Neighboring Carboxyl Group Participation in the Hydrolysis of Acetals. Hydrolysis of *o*-Carboxybenzaldehyde *cis*- and *trans*-1,2-Cyclohexanediyl Acetals

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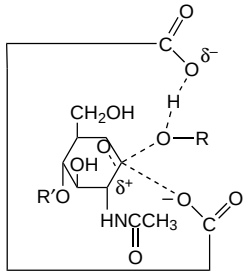
Abstract: The plot of log k_{obsd} *vs* pH for the hydrolysis of *o*-carboxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal at 50 °C in H_2O has four unit changes of slope in the pH range 2−9. The plot is here described by proceeding from low pH to high pH. The observed hydronium ion- and water-catalyzed reactions at pH < 6 have rate constants that are similar, but not identical, to those for hydrolysis of the acylal 3-[(*trans*-2-hydroxycyclohexyl)oxy]phthalide, which was isolated from the reaction at pH 3, and synthesized independently. The pH−log rate constant profile for hydrolysis of the acetal bends downward near pH 6 to give a slope of −1.0. Oxocarbonium ion hydrolysis is then a water reaction. At pH 7 the mechanism of the reaction changes to attack of OH^- on the oxocarbonium ion intermediate. A change in rate-determining step takes place at pH 8 to hydronium ion-catalyzed ring opening of the anionic species of the acetal, or the kinetically equivalent intramolecular general acid catalysis in ring opening of the neutral species. The mechanism involving general acid catalysis by the neighboring carboxyl group is strongly supported by the D_2O solvent isotope effect. The *o*-carboxyl group enhances the rate of the acetal ring-opening reaction by a factor of 220 in comparison with the exactly analogous *p*-carboxyl-substituted acetal. In contrast, the analogous *p*-OCH_3-, *p*-NO_2-, *o*- and *p*-$COOCH_3$-, and *p*-COOH-substituted derivatives have uncomplicated linear pH−log rate constant profiles with slopes of −1.0. A neighboring carboxyl group can participate in the hydrolysis of an acetal of an aliphatic alcohol if the C−O bond breaking process is facilitated by the release of steric strain. The implications of these results for the mechanism of lysozyme-catalyzed reactions are discussed.

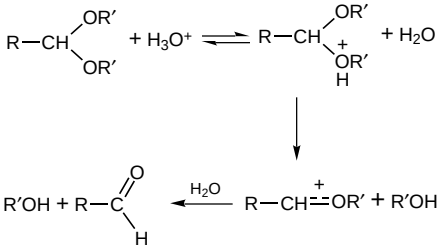
The mechanism of action for the glycosidase enzyme lysozyme that has received the most attention involves intracomplex general acid catalysis by Glu-35 and electrostatic stabilization of the developing oxocarbonium ion by Asp-52 (**I**).[1] This mechanism represented unknown chemistry when first



I

proposed; the generally accepted A-1 mechanism for the hydrolysis of glycosides and simple acetals utilizes equilibrium protonation of the acetal by hydronium ion followed by rate-determining, unimolecular breakdown of the protonated species to a resonance-stabilized oxocarbonium ion (eq 1).[2]

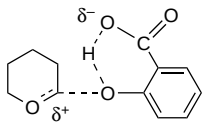
Bimolecular general acid catalysis, in which there is concerted proton transfer and C−O bond breaking, can be observed in acetal hydrolysis reactions when the leaving group is very good (a phenol),[2a,b,3−6] or the intermediate oxocarbonium ion is highly



(1)

stabilized internally.[7] The ease of C−O bond breaking is a key factor in the occurrence of general acid catalysis in acetal hydrolysis;[2a,b] buffer acid catalysis does not occur in the hydrolysis of acetals of aliphatic alcohols, unless there is a structural feature that promotes the ease of C−O bond breaking.

Intramolecular general acid catalysis by a neighboring carboxyl group occurs with phenolic acetals, e.g., **II**,and the large



II

rate enhancements of 10^4−10^6-fold are obtained.[8−10] The two carboxyl groups of benzaldehyde bis(2-carboxyphenyl) acetal

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(1) (a) Phillips, D. C. *Sci. Am.* **1969**, *215*, 78. (b) Phillips, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 484. (c) Strynadka, N. C. J.; James, M. N. G. *J. Mol. Biol.* **1991**, *220*, 401. (d) McCarter, J. D.; Withers, S. G. *Curr. Opin. Struct. Biol.* **1994**, *4*, 885.

(2) (a) Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264; (b) Fife, T. H. *Adv. Phys. Org. Chem.* **1975**, *11*, 1. (c) Capon, B. *Chem. Rev.* **1969**, *69*, 407.

(3) Fife, T. H.; Jao, L. K. *J. Am. Chem. Soc.* **1968**, *90*, 4081.

(4) Fife, T. H.; Brod, L. H. *J. Am. Chem. Soc.* **1970**, *92*, 1681.

(5) Anderson, E.; Capon, B. *J. Chem. Soc. B* **1969**, 1033.

(6) Capon, B.; Nimmo, K. *J. Chem. Soc., Perkin Trans. 2*, **1975**, 1113.

(7) Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1969**, *91*, 7163.

(8) Fife, T. H.; Anderson, E. *J. Am. Chem. Soc.* **1971**, *93*, 6610.

(9) Anderson, E.; Fife, T. H. *J. Am. Chem. Soc.* **1973**, *95*, 6437.

(10) Fife, T. H.; Jaffe, S.; Natarajan, R. *J. Am. Chem. Soc.* **1991**, *113*, 7646.

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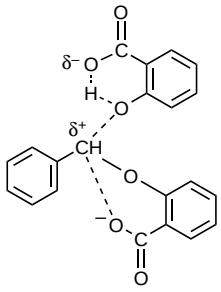
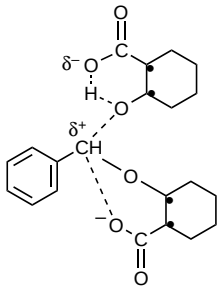
(**III**) give rise to a bell-shaped pH−rate constant profile and provide an enhancement in k_{obsd} of 3×10^9 in comparison with



III

hydrolysis of the corresponding dimethyl ester.[9] Electrostatic stabilization of a developing oxocarbonium ion by a neighboring carboxylate anion was unambiguously observed in the pH-independent breakdown of a phenolic acetal.[11] Intramolecular carboxyl group participation in acetal hydrolysis has been observed in only one case when the leaving group is an aliphatic alcohol, that of benzaldehyde bis(*cis*-2-carboxycyclohexyl) acetal (**IV**).[12] The second carboxyl group of **IV** is necessary

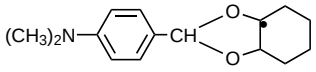


IV

to electrostatically promote C−O bond breaking sufficiently for general acid participation to occur.

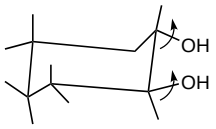
The natural substrates for lysozyme have poor leaving groups and generate an oxocarbonium ion that is relatively unstable. The question then arises as to how C−O bond breaking can be enhanced so that the functional groups in the active site of the enzyme can participate in the reaction, as in **I**. Phillips[1] originally suggested that the hexose unit binding in subsite D, where the cleavage reaction occurs, is distorted into a half-chair, which resembles the oxocarbonium ion intermediate, and presumably the transition state of the reaction. In that manner C−O bond breaking would be facilitated.

The relief of steric strain in the hydrolysis of *p*-(dimethylamino)benzaldehyde *trans*-1,2-cyclohexanediyl acetal (**V**) has



V

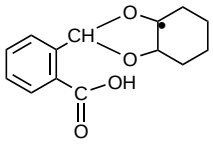
a profound kinetic effect, and bimolecular general acid catalysis is observed in the ring-opening step.[13] This results because the C−O bond breaking reaction of **V** is so facile. When a cyclic acetal is formed from either *cis*- or *trans*-1,2-cyclohexanediol, the hydroxyl groups must be forced closer together. The cyclohexane ring of *cis*-1,2-cyclohexanediol must become more planar upon formation of a cyclic acetal. However, the cyclohexane ring of the *trans*-1,2-diol derivative will become more puckered (VI),[14] which will thereby produce steric strain



VI

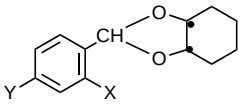
in the cyclic acetal.[13] This strain will be released when the acetal ring opens during hydrolysis. With **V** there is also a high degree of internal oxocarbonium ion stabilization by the *p*-dimethylamino group, which will increase the ease of C−O bond breaking.

In the present work, we have investigated the hydrolysis of *o*-carboxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**VII**) to determine whether relief of strain will allow neighboring



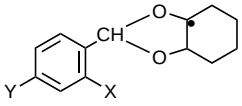
VII

carboxyl group participation when the leaving group is an aliphatic alcohol, and when there is little internal stabilization of the developing oxocarbonium ion in the transition state. For comparison, the corresponding *cis* derivative **VIII** has also been studied, as well as the *p*-carboxyl-substituted acetals **IX** and **X**, the corresponding *o*- and *p*-methyl esters **XI**−**XIV**, and the *p*-methoxy (**XV**) and *p*-NO_2 (**XVI**) derivatives.



cis

VIII: Y = H; X = COOH
X: Y = COOH; X = H
XII: Y = H; X = $COOCH_3$
XIV: Y = $COOCH_3$; X = H



trans

IX: Y = COOH; X = H
XI: Y = H; X = $COOCH_3$
XIII: Y = $COOCH_3$; X = H
XV: Y = OCH_3; X = H
XVI: Y = NO_2; X = H

Experimental Section

Materials. Phthalaldehydic acid was converted to methyl *o*-formylbenzoate by the method of Brown and Sargent.[15] The product was converted to the corresponding dimethyl acetal by mixing the ester with 1 equiv of trimethylorthoformate and excess methanol, and then adding a few crystals of *p*-toluenesulfonic acid. The mixture was allowed to stand for 24 h. Sodium carbonate was then added. The mixture was filtered, the solvent was rotary evaporated, and the residue was distilled (bp 80 °C at 0.3 mmHg).

The *o*-carbomethoxybenzaldehyde *trans*- and *cis*-1,2-cyclohexanediyl acetals were prepared by mixing equivalent amounts of the appropriate diol and the dimethyl acetal of methyl *o*-formylbenzoate and adding 1 drop of benzoyl chloride (to introduce a trace amount of HCl). The mixture was then heated by means of an oil bath, and the methanol formed in the reaction was removed continuously by distillation. The residue was then vacuum distilled. *o*-Carbomethoxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**XI**) boiled at 148 °C (0.1 mmHg): IR ν 1725 cm^{-1} (C═O); ^{13}C NMR δ 103.0. Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.70; H, 6.87. Found: C, 68.39; H, 6.93. *o*-Carbomethoxybenzaldehyde *cis*-1,2-cyclohexanediyl acetal (**XII**) boiled at 140 °C (0.2 mmHg): IR ν 1725 cm^{-1} (C═O); ^{13}C NMR δ 102.1. Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.70; H, 6.87. Found: C, 69.08; H, 6.92.

(11) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1977**, *99*, 6693.
(12) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1979**, *101*, 1202.
(13) Fife, T. H.; Natarajan, R. *J. Am. Chem. Soc.* **1986**, *108*, 8050.
(14) Kuhn, L. P. *J. Am. Chem. Soc.* **1952**, *74*, 2492.
(15) Brown, C.; Sargent, M. V. *J. Chem. Soc. C* **1969**, 1818.

p-Carbomethoxybenzaldehyde dimethyl acetal was prepared from *p*-carbomethoxybenzaldehyde by the same method utilized for the *ortho* derivative. The product boiled at 90 °C (0.01 mmHg), mp 30−32 °C. The *p*-carbomethoxybenzaldehyde *trans*- and *cis*-1,2-cyclohexanediyl acetals were prepared by the same method employed for the *o*-carbomethoxy derivatives. *p*-Carbomethoxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**XIII**) boiled at 140 °C (0.03 mmHg) and melted at 93−94 °C: IR ν 1720 cm^{-1} (C═O); ^{13}C NMR δ 102.7. Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.70; H, 6.87. Found: C, 68.73; H, 6.76. *p*-Carbomethoxybenzaldehyde *cis*-1,2-cyclohexanediyl acetal (**XIV**) melted at 83−84 °C: IR ν 1725 cm^{-1} (C═O); ^{13}C NMR δ 102.3. Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.70; H, 6.87. Found: C, 68.56; H, 6.72.

The *p*-methoxy- and *p*-nitro-substituted benzaldehyde acetals of *trans*-1,2-cyclohexanediol were prepared by the same method as the carbomethoxy derivatives. *p*-Methoxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**XV**) had bp 114 °C (0.007 mm). Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.79; H, 7.69. Found: C, 71.90; H, 7.69. *p*-Nitrobenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**XVI**) had mp 109 °C. Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.65; H, 6.02; N, 5.62. Found: C, 62.75; H, 6.04; N, 5.59.

3-[(*trans*-2-Hydroxycyclohexyl)oxy]phthalide (**XVII**) was prepared by dissolving phthalaldehydic acid (3.0 g, 0.02 mol) in 100 mL of dry 1:1 benzene−ethyl acetate, passing in dry HCl gas for 10 min, and adding *trans*-1,2-cyclohexanediol (2.3 g, 0.02 mol). The mixture was refluxed for 4 h under a Dean Stark trap. The solvent was removed by rotary evaporation, and the residue was distilled. The product boiled at 125−130 °C (1.5 mmHg), as a colorless viscous liquid: IR ν 1774 cm^{-1} (C═O); ^{13}C NMR δ 103.5. Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.61; H, 6.63.

Infrared spectra were obtained with a Perkin-Elmer Paragon 1000 FTIR spectrometer. Proton NMR spectra were obtained with a Brucker AC-250 spectrometer, and $CDCl_3$ was employed as the solvent. Proton decoupled carbon-13 spectra were also obtained with the Brucker AC-250 instrument. Chemical shifts are in reference to TMS. The spectra were consistent with the expected structures.

Kinetic Measurements. The rates of hydrolysis of compounds **VII**−**XVII** were measured spectrophotometrically with a Pye-Unicam SP8-100 or a Beckman DU-7500 spectrophotometer by following the absorbance increase due to the appearance of aldehyde at 295 nm (**VII**, **VIII**, and **XI**−**XIV**), 260 nm (**IX** and **X**), 280 nm (**XV**), and 360 nm (**XVI**). The ionic molarity of all buffers was maintained constant at 0.5 M with KCl. Buffer catalysis was not observed with any acetal at buffer concentrations ranging from 0.02 to 0.25 M. Stock solutions of substrate (**XI**−**XVII**) were prepared in anhydrous acetonitrile. Kinetic runs were initiated by injecting 15 μL of substrate stock solution into 3 mL of temperature-equilibrated buffer in the cuvette. For the hydrolysis of **VII**−**X**, the corresponding methyl ester was added to an 80% ethanol−H_2O solution containing 0.5 M NaOH. The resulting solution was allowed to stand for a sufficient length of time to hydrolyze the ester to the carboxylic acid. An aliquot of this solution was then added to the appropriate buffer, and the reactions were followed to completion. The values of k_{obsd}, the pseudo-first-order rate constant, were computer calculated, and had a precision of 2−4%. Reaction mixture pH values were measured with a Beckman 3500 digital pH meter. The values of pD were determined by employing the glass electrode temperature correction equation.[16]

Molecular modeling of the acetals was carried out with a Silicon Graphics Indigo 2 workstation. The software programs that were employed were Spartan 3.1 from Wavefunction, Inc., and Quanta 4.0-Charmm from Molecular Simulations.

Intermediate Determination. *o*-Carbomethoxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal was hydrolyzed to the corresponding carboxylic acid as above. Water was added, and the pH was adjusted to 3.0. The solution was allowed to stand for 2 min at room temperature, and was then extracted twice with ether. The ether extract was dried with sodium sulfate and filtered. Rotary evaporation of the ether left a residue that had physical properties and an infrared spectrum identical to those of an authentic sample of the acylal **XVII**. Ether extraction of an authentic sample of **XVII** from aqueous pH 3 solution gave identical material.

(16) Fife, T. H.; Bruice, T. C. *J. Phys. Chem.* **1961**, *65*, 1079.

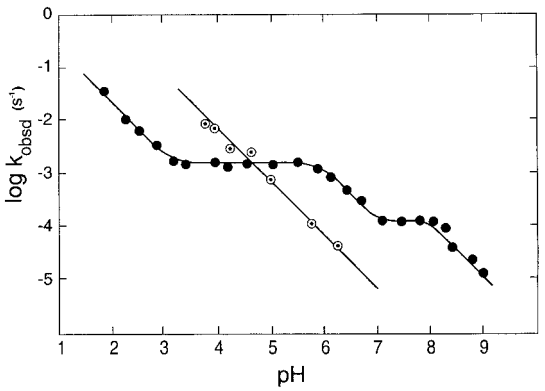


Figure 1. log k_{obsd} *vs* pH for hydrolysis of *o*-carboxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (●) and *p*-carboxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (⊙) in H_2O at 50 °C and μ = 0.5 M (with KCl).

Table 1. Rate Constants for Hydrolysis of *o*- and *p*-Carboxybenzaldehyde *cis*- and *trans*-1,2-Cyclohexanediyl Acetals in H_2O at 50 °C (μ = 0.5 M with KCl)

compd	k_H (M^{-1} s^{-1})	$k_o' \times 10^3$ (s^{-1})	$k_H' \times 10^{-3}$ (M^{-1} s^{-1})	$k_o'' \times 10^4$ (s^{-1})	k_H'' (M^{-1} s^{-1})
VII	2.2	1.5	1.1	1.1	1.4×10^4
VIII	12.6	0.2	0.2		
IX	63				
X	35				

Results

In Figure 1, the plot is shown of log k_{obsd} *vs* pH for hydrolysis of *o*-carboxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**VII**) at 50 °C (μ = 0.5 M with KCl) in H_2O. The reactions are pseudo-first-order with correlation coefficients equal or better than 0.999. Hydronium ion catalysis occurs at pH less than 3, as indicated by the slope of −1.0. The value of k_H, the second-order rate constant, is 2.2 ± 0.2 M^{-1} s^{-1}. There are 4 unit changes of slope in the profile. A pH-independent region in the profile from pH 3 to pH 6 has a rate constant, k_o', of (1.5 ± 0.07) × 10^{-3} s^{-1}. At pH 6 the plot bends downward, thereby indicating another hydronium ion-catalyzed reaction with k_H' = (1.1 ± 0.1) × 10^3 M^{-1} s^{-1}. This reaction is followed by another pH-independent reaction from pH 7 to pH 8 (k_o'' = 1.1 × 10^{-4} s^{-1}), and a further hydronium ion-catalyzed reaction at pH > 8 (k_H'' = (1.4 ± 0.1) × 10^4 M^{-1} s^{-1}). The values of k_{obsd} in the latter reaction are similar in D_2O and in H_2O at pH = pD, k_D'' = (1.5 ± 0.2) × 10^4 M^{-1} s^{-1} (k_D''/k_H'' = 1.1). Also included in Figure 1 is the plot of log k_{obsd} *vs* pH for hydrolysis of *p*-carboxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (**IX**). In contrast to the plot for hydrolysis of **VII**, that for hydrolysis of **IX** is linear with a slope of −1.0 (k_H = 63 ± 7 M^{-1} s^{-1}). The rate constants for the hydrolysis of **VII** and **IX** are summarized in Table 1.

The acylal 3-[(*trans*-2-hydroxycyclohexyl)oxy]phthalide, which is an intermediate in the hydrolysis of **VII**, hydrolyzes with hydronium ion catalysis at low pH, hydroxide ion catalysis at high pH, and a pH-independent reaction from pH 3.5 to pH 9. The plot of log k_{obsd} for hydrolysis *vs* pH is shown in Figure 2. The rate constants for these reactions at 50 °C (μ = 0.5 M with KCl) are k_H = 1.8 ± 0.2 M^{-1} s^{-1}, k_{OH} = 5.8 M^{-1} s^{-1}, and k_o' = (9.5 ± 0.68) × 10^{-4} s^{-1}, respectively. The D_2O solvent isotope effect in the pH-independent reaction is unity ($k_o'(D_2O)/k_o'(H_2O)$ = 1.0).

The hydrolysis of *o*-carboxybenzaldehyde *cis*-1,2-cyclohexanediyl acetal (**VIII**) and the corresponding *p*-carboxyl-substituted acetal **X** at 50 °C in H_2O (μ = 0.5 M) is characterized

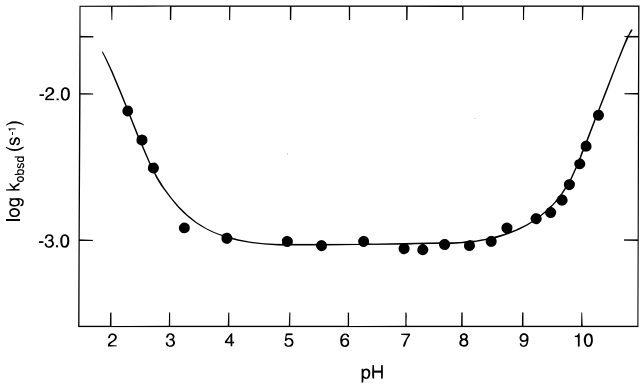


Figure 2. log k_{obsd} *vs* pH for the hydrolysis of 3-[(*trans*-2-hydroxycyclohexyl)oxy]phthalide in H_2O at 50 °C and $\mu = 0.5$ M (with KCl).

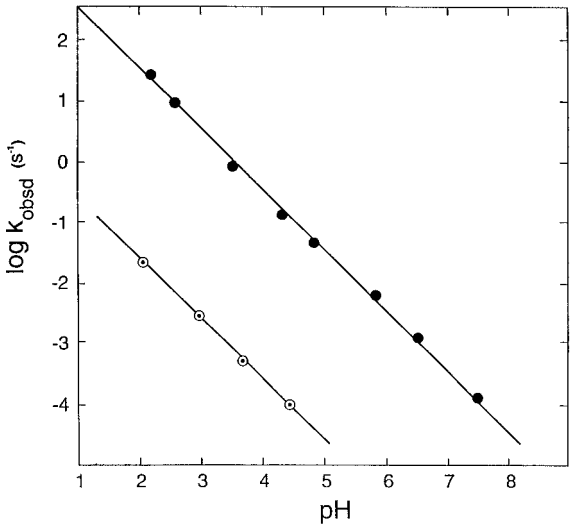


Figure 3. log k_{obsd} *vs* pH for hydrolysis of *p*-methoxybenzaldehyde *trans*-1,2-cyclohexanediyl acetal (●) and *p*-nitrobenzaldehyde *trans*-1,2-cyclohexanediyl acetal (⊙) in H_2O at 30 °C and $\mu = 0.5$ M (with KCl).

Table 2. Second-Order Rate Constants for Hydronium Ion-Catalyzed Hydrolysis of *o*- and *p*-Substituted Benzaldehyde *cis*- and *trans*-1,2-Cyclohexanediyl Acetals in H_2O at 50 °C ($\mu = 0.5$ M with KCl)

compd	k_H (M^{-1} s^{-1})	compd	k_H (M^{-1} s^{-1})
XI	48 ± 1.3	**XV**	3700 ± 380^a
XII	0.85 ± 0.12	**XVI**	2.7 ± 0.02^a
XIII	100 ± 2		

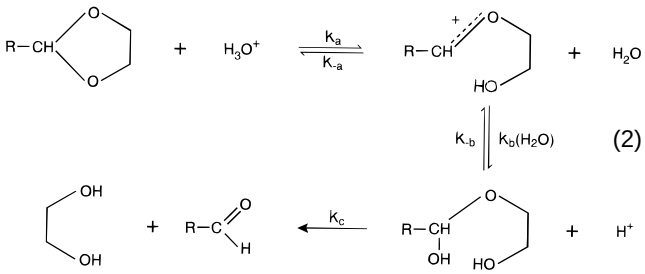
[a] At 30 °C.

by hydronium ion-catalyzed reactions at low pH that proceed with similar rate constants. There is only a 2.8-fold difference in the values of k_H for **VIII** and **X**. The plot of log k_{obsd} *vs* pH for hydrolysis of **X** is linear with a slope of −1.0. A pH-independent reaction occurs from pH 4.5 to pH ∼5.5 in the hydrolysis of **VIII** and is followed by a downward bend in the log k_{obsd} vs pH profile to give a further apparent hydronium ion-catalyzed reaction. The rates of the reactions at pH > 7 are too slow to measure conveniently at 50 °C. Rate constants for these reactions are summarized in Table 1.

The *o*- and *p*-carbomethoxy derivatives **XI**−**XIV** and the *p*-methoxy (**XV**) and *p*-nitro (**XVI**) derivatives of benzaldehyde *trans*-1,2-cyclohexanediyl acetal give linear plots of log k_{obsd} *vs* pH for hydrolysis in H_2O at 50 or 30 °C ($\mu = 0.5$ M) with slopes of −1.0, as seen in Figure 3. Values of k_H are provided in Table 2.

Discussion

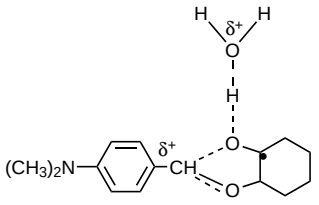
The hydronium ion-catalyzed hydrolysis of cyclic acetals proceeds as in eq 2.[17−20] In the hydrolysis of cyclic acetals,



(2)

the initial C−O bond breaking step does not result in the departure of the alcohol from the molecule, so reversibility (k_{-a}) is a possibility (eq 2). Breakdown of a hemiacetal (k_c) can then be rate determining, or attack of a water molecule on the oxocarbonium ion (k_b) can be rate determining if hemiacetal hydrolysis is fast.[13,20,21] The relative magnitudes of the rate constants of eq 2 will determine the identity of the rate-determining step.

The pH−rate constant profile for hydrolysis of *p*-(dimethylamino)benzaldehyde *trans*-1,2-cyclohexanediyl acetal (**V**) has 7 unit changes in slope, only one of which is due to a pK_a (the *p*-dimethylamino group conjugate acid at pH 4).[13] The other inflections in the profile represent changes in the rate-determining step or mechanism. The ring-opening reaction is rapid because of the release of the steric strain due to the five-membered cyclic acetal ring spanning the equatorial 1,2-positions of cyclohexane, and also because of the presence of the *p*-dimethylamino group. At low pH, the hydrolysis of the hemiacetal intermediate is rate determining (k_c in eq 2). A change in the rate-determining step occurs near pH 6 to attack of H_2O on the oxocarbonium ion intermediate, because hemiacetal hydrolysis is OH^- catalyzed and becomes rapid as the pH is increased. At pH 8 a further change in the rate-determining step occurs to hydronium ion-catalyzed ring opening (**XVIII**).



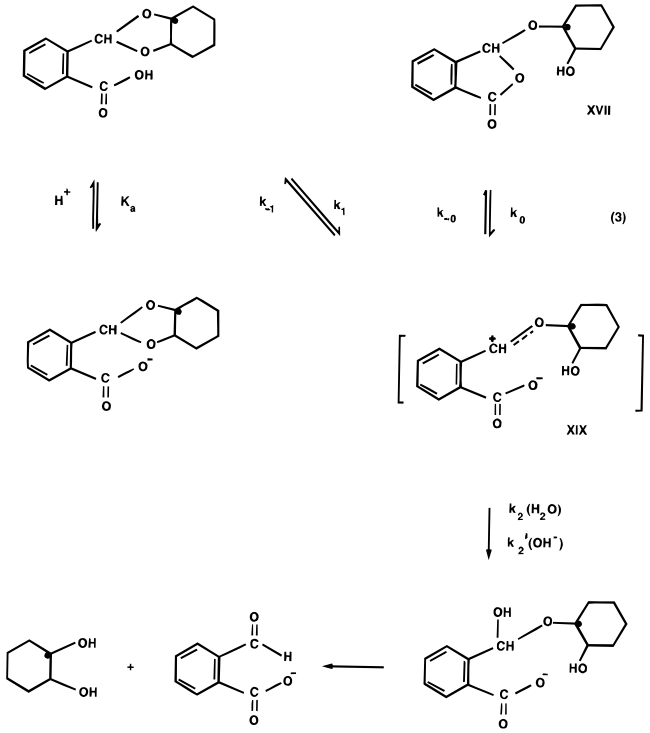
XVIII

The stabilization that the developing oxocarbonium ion receives from the *p*-dimethylamino group of **V** appears to be of critical importance. The pH−log rate constant profiles for the corresponding *p*-methoxy and *p*-nitro derivatives **XV** and **XVI** in Figure 3 are linear with slopes of −1.0. Thus, even a *p*-methoxy group does not provide enough electron release that steps subsequent to ring opening become rate limiting. The oxocarbonium ion stabilization provided by the *p*-dimethylamino group is required so that C−O bond breaking is sufficiently rapid.

(17) Fife, T. H.; Jao, L. K. *J. Org. Chem.* **1965**, *30*, 1492. Fife, T. H.; Hagopian, L. *J. Org. Chem.* **1966**, *31*, 1772.
(18) Ceder, O. *Ark. Kemi* **1954**, *6*, 523.
(19) Salomaa, P.; Kankaanpera, A. *Acta Chem. Scand.* **1961**, *15*, 871.
(20) Fife, T. H.; Natarajan, R. *J. Am. Chem. Soc.* **1986**, *108*, 2425.
(21) Willi, A. V. In *Comprehensive Chemical Kinetics*; Bamford, C. H., Tipper, C. F. H., Eds.; Elsevier: Amsterdam, 1977; pp 49−52.

The neighboring carboxyl group of **VII** enhances C−O bond breaking greatly in view of the pH−log k_{obsd} profile in Figure 1, which is quite dissimilar to the linear profile of the *p*-methoxy ($\sigma = -0.268$) substituted acetal. The profile for hydrolysis of the *p*-carboxyl-substituted acetal **IX** is also reasonably linear with a slope of −1.0; ionization of the carboxyl group has little effect in the hydronium ion-catalyzed reaction of **IX**. An unionized carboxyl group is electron withdrawing, and a carboxylate anion has a σ of ∼0. Therefore, on the basis of electronic effects, a linear profile of slope −1.0 would also be expected in the hydrolysis of **VII**. Thus, the neighboring carboxyl group of **VII** participates in the acetal ring-opening reaction. Carboxyl participation is also evident in the much faster rate of reaction of **VII** than that of **IX** at pH > 5.

Steps subsequent to acetal ring opening are clearly rate determining in the hydrolysis of **VII** at pH < 8. However, it can be seen in Figure 1 that the corresponding acetal with the carboxyl group *para*, in fact, hydrolyzes much faster at pH < 4.5 in a hydronium ion-catalyzed reaction than the *o*-carboxyl-substituted acetal. There is no apparent reason why an oxocarbonium ion or a hemiacetal intermediate would react more slowly with water in the case of **VII** than **IX**. The *o*-carboxyl group of **VII** very likely captures the developing or fully formed oxocarbonium ion, as in eq 3, to give **XVII**, which



breaks down slowly relative to **IX** at low pH. Acylal **XVII** is formed at high concentration at low pH and was isolated from the reaction. The final products (phthalaldehydic acid and *trans*-1,2-cyclohexanediol) are then produced more slowly from **VII** than the corresponding products from **IX** at pH < 4.5.

The hydrolysis of acetal-acylals is characterized by hydronium ion and hydroxide ion reactions, and a rapid pH-independent reaction through the neutral pH range.[22−25] The hydronium ion-catalyzed reaction at low pH can also be slow in comparison with the hydrolysis of corresponding acetals.[26,27] Acylals derived from phthalaldehydic acid also give the typical log k_{obsd} *vs*. pH profiles for hydrolysis,[26] and 3-[(*trans*-2-hydroxycyclohexyl)oxy]phthalide (**XVII**), the acylal intermediate in the reaction of **VII**, does likewise (Figure 2). The D_2O solvent isotope effect near unity in the pH-independent reaction of the acylal **XVII** is in accord with rate-determining unimolecular breakdown to an oxocarbonium ion (**XIX**),[28] although solvent interaction with the developing charges may also occur in the transition state. A unimolecular ring-opening reaction of **XVII** should be markedly reversible, and therefore, solvent participation may be required, so the reaction can proceed readily to products.

The concentration of acylal **XVII** builds up rapidly in the reaction of **VII** at low pH. Since k_1 (eq 3) is large (as follows from the large k_H'' at high pH),[29] **VII** will be rapidly converted to the oxocarbonium ion **XIX** at low pH. The k_{-1} step of eq 3 should be fairly slow in comparison with the step governed by k_{-o}, because reversal to **VII** would require the reintroduction of strain. The oxocarbonium ion will then partition between the acylal **XVII** and the hemiacetal. If $k_{-o} \geq k_2$, and the acylal breakdown (k_o) is relatively slow ($k_{-o} > k_o$), **XVII** will quickly build up. Thus, the formation of **XVII** will slow the observed reaction because the k_{-o} step traps the oxocarbonium ion.

Equilibrium between **VII** and **XVII** at pH < 6 would require the pH−rate constant profiles for hydrolysis in that pH region to be the same for each, and the rate constants to be identical. The profiles for hydrolysis of **VII** and **XVII** at pH < 6 are closely similar in shape, but the rate constants for hydrolysis of **VII** are larger than those for hydrolysis of **XVII** by ∼30%. The k_{obsd} values have a precision of 2−4%, but the uncertainty in the derived rate constants is somewhat larger (7−9%). The pH-independent reaction of the acetal **VII** from pH 3−6 has $k_o' = (1.50 \pm 0.07) \times 10^{-3}$ s^{-1} (6 points), whereas k_o' for hydrolysis of **XVII** is $(9.5 \pm 0.68) \times 10^{-4}$ s^{-1} (15 points from pH 3 to pH 8.5). Thus, the differences in the rate constants for **VII** and **XVII** at pH < 6 are outside the limits of uncertainty. Lack of equilibrium between **VII** and **XVII** could result from a relatively small k_{-1} and/or the rapid reactions of the oxocarbonium ion. The rate of product appearance, which will depend upon the concentration of the oxocarbonium ion **XIX**, need not be the same in the reactions of **VII** and **XVII** if they are not in complete equilibrium. There is no observable deviation from first-order kinetics; the reactions are nicely first-order upon commencement of the rate measurements after mixing. Clearly, **XIX** attains its maximum concentration very rapidly in the ring opening of **VII** at low pH,[29] and reacts via both the k_{-o} and k_2 steps.[30,31]

The plot of log k_{obsd} *vs* pH for the hydrolysis of **VII** in Figure 1 bends downward near pH 6, in contrast with the pH independence of the plot for hydrolysis of **XVII** (Figure 2). Thus, **VII** and **XVII** cannot be in equilibrium at pH > 6. The presence of a proton is required for rapid cleavage of the acetal

(22) Fife, T. H. *J. Am. Chem. Soc.* **1965**, *87*, 271.
(23) Fife, T. H.; Bembi, R. *J. Org. Chem.* **1992**, *57*, 1295.
(24) Fife, T. H.; De, N. C. *J. Am. Chem. Soc.* **1974**, *96*, 6158.
(25) Salomaa, P.; Laiho, S. *Acta. Chem. Scand.* **1963**, *17*, 103. Salomaa, P. *Suom. Kemistil.* **1964**, *37*, 86. Salomaa, P. *Acta Chem. Scand.* **1965**, *19*, 1263.
(26) The value of k_H for hydrolysis of γ-ethoxyphthalide at 80 °C in 50% dioxane−H_2O (v/v) is 0.4 M^{-1} s^{-1}; k_o is 3×10^{-4} s^{-1}. Fife, T. H.; Bembi, R. Unpublished data.
(27) Weeks, D. P.; Crane, J. P. *J. Org. Chem.* **1973**, *38*, 3375.
(28) A D_2O solvent isotope effect near unity has been found in the pH-independent reactions of other mixed acetal-acylals (see refs 22 and 23).
(29) Assuming a pK_a of the carboxyl group of 4, from the value of k_H'' and the relationship $k_1 = k_H''K_a$, k_1 may be calculated to be 1.4 s^{-1}.
(30) The k_2 should be >10^3 s^{-1}. See: Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 8238. See also ref 13. Carboxylate stabilization of **XIX** would reduce k_2 and k_{-1}.
(31) There are possible mechanisms for the direct formation of **XVII** from **VII**, without the intermediacy of an oxocarbonium ion, but such mechanisms are ruled out at pH > 8 because of the D_2O solvent isotope effect.

ring, so the rate of ring opening to **XIX** will decline with increasing pH at pH values greater than the pK_a of the carboxyl group. As the pH increases above the pK_a, k_{obsd} for hydrolysis of the acetal must bend downward, whereas k_{obsd} for acylal hydrolysis is pH independent. The downward bend in k_{obsd} in Figure 1 will occur near the pH value where the calculated k_{obsd} values for acetal and acylal hydrolysis are equal (pH 6).[32] The attack of water on the oxocarbonium ion (k_2) is kinetically important in the pH range 6−7, in view of the further downward bend in the profile of Figure 1 at pH 8 that can only be attributed to a change in rate-determining step to acetal ring opening. From the profile at pH > 6, it can be calculated that the ratio of the rate of acetal ring opening to the rate of hydrolysis is 12.7 (k_H''/k_H'). But the oxocarbonium ion is not trapped by the formation of **VII** in the hydrolysis of **XVII** at pH 6−7.

A mechanism change occurs in the hydrolysis of **VII** at pH 7 to attack of OH^- on the oxocarbonium ion arising in the acid-catalyzed acetal ring opening, which thereby produces another pH-independent reaction from pH 7−8. The OH^- reaction must involve attack on the oxocarbonium ion since OH^- attack on the acylal **XVII** does not occur until pH 9. Therefore, the hydrolysis of **XIX** is rate determining in the pH range 7−8. As the pH increases, the hydrolysis of the oxocarbonium ion will become fast due to OH^- catalysis, and $k_2'[OH^-]$ will become large. The formation of **XVII** will be inappreciable when $k_2'[OH^-] \gg k_{-o}$.

An OH^- reaction of the oxocarbonium ion ($k_2'[OH^-]$) is not apparent in the log k_{obsd} vs pH profile of Figure 2 for the hydrolysis of **XVII**. Therefore, the ring-opening step governed by k_o is partly or completely rate determining, which is in agreement with the D_2O solvent isotope effect near unity. The rate-determining step in the hydrolysis of **XVII** is determined by the relative magnitudes of k_2 and k_{-o} (eq 4),so both k_2 and

$$k_{obsd} = \frac{k_o k_2}{k_{-o} + k_2} \qquad (4)$$

k_o could contribute to k_{obsd}. At higher pH, k_o must, of course, be solely rate determining when $k_2'[OH^-] \gg k_{-o}$. Equation 4 considers the mechanism of acylal ring opening to be the microscopic reverse of acylal formation from the oxocarbonium ion. Water attack on the acylal carbonyl would avoid the reactive oxocarbonium ion intermediate, but a large D_2O solvent isotope effect would then be expected, analogous to other water reactions in ester hydrolysis.[2b,24] The OH^- reaction observed at pH > 9 undoubtedly reflects attack of OH^- at the carbonyl.

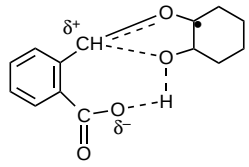
Intramolecular Carboxyl Group Participation. At pH 8 the plot of log k_{obsd} *vs* pH for the hydrolysis of **VII** again bends downward to give a slope of −1.0. The downward bend must correspond to a change in the rate-determining step, since there are no ionizable functional groups that would have a pK_a close to 8. In view of the pH-independent reaction at pH > 7, which must be attributed to oxocarbonium ion hydrolysis, a slope of −1.0 at pH > 8 is not consistent with the known mechanisms for reaction of any of the possible intermediates in the hydrolysis of **VII**.[13,22−25,33] Therefore, the profile of slope −1.0 at pH > 8 indicates that the rate-determining step changes to hydronium ion-catalyzed ring opening of the anionic acetal, or the kinetically equivalent intramolecular general-acid-catalyzed hydrolysis of the neutral species, as the OH^- reaction of the oxocarbonium ion becomes more rapid with increasing pH. Ring opening (k_1) will become rate determining in the hydrolysis of **VII** when $k_2'(OH^-) > k_{-1}$ and k_{-o}. The ring opening will in general be rate determining in the hydrolysis of carboxyl-substituted cyclic acetals if k_2 or $k_2'[OH^-] > k_{-1}$ (eq 5) in cases where k_{-1} is

$$k_{obsd} = \frac{k_1 k_2'[OH^-] a_H}{(k_{-1} + k_2'[OH^-])K_a} \qquad (5)$$

significant. In comparison with the hydronium ion-catalyzed reaction of the *p*-carboxyl-substituted acetal **IX**, the rate enhancement in the ring-opening reaction of **VII** due to carboxyl group participation is a factor of 220.

The *cis*-1,2-cyclohexanediyl acetal **VIII** hydrolyzes with a rate constant for the hydronium ion-catalyzed reaction at low pH, k_H, that is 6-fold larger than the k_H in the reaction of **VII**, and only ∼3-fold less than the k_H for the reaction of the *p*-carboxyl-substituted acetal **X**. At pH > 7 the hydrolysis of **VIII** becomes very slow at 50 °C. Thus, the magnitude of the neighboring carboxyl group participation depends markedly on the *cis* or *trans* configuration; the effect is most pronounced for the *trans* acetal. The puckered ring that results from the *trans*-fused cyclic acetal ring is perhaps more strained than the more planar ring resulting from a *cis* ring juncture (see also ref 13).

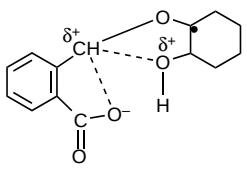
There are kinetically equivalent mechanisms for the carboxyl group participation of **VII**. Ring opening could involve intramolecular general acid catalysis by the un-ionized carboxyl group (**XX**). A seven-membered hydrogen-bonded ring would



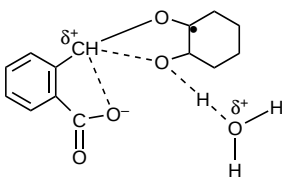
XX

be required, but it has been shown that a precise steric fit is not a requirement for the occurrence of intramolecular general acid catalysis.[10,11] The carboxyl group to acetal oxygen O−O interatomic distance in **VII**, calculated from molecular modeling, is 2.99 Å.[34] Therefore, proton transfer may not occur directly from the un-ionized carboxyl group to the leaving group oxygen in the transition state; the ease of proton transfer will depend on the extent of C−O bond breaking. Concerted proton transfer from the carboxyl group could also be mediated by a water molecule,[10] with a consequent reduction in the observed rate enhancement.

Other possible mechanisms involve electrostatic stabilization of the developing oxocarbonium ion by the ionized carboxylate anion (**XXI** and **XXII**). Mechanism **XXII**, in which there is



XXI



XXII

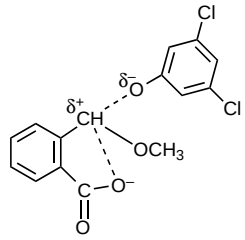
proton transfer to the anionic species from hydronium ion in the transition state, is not likely in view of the lack of buffer

(32) Equilibrium between **VII** and **XVII** at pH < 6 would require a downward bend at p(K_a/K_{eq}) where K_{eq} is the equilibrium constant for **VII** ⇌ **XVII** and is larger than unity.

(33) Przystas, T. J.; Fife, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 4884.

(34) The O−O interatomic distance of 2.99 Å was obtained with the Quanta 4.0-Charmm program in which the methods of steepest descent and Newton−Raphson were employed in the minimization process. An O−O distance of 2.67 Å was obtained with Spartan 3.1 employing a Sybyl minimizer; MM2 and MM3 failed.

acid catalysis. Mechanisms **XX** and **XXI** differ in the extent and timing of the proton transfer to the leaving group oxygen, and in the stabilization afforded the oxocarbonium ion. In contrast with the concerted proton transfer and C−O bond breaking of mechanism **XX**, proton transfer to the acetal oxygen in mechanism **XXI** is an equilibrium process that is established prior to the C−O bond-breaking step. Electrostatic stabilization effects by a neighboring carboxylate anion have been observed in the pH-independent unimolecular breakdown of phthalaldehydic acid methyl 3,5-dichlorophenyl acetal in the solvent 50% dioxane−H_2O (**XXIII**).[11] In that reaction, there is extensive



XXIII

C−O bond breaking in the transition state, and accordingly, there is considerable oxocarbonium ion development. The rate enhancement in reaction **XXIII** in comparison with the *p*-carboxyl-substituted compound is 100-fold. Electrostatic stabilization effects, however, have not been observed in A-1 hydronium ion-catalyzed reactions where there is less C−O bond breaking in the transition state.

Mechanisms **XX** and **XXI** should be easily distinguished by means of the D_2O solvent isotope effect. An A-1 mechanism, in which a proton is completely transferred from hydronium ion to an acetal oxygen in an equilibrium step, as in **XXI**, would proceed considerably faster in D_2O than in H_2O, because of the increased concentration of the conjugate acid in D_2O. Solvent isotope effects, k_D/k_H, of 2.7−3.0 are commonly observed in A-1 acetal hydrolysis reactions.[17] In contrast, a reaction involving general acid catalysis, in which the proton transfer occurs in the transition state (**XX**), should proceed more slowly in D_2O than in H_2O.

The hydrolysis reaction of **VII** at pH or pD > 8, where ring opening is rate determining, has a D_2O solvent isotope effect near unity ($k_D''/k_H'' = 1.1$). This value is not consistent with an equilibrium protonation step, as in **XXI**. Also, a k_D''/k_H'' ratio of 0.3−0.5 would be expected for mechanism **XXII**. However, if the reaction is considered to proceed via the neutral species (**XX**), then the calculated rate constant for general acid catalysis is considerably less in D_2O than in H_2O. For a neutral species reaction at pH > 8, $k_H'' = k_1/K_a$, where k_1 is the rate constant for the step involving concerted proton transfer and C−O bond breaking. The pK_a of the carboxyl group will be increased in D_2O as compared with H_2O by ∼0.5 pK_a unit,[35] so $k_1(D_2O)$ is less than $k_1(H_2O)$. Therefore, the most likely mechanism is **XX**, in which the neighboring un-ionized carboxyl group acts as an intramolecular general acid. The large effective molarity of the carboxyl group general acid outweighs the increased oxocarbonium ion stabilization provided by **XXI**. Consequently, such a mechanism (**XX**) can occur in the hydrolysis of sterically strained acetals of aliphatic alcohols because the C−O bond breaking reaction is facilitated.

Lysozyme. It is clear that the release of steric strain in the transition state will allow intramolecular carboxyl group participation as a general acid in acetal hydrolysis even when the leaving group is an aliphatic alcohol of high pK_a. Thus, one of the suggested features of the lysozyme mechanism[1] has now been shown to be chemically feasible.

The rate enhancement provided by mechanism **XX** does not, of course, account for the rate enhancements due to the enzyme in the hydrolysis of its substrates (10^{10}).[36,37] The rate facilitation factor of 220, due to carboxyl group participation with **VII** in comparison with **IX**, must be multiplied by the factor due to the release of steric strain as the C−O bond breaks (10−100),[38] but the result is still far less than required. If it were possible to improve the steric fit of the carboxyl group, so that a five- or six-membered hydrogen-bonded ring could be formed, then the observed rate enhancement would be increased, but only an additional factor of 10^2−10^3 would be expected.[12] In the only other example of carboxyl group participation in the hydrolysis of an aliphatic alcohol acetal, a second ionized carboxyl group was required to electrostatically enhance the ease of C−O bond breaking.[12] The observed rate enhancement in the reaction of **IV**, in comparison to the hydrolysis of the corresponding dimethyl ester, is 4×10^4-fold. The rate enhancement of 3×10^9-fold for salicylic acid release from benzaldehyde bis(2-carboxyphenyl) acetal (**III**)[9] is sufficiently large, but the leaving group in that case is very good (a phenol), in contrast with the poor leaving groups of natural substrates for lysozyme.[1] If Glu-35 functions as a general acid, then the enzyme must make C−O bond breaking easier. Therefore, the effects of intramolecular general acid catalysis are combined with other features of the enzymatic reaction to obtain rate constants of the proper magnitude. Such features could be electrostatic stabilization of the developing oxocarbonium ion, as in mechanism **I**,[1,9−11] or nucleophilic participation by functional groups, e.g., a neighboring acetamido group.[39,40]

Considerably more strain energy could be released in the transition state of the enzymatic reaction than in the reaction of **VII** if the substrate is distorted to resemble a half-chair.[1b,36,41] The magnitude of the enzymatic rate constants should then be explainable in terms of currently recognized mechanistic factors. An increase in the ease of C−O bond breaking in lysozyme substrates to an extent comparable to that produced by a reduction in leaving group pK_a of 3−4 pK_a units would be required to give the rate enhancement provided by mechanism **III**, assuming comparable stabilization of the developing oxocarbonium ion in **I** and **III**.

An acylal intermediate has been suggested as a possibility in lysozyme-catalyzed reactions via nucleophilic attack by Asp-52.[36,40] However, no clear evidence for such an intermediate has been obtained. Vernon considered an acylal intermediate unlikely because the distance between the C-1 reaction center

(35) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 250−253. Hogfeldt, E.; Bigeleisen, J. *J. Am. Chem. Soc.* **1960**, *82*, 15.

(36) Kirby, A. J. *CRC Crit. Rev. Biochem.* **1987**, *22*, 283.

(37) But note that the value of the rate constant for the rate-determining step in lysozyme-catalyzed reactions has been calculated to be 1.75 s^{-1}: Chipman, D. M. *Biochemistry* **1971**, *10*, 1714. That value is not greatly different from the value of k_1 in the hydrolysis of **VII** (see ref 29).

(38) The k_H' for hydrolysis of 2-(*o*-carboxyphenyl)-1,3-dioxolane in water at 80 °C is 560 M^{-1} s^{-1}, 100-fold less than k_H'' for **VIII** at 80 °C. There is no carboxyl participation in the hydrolysis of the dioxolane. Fife, T. H.; Bembi, R. Unpublished data.

(39) Piszkiewicz, D.; Bruice, T. C. *J. Am. Chem. Soc.* **1968**, *90*, 2156. Nucleophilic attack by the neighboring acetamido group of 2-deoxy-2-acetamido-β-D-glucopyranosides provide large rate enhancements in the hydrolysis reactions. Substrates for lysozyme lacking the 2-acetamido group hydrolyze readily in the enzymatic reaction,[40] but 2-deoxy derivatives should hydrolyze rapidly because of reduced electron withdrawal from the reaction center.

(40) Raftery, M. A.; Rand-Meir, T. *Biochemistry* **1968**, *7*, 3281.

(41) It should be pointed out that there is no general agreement on substrate distortion in binding or on the role of conformational changes involving the active site; see refs 1c and 36 and references therein.

and the oxygen of the Asp-52 carboxyl group, as revealed by X-ray crystallographic analysis, is greater than the covalent bond distance.[42] A conformational change of the enzyme might allow C−O bond formation, but should require energy. In the present study of **VII**, either the formation of an acylal has no effect on the observed kinetics because acylal formation or breakdown is not rate limiting (pH > 8), or acylal formation is inhibitory in the overall hydrolysis reaction (pH < 5). Thus, in evaluating mechanistic factors that will provide large rate enhancements in acetal hydrolysis reactions and give insight into lysozyme-catalyzed reactions, it is clear that factors other than nucleophilic carboxyl group reactions[43] (analogous to those possible for Asp-52) should be stressed.

Acknowledgment. This work was supported by research grants from the National Science Foundation and National Institutes of Health.

JA953362T

(42) Vernon, C. A. *Proc. R. Soc. London, B* **1967**, *167*, 389.

(43) Nucleophilic reactions are considered to involve complete covalent C−O bond formation.