues (10–16). Thus the functional groups available to participate in the catalytic process are the two carboxyl groups of the protein and the 2-acetamido group of the substrate. The following discussion will consider first the character of the enzymatic reaction, and second the various model systems designed to ascertain the magnitude of catalysis available from participation of these functional groups.

## II. Characterization of the Enzymatic Reaction

Following the elucidation of the structure of the crystalline protein it was also possible to determine the structure of a crystalline complex of lysozyme and the ineffective substrate tri-(N-acetylglucosamine) or chitotriose. In the case of the crystalline derivative employed for X-ray examination chitotriose occupies the binding sites A, B, and C of the active site cleft. By assuming that the binding of longer oligomers should be identical to that of the trimer in the region where the trimer binds, Phillips built a Kendrew model of the binding of a hexasaccharide by fitting additional sugar residues on the enzyme–trimer Kendrew model (4–7). It was explicitly assumed that extension of the trimer to the hexamer by addition of sugar residues D, E, and F was not accompanied by any change in the tertiary structure of the protein in these regions. With this assumption the contact points of protein to substrate and the conformation of substrate moieties at sites D, E, and F were assigned on the basis of the most reasonable means of binding. For this reason mechanisms which incorporate these features rest on model building (i.e., no direct X-ray studies can be carried out with the actual pro-

ductive ES complex). Figure 1 gives the approximate orientation of the hexasaccharide in the active site cleft with relative positions of some of the amino acid residues. Residues E and F of the sugar chain make nonpolar contact with some protein side chains and a scheme of hydrogen bonding could be proposed involving residues Phe 34, Glu 35, Asn 37, Asn 44, and Arg 114. The following observations have been made from examination of this model (4–7): The sugar ring occupying subsite D must be distorted to a half chair conformation due to the interaction of the 6-OH and residues 52 and 108 of the enzyme and the 2-N-acetyl group of the sugar residue in subsite C. This distortion is required to allow the hexasaccharide to fit the curved enzyme surface. Because of the orientation of the sugar rings, the bulky lactyl groups at C-3 of the NAM residues of the natural substrate cannot be accommodated at subsites A, C, or E (assuming NAG–NAM oligomers bind the same as NAG oligomers). Thus NAM residues must be at subsites B, D, and F. Since cleavage of the natural substrate occurs only on the reducing end of NAM residues, the linkage affected by the enzyme must be between sugars B and C, or D and E. Since the trisaccharide complex in subsites A, B, and C is stable, the cleavage must occur between residues D and E. Examination of this region of the protein for functional groups able to participate in the catalytic reaction has revealed only the side chain carboxyl groups of Glu 35 and Asp 52.

Based on these observations and a consideration of the features of glycoside hydrolysis, as understood at that time, a mechanism was proposed to account for the catalytic efficiency of lysozyme (Fig. 2). In this mechanism, the proposed distortion of the sugar ring at subsite D to the half-chair conformation moves the substrate toward the transition state for bond cleavage. Oxocarbonium ion formation is favored in the strained half-chair conformation where overlap of the neighboring oxygen lone pair electrons with the developing positive charge at $C_1$ is maximized. General acid catalysis via protonation of the leaving oxygen atom by Glu 35 is suggested to facilitate $C_1$–O bond cleavage. Stabilization of the positively charged intermediate oxocarbonium ion is presumed to occur by electrostatic interaction with the ionized carboxyl group of Asp 52 (Fig. 2).

Since this mechanism is based on interactions of functional groups of the enzyme side chains with the substrate it should be possible to examine these hypotheses in relevant model compounds. In Section
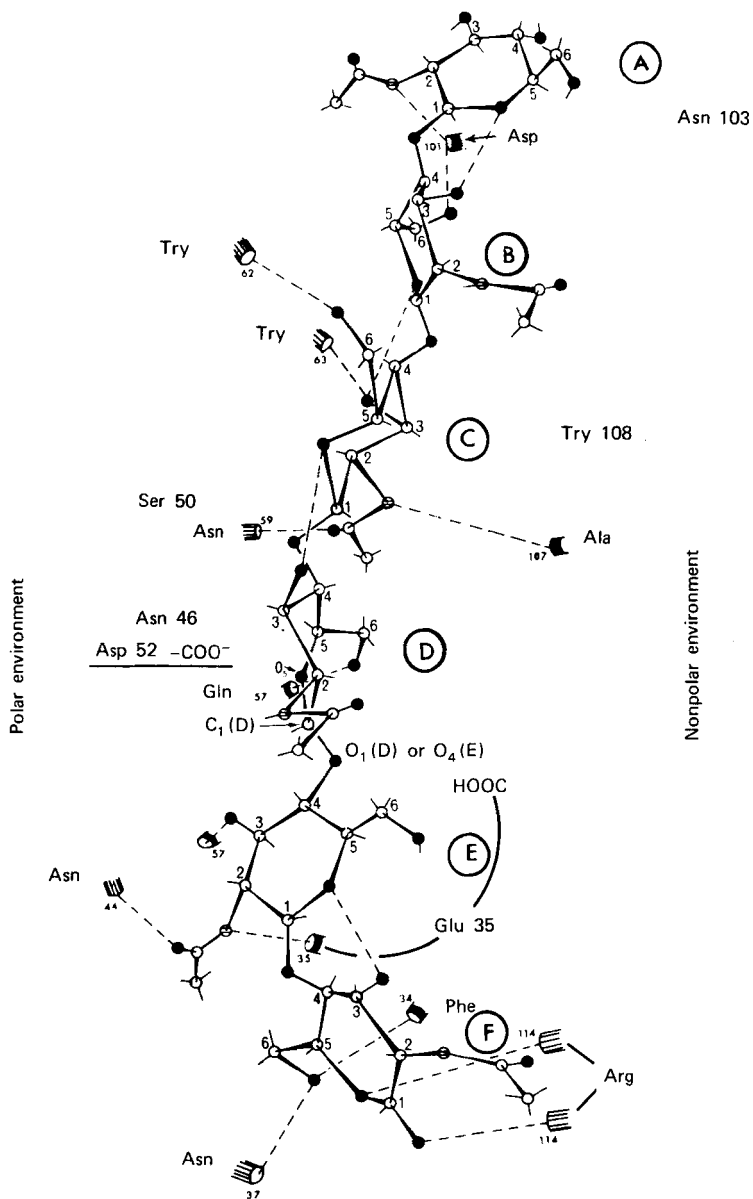
Fig. 1. Orientation of hexasaccharide in the active site as deduced from X-ray structure of trimer enzyme complex and model building (after Blake et al.,

III we will examine in detail the chemistry of hydrolytic reactions related to the process catalyzed by lysozyme.

Many investigations of a chemical nature have probed the environment of the enzyme-active site. The intimate knowledge of the protein structure obtained by Phillips and collaborators has facilitated the interpretation of these results.

A variety of studies have examined the substrate specificity of lysozyme. The enzyme cleaves $\beta(1 \rightarrow 4)$ linked glycosidic bonds and shows no activity toward naturally occurring $(1 \rightarrow 2)$, $(1 \rightarrow 3)$, $(1 \rightarrow 6)$, or $\alpha(1 \rightarrow 4)$ glycosidic bonds. However, Sharon has presented evidence that transglycosylation sometimes results in formation of bonds other than $\beta$ $(1 \rightarrow 4)$ with sugars other than NAG as acceptors (17,27).

The sugar residue that contributes the anomeric carbon undergoing substitution (at subsite D of the active site) may be glucose, 2-deoxyglucose, NAM, as in the natural substrate, or, since chitin oligomers are hydrolyzed by lysozyme, NAG.

X-ray studies combined with model building, based on these studies (discussed above), have shown that the active site consists of a cleft in one side of the molecule which can accommodate up to six sugar residues and that the strongest binding occurs in that portion of the cleft remote from the catalytic site. Thus the best substrates for the enzyme should be those that contain enough residues to span the catalytic site. Rupley et al., have examined the binding of NAG oligomers ($NAG_n$, $n = 1-6$) and found that binding increases with increasing chain length up to the trimer. Further extension of the chain does not increase the binding constant above that observed with $NAG_3$ (29). This behavior has been attributed to the distortion resulting from binding in site D which increases the free energy of the bound state. Thus subsite D is only occupied when binding in site E or sites E and F adds a compensating negative term to the free energy of binding.

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ref. 6). Some positions of the amino acid side chains are indicated. The sensitive linkage is between residues D and E. Note the proximity of Glu 35 and Asp 52 carboxyl groups and the distortion of ring D to a half-chair form postulated to arise by interaction of the 6-OH of D with the enzyme. In the natural substrate the 3- oxygen of residues B, D, and F would be substituted with a lactyl group ($CH_3CHCOOH$) and thus these positions are constrained to point away from the bulk of the enzyme surface. Asp 52 is in close proximity with Asn 46 and Asn 59 and is believed to form hydrogen bonds through these to Ser 50 (5,6).
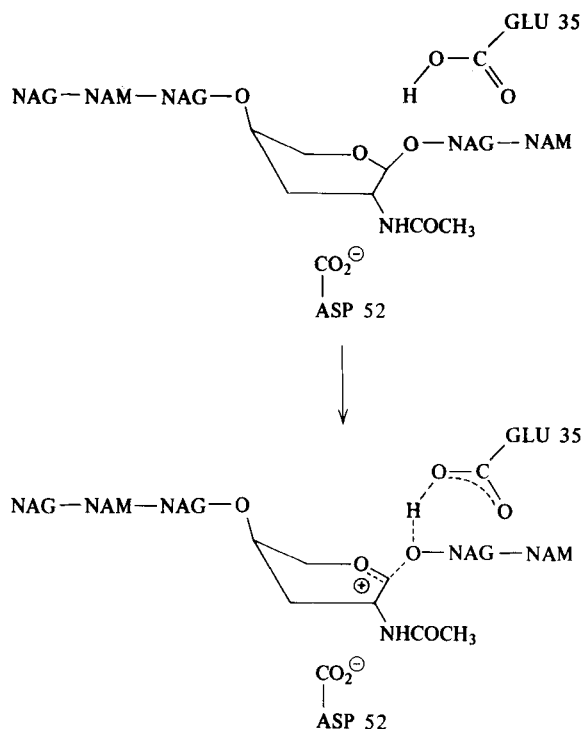
Fig. 2. The Phillips-Vernon mechanism for lysozyme action.

Raftery and co-workers have determined the orientation of a wide variety of oligosaccharides bound to lysozyme by taking advantage of the perturbing influence of the various side chains on the proton magnetic resonance (PMR) spectra of the small molecules (18–22). By examining the dependence of the chemical shifts on pH (20) and the changes in the spectra of the tryptophan side chains (23), they have identified the $pK_a$s of the various carboxyl groups in the upper portion of the active site (see below). It has been possible to correlate the majority of these results with the X-ray structure (4–7) of crystalline inhibitor–enzyme complexes.

Lysozyme catalyzes transglycosylation reactions, in other words, transfer of saccharide units to acceptors other than water (1,17,24–

28), and this reaction has been utilized to examine binding specificity in the lower portion of the cleft.

The $N$-acetyl group contributes greatly to the binding as glucose oligomers do not bind (29). Hydrolytic rates (30,31) and substrate modification (32) have also been employed to demonstrate the essentiality of the $N$-acetyl group. Piszkiewicz and Bruice obtained the cellodextrins $Glu_2$, $Glu_3$, $Glu_4$, $Glu_5$, and $Glu_6$ by partial hydrolysis of cellulose and determined that none of these are hydrolyzed by lysozyme (33). Also, the hydrolysis of a known lysozyme substrate (chitotriose) was not affected by the addition of $Glu_6$ to the assay medium. Furthermore, equilibrium dialysis studies suggest an upper limit of 200 $M^{-1}$ for the association constant of all the cellodextrins, which is below the productive binding constant for chitopentose or chitohexose by about 3 powers of ten.

In addition, recent isolation of a disaccharide from the cell wall peptoglycan of lysozyme-resistant bacteria has revealed the absence of the acetyl group on the glucosamine residue (34). Upon acetylation of the cell walls or the peptoglycan with acetic anhydride, the material becomes sensitive to lysozyme (35). This result may find explanation either in the enhancement of rate brought about by the $N$-acetyl group or in mere cancellation of the positive charge of the 2-$NH_3^+$ upon acetylation.

By following the hydrolysis of chitotriose in $^{18}O$-enriched water (36), Rupley has shown that the enzymatic reaction proceeds with fission of the anomeric carbon–oxygen bond ($C_1$–$O$). Raftery and co-workers demonstrated that the configuration at the anomeric carbon is unchanged in hydrolysis and transglycosylation reactions catalyzed by lysozyme (37,38). This result implies that either ($a$) an even number of displacement reactions has taken place (i.e., the double-displacement mechanism of Koshland); ($b$) the enzyme provides an asymmetric environment that prevents "backside" quenching of an intermediate, or ($c$) the neighboring acetamido group provides stereochemical control. Sharon and co-workers have presented evidence that $\beta$ linkages are formed in transglycosylation reactions by isolating the products and using an enzyme of known specificity to degrade them (25). Rupley and Gates examined the products of transfer with chitin oligosaccharides and found they were identical to the naturally occurring $\beta$-linked oligomers in their chromatographic behavior (28).

Koshland has developed a procedure for the modification of carboxylic acid residues in proteins via treatment with a carbodiimide and glycine methyl ester, glycine amide, or aminomethanesulfonic acid (39,40). It was determined that in the presence of substrate or inhibitors the Asp 52 and Glu 35 carboxyl groups of lysozyme are not modified while all other carboxyl groups are converted to —CONH—CH$_2$—SO$_3$⁻ groups. This preparation had 50% activity toward cell walls. Upon removal of the protecting agent, further treatment leads to derivatization of Asp 52 with concomitant total loss of activity, implying that Asp 52 is essential for activity.

Parsons et al., applied a different modification procedure (41*a*) and obtained a singly esterified lysozyme derivative that was completely inactive. This modified residue was identified as Asp 52 (42). The lysozyme ester retains the capacity to bind substrate but is catalytically inactive using chitotriose as substrate. This result nicely confirms that of Koshland. It should be pointed out that Asp 52 is proposed to be involved in a network of hydrogen bonds to other amino acids (5,6) and modification with either of the reagents might disrupt secondary structure in the vicinity of the active site in such a way as to prevent catalysis while not seriously affecting the binding.

Sharon and co-workers have recently prepared active-site-directed inhibitors of lysozyme utilizing an epoxide as an alkylating reagent. Compounds 2 and 3 provide a time-dependent irreversible inhibition of the enzyme with protection against this inhibition provided by the reversible inhibitor, methyl-β-NAG–NAG. Available evidence indicates that this reagent also modifies Asp 52 (41*b*).
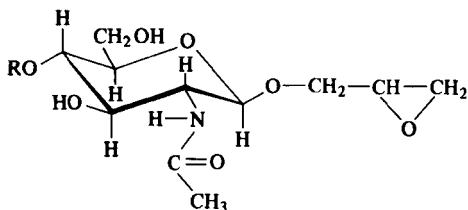


1   R = H

2   R = GlcNAc

3   R = GlcNAc–β–(1→4)–GlcNAc

Because of the insolubility of the natural substrate, the complexities involved in hydrolysis of chitin oligosaccharides (i.e., transgly-

cosylation and nonproductive complexing), experimental difficulties in following the rates, and the desire for quantitative separation of binding and catalytic parameters, several groups have searched for low molecular weight colorimetric substrates. Osawa and Nakayawa prepared β-p-nitrophenyl-chitotrioside and observed the formation of p-nitrophenoxide, chitotriose, chitobiose, and NAG upon incubation with lysozyme (43) indicating nonspecific cleavage. Zehavi and Jeanloz also observed complex results when β-benzyl-chitobioside was incubated with lysozyme in the presence of oligomers of NAG (44).

Osawa followed p-nitrophenol release from the transglycosylation of β-p-nitrophenyl-NAG and chitotetrose in the presence of lysozyme (45). Transglycosylation products were detected by chromatography. Interestingly, the 6-deoxy analog of β-p-nitrophenyl-NAG yielded transglycosylation products with chitotetrose and lysozyme but these were not hydrolyzed to yield nitrophenol. Since the 6-OH group is postulated to cause distortion of ring D by interaction with the enzyme surface, this result suggests that the same distortion is not occurring in the absence of the —OH group and thus that this distortion is in fact essential for catalysis. However, in reactions where the leaving group is at the end of a polysaccharide chain rather than in the middle, one may question what forces are responsible for holding the sugar residue in subsite D in a distorted conformation. Without the binding of sugar residues in subsites E and F, the predominant mode of binding could be nonproductive even if subsite D is occupied.



4a



4b

Lowe, Sheppard, Sinnott, and Williams prepared substituted phenyl β-glycosides of chitobiose and observed Michaelis-Menten kinetics (46). The observed $K_m$ values were nearly invariant but $k_{cat}$ yielded a Hammett ρ value of 1.23. In an extension of this work, Lowe and Sheppard prepared p-nitrophenyl-β-NAG–Glu and found its enzymic decomposition to be slower than that for p-nitrophenyl-β-NAG–NAG (47). On the basis of considerations of the ratio of

productive to nonproductive binding for these derivatives, these authors calculate a rate difference of 100-fold which, in their estimation, supports the involvement of the N-acetyl group of the substrate in the mechanism. More recently, Rand-Meir et al. investigated the products obtained from p-nitrophenyl-β-NAG–NAG (5), p-nitrophenyl-β-NAG–Glu (6), and p-nitrophenyl-β-NAG–2-deoxyGlu (7) when hydrolyzed by lysozyme (48). Considerable cleavage between the NAG residues of 5 and transglycosylation products were observed. This result casts serious doubt on the validity of the kinetic parameters obtained by Lowe and Sheppard.

At the present time there is no simple substrate for lysozyme that yields an uncomplex reaction and a simple assay procedure while at the same time incorporating all the features of the natural substrate. The pH dependence of binding and activity of lysozyme has received some study although the results are subject to the same limitations as observed in the preceding paragraphs. Rupley et al. determined the pH dependence of binding of chitin oligomers by difference spectroscopy and found inflections at pHs 1.2, 3.5, and 6.7 (29). In a kinetic study, Rupley and Gates report that the activity toward chitopentose exhibits a dependence on the acidic form of a species of $pK_a$ 6.7 (28).

Utilizing difference spectra and the Dixon plotting technique ($pK$s vs. pH), Dahlquist, Jao, and Raftery found two $pK_a$s for the binding of chitotriose (23). A $pK_a$ of 4.2 in the free enzyme is perturbed to 3.55 in the enzyme–chitotriose complex and a $pK_a$ of 5.8 is perturbed to 6.25. Using NMR techniques, Dahlquist and Raftery examined the binding of methyl-β-NAG as a function of pH (20). The pH dependence of $pK_s$ revealed a $pK_a$ of 6.1 in the free enzyme changed to 6.6 in the complex. The chemical shift of the acetamido methyl protons was also determined to depend on pH, exhibiting $pK_a$s of 4.7 and 7.0. Since the Asp 101 carboxyl group interacts with the trimer but not with the monosaccharide, its $pK_a$ is assigned as 4.2. The higher $pK_a$ (6 to 7) is assigned to Glu 35 since the environment of this residue is very hydrophobic, a situation known to raise the $pK_a$s of carboxyl groups. In a kinetic study, Raftery and Rand-Meir found $pK_a$s of 3.5 and 6.5 for the dependence of the rate of release of p-nitrophenol from p-nitrophenyl-β-glucopyranoside in the presence of chitotetrose and lysozyme (37). Since lysozyme-catalyzed hydrolysis of p-nitrophenyl-β-NAG-Glu to produce p-nitrophenol and NAG-Glu is not complicated by transglycosylation or

multiple bond cleavages. Rand-Meir et al. used this substrate to determine kinetic parameters and their dependence on pH (48). A plot of $pK_m$ versus pH shows a change in $pK_a$ from 5.6 in the free enzyme to 6.2 in the complex. A plot of log $V_{max}/K_m$ versus pH exhibited an inflection near pH 6 as well as inflections in the region pH 2.0–4.5. Parsons and Raftery have titrated the lysozyme ester mentioned above and assigned $pK_a$s of 4.5 to Asp 52 and 5.9 to Glu 35 (49). The reports of various pH dependencies of lysozyme behavior must be accepted only with the understanding that these dependencies are influenced by the nature or concentration of cations in solution (124). The mechanistic implications of the finding that the $pK_{app}$ values are drastically influenced by cations is not clear.

Dahlquist, Rand-Meir, and Raftery have examined the secondary isotope effect upon lysozyme catalyzed hydrolysis of phenyl-$\beta$-NAG–Glu (50,51) in an attempt to probe the transition state character of the reaction. This was accomplished by substitution of deuterium for hydrogen at the $C_1$ undergoing substitution. Acid-catalyzed nonenzymatic hydrolysis of phenyl-$\beta$-D-glucopyranoside yielded a $k_H/k_D$ ratio of 1.13, in accord with carbonium ion formation. Base-catalyzed hydrolysis of the same species, which does not involve carbonium ion formation, gave a $k_H/k_D$ ratio of 1.03. The lysozyme-catalyzed hydrolysis of phenyl-$\beta$-NAG–Glu exhibited, at pH 3.1, 5.5, and 8.3, a $k_H/k_D$ ratio of 1.11, which is in accord with the enzymatic reaction proceeding through the formation of an oxocarbonium ion in the rate determining step. It must be noted that this result is somewhat clouded by the absence of an N-acetyl substituent in the 2 position. Furthermore, this result does not eliminate the possibility of collapse to a covalently bound glycosyl enzyme in a post-rate-determining step.

Rupley, Gates, and Bilbrey (52) have taken up the problem of the nature of the intermediate produced by lysozyme by examining the relative rates ($k_A{}^{rel}$) of capture of this species by a series of alcohols. The values of $k_A{}^{rel}$ were found to be nearly constant.

$$k_A{}^{rel} = \frac{(\text{transfer products})(\text{H}_2\text{O})}{(\text{hydrolysis product})(\text{acceptor})}$$

Mechanistic implications of these results are beclouded by the fact that the pH employed for the study was many units below that of the $pK_a$ of the most acidic alcohol. Thus constant relative rates

would be obtained if: $(a)$ the intermediate was indiscriminate and reaction with ROH was diffusion controlled (i.e., Brønsted $\beta = 0.0$); or $(b)$ the intermediate was highly discriminate and the reaction was with the RO$^-$ species (Brønsted $\beta = 1.0$). For the latter, $\beta$ being 1.0 for attack would mean that $\alpha = 0.0$ for departure so that the leaving group would be unprotonated, which implies the highly unlikely spontaneous decomposition of the glycoside to an oxocarbonium ion and an alkoxide ion at the active site. The most acceptable explanations of the experimental data are that $(a)$ the intermediate is indiscriminate and the reactive species is ROH so that by microscopic reversibility hydrolysis occurs via dissociation of a completely protonated glycoside to oxocarbonium ion and ROH the former being stabilized electrostatically by the anionic form of Glu 35; or $(b)$ the reactive species is ROH, the intermediate (oxocarbonium ion) is discriminatory but general base removal of H from ROH by the anion of Glu 35 has a levelling effect on the nucleophilicity of ROH. The latter mechanism, which is the retrograde of the Phillips-Vernon proposal, was favored by the authors. In the same study it was found that $k_{\mathrm{A}}^{\mathrm{rel}}$ for RSH was somewhat less than for ROH. This result is inconsistent with attack on a covalently linked intermediate although not necessarily evidence for the presence of a carbonium ion. In summary, Rupley's results are accorded most simply by lysozyme-catalyzed hydrolysis of glycosides proceeding through the involvement of an oxocarbonium ion intermediate with carboxyl group general acid catalysis or carboxylate electrostatic stabilization of a protonated substrate intermediate (for a possible model of this process see p. 46).

## III. Physical Organic Studies of Model Systems

Glycosides are members of a general class of compounds represented by 8:



8
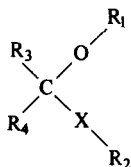
X = O

$R_1$ and $R_2$ = alkyl or aryl

| | |
|---|---|
| $R_3 = R_4 = H$ | formals |
| $R_3$ = alkyl or aryl, $R_4$ = H | acetals (glycosides) |
| $R_3 = R_4$ = alkyl or aryl | ketals |
| $R_3$ = H, $R_4$ = —O—alkyl or | orthoformate |
| —O—aryl | |
| $R_3$ = alkyl or aryl, $R_4$ = —O— | |
| alkyl or —O—aryl | orthoester |

X = N

| | |
|---|---|
| $R_1 = R_3$ = alkyl, $R_4$ = H, $R_2$ = purine | |
| or pyrimidine | nucleoside |

X = S

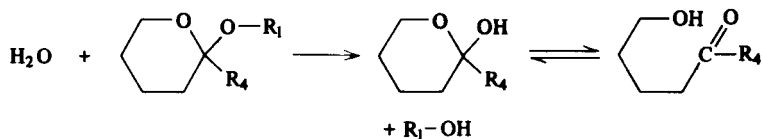| | |
|---|---|
| $R_1 = R_2 = R_3$ = alkyl or aryl, $R_4$ = H | thioacetal, etc. |

Thus all members of this class contain a tetrahedral carbon bonded to at least two electronegative atoms.

Hydrolysis of compounds of this type results in formation of a carbonyl group and liberation of the two electronegative functions, in other words, 9:

$$H_2O \quad + \quad \underset{R_4}{\overset{R_3}{\diagdown}}C\underset{O-R_2}{\overset{O-R_1}{\diagup}} \quad \longrightarrow \quad \underset{R_4}{\overset{R_3}{\diagdown}}C{=}O \quad + \quad HOR_1 \quad + \quad HOR_2$$
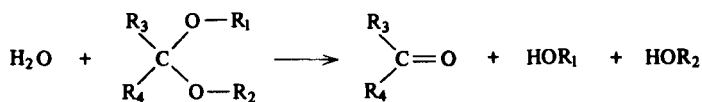
9

If $R_2$ is part of a carbocyclic ring with $R_3$, then the hydrolysis may result in a hemiacetal which will exist in equilibirum with its acyclic form (10).

$$H_2O \quad + \quad \text{[ring structure with O-R_1, R_4]} \quad \longrightarrow \quad \text{[ring structure with OH, R_4]} \quad \rightleftharpoons \quad \text{[ring structure with OH, C-R_4]}$$

+ $R_1$-OH

10

Mechanisms for C–O bond scission include: ($a$) protonation of oxygen followed by rate determining ROH and $\overset{+}{\diagup}C{=}O$ -- formation

(specific acid catalysis); (b) spontaneous formation of $RO^-$ and $\overset{+}{>}C\overset{+}{\doteq}O-$ in the rate determining step; (c) concerted proton transfer and C—O bond scission to provide ROH and $>C\overset{+}{\doteq}O-$ in the rate determining step (general acid catalysis); and (d) Attack by nucleophiles (N:) to provide $RO^-$ and $N\overset{|}{-}\overset{|}{C}-O$ in the rate determining step.

In what follows, the literature dealing with mechanisms (a) through (d) will be discussed with special attention accorded to the dependence of the type mechanism upon the structure of the substrate and the nature of the transition state.

## A. SPECIFIC ACID CATALYSIS

Because of the strength of the carbon–oxygen bond, hydronium ion catalysis, which aids greatly in polarizing this bond, is ubiquitous in the hydrolysis of compounds of type 8. Cordes has discussed at length the experimental findings which permit delineation of the essential features of this pathway (53). Some of these features may be summarized as follows.

For the majority of cases examined, the site of carbon–oxygen bond cleavage has been established as 11a. Thus, if $R_1$ contains an optically active center at the point of oxygen attachment, the prod-



11          11a          11b
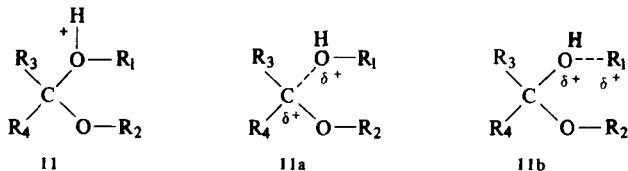
uct alcohol is of the same optical rotation (54–56). Also, hydrolysis of acetals in $^{18}O$-enriched water produced alcohols of normal $^{18}O$ content (57). The one exception to this general process is the finding of Armour et al. that t-butyl-β-D-glucopyranoside reacts with t-butyl-oxygen cleavage (58), indicating, most importantly, that the t-butyl cation is more stable than the glycosyl cation. Because of the unsymmetrical nature of glycoside acetals, two pathways exist for their hydrolysis: either a cyclic oxocarbonium ion (12a); or a noncylic oxocarbonium ion (12b) may be formed as in equation 12.
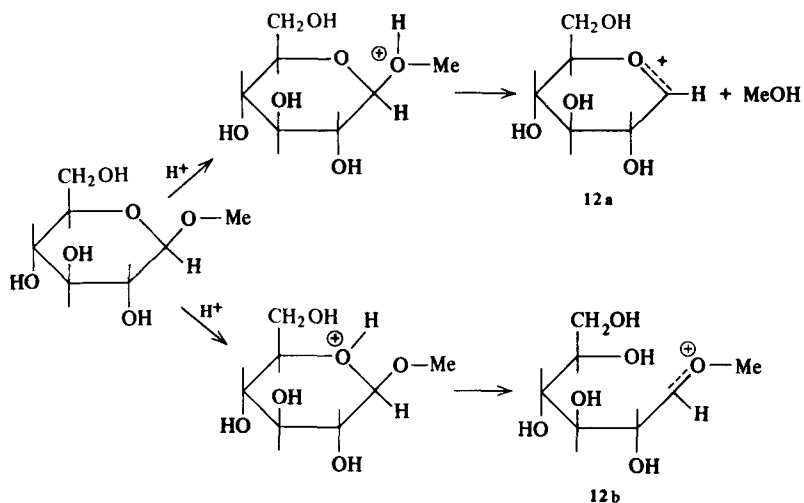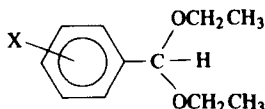
Banks et al. measured the $^{18}O$-content of the methanol product from hydrolysis of methyl-$\beta$-D-glucopyranoside after 7% and 100% hydrolysis (59). The value at 100% reaction represents the amount of $^{18}O$ present in the sample and the value at 7% of reaction may be taken as a measure of the relative rate of reaction of the $^{16}O$ versus the $^{18}O$ compound. The value obtained ($k_{16}/k_{18} = 1.03$) is consistent with exocyclic bond cleavage (12a) in the rate-determining step. Since the following discussion will show that all reactions after the initial bond cleavage are fast, this evidence is sufficient to favor the pathway leading to 12a as the correct description of the reaction for this representative glycoside.

Nucleophilic attack by a water molecule on a protonated acetal (A-2 mechanism, 13) would yield the same kinetic expression and the same products as a mechanism involving unimolecular decomposition of protonated acetal to yield an alcohol and an oxocarbonium ion (A-1 mechanism, 14). Distinction between these two processes
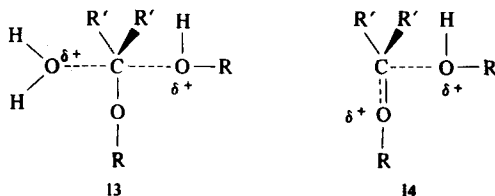
may best be based on structure–reactivity correlations, entropies of activation, isotope effects, and trapping studies.

Consistent with the A-1 mechanism is the great sensitivity to electronic effects upon the carbonyl moiety of the acetal, ketal, and so on, and the lack of sensitivity to electronic effects upon the alcohol moiety. Thus for a series of substituted benzaldehyde diethyl acetals (15), a plot of the log of the second order rate constants for specific

$$X \text{—} \underset{\phantom{x}}{\bigcirc} \text{—} \overset{\displaystyle OCH_2CH_3}{\underset{\displaystyle OCH_2CH_3}{\overset{\diagup}{\underset{\diagdown}{C}}} \text{—} H}$$

**15**

acid catalysis ($\log k_{\mathrm{H}}$) versus the Hammett $\sigma$ constants yielded a $\rho$ value of $-3.35$ indicating a large amount of positive charge build-up at the reaction center in the transition state. This would be consistent with formation of a carbonium ion (A-1) (60). Similarly, acetals of the structure $RCH(OCH_2CH_3)_2$ and ketals of the structure $RC(OCH_2CH_3)_2$ yielded a $\rho^*$ value of $-3.60$ in a plot of the
$\phantom{xxx}|$
$\phantom{xx}CH_3$
rate constants versus the aliphatic substituent constants, $\sigma^*$ (61). In contrast, in the hydrolysis of substituted phenyl glycosides, where structural variation is in the leaving group, the $\rho$ is but $-0.66$ (62). This value presumably represents a compensation between leaving group capacity (enhanced by electronegative substitution) and decrease in concentration of protonated species due to a decrease in basicity (reduced by similar substitution). Based on consideration of bond angle straining, steric factors, and electronic effects, Kreevoy, Morgan, and Taft (64) presented evidence for a transition state in ketal hydrolysis with substantial carbonium ion geometry.

The entropy of activation of an A-1 reaction is usually zero or slightly positive. A-2 reactions, due to their higher kinetic order, are associated with $\Delta S^{\ddagger}$ values considerably more negative. This distinction allows many acid catalyzed reactions of the type under consideration to be classed as A-1. Hydrolysis of $CH_2(OCH_2CH_3)_2$ is associated with an entropy of activation of $+7.0$ eu, clearly in the A-1 range. For A-2 reactions $\Delta S^{\ddagger}$ is of less value as a diagnostic tool. The acid-catalyzed hydrolysis of methyl acetate, which involves $H_2O$ attack on protonated substrate ($A_{AC}2$) and is therefore akin to the A-2 mechanism of acetal hydrolysis, has a $\Delta S^{\ddagger}$ *of* $-21.3$ eu. In

comparison to the $A_{AC}2$ mechanism of ester hydrolysis, the A-2 mechanism of acetal hydrolysis may involve fewer water molecules (126) and proton transfer may not be important so that a less negative value of $\Delta S^{\ddagger}$ may apply to this process. The acid-catalyzed hydrolysis of 2-phenyl-4,4,5,5-tetramethyldioxolane has a $\Delta S^{\ddagger}$ of $-14.2$ eu (63) and is most likely A-2 (see also Capon and Page, ref. 127.)

Fife and Jao studied the hydrolysis of the two series of benzaldehyde acetals (16 and 17) (60). Within the two series, the variation in rate with changing substituents is due entirely to changes in $\Delta H^{\ddagger}$,
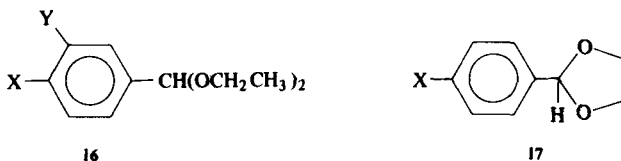


the potential energy term. Compounds represented by 16 were hydrolyzed 30–35 times faster than the series represented by 17. This difference is totally accounted for by the difference in $\Delta S^{\ddagger}$, with series 16 exhibiting $\Delta S^{\ddagger}$ values about 9 eu more positive than the $\Delta S^{\ddagger}$ values of the similarly substituted compound in series 17 while the $\Delta H^{\ddagger}$ values are nearly equivalent. A possible explanation for the more negative kinetic energy terms for series 17 is that the rotation about the bond being broken is restricted in the transition state due to the cyclic nature of the substrate.

Typical A-1 reactions exhibit an "inverse" isotope effect ($k_{D_2O}/k_{H_2O}$) of 2.4 to 3.0. This results from the greater acidity of $D_3O^+$ compared to $H_3^+O$ and the resultant more favorable equilibrium constant for formation of the conjugate acid of an acetal in $D_2O$. Thus, in the benzaldehyde acetals discussed above (16 and 17), $k_{D_2O}/k_{H_2O}$ for both series is between 2.7 and 3.0 (60). For A-2 ester hydrolysis, the isotope effect is considerably less, $k_{D_2O}/k_{H_2O}$ generally falling around unity.

Finally, even more convincing evidence for the unimolecular nature of the rate determining step stems from the work of Cordes et al. which demonstrates that the addition of nucleophilic reagents has no effect on the rate of acid-catalyzed decomposition of methyl orthobenzoate although the ratio of products depends strongly on the amount of added nucleophile (65,66). This is clear evidence for the rate determining formation of a high energy intermediate (oxo-

carbonium ion) that is subsequently trapped by nucleophilic species. In a similar study, Kresge and Preto found the addition of iodide had no effect on the hydrolysis of ethyl orthocarbonate (67).

Thus these combined data strongly suggest the mechanism shown in equation 1. Evidence relative to the choice of the rate determining



$$+ R_1—OH \qquad + R_2—OH$$
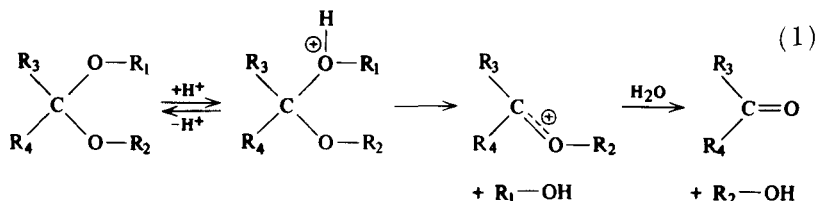
step of equation 1 for orthoester hydrolysis has been accumulated by Wenthe and Cordes (68). By utilizing the distinctive chemical shifts of the protons of reactants and products of methyl orthobenzoate reaction in $d_4$-methanol–deuterium oxide mixtures, they have determined that the rate constants for disappearance of orthoester, for appearance of methanol product, and for appearance of methyl benzoate product are identical. In addition; the product methyl benzoate still contained protons (i.e., the protonated methanol of the substrate had not been exchanged for deuterated methanol from the medium). This result provides strong evidence for the rate determining formation of the oxocarbonium ion with its subsequent rapid collapse to the final products (18a–c).



$$- \frac{d(a)}{dt} = + \frac{d(b)}{dt} = + \frac{d(c)}{dt}$$
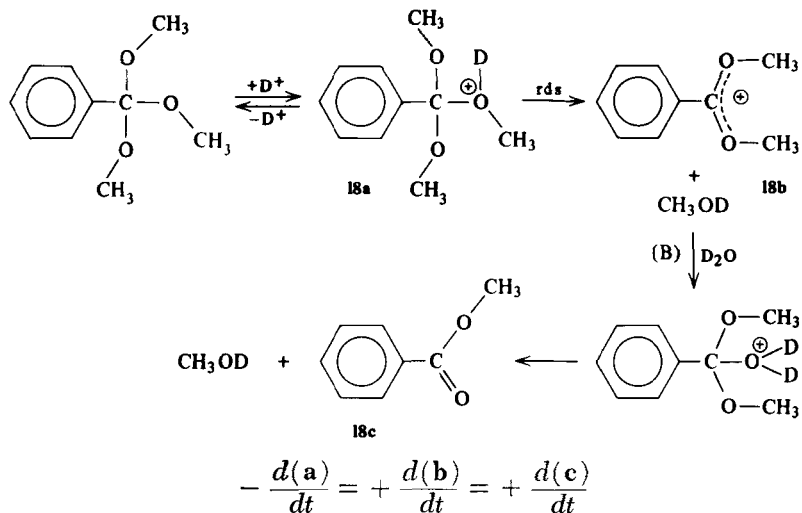
Finally, Bull et al. have examined the secondary deuterium kinetic isotope effect on the hydrolysis of some acetals and an orthoester (85). The results for ethyl orthoformate and propionaldehyde diethyl acetal, equations 2 and 3, respectively (both of which do not

$$\frac{k_{H-C(O-CH_2CH_3)_3}}{k_{D-C(O-CH_2CH_3)_3}} = 1.05 \tag{2}$$

$$\frac{k_{CH_3CH_2-C(O-CH_2CH_3)_2} \atop \overset{|}{H}}{k_{CH_3CH_2-C(O-CH_2CH_3)_2} \atop \overset{|}{D}} = 1.17 \tag{3}$$

exhibit general acid catalysis), are particularly relevant. The large ratio for the acetals of equation 2 is in accord with a large amount of carbonium ion formation in the critical transition state. The smaller effect observed with the orthoesters (eq. 3) is fully in agreement with a transition state much closer to reactants. Thus available evidence indicates that for hydrolysis of orthoesters the transition state occurs earlier along the reaction coordinate than an analogous acetal hydrolysis.

This study also provides evidence for variation in transition state position within a series of A-1 reactions. These authors determined the secondary deuterium isotope effect on $H_3O^+$ hydrolysis of benzaldehyde acetals (85). In this series, electron withdrawal destabilizes the carbonium ion and the results indicate that with the most stable carbonium ion the transition state is more remote from the positively charged intermediate. This is in complete accord with the Hammond postulate. The compounds of Table 1 are not subject to general acid catalysis by acetic acid (60).

Although available evidence eliminates the participation of water and other nucleophiles in the rate determining step for the acid-catalyzed hydrolysis of all but a few of these compounds, there are several examples of intramolecular nucleophilic participation providing kinetically important pathways. These examples serve to illustrate the great rate enhancements available on conversion of bimolecular reactions to intramolecular reactions (and enzymatic reactions) (128). Capon and Thacker reported that the acid-catalyzed ring closure of dimethyl acetals of glucose (acyclic form) proceeds faster than the rate predicted on the basis of inductive effects alone.
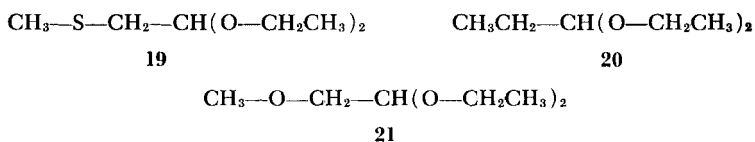
TABLE I.



| k | | | | | k | | | | |
|---|---|---|---|---|---|---|---|---|---|

| | X |
|---|---|
| 1.15 | —NO$_2$ |
| 1.09 | —H |
| 1.04 | —O—CH$_3$ |

In addition, the rate is dependent on the configuration at the 4 carbon in accord with equation 4 (69).



(4)
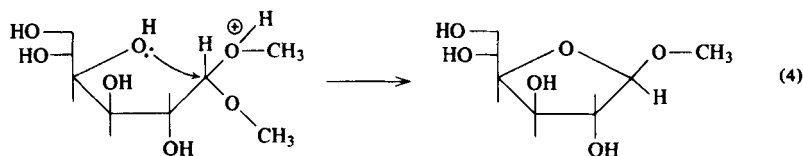
Another case of intramolecular participation was presented by Speck, Rynbrandt, and Kochevar (70). The acid-catalyzed hydrolysis of 21 was 10³ times slower than 20, presumably reflecting the known

CH$_3$—S—CH$_2$—CH(O—CH$_2$CH$_3$)$_2$          CH$_3$CH$_2$—CH(O—CH$_2$CH$_3$)$_2$

          19                                                         20

CH$_3$—O—CH$_2$—CH(O—CH$_2$CH$_3$)$_2$

          21

inductive effect in these reactions. The hydrolysis of 19, however, was only 10 times slower than 20 even though $\sigma^*$ for CH$_3$—O— and CH$_3$—S— groups are similar. This rate enhancement ($k19/k21 = 100$) was suggested to arise from nucleophilic participation of the alkylthio function (eq. 5).



(5)

CH$_3$—S—CH$_2$CHO
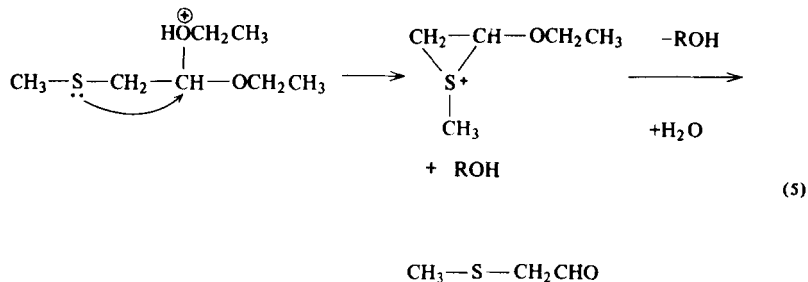
More recently, Piszkiewicz and Bruice have presented evidence that the N-acetyl functional group is able to provide anchimeric assistance in the specific acid-catalyzed hydrolysis of methyl 2-acet-amido-2-deoxy-β-D-glucopyranoside (71). This is based on the accelerated rate of hydrolysis of methyl-NAG relative to methyl-β-D-glucopyranoside. The rate enhancement for this process is about 50-fold, and is observed for the methyl β-D-glucopyranoside and not for glycosides of bulkier aglycones because the reaction requires an axial–axial disposition of the two groups (nucleophile and leaving group). The larger groups exist overwhelmingly in the equatorial–equatorial form and the participation is not seen.

## B. SPONTANEOUS HYDROLYSIS

Unimolecular hydrolysis has been observed for a number of systems. These may be classed into two types: those reactions which involve the formation of a very stable oxocarbonium ion, and those reactions that involve the intramolecular participation of a neighboring nucleophile in an otherwise uncatalyzed pathway.

Several examples of the first type are provided by the work of Fife and coworkers. The hydrolysis of 2-($p$-nitrophenoxy)-tetrahydropyran is insensitive to pH above pH 4 (72,73). Evidence for the unimolecular nature of the reaction is as follows. The rate of reaction is unchanged from pH 4.0 to 0.05 $M$ NaOH, arguing against nucleophilic attack by water on an unprotonated reactant. The solvent istotope effect, $k_{D_2O}/k_{H_2O} \approx 1.0$, and the fact that $H_2O$ if acting as a general acid would have a positive deviation of 8 to 9 log units from a Brønsted plot, argues against the involvement of water in the transition state. In addition, the entropy of activation, $\Delta S^{\ddagger} = +2.2$ eu, is in accord with a unimolecular decomposition. Fife considers this process to be aided by the good leaving ability of the $p$-nitrophenolate ion and the reasonable stability of the oxocarbonium ion.

In another study, Anderson and Fife reported that tropone diethyl ketal exhibits a pH independent reaction above pH 10 (74). The great reactivity of this species is due to the exceedingly stable carbonium ion that is formed, overcoming the poor nature of the leaving group. The reaction exhibits a solvent isotope effect of 0.86 in the plateau region.

The hydrolysis of **22** was also reported to be pH independent from pH 1 to 11 (75). The unimolecular decomposition is greatly aided by the electron withdrawing nitro groups, while the benzyl-oxocarbonium ion formed is fairly stable.

22
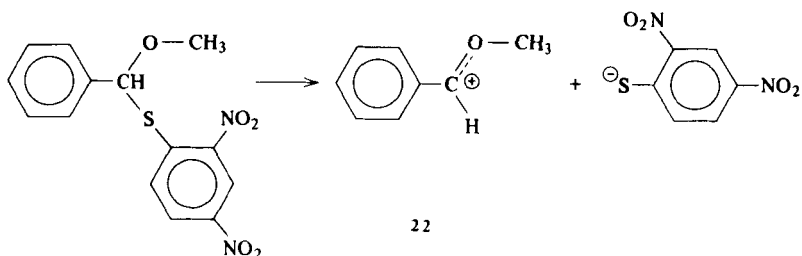
The second class of spontaneous reactions is represented by the facile reaction of equation 6 (Fig. 3). This process has been observed by Piszkiewicz and Bruice and results in large plateau re-



(6)
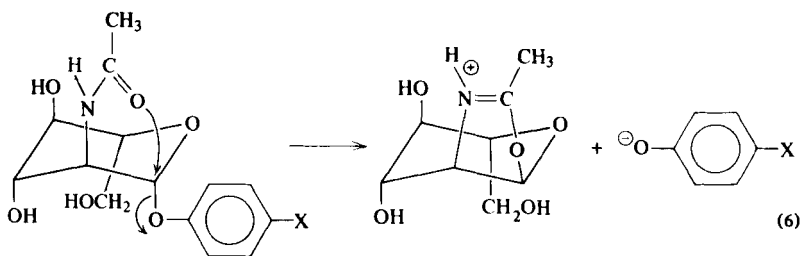
gions in the pH–log $k_{obs}$ profiles (76). Experimental evidence in support of this mechanism includes the absence of rate enhancement in the case of the $\alpha$-linked compounds where the necessary trans–diaxial configuration cannot be achieved. There is no catalysis by buffer species in the plateau region and no solvent isotope effect. The possibility of reaction through a zwitterion species as in equation 7 has been eliminated by the calculated magnitude of the rate constant ($k_0' \approx 10^{18}$ min$^{-1}$) for this process.



(7)
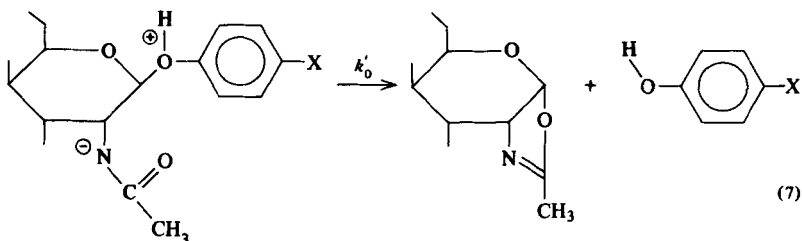
Similar plateau rates are evident in the hydrolysis of nitrophenyl-$\beta$-glucopyranosides thus suggesting that the 2—OH group is also
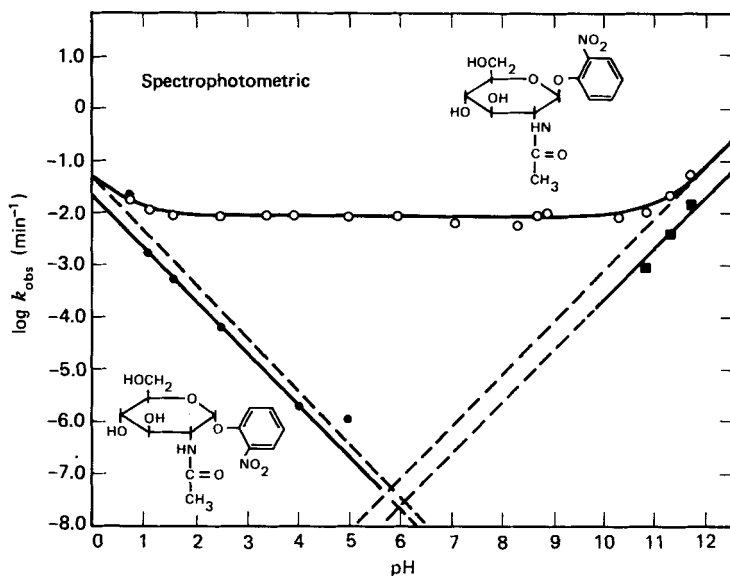
Fig. 3. Spectrophotometrically determined pH–log $k_{obs}$ profiles for the hydrolyses of o-nitrophenyl-2-acetamido-2-deoxy-$\beta$-D-glucopyranoside and o- nitrophenyl-2-acetamido-2-deoxy-$\alpha$-D-glucopyranoside.

capable of participating in a similar manner. The ratio of rate constants for participation by the N-acetyl group and the —OH group ($k_{NAC}^{spon}/k_{OH}^{spon}$) is $10^3$. The plateau rates for the phenyl-$\beta$-NAGs obey a Hammett relationship when plotted against $\sigma^-$ and yield a slope of +2.28, indicating that electron withdrawal in the leaving group greatly favors this intramolecular attack (77,78).

Finally, a process not strictly spontaneous, but independent of lyate species, has been observed by Clark et al. (79). The hydrolysis
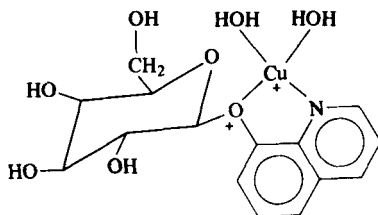
of 8-hydroxyquinolinoly-$\beta$-D-glucopyranoside is catalyzed by Cu(II) ions yielding, at pH 5.5 − 6.0 and 2.5 × $10^{-4}$ M Cu(II), a rate comparable to that brought about by 4.18 M HCl. This observation suggests the chelate 23 is reacting at a rate comparable to the rate of reaction of the conjugate acid of the glycoside.
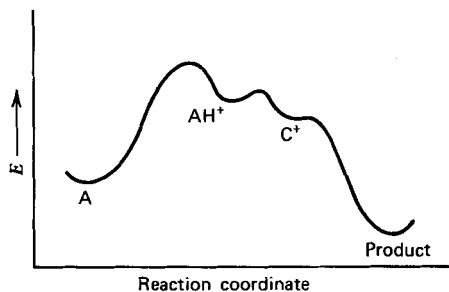
## C. GENERAL ACID CATALYSIS

The presence of a protonated carboxyl group at the active site of the productive lysozyme substrate complex (Glu 35) had led (loc. cit.) to the proposal of general acid catalysis as a logical explanation for at least part of the catalytic efficiency of this enzyme. This and other suggestions of the involvement of carboxyl groups at the active site of certain glycosidases has led to the study of the structural requirements for general acid catalysis of hydrolysis of acetals and related compounds and the magnitude of possible rate enhancements obtained via this process. For a typical A-1 hydrolytic process the reaction coordinate diagram of equation 8 is sufficient to describe the mechanism. It is seen that proton transfer occurs in a pre-



$$A + H^+ \rightleftharpoons AH^+ \longrightarrow C^+ \longrightarrow \text{Product} \qquad (8)$$
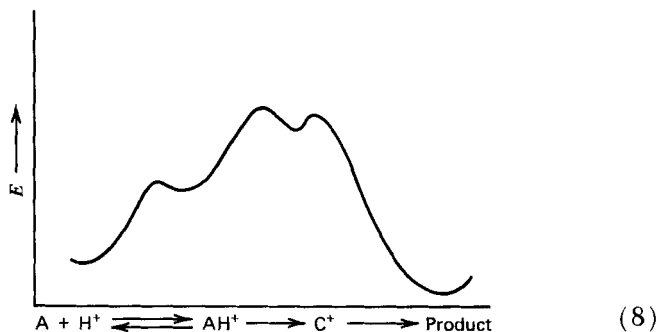
equilibrium step which is followed by rate determining decomposition to the oxocarbonium ion. The experimental findings presented under specific acid catalysis are fully in accord with this description.

Kresge and Preto have pointed out that proton transfer between oxygen bases (i.e., a carboxylic acid and an acetal), which has been considered to be very fast, could easily be the rate determining step of a reaction measurable by conventional methods since the activation energy for this process must be at least as great as the difference

in free energy of the two conjugate acids in question (COOH vs. AH$^+$) (67). For protonation to become a part of the rate determining step of the reaction depicted by equation 8, it is necessary to lower the activation energy of the bond cleavage step or to raise the activation energy of protonation or to do both, so that the reaction coordinate diagram resembles Scheme I or II. In Scheme II, AH$^+$



Scheme I
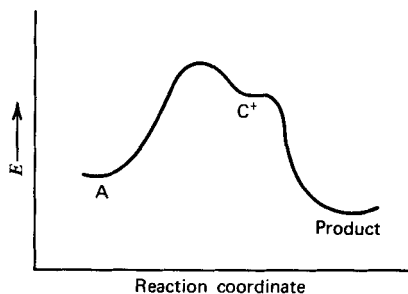


Scheme II

is indicated as an intermediate while in II protonation and C–O bond scission are concerted. In accord with the Hammond postulate, the transition state for general acid catalysis is moved closer to reactants, than in the case of the A-1 process, if the intermediate oxocarbonium ion has been stabilized.

We will now consider some of the cases in which intermolecular general acid catalysis has been observed.

## D. INTERMOLECULAR GENERAL ACID CATALYSIS OF ORTHOESTER HYDROLYSIS

General acid catalyzed hydrolysis of orthoesters was originally observed by Brønsted and Wynne-Jones in 1929 (80). This reaction has proved very useful in delineating our current concepts of catalysis in aqueous solution and a discussion of the accumulated results is therefore justified. Examination of structures 24 and 25 reveals why the mechanism for orthoesters is often general acid (Scheme I or II)
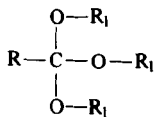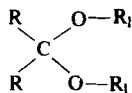


while for acetals the A-1 (eq. 8) process prevails. The additional electron withdrawing alkoxy group will be expected to reduce basicity and thus raise the activation energy for protonation. This should be compensated for by the increased stability of the carbonium ion formed upon bond cleavage due to the additional possible resonance forms.

Kwart and Price reported that the hydrolysis of methyl orthobenzoate was catalyzed by weak acids in addition to hydronium ion in 30% methanol–water (81). A plot of the second order rate constants for weak acid catalysis versus the $pK_a$ of the acid in water yielded a linear plot with a slope ($\alpha$) of $-0.74$. The Hammett plot for hydronium ion catalysis was linear with a $\rho$ of $-2.02$ but the analogous plot for catalysis by chloroacetic acid was curved. In addition, a correlation between the rate, acidity, and an empirical function of the solvent composition as suggested by Grunwald yielded a curved plot which is indicative of the involvement of water in the transition state. On this basis Kwart and Price favored an A-2 mechanism for these substrates. However the establishment of a lack of influence of added nucleophilic reagents on the rate of hydrolysis of methyl orthobenzoate by Fullington and Cordes (see acid-catalyzed hydrolysis) eliminates an A-2 pathway for this reaction (65,66). The observations of Kwart and Price probably find explanation in specific salt effects (see p. 28).

Bunton and DeWolfe have examined the question of rate deter-

mining proton transfer in the hydronium ion catalyzed hydrolysis of acetals and orthoesters (82). For an A-1 reaction, equation 9 de-

$$S + H_3O^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SH^+ + H_2O \tag{9}$$

$$SH^+ \xrightarrow{k_2} Product$$
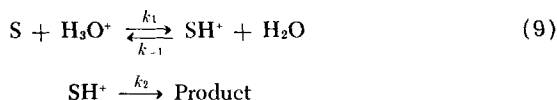
scribes the kinetic steps, with $k_2$ as the rate determining process. Application of a steady-state treatment in $SH^+$ yields equation 10.

$$k_{obs} = \frac{k_1 k_2 [H_3O^+]}{(k_{-1}[H_2O] + k_2)} \tag{10}$$

Dividing through by $[H_3O^+]$ yields the second order constant, $k_{H^+}$ (eq. 11). If the A-1 mechanism is followed, $k_{-1}[H_2O] \gg k_2$, (i.e.,

$$k_{H^+} = \frac{k_1 k_2}{(k_{-1}[H_2O] + k_2)} \tag{11}$$

rate of proton loss from a strong acid is faster than the rate determining step of reaction).

$$k_{H^+} = \frac{k_1 k_2}{k_{-1}[H_2O]} = \frac{k_2}{K_a[H_2O]} \tag{12}$$

Since $k_{H^+}$ is the experimentally determined second order rate constant for hydronium ion catalysis, this expression allows the calculation of $k_2$ provided that $K_a$ is known. Bunton and DeWolfe estimated this number using the known $pK_a$s of methyl and ethyl ethers, the sensitivity to electronic effects for the ionization of aliphatic amines, and the substitutent constants for the various groups. Although this computation requires a lengthy extrapolation the assumptions are sound and experimental measurements have yielded values for analogous compounds (83) that are reassuringly close to those calculated by Bunton and DeWolfe. The value of $k_{-1}$ may be estimated as $10^8$ sec$^{-1}$ M$^{-1}$. When the calculated values of $k_2$ are compared to this number, the following conclusions may be reached. For dimethyl formal, $[CH_2(OCH_3)_2]$,* $k_2 \approx 7$ sec$^{-1}$ and

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* Capon's comment (84) is in error on this point. Bunton and DeWolfe used the correct structure and the appropriate constants in their calculation and discussion but simply misnamed this species dimethyl acetal. Cordes apparently inferred that this meant dimethoxyethane (53). The conclusions of Bunton and DeWolfe are not altered.

thus the condition $k_{-1} \gg k_2$ holds and the mechanism is confirmed as A-1 for this acetal. The situation changes drastically for the hydrolysis of orthoesters. As an example, for ethyl orthoacetate, [$CH_3C(OCH_2CH_3)_3$], $k_2$ is calculated to be $2 \times 10^{11}$ $sec^{-1}$. It is clear that the assumption of $k_{-1} \gg k_2$ is no longer valid. Thus for orthoesters, protonation is not a preequilibrium process and the first step of the reaction $k_1$ must be rate controlling. Therefore hydronium ion catalysis of orthoester hydrolysis may be considered as general acid catalysis.

Not all orthoesters are subject to general acid catalyzed hydrolysis. DeWolfe and Roberts reported that ethyl orthoformate hydrolysis was catalyzed by buffer acids in aqueous dioxane (86). Recently Lahti and Kankaanpera have reexamined this reaction and found it subject to a large salt effect which varies in magnitude with salt type (87,88). These authors conclude that the apparent general acid catalysis in this system is in fact the result of the exchange of acetate ions for chloride ions in performing a buffer dilution using NaCl to hold ionic strength constant. In fact, the apparent catalysis disappears when the ionic strength is increased to 2.0 $M$ (LiClO$_4$).

## E. INTERMOLECULAR GENERAL ACID CATALYSIS OF ACETAL HYDROLYSIS

Despite the early observation of general acid catalysis in the hydrolysis of orthoesters, no unambiguous observation of buffer catalysis of acetal hydrolysis appeared in the literature prior to 1968. In that year, Fife and Jao reported that 2-aryloxytetrahydropyrans with electron-withdrawing leaving groups in the phenyl ring are subject to marked catalysis of hydrolysis by formic acid (72). In the hydrolysis of a series of acetals of general structure 26, in which X ranged from electron donating to electron withdrawing, the ob-
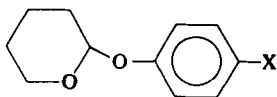


26

served ratios of $k_{D_3O^+}/k_{H_3O^+}$ decreased from 2.48 (X = OCH$_3$) to 1.33 (X = NO$_2$) which was suggested to indicate a change in mechanism from preequilibrium protonation to protonation becoming concerted with C–O bond breaking. The isotope information by

itself, however, is probably mechanistically inconclusive since it can be explained as a difference in ground state $pK_a$ of the substrates in $H_2O$ and $D_2O$ (Rule and LaMer relationship, ref. 125). However, when combined with the finding of general acid catalysis by HCOOH ($k^D/k^H = 0.29$; $X = -NO_2$), the suggestion of proton transfer becoming concerted with C–O scission is most reasonable. Since the rate constant for HCOOH catalysis increases with increase in leaving ability of HOR (i.e. $k$ rate greater with $X = NO_2$ than with $X = Cl$), Fife reasoned that proton transfer from a weak acid is most effective when C–O bond scission is appreciable. In an amplification of this original study, Fife and Brod have determined the Brønsted coefficient for general acid catalysis by four acids (including $H_3O^+$) to be $-0.5$ for hydrolysis of the nitro-substituted compound (73). Since HCOOH is a much weaker acid than protonated **26** ($X = -NO_2$), the proton in the transition state should lie closer to **26** (i.e., the weakest base) than to $HCOO^-$. That $\alpha = -0.5$ indicates the proton lies between catalyst and substrate which in turn suggests the proton to be transferred in the transition state, when the basicity of substrate is increased due to C–O bond cleavage. Increase in basicity of substrate in the transition state for general acid catalysis has been considered an important factor in other hydrolytic reactions (128). In addition, the Hammett $\rho$ value was determined to be $-0.9$ for hydronium ion catalysis (basicity of $-O^-$ more important than leaving ability of HOR) (72) and $+0.9$ for formic acid catalysis (leaving ability more important than basicity of **26** since the importance of basicity is in the transition state) (73). According to Fife's discussion of these results, the C–O bond is presumably broken to a greater extent in the case of $X = NO_2$ than in the case of $X = Cl$ or $X = H$. An alternative to this description of the reaction would envision a two-step mechanism (proton transfer followed by C–O bond cleavage) with the protonation as the rate determining step for **26** when $X =$ electron withdrawing group and C–O bond cleavage as rate determining when $X =$ electron donating group. The greater catalytic effect of HCOOH in the case of electronegative substituents in **26** (i.e., positive $\rho$ for formic acid catalysis) would follow from the greater importance of protonation in the transition state for those substrates (catalysis seen where most needed). Of possible utility in differentiating these alternatives would be the determination of Brønsted $\alpha$ values for several different substrates ($X$ in **26**).

Several recent studies have addressed the question of structural variations that are necessary for the observation of intermolecular general acid catalysis in acetal hydrolysis. DeWolfe and co-workers first discussed this problem in terms of basicity of the oxygen of the leaving group and stability of the intermediate oxocarbonium ion (90). These authors offered evidence for buffer catalysis in the hydrolysis of 2,2-diphenyl-dioxolanes and benzophenone diethyl ketals. More recent studies have disputed this claim.

A recent study by Anderson and Fife demonstrates the dependence of the position of the proton in the transition state on carbonium ion stability. Compounds 27 and 28 are both hydrolyzed with loss of phenol but the oxocarbonium ion intermediate for 28 is much



27

28
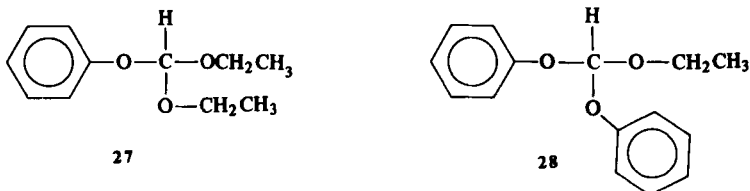
less stable because the phenoxy group cannot stabilize the positive charge as well as a second ethoxy group (89). This is realized in the slower rates of hydrolysis of 28 relative to 27. General acid catalysis is observed for both compounds with the $\alpha$ value for 27 equal to $-0.47$ and for 28 equal to $-0.68$. Thus the proton is transferred to a greater extent in the transition state in the case of 28, an expectation of the Hammond postulate.

Capon and Smith examined 2,5-anhydro-$\alpha$ L-arabinofuranoside, benzaldehyde diethyl acetal, and benzophenone diethyl ketal and found no buffer catalysis by acetate or imidazole buffers (91). Anderson and Capon have presented evidence for intermolecular general acid catalysis of the hydrolysis of a mixed aryl alkyl acetal of benzaldehyde (93,94). Here the basicity of the leaving group (the phenoxy group in 29) is reduced relative to a dialkyl acetal. That phenol leaves in the rate determining step (rather than methanol) is



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