Intramolecular General-Acid and Electrostatic Catalysis in Acetal Hydrolysis. Hydrolysis of 2-(Substituted phenoxy)-6-carboxytetrahydropyrans and 2-Alkoxy-6-carboxytetrahydropyrans

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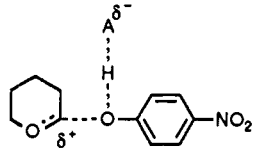
Abstract: Rate constants have been determined for hydrolysis of a series of *trans*-2-(substituted phenoxy)-6-carboxytetrahydropyrans (e,a) in H_2O and 50% dioxane–H_2O. The second-order rate constant (k_2) for the apparent hydronium ion catalyzed reaction of the anionic species of the *p*-nitro derivative in 50% dioxane–H_2O (50 °C) is 620-fold larger than the second-order rate constant for hydrolysis of 2-(*p*-nitrophenoxy)tetrahydropyran. The slope (ρ) of the Hammett plot of log k_2 vs σ is approximately 0, which supports a mechanism involving intramolecular general-acid catalysis by the un-ionized carboxyl group with proton transfer to the leaving group oxygen. Sizable catalytic effects can therefore be obtained in an intramolecular general-acid-catalyzed reaction when the leaving group is sufficiently good, even though the steric fit is poor and proton transfer may necessarily occur via an intervening water molecule. Intramolecular general-acid-catalyzed ring opening is not a favorable process in the hydrolysis of tetrahydropyranyl acetals; such a mechanism does not occur in the hydrolysis of 2-alkoxy-6-carboxytetrahydropyrans. In the pH-independent decomposition of 2-(*p*-nitrophenoxy)-6-carboxytetrahydropyran at pH >6, the neighboring carboxylate anion participates electrostatically in 50% dioxane–H_2O and gives a 10-fold rate enhancement. The plot of log k_{obsd} vs pH for hydrolysis of 2-(*o*-carboxyphenoxy)-6-carboxytetrahydropyran is bell shaped, which indicates that the monoanionic species is maximally reactive. The rate enhancement in that reaction in comparison with the hydrolysis of 2-(*p*-carboxyphenoxy)-6-carboxytetrahydropyran or 2-(*p*-carboxyphenoxy)tetrahydropyran is a factor of (2–4) × 10^3 in H_2O but is 10^4–10^5 in 50% dioxane–H_2O. The *o*-carboxyl group acts as an intramolecular general acid. However, bifunctional catalysis does not take place in this system. The lack of efficient electrostatic facilitation of these reactions by a neighboring carboxylate anion is most likely due to the energy expenditure required for the 6-carboxylate group to approach the developing oxocarbonium ion closely. There is no chemical support for proposed mechanisms of lysozyme-catalyzed reactions that involve intracomplex general-acid-catalyzed opening of an unstrained hexose ring or large electrostatic stabilization effects by Asp-52.

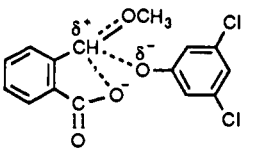
There has been considerable interest in the mechanisms of hydrolysis of glycosides and structurally simpler acetals because of the chemical and biochemical importance of such reactions.[1-4] The generally accepted A-1 mechanism involves equilibrium protonation of the acetal by hydronium ion followed by a unimolecular rate-determining breakdown of the protonated acetal to an alcohol and a resonance-stabilized oxocarbonium ion.[2-6] However, general-acid catalysis involving proton transfer in the transition state concerted with C–O bond breaking, as in I, can



I

be observed when C–O bond breaking is facile because of a very good leaving group (a phenol) and an oxocarbonium ion intermediate of moderate stability[7-10] or, if the leaving group is an aliphatic alcohol, because the oxocarbonium ion is highly stabilized (an alkoxytropylium ion).[11] The first observation of buffer acid catalysis in acetal hydrolysis was in reactions of 2-(aryloxy)-tetrahydropyrans.[7] It was also found with 2-(*p*-nitrophenoxy)-tetrahydropyran that a facile pH-independent reaction occurs at pH >5. That reaction was subsequently shown[8] to be a unimolecular decomposition brought about by the good leaving group and the moderately stable oxocarbonium ion intermediate.

A neighboring carboxyl group will participate readily in the hydrolysis of acetals,[12-20] and extremely large rate enhancements have been obtained in comparison with the hydrolysis of suitable reference compounds. For example, in the hydrolysis of benzaldehyde disalicyl acetal[13] the maximum enhancement in k_{obsd} in comparison with the corresponding dimethyl ester is 2 × 10^9. The mechanism of hydrolysis of benzaldehyde methyl salicyl acetal must involve intramolecular general-acid catalysis since the kinetically equivalent possibilities can be ruled out.[12] A neighboring carboxylate anion will also act as an electrostatic catalyst in reactions that require extensive C–O bond breaking in the transition state.[19,20] The pH-independent unimolecular reaction of phthalaldehydic acid methyl 3,5-dichlorophenyl acetal is electrostatically enhanced 100-fold by the neighboring carboxylate group in 50% dioxane–H_2O (II).[19] Bifunctional catalysis, in which



II

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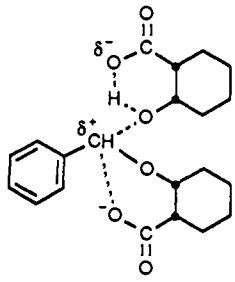
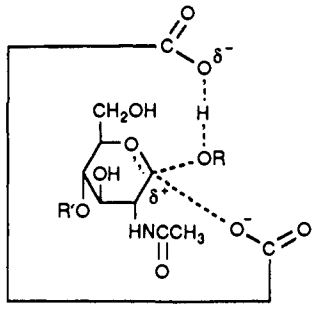
an un-ionized carboxyl group acts as an intramolecular general acid and a neighboring carboxylate ion electrostatically stabilizes the developing oxocarbonium ion in the transition state, occurs in the hydrolysis of benzaldehyde bis(*cis*-2-carboxycyclohexyl) acetal (III).[14]



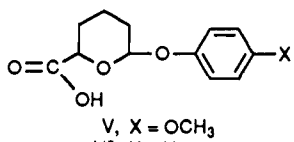
III

An understanding of the factors influencing intramolecular carboxyl group participation in acetal hydrolysis is critical to understanding the mechanism of action of glycosidase enzymes such as lysozyme with which intracomplex carboxyl group participation takes place.[2,4] A mechanism for lysozyme-catalyzed reactions has been proposed (IV),[21,22] which utilizes concerted

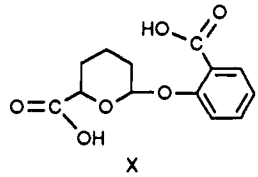


IV

bifunctional catalysis analogous to III. Electrostatic stabilization effects by Asp-52 have been stressed.[23,24] The Asp-52 carboxyl group is too far away from C-1 of the hexose unit in subsite D for covalent bond formation.[23] Thus, a conformation change of the enzyme would be required for such bond formation. In contrast, a covalent bond can readily form in the neighboring carboxylate facilitated reactions of II and III. There is little information available concerning the effect of structural changes on intramolecular electrostatic and general-acid catalysis. It is especially important to determine the influence of the stability of the oxocarbonium ion intermediate and the precision of the steric fit on the efficiency of these catalytic processes. Consequently, we have studied the hydrolysis reactions of a series of 2-(substituted phenoxy)-6-carboxytetrahydropyrans (V–X). The



V, X = OCH_3
VI, X = H
VII, X = Cl
VIII, X = NO_2
IX, X = COOH



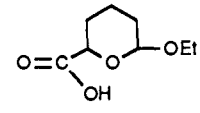
X

reactions of 2-(*p*-carboxyphenoxy)-6-carboxytetrahydropyran (IX) and 2-(*p*-carboxyphenoxy)tetrahydropyran (XI) have been investigated so that X can be compared with these analogous derivatives having the carboxyl substituent in the para position of the leaving group.

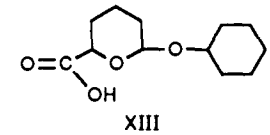
An ionized carboxyl group at the 6-position of the tetrahydropyran acetals could electrostatically stabilize the developing oxocarbonium ion at C-2 or participate as a nucleophile in the hydrolytic reactions, and an un-ionized carboxyl group at C-6 might function as an intramolecular general acid in assisting leaving group expulsion. These processes would be sensitive to the steric factors inherent in the tetrahydropyran ring system; e.g., the formation of a covalent bond between the carboxylate oxygen and C-2 would be sterically possible but would result in a strained ring system. Electrostatic catalysis will depend upon the closeness of approach of the negatively charged oxygen to the developing positive charge, and it has not been established whether such catalysis can be significant in acetal hydrolysis reactions when the steric situation is unfavorable for covalent bond formation,[25] as is apparently the case in the ES complexes of lysozyme.[23] The intramolecular reactions of the acetals V–X have provided information on this important point.

There has been considerable discussion in the literature on whether participation by a neighboring carboxylate anion in the hydrolysis of acetals and glycosides should be viewed as electrostatic stabilization of a developing oxocarbonium ion or as nucleophilic attack at the reaction center.[26] However, it should be recognized that the terms "electrostatic catalysis" and "nucleophilic participation" are equivalent in systems where complete covalent bond formation can readily occur. Whether the carboxylate participation is viewed as stabilization of the developing positive charge on carbon, or whether participation is considered to provide a push for leaving group departure, the net effect will be to provide a transition state that more closely resembles the reactants; i.e., in both cases, there will be less C–O bond breaking required to reach the transition state and the transition states will be similar or identical. Formation of a covalent bond is not a suitable criterion since bond formation will only be complete *after* the transition state has been achieved. Thus, the two terms are meaningfully distinct only in a system where complete C–O bond formation is difficult or precluded.

An un-ionized carboxyl group in the 6-position might also catalyze an initial opening of the tetrahydropyran ring as a general acid via a sterically favorable five-membered ring transition state. Such a process would be most likely to occur in reactions of derivatives of aliphatic alcohols with which the remaining OR group could provide sufficient stabilization of the developing oxocarbonium ion.[27] 2-Alkoxytetrahydropyran acetals may undergo some ring opening in hydronium ion catalyzed hydrolysis reactions,[28] even though ring opening should be entropically less favorable than expulsion of the exocyclic alcohol group. A possible mechanism for lysozyme-catalyzed reactions in which Glu-35 promotes opening of the glycosyl ring in subsite D via general-acid catalysis has been discussed.[2,29] To assess such a mechanism in the reactions of tetrahydropyran acetals, we have also investigated the hydrolysis reactions of the aliphatic acetals XII and XIII.



XII



XIII

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Experimental Section

Materials. 2-Ethoxy-6-carbethoxytetrahydropyran was prepared from 3,4-dihydro-2*H*-pyran-2-carboxylate sodium salt (Aldrich) by the method employed by Dyer et al.[30] for synthesis of the analogous methoxy derivative. The clear liquid boiled at 98 °C (1.5 mm): ^{1}H NMR δ 5.0 (narrow, 1 H). This material was dissolved in diethyl ether, and dry HCl gas was bubbled through the solution for 30 min. The ether was rotary evaporated, and the chloro ether residue was then added to a solution of the sodium salt of the appropriate phenol in DMF. The mixture was added to 100 mL of benzene. The benzene solution was washed with a solution of K_2CO_3 in H_2O. The benzene layer was dried over Na_2SO_4. The benzene was then rotary evaporated, and the residue was either distilled or recrystallized from hexane and ether in the case of the *p*-nitro derivative.

2-(*p*-Methoxyphenoxy)-6-carbethoxytetrahydropyran: bp 150 °C (0.02 mm); n^{24}_D 1.5102; ^{1}H NMR δ 4.95 (1 H). Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.28; H, 7.14. Found: C, 64.10; H, 7.30.

2-Phenoxy-6-carbethoxytetrahydropyran: bp 108 °C (0.02 mm); n^{24}_D 1.5100; ^{1}H NMR δ 5.30 (1 H). Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.20; H, 7.20. Found: C, 66.71; H, 7.11.

2-(*p*-Chlorophenoxy)-6-carbethoxytetrahydropyran: bp 125 °C (0.02 mm); n^{24}_D 1.5147; ^{1}H NMR δ 5.15 (1 H). Anal. Calcd for $C_{14}H_{17}ClO_4$: C, 59.05; H, 6.02. Found: C, 58.96; H, 6.23.

2-(*p*-Nitrophenoxy)-6-carbethoxytetrahydropyran: mp 94–95 °C; ^{1}H NMR δ 6.25 (1 H), 5.0 (mult); ^{13}C NMR δ 170.5 (C=O), 98.6 (C-2), 73.6 (C-6), 19.1 (C-4). Anal. Calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.76; N, 4.74. Found: C, 56.85; H, 5.77; N, 4.64.

2-(*o*-Carbomethoxyphenoxy)-6-carbethoxytetrahydropyran: bp 140–141 °C (0.1 mm); n^{21}_D 1.5072; ^{1}H NMR δ 5.20 (1 H); ^{13}C NMR δ 169.6, 165.8 (C=O), 99.0 (C-2), 72.5 (C-6), 18.5 (C-4). Anal. Calcd for $C_{16}H_{20}O_6$: C, 62.33; H, 6.49. Found: C, 61.95; H, 6.81.

2-(*p*-Carbomethoxyphenoxy)-6-carbethoxytetrahydropyran: mp 94–95 °C; ^{13}C NMR δ 170.3, 165.7 (C=O), 98.4 (C-2), 73.5 (C-6), 19.3 (C-4). Anal. Calcd for $C_{16}H_{20}O_6$: C, 62.33; H, 6.49. Found: C, 62.30; H, 6.48.

2-(*p*-Carbomethoxyphenoxy)tetrahydropyran was prepared from 2-chlorotetrahydropyran and the sodium salt of methyl *p*-hydroxybenzoate in the same manner as the other compounds in the series. The compound was distilled (bp 110–112 °C (0.1 mm)). The material then crystallized and after recrystallization from cyclohexane melted at 63–64 °C: ^{13}C NMR δ 166.7 (C=O), 95.9 (C-2).

2-(*p*-Carboxyphenoxy)tetrahydropyran (XI), which is produced by alkaline hydrolysis of the ester, has been previously reported.[12]

2-(Cyclohexyloxy)-6-carbethoxytetrahydropyran: bp 115–117 °C (0.3 mm); n^{22}_D 1.4668. Anal. Calcd for $C_{14}H_{24}O_4$: C, 65.63; H, 9.38. Found: C, 65.99; H, 9.34. **2-(Cyclohexyloxy)tetrahydropyran**: bp 90 °C (2.5 mm); n^{23}_D 1.4687. Anal. Calcd for $C_{11}H_{20}O_2$: C, 71.74; H, 10.87. Found: C, 71.55; H, 10.67.

Dioxane was spectral grade (Mallinckrodt) and was refluxed over sodium borohydride for at least 3 h and freshly distilled prior to use. All other chemicals were reagent grade. Amine buffer components were freshly distilled or recrystallized prior to use.

Proton NMR spectra were obtained with a Varian XL-200 spectrometer, and $CDCl_3$ was employed as the solvent. Proton-decoupled carbon-13 NMR spectra were obtained with a Brucker AC-250 spectrometer at ambient and low temperatures. Chemical shifts are reported in reference to TMS. Signal assignments in the ^{13}C NMR studies were based on the previously assigned spectra of a large series of tetrahydropyran derivatives.[31] The carbon-13 NMR spectra of the esters of acetals V–XI are simple and give conclusive evidence that one isomer is present (within the limits of detectability). For example, 2-phenoxytetrahydropyran has a spectrum with nine peaks, as expected. 2-(*p*-Carbomethoxyphenoxy)tetrahydropyran has a spectrum with 11 peaks. 2-(*p*-Carbomethoxyphenoxy)-6-carbethoxytetrahydropyran has a spectrum with three additional peaks: 170.3 (C=O), 61.1 (OCH_2), and 13.8 ppm (CH_3). All of the peaks can be accounted for on the basis of structure.

Kinetic Measurements. The rates of hydrolysis of the acetals were measured spectrophotometrically with a Beckman Model 25 or Pye-Unicam SP8-100 recording spectrophotometer. Stock solutions of acetals V–XI were 5×10^{-3} M in 80/20 EtOH–H_2O containing 0.2 M NaOH. This solution was employed to hydrolyze the esters to the corresponding carboxylates. At least 2 h was allowed for the ester hydrolysis to proceed to completion. Stock solutions of the esters were 10^{-2} M in THF. To initiate a kinetic run, 10–30 μL of the stock solution was injected into 3 mL of the reaction solution maintained at the desired temperature. The hydrolysis reaction was then monitored by following the release of the phenolic product at 300 nm (V), 280 nm (VI, IX, XI), 288 nm (VII), 340 or 400 nm (VIII), and 310 nm (X). The reactions were pseudo-first-order for at least 4 half-lives. The values of k_{obsd} and subsequent kinetic parameters were calculated with an IBM-370 computer. The hydrolysis reactions of the acetals were quantitative; i.e., a theoretical amount of *p*-nitrophenol is released from VIII. Only a single kinetic process is observed as would be the case if only one species is hydrolyzing.

In the hydrolysis of 2-ethoxy-6-carboxytetrahydropyran (XII), 2-(cyclohexyloxy)-6-carboxytetrahydropyran (XIII), and the corresponding ethyl ester, the appearance of the aldehyde addition compound with 0.01 M semicarbazide at pH >3.4 or with 0.04 M thiosemicarbazide at pH <3.4 was followed at 225 or 287 nm, respectively. This method has been described previously[32] and its accuracy verified.[33] Reaction mixture pH values were measured with a Beckman Model 3500 pH meter. The glass electrode gives the correct pH reading in concentrated dioxane–water mixtures.[34]

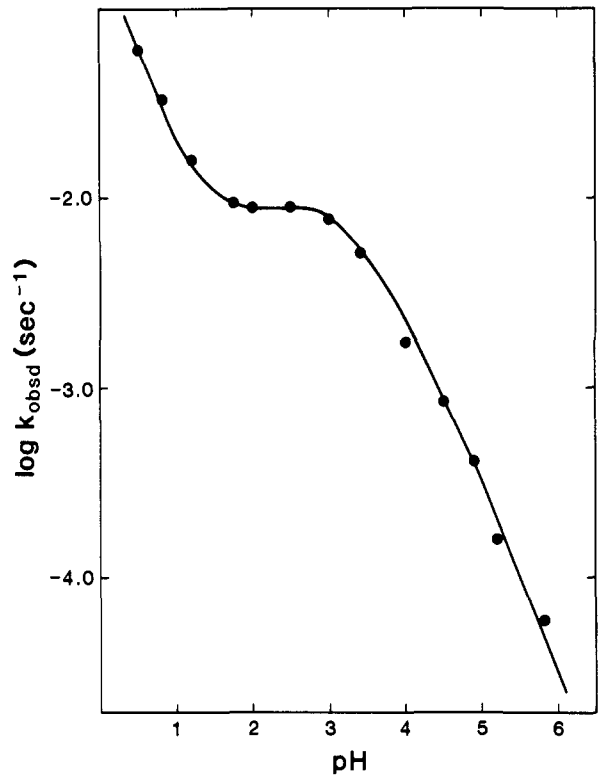

Figure 1. Plot of log k_{obsd} vs pH for hydrolysis of 2-(*p*-chlorophenoxy)-6-carboxytetrahydropyran (VII) in H_2O at 50 °C and μ = 0.5 M.

Table I. Rate Constants for Hydrolysis of 2-(Substituted phenoxy)-6-carboxytetrahydropyrans in H_2O at 50 °C, μ = 0.5 M (with KCl)

compd	k_1, M^{-1} s^{-1}	k_2, M^{-1} s^{-1}	$k_0 \times 10^4$, s^{-1}	pK_{app}
V	0.16	27.2		3.5
VI	0.11	35.3		3.6
VII	0.15	29.6		3.6
VIII	0.014	22.1	1.9	3.4
		97.1[a]	0.4[a]	4.8[a]

[a] In 50% dioxane–H_2O (v/v) at 50 °C, μ = 0.1 M.

Results

The values of k_{obsd} for hydrolysis of the series of 2-(substituted phenoxy)-6-carboxytetrahydropyrans in H_2O at 50 °C and μ = 0.5 M follow eq 1, where k_1 and k_2 are second-order rate constants

$$k_{obsd} = k_1 a_H \left[\frac{a_H}{K_a + a_H} \right] + k_2 a_H \left[\frac{K_a}{K_a + a_H} \right] \quad (1)$$

for hydronium ion catalyzed hydrolysis of the neutral and the

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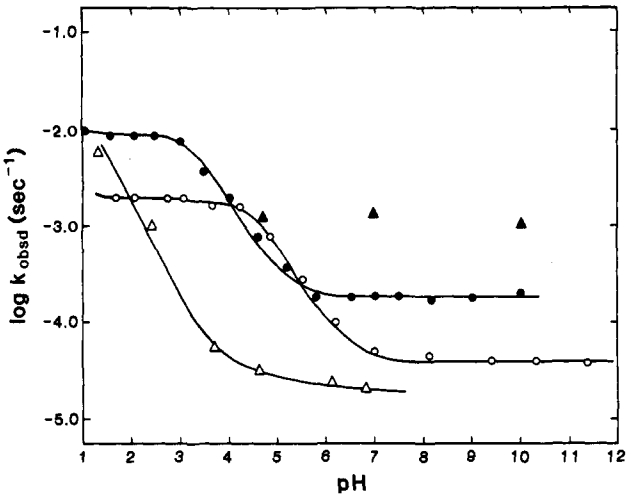


Figure 2. Plot of log k_{obsd} vs pH for hydrolysis of 2-(*p*-nitrophenoxy)-6-carboxytetrahydropyran (VIII) in H_2O (●) and in 50% dioxane–H_2O (v/v) (○) at 50 °C and μ = 0.5 M. For comparison purposes, plots of log k_{obsd} vs pH are also included for hydrolysis of 2-(*p*-nitrophenoxy)-tetrahydropyran in H_2O (▲) and in 50% dioxane–water (v/v) (△) at 50 °C, μ = 0.5 M.[7,8]

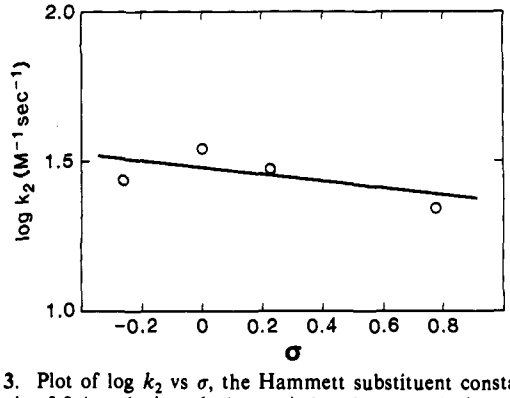


Figure 3. Plot of log k_2 vs σ, the Hammett substituent constant, for hydrolysis of 2-(*p*-substituted phenoxy)-6-carboxytetrahydropyrans in H_2O at 50 °C, μ = 0.5 M.

anionic species, respectively, and K_a is the dissociation constant of the 6-carboxyl group. Intramolecular general-acid catalysis in the neutral species would be kinetically equivalent to hydronium ion catalyzed hydrolysis of the species with the carboxyl group ionized. Equation 2 would then be followed, where k_{ga} is the rate

$$k_{obsd} = [k_1 a_H + k_{ga}]\left[\frac{a_H}{K_a + a_H}\right] \quad (2)$$

constant for the intramolecular general-acid-catalyzed reaction. From eqs 1 and 2 it can be seen that $k_{ga} = k_2 K_a$. A typical plot of log k_{obsd} vs pH is presented in Figure 1 for hydrolysis of the *p*-chloro derivative (VII). The *p*-nitro-substituted acetal (VIII) also has a pH-independent hydrolysis reaction at pH >6. The plot of log k_{obsd} vs pH for that compound is shown in Figure 2. An additional term must therefore be added to the equation for k_{obsd} (eq 3), where k_0 is the rate constant for the pH-independent

$$k_{obsd} = k_1 a_H\left[\frac{a_H}{K_a + a_H}\right] + [k_0 + k_2 a_H]\left[\frac{K_a}{K_a + a_H}\right] \quad (3)$$

reaction. Values of the rate constants are given in Table I. The plot of log k_{obsd} vs pH for hydrolysis of VIII in 50% dioxane–H_2O is also shown in Figure 2. The pH-independent reaction is ~5-fold faster in the more polar solvent. The plot of log k_2 for hydrolysis of the series V–VIII vs σ, the Hammett substituent constant,[35] is shown in Figure 3. The value of ρ is –0.1.

(35) Hammett, L. P. *Physical Organic Chemistry*; Mc-Graw-Hill: New York, 1940; Chapter 7.

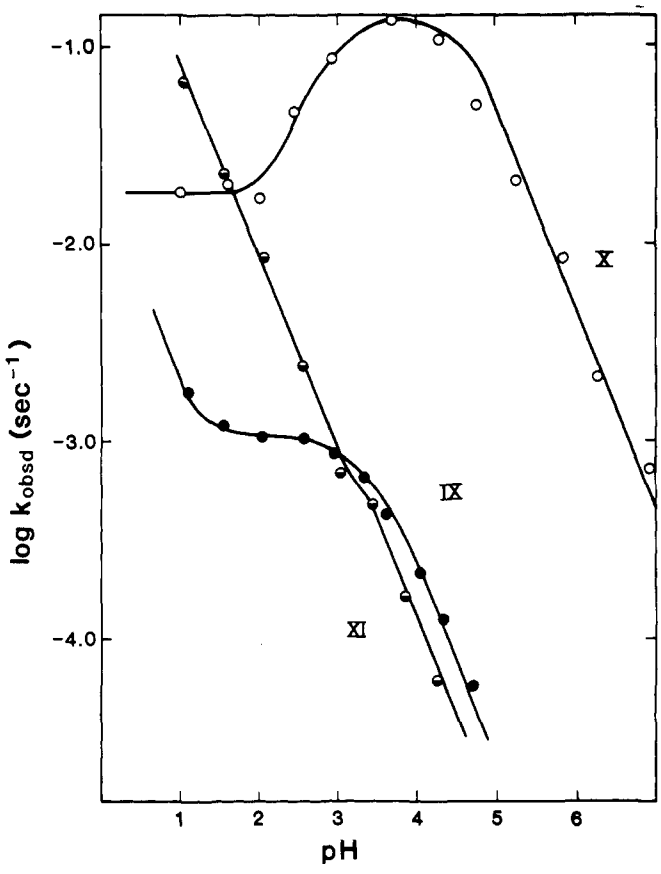


Figure 4. Plot of log k_{obsd} vs pH for the release of salicylic acid from 2-(*o*-carboxyphenoxy)-6-carboxytetrahydropyran (○) in H_2O at 30 °C, μ = 0.1 M. Also shown are plots of log k_{obsd} vs pH for hydrolysis of 2-(*p*-carboxyphenoxy)-6-carboxytetrahydropyran (●) and 2-(*p*-carboxyphenoxy)tetrahydropyran (⊖) in H_2O at 30 °C, μ = 0.1 M.

Experimentally significant buffer catalysis was not observed in the hydrolyses of V–VIII in formate and acetate buffers at total buffer concentrations of 0.5 M. Buffer catalysis was observed with VIII only at pH 5.8 in cacodylate buffer (k_{HA} = 4.4 × 10^{-4} M^{-1} s^{-1}). Only hydronium ion catalysis was observed in the hydrolysis of the ethyl esters of V–VIII; the second-order rate constants k_H at 50 °C are 0.11, 0.057, 0.058, and 0.02 M^{-1} s^{-1}, respectively.

The hydrolysis of 2-ethoxy-6-carboxytetrahydropyran (XII) and 2-(cyclohexyloxy)-6-carboxytetrahydropyran (XIII) in water at 70 °C, μ = 0.1 M, also gives values of k_{obsd} that follow eq 1, with k_1 = 1.2 M^{-1} s^{-1} and k_2 = 12 M^{-1} s^{-1} with XII and k_1 = 4.0 M^{-1} s^{-1} and k_2 = 33.0 M^{-1} s^{-1} with XIII. The hydrolysis of the ethyl ester of XIII under the same conditions proceeds with hydronium ion catalysis and a second-order rate constant k_H of 3.0 M^{-1} s^{-1}. The value of k_H for hydrolysis of 2-(cyclohexyloxy)-tetrahydropyran in water (70 °C) is 74 M^{-1} s^{-1}.

The plot of log k_{obsd} vs pH for release of salicylic acid from 2-(*o*-carboxyphenoxy)-6-carboxytetrahydropyran in H_2O at 30 °C (μ = 0.1 M) is shown in Figure 4. The plot is bell shaped, which indicates that the monoanionic species (or a kinetic equivalent) is maximally reactive. The equation for k_{obsd} is given in eq 4, where K_1 and K_2 are the first and second acid dissociation

$$k_{obsd} = \frac{k_{H_2A} a_H^2 + k_{H_1A} K_1 a_H}{a_H^2 + K_1 a_H + K_1 K_2} \quad (4)$$

constants and k_{H_2A} and k_{H_1A} are the rate constants for intramolecular general-acid catalysis in the neutral and the monoanionic species. A kinetic equivalent for this reaction is hydronium ion catalyzed hydrolysis of the monoanion and the dianion (eq 5). The

$$k_{obsd} = \frac{k_2 K_1 a_H^2 + k_3 K_1 K_2 a_H}{a_H^2 + K_1 a_H + K_1 K_2} \quad (5)$$

Table II. Rate Constants for the Hydrolysis of 2-(*p*-Carboxyphenoxy)-6-carboxytetrahydropyran (IX), 2-(*o*-Carboxyphenoxy)-6-carboxytetrahydropyran (X), and 2-(*p*-Carboxyphenoxy)tetrahydropyran (XI) in H_2O at 30 °C, μ = 0.1 M (with KCl)

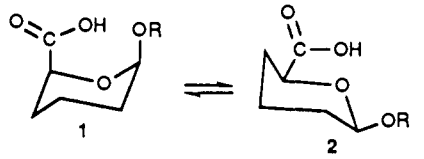
compd	k_1, M^{-1} s^{-1}	k_2, M^{-1} s^{-1}	k_3, M^{-1} s^{-1}	pK_{a1}	pK_{a2}
IX	0.02	2.5	2.5	3.4	
			0.1[a]		
X		26.9	4810	3.3	4.4
	0.004[a]	6.3[a]	2000[a]	4.4[a]	5.6[a]
XI	0.8	1.1			
	0.0092[a,b]	0.02[a,b]			

[a] In 50% dioxane–H_2O (v/v) at 15 °C, μ = 0.1 M. [b] Reference 12.

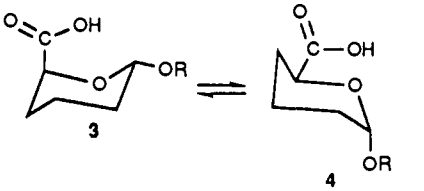
rate constants for the reactions in H_2O at 30 °C and in 50% dioxane–H_2O at 15 °C are given in Table II. For comparison purposes, the plots of log k_{obsd} vs pH for hydrolysis of 2-(*p*-carboxyphenoxy)tetrahydropyran and 2-(*p*-carboxyphenoxy)-6-carboxytetrahydropyran are included in Figure 4 and rate constants are given in Table II.

Discussion

From the method of preparation of compounds V–X, a mixture of cis and trans isomers could be produced (eqs 6 and 7). The

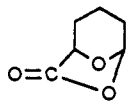


(6)



(7)

cis (a,a) and (e,e) and the trans (a,e) derivatives are each interconvertible. Steric interactions should be minimized in the cis (e,e) conformation (**2**). However, alkoxy or aryloxy groups at C-2 of the tetrahydropyran ring prefer the axial position (the anomeric effect).[36] Stuart–Briegleb models indicate that intramolecular general-acid catalysis involving proton transfer to the leaving group oxygen should be sterically difficult in the cis (e,e) and trans (a,e) conformations but could take place via the diaxial form (**1**), in which the ring has a chair conformation. On the other hand, electrostatic stabilization effects by the carboxylate anion should be possible in either the cis or trans configuration. For closest approach to the developing oxocarbonium ion, the carboxylate group should be axial. There will, of course, be an increase in planarity in the transition state, which will depend on the amount of C–O bond breaking. The Stuart–Briegleb model further indicates that moderate distortion would be required for the formation of a full covalent bond between the axial carboxyl oxygen and the reaction center, and the acylal XIV would then



XIV

be relatively inflexible. Such covalent bond formation can in fact occur.[37,38] The half-life for pH-independent hydrolysis of XIV in H_2O at 30 °C[39] is 30 s in comparison with 34 min for the cyclic acylal γ-ethoxy-γ-butyrolactone.[40] The rapid rate of hydrolysis of XIV can be most simply explained in terms of relief of strain.

The proton NMR spectra of the ethyl esters of V–X in $CDCl_3$ revealed that the substituent group at C-2 is predominantly axial (equatorial proton). Eliel and Giza[41] considered that an equatorial proton at C-2 of a tetrahydropyran derivative would give a sharp peak at 4.53–5.52 ppm, whereas an axial proton would give broadly split peaks at 4.15–4.72 ppm. The NMR spectra of the esters of V–VII, and X, have in each case a peak in the range for an equatorial proton at C-2 (1 H), and the spectrum of the ester of VIII has a peak at 6.2 ppm (1 H).

The carbon-13 NMR spectra of the esters of acetals V–X are simple and give conclusive evidence that only one of the possible cis–trans isomers is present within the limits of detectability (see the Experimental Section). A carbomethoxy group in the 2-position of tetrahydropyran has a marked preference for the equatorial position; $-\Delta G°$ values of 1.62 and 1.38 kcal/mol have been reported.[31,42] The equatorial preference of the carbethoxy group at C-6 would be increased by the presence of an axial substituent at C-2. Consequently, the trans (e,a) conformation (**4**) would be expected. An equatorial conformation for the carbethoxy substituent at C-6 is confirmed by the ^{13}C NMR spectra.

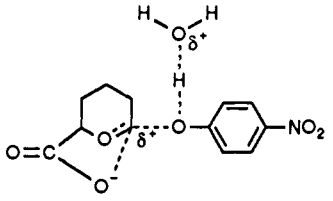
In conformation **1** there will be axial–axial interactions of the substituent group at C-6 with the –OR group at C-2 and H at C-4. Such γ-axial effects should produce upfield shifts at C-2 and C-4.[31,43] However, that is not the case with the 6-carbethoxy derivatives. Introduction of an axial –OR group into the C-2 position of tetrahydropyran[31] produces an upfield shift for C-4 and C-6 and a downfield shift for C-2. A carbethoxy group at C-6 in the ester acetals then produces either a small downfield shift or no change in the shift of C-4 and a downfield shift of C-2 and C-6.[44] On the basis of similar chemical shifts, Eliel et al.[31] concluded that in *trans*-2-methoxy-6-(hydroxymethyl)tetrahydropyran and the *trans*-2-methoxy-6-methyl derivative the substituent groups at C-2 and C-6 are axial and equatorial, respectively. Consequently, the NMR results given by the aromatic acetal esters strongly support the trans (e,a) conformation (**4**) as the only detectable species.[45]

The log k_{obsd} vs pH profiles for hydrolysis of the 2-(substituted phenoxy)-6-carboxytetrahydropyrans have regions with slopes of −1.0 separated by a plateau region at pH values near the pK_a of the carboxyl group substituent. The reactions correspond with hydronium ion catalyzed hydrolysis of the neutral species and a faster hydronium ion catalyzed hydrolysis of the anionic species or a kinetic equivalent. An un-ionized carboxyl group in the 6-position will exert an electron-withdrawing effect (σ_I = 0.39),[46] which will decrease the basicity of the acetal oxygens and reduce the stability of the intermediate oxocarbonium ion. In contrast, the inductive effect of an ionized carboxyl group is nearly 0 (σ_I = 0.06).[46] Therefore, on the basis of a changing inductive effect due to carboxyl group ionization, there should be a considerable difference in k_1 and k_2 (see eq 1). However, the k_2 value of VIII is 620-fold larger than the second-order rate constant for hy-

(36) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: New York, 1983.
(37) Brezinski, J. J.; Kubler, D. G.; Montagna, A. E. *J. Org. Chem.* **1959**, *24*, 1807.
(38) Stoner, G. G.; McNulty, J. S. *J. Am. Chem. Soc.* **1950**, *72*, 1531.
(39) Fife, T. H.; Przystas, T. J. Unpublished data.
(40) Fife, T. H. *J. Am. Chem. Soc.* **1965**, *87*, 271.
(41) Eliel, E. L.; Giza, C. A. *J. Org. Chem.* **1968**, *33*, 3754.
(42) Anderson, C. B.; Sepp, D. T. *J. Org. Chem.* **1968**, *33*, 3272. Eliel, E. L.; Hargrave, K. D.; Pietrusiewicz, K. M.; Manoharan, M. *J. Am. Chem. Soc.* **1982**, *104*, 3635.
(43) Duddeck, H. In *Topics in Stereochemistry*; Eliel, E. L., Wilen, S. H., Allinger, N. L., Eds.; Wiley Interscience: New York, 1986; Vol. 16, p 219.
(44) For example, the chemical shifts of C-2 for 2-phenoxytetrahydropyran, 2-(*p*-carbomethoxyphenoxy)tetrahydropyran, 2-(*p*-carbomethoxyphenoxy)-6-carbethoxytetrahydropyran, and 2-(*o*-carbomethoxyphenoxy)-6-carbethoxytetrahydropyran are 95.7, 95.9, 98.4, and 99.0 ppm, respectively, and the shifts for C-4 are 18.3, 18.4, 19.3, and 18.5 ppm. Likewise, the chemical shifts for C-2 and C-4 of the ethyl ester of VIII are 98.6 and 19.1 ppm as compared with 96.0 and 18.6 ppm for C-2 and C-4 of 2-(*p*-nitrophenoxy)tetrahydropyran. The chemical shifts of C-2 and C-4 of tetrahydropyran are 68.7 and 23.8 ppm, respectively.[31]
(45) The ^{13}C NMR spectra of the aliphatic acetals 2-ethoxy-6-carbethoxytetrahydropyran and 2-(cyclohexyloxy)-6-carbethoxytetrahydropyran indicate that one isomer is predominant but is not present exclusively.
(46) Charton, M. *J. Org. Chem.* **1964**, *29*, 1222. Note that σ_I for COO^- can vary with solvent.

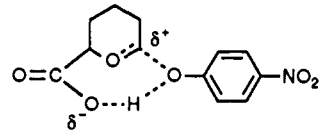
dronium ion catalyzed hydrolysis of 2-(*p*-nitrophenoxy)tetrahydropyran in 50% dioxane–H_2O at 50 °C.[7] This difference is larger than expected for a steric or electronic effect;[47] note that the k_0 values for VIII and 2-(*p*-nitrophenoxy)tetrahydropyran differ by only a factor of 2 in the same solvent. Thus, the neighboring carboxyl group of VIII is participating in the step governed by k_2 (or k_{ga}) and is thereby producing a sizable rate enhancement.

The carboxylate anion could be electrostatically stabilizing the developing oxocarbonium ion in the transition state XV. Nev-



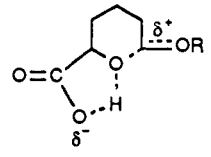
XV

ertheless, the much smaller effect of the carboxylate ion in the pH-independent reaction of VIII, where the extent of C–O bond breaking in the transition state must be greater than in the hydronium ion catalyzed reaction, argues strongly against that type of participation. A kinetically equivalent possibility would be intramolecular general-acid catalysis by an un-ionized carboxyl group in the 6-position. Such catalysis, in which proton transfer to the phenolic oxygen occurs in the transition state (XVI), would



XVI

only be sterically favorable in the a,a conformation (**1**) and would require a seven-membered ring transition state. A five-membered ring transition state could be achieved with partial proton transfer to the ring oxygen (XVII), and that could occur with the carboxyl

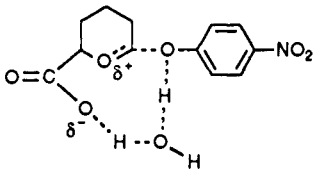


XVII

group in the equatorial position. However, an adjoining *p*-nitrophenoxy group could not appreciably stabilize the developing oxocarbonium ion. Also, the ΔS^* advantage of exocyclic leaving group expulsion would then be lost. A ring-opening reaction of that type would be most favorable with electron-donating OR groups, but intramolecular general-acid catalysis does not take place in the hydrolysis of 2-ethoxy-6-carboxytetrahydropyran (at 70 °C in H_2O, k_2 is only 10-fold larger than k_1). Clearly, general-acid-catalyzed ring opening (endocyclic displacement) is not a favorable process. Intramolecular general-acid catalysis is, however, strongly supported in the reactions of VIII by the lack of significant buffer catalysis in the hydrolysis of V–VIII, in contrast with the pronounced general-acid catalysis in the hydrolysis of the corresponding 2-(substituted phenoxy)tetrahydropyrans;[7] electrostatic stabilization effects, as in XV, should enhance general-acid catalysis by buffer acids.

The Hammett ρ value for the step governed by k_2 or the kinetically equivalent k_{ga} is ~0. From eqs 1 and 2 $k_{ga} = k_2K_a$, but since K_a is not significantly affected by the X substituent (see Table I), ρ will be the same for k_2 and k_{ga}. The ρ values for hydronium ion catalyzed hydrolysis of the 2-(substituted phenoxy)tetrahydropyrans[7] and the ethyl esters of V–VIII are −0.9 and −0.7, respectively. Electron withdrawal in the leaving group of these acetals will lower the basicity of the acetal oxygens but will increase the ease of C–O bond breaking. The negative values of ρ for the latter two series of compounds indicate that in the hydronium ion catalyzed reaction basicity considerations are of predominant importance. However, in the general-acid-catalyzed hydrolysis of the 2-(substituted phenoxy)tetrahydropyrans by formic acid, ρ is +0.9, which shows that ease of C–O bond breaking is of greater importance than basicity in that reaction.[8] This is undoubtedly because of an increase in the amount of C–O bond breaking required to attain the transition state as the catalyzing acid becomes weaker. In the hydrolyses of V–VIII, the $\rho = 0$ indicates that basicity and ease of bond breaking are of nearly equal importance in regard to the magnitude of the rate constants. Thus, the ρ value is in accord with carboxyl group participation according to mechanism XVI, even though the steric fit must be poor.

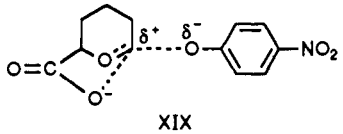
Proton transfer from the neighboring carboxyl group to the leaving group oxygen of VIII in the transition state could be facilitated by an intervening water molecule, as in XVIII.



XVIII

Therefore, contrary to generally accepted concepts, a precise steric fit should not be required for the occurrence of intramolecular general-acid catalysis, although the restriction of water would reduce the rate enhancement that can be achieved. When C–O bond breaking is facile because of a good leaving group, then a moderately large rate enhancement might still be obtained at pH >3 because of the greater effective concentration of the carboxyl proton than hydronium ion.

The hydrolysis of the *p*-nitro-substituted derivative VIII is characterized by a pH-independent reaction at pH >6. This reaction very likely proceeds by unimolecular decomposition and is similar to the analogous reaction of 2-(*p*-nitrophenoxy)tetrahydropyran.[7,8] Electrostatic stabilization of the developing oxocarbonium ion by the neighboring carboxylate anion does not appear to occur when H_2O is the solvent; k_0 for VIII is, in fact, 5-fold less than with 2-(*p*-nitrophenoxy)tetrahydropyran. However, in 50% dioxane–H_2O, VIII reacts 2-fold more rapidly than 2-(*p*-nitrophenoxy)tetrahydropyran. The pH-independent reaction of VIII is only 5-fold slower in 50% dioxane–H_2O than in H_2O, whereas the difference is a factor of 50 for the acetal unsubstituted at the 6-position.[8] Thus, the neighboring carboxylate anion is enhancing the rate of the pH-independent reaction in 50% dioxane–H_2O by a factor of approximately 10. Electrostatic stabilization of the developing oxocarbonium ion (XIX) would, of



XIX

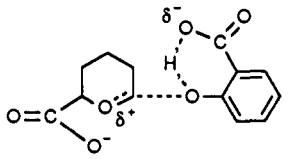
course, be more important in the solvent of lower dielectric constant. However, the rate enhancement may be contrasted with the factors of over 100 that have been found with other acetals when the leaving groups had pK_a values comparable to that of VIII.[19,20,48] That only a modest 10-fold rate enhancement is achieved in the pH-independent reaction of VIII may be due in part to the equatorial conformation of the carboxylate ion in the 6-position so that electrostatic stabilization effects would require

(47) Ortho-substituted phenyl β-D-glucopyranosides hydrolyze faster than corresponding para-substituted derivatives, but the effects are small (5-fold or less). Nath, R. L.; Rydon, H. N. *Biochem. J.* **1954**, *57*, 1.

(48) An 860-fold enhancement by a favorably located carboxylate ion has recently been observed in the rate of pH-independent hydrolysis of a ketal derivative of a *p*-nitrophenyl riboside. This difference is due primarily to the slow hydrolysis of the reference ketal. Cherian, X. M.; Van Arman, S. A.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 6566.

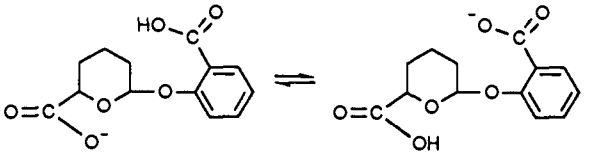
an e → a conformation change for closest approach of the ions.[49,50] The reduced oxocarbonium ion stability with the tetrahydropyran acetals in comparison with that from phthalaldehydic acid acetals apparently does not make an appreciable difference in regard to the magnitude of electrostatic stabilization effects.[51] This reduction in stability cannot overcome the moderate difficulties imposed by the steric situation in the 6-carboxytetrahydropyran acetals. Thus, electrostatic effects in acetal hydrolysis will be small unless the polarity of the solvent is reduced even further than that of 50% dioxane–H_2O, in which case the pK_a of the neighboring carboxyl group will be quite high.[52]

2-(*o*-Carboxyphenoxy)-6-carboxytetrahydropyran. The pH–rate constant profile for release of salicylic acid from X is pH independent at low pH and then bell shaped with pK_{app} values of 3.3 and 4.4 in H_2O. The enhancement in the k_3 step in H_2O (see eq 5) in comparison with the hydrolysis of 2-(*p*-carboxyphenoxy)-6-carboxytetrahydropyran (IX) is 2×10^3. However, in 50% dioxane–H_2O at 15 °C the rate enhancement in comparison with hydronium ion catalyzed hydrolysis of IX is 2×10^4 (10^5 in comparison with XI). The *o*-carboxyl group is very likely participating in the reaction as an intramolecular general acid (XX), analogous to the other salicyl acetals that have been



XX

studied.[2,12-14] The bell-shaped pH–rate constant profile for the reaction of X indicates that the monoanionic species is maximally reactive. However, significant bifunctional catalysis does not occur. Even in 50% dioxane–H_2O as the solvent, the monoanion reaction is 7-fold slower than the comparable reaction of 2-(*o*-carboxyphenoxy)tetrahydropyran.[12] Therefore, the bell-shaped profile for the reaction of X is probably reflecting intramolecular general-acid catalysis by the salicyl carboxyl group and a changing inductive effect produced by ionization of the 6-carboxyl group. Since an un-ionized carboxyl group is electron withdrawing in comparison with the corresponding carboxylate anion, such ionization will facilitate the general-acid-catalyzed reaction. The monoanion reaction of X must reflect in part the equilibrium of eq 8, which will reduce the concentration of the reactive monoanion



(8)

at any pH. It appears from the data of Table I that the pK_a of the 6-carboxyl group must be near 3.3 in water, corresponding closely with pK_1. As a consequence, the equilibrium of eq 8 could only reduce the reactive monoanion concentration by ~30%. The major reason for the slower reaction of X in comparison with 2-(*o*-carboxyphenoxy)tetrahydropyran must be steric or inductive. The intermediate oxocarbonium ion of X is much less stable than that derived from benzaldehyde acetals[14] or from phthalaldehydic acid acetals,[19,20] so that electrostatic stabilization effects would have been expected to be more important. Thus, the lack of bifunctional catalysis strongly indicates that the nature of the leaving group and the steric situation is much more important in regard to the mechanism than is the stability of the developing oxocarbonium ion.

The 10–50-fold larger rate enhancement for the hydrolysis of X in comparison with IX or XI in 50% dioxane–H_2O than in H_2O must reflect in part the higher pK_a of the salicyl carboxyl group in the less polar solvent (Table II). The carboxyl group can then function with maximum efficiency as an intramolecular general acid at pH values where the concentration of hydronium ion is less than is the case for the reaction in water. The rate enhancement will then be greater because of the reduced rate of hydrolysis of the reference compound. A less polar solvent should also be relatively favorable for the general-acid-catalyzed reaction, in which a positively charged conjugate acid intermediate is avoided.

The structural features that will promote electrostatic and general-acid catalysis are in opposition. Electrostatic catalysis requires extensive oxocarbonium ion development in the transition state, i.e., considerable C–O bond breaking,[19] whereas general-acid catalysis requires a good leaving group so that the C–O bond can begin to break when the proton is still at a distance from the oxygen.[7,8] There will then be little bond breaking in the transition state. It is probably this feature that is responsible for the rarity of bifunctional mechanisms; structural features that will enhance one type of catalytic effect will make the other more difficult. A rather precise compromise of effects is apparently required for bifunctional catalysis to be effective.[14,20] Bifunctional catalysis has been observed in the hydrolysis of benzaldehyde bis(*cis*-2-carboxycyclohexyl) acetal (III)[14] and phthalaldehydic acid *O*-methyl *S*-(*o*-carboxyphenyl) thioacetal.[20] In these examples the poor leaving group necessitates significant bond breaking to attain the transition state and thereby makes electrostatic stabilization effects by the second carboxyl group important. General-acid catalysis in these systems is less favorable than with a salicylic acid leaving group, but electrostatic stabilization effects are correspondingly more important. Such a compromise of effects is clearly not attained with the salicyl acetal X in which the oxocarbonium ion stability has been greatly reduced in comparison with corresponding benzaldehyde or phthalaldehydic acid acetals. Likewise, bifunctional catalysis does not occur in the hydrolysis of 2-(β-*cis*-carboxycyclohexyl)-6-carboxytetrahydropyran,[39] in which case the leaving group is an aliphatic alcohol (the same as in the hydrolysis of benzaldehyde bis(*cis*-2-carboxycyclohexyl) acetal). It is clear that the stabilization of the developing oxocarbonium ion that might be provided by the 6-carboxylate anion is not sufficient to overcome the energy required to bring the ions into close proximity even in a solvent of relatively low polarity (50% dioxane–H_2O).

Lysozyme. The mechanistic possibility for the glycosidase enzyme lysozyme that has received the most attention[21,22] is that of IV in which glutamic acid-35 functions as an intracomplex general acid. A mechanism involving general-acid-catalyzed ring opening has also recently been reiterated.[29] Such a mechanism had been previously discussed considering that opening of a strained hexose ring in subsite D would release all of the strain energy (see ref 2, pp 103–104). There would appear to be little basis for such a mechanism if the ring in subsite D is not strained;[53] i.e., the hexose unit has the unstrained chair conformation recently proposed.[29] Note that the neighboring carboxyl group of XII and XIII does not catalyze the hydrolysis reaction, even though the steric fit is excellent and the intermediate oxocarbonium ion

(49) In cyclohexane derivatives the best value of $-\Delta G°$ for an axial–equatorial change with a COOH substituent group is 1.35 kcal/mol, while that with COO^- is 1.92 kcal/mol. Hirsch, J. A. In *Topics in Stereochemistry*; Allinger, N. L., Eliel, E. L., Eds.; Wiley Interscience: New York, 1967; Vol. 1, p 199.

(50) The equatorial isomer of 2-(4-nitrophenoxy)-*trans*-1-oxadecalins spontaneously decomposes about 3-fold faster than the axial isomer. Chandrasekhar, S.; Kirby, A. J.; and Martin, R. J. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1619.

(51) The plot of log k_{obsd} vs pH for the hydrolysis of 2-naphthyl β-D-glucopyranosiduronic acid, with which the carboxyl group substituent is presumably equatorial, is sigmoidal at 90 °C in the range pH 1–5. The relative rate of hydrolysis of the ionized to the un-ionized species was found to be 1580/1. It was concluded that the carboxyl group is not participating in the reaction. Capon, B.; Ghosh, B. C. *Chem. Commun.* **1965**, 586.

(52) The effect of the neighboring carboxyl group of phthalaldehydic acid diethyl acetal is ~1000-fold larger in 82% dioxane–water (w/w) than in water. The pK_a of the carboxyl group is 10–11 in the aqueous organic solvent. Anderson, E.; Capon, B. *J. Chem. Soc., Perkin Trans. 2* **1972**, 515.

(53) The ΔS^* for hexose ring opening would be less favorable than the ΔS^* change associated with exocyclic displacement. Also ring opening would be readily reversible, which would greatly slow the overall reaction.

resulting from ring opening would be considerably more stable than that from a substrate for lysozyme. The natural substrates for lysozyme have poor leaving groups and give rise to unstable oxocarbonium ions.[21,22] However, general-acid catalysis is only detected in acetal hydrolysis reactions when the C–O bond breaking process is very favorable because of a good leaving group or a highly stabilized oxocarbonium ion.[2,7-11] Consequently, if Glu-35 is functioning as a general acid in lysozyme-catalyzed reactions, as in the postulated mechanisms,[21,22] then the enzyme must in some manner make C–O bond breaking easier. This might be achieved through electrostatic stabilization of the developing carbonium ion and/or relief of strain in the hexose unit bound in subsite D. It has been estimated that lysozyme must stabilize the oxocarbonium ion by 5–7 kcal/mol.[6]

A key question is assessing the electrostatic role of Asp-52 in the lysozyme mechanism (IV) concerns the closeness of approach of the carboxylate ion to the developing oxocarbonium ion required to produce a significant stabilization effect. The energy involved in a charge–charge interaction will be inversely proportional to the square of the distance between the charges and to the dielectric constant of the medium. Thus, in a water medium or one that is moderately hydrophobic,[54] a reasonably close approach would be required, considering that the positive charge will be only partially developed in the transition state. Since the Asp-52 carboxyl group is too far from the reaction center of the hexose unit in subsite D for covalent bond formation,[23] an energy requiring conformational change would be required of the ES complex for either nucleophilic or electrostatic catalysis to be effective. The rate enhancements due to electrostatic catalysis are only 100–800-fold in the most favorable cases where extensive bond breaking must occur to attain the transition state and the steric situation is excellent.[19,20,48] As shown in the present work, the rate enhancement due to electrostatic stabilization will be substantially reduced or abolished if there is even moderate difficulty in the close approach of the ions, even though other features, e.g., less internal oxocarbonium ion stabilization, are more favorable. It can be concluded that electrostatic stabilization effects by Asp-52 should not be of great significance in lysozyme-catalyzed reactions unless the environment is much less polar than the macroscopic pK_a values attributed to Glu-35 and Asp-52 in both the free enzyme and the ES complex would indicate.[54] It is, of course, the local environment near the charges that is of crucial importance. In this regard, Warshel[55] has suggested that a system of local dipoles might lead to low pK_a values for the carboxyl groups of lysozyme *and* a large charge–charge interaction. However, the steric factors involved in such an interaction clearly cannot be neglected and could be of predominant importance, especially if there is little positive charge development in the transition state.

Intramolecular general-acid catalysis has been found to give rate enhancements of 10^3–10^7 in acetal hydrolysis reactions,[2,12-14] and the efficiency of such catalysis increases in a solvent of reduced polarity. Nevertheless, an additional factor such as relief of strain, enzyme-solvation effects,[56] or nucleophilic acetamido group participation[57] appears to be necessary to account for the rate enhancements achieved in the lysozyme-catalyzed reactions (10^9–10^{10}).[59]

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Registry No. V, 133754-16-2; VI, 133754-17-3; VII, 133754-18-4; VIII, 133754-19-5; IX, 133754-22-0; X, 133754-23-1; XI, 35486-97-6; XII, 133754-20-8; XIII, 133754-21-9; *p*-$MeOC_6H_4OH$·Na, 1122-95-8; PhOH·Na, 139-02-6; *p*-ClC_6H_4OH·Na, 1193-00-6; *p*-$O_2NC_6H_4OH$·Na, 824-78-2; *o*-$MeO_2CC_6H_4OH$·Na, 7631-93-8; *p*-$MeO_2CC_6H_4OH$·Na, 5026-62-0; *p*-$MeO_2CC_6H_4OTHP$, 106342-09-0; 2-ethoxy-6-carbethoxytetrahydropyran, 27172-92-5; 2-chlorotetrahydropyran, 3136-02-5; *trans*-2-(*p*-methoxyphenoxy)-6-carbethoxytetrahydropyran, 133754-24-2; *trans*-2-phenoxy-6-carbethoxytetrahydropyran, 133754-25-3; *trans*-2-(*p*-chlorophenoxy)-6-carbethoxytetrahydropyran, 133754-26-4; *trans*-2-(*p*-nitrophenoxy)-6-carbethoxytetrahydropyran, 133754-27-5; *trans*-2-(*o*-carbomethoxyphenoxy)-6-carbethoxytetrahydropyran, 133754-28-6; *trans*-2-(*p*-carbomethoxyphenoxy)-6-carbethoxytetrahydropyran, 133754-29-7; 2-(cyclohexyloxy)tetrahydropyran, 709-83-1; lysozyme, 9001-63-2.

(54) In 50% dioxane–H_2O the pK_a of acetic acid is 6.1. This corresponds reasonably with the pK_a attributed to Glu-35 in the active site of lysozyme (5.9–6.1). The pK_a of Asp-52 in the free enzyme is 4.2–4.5. The pK_a values are not greatly different in the free enzyme and in enzyme–substrate complexes. Parsons, S. M.; Raftery, M. A. *Biochemistry* **1972**, *11*, 1623, 1633. Banerjee, S. K.; Kregar, I.; Turk, V.; Rupley, J. A. *J. Biol. Chem.* **1973**, *248*, 4786. These constants are, of course, macroscopic constants, which could differ from the microscopic constants.

(55) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5250.

(56) Warshel, A. *Biochemistry* **1981**, *20*, 3167.

(57) Since nucleophilic attack by the neighboring acetamido group of 2-deoxy-2-acetamido-β-D-glucopyranosides provides large rate enhancements in the hydrolysis reactions,[17] the possibility that the 2-acetamido group of lysozyme substrates acts as a nucleophile must be considered. Substrates lacking this group readily hydrolyze in the enzymatic reaction,[58] but 2-deoxy derivatives should hydrolyze rapidly because of reduced electron withdrawal from the reaction center. Therefore, acetamido group participation *in* the enzyme reaction should not be ruled out.

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

(59) It has been calculated that the value of the rate constant for the rate-determining step in lysozyme-catalyzed reactions is 1.75 s^{-1}. Chipman, D. M. *Biochemistry* **1971**, *10*, 1714. The value of k_{HIA}, the rate constant for the monoanion reaction, in the hydrolysis of X at 30 °C is 0.19 s^{-1}, i.e., 9-fold less. This rate constant will, of course, be altered by structural changes affecting the leaving group pK_a and the oxocarbonium ion stability; k_{HIA} for *o*-carboxybenzaldehyde methyl salicyl acetal is 45.2 s^{-1}, whereas that for benzaldehyde bis(*cis*-2-carboxycyclohexyl) acetal, where the leaving group is an aliphatic alcohol, is 0.008 s^{-1} (50% dioxane–H_2O).[14]