solution of **4** in nonane, of known composition, was placed in the capillary tubes. The contents of the tubes were frozen in Dry Ice–acetone, evacuated to pressures of 1 mm or less, and sealed off while frozen. For kinetic runs at each temperature, five sealed capillaries were placed in individual perforated copper casings and immersed in the bath at measured intervals. Reactions were quenched by quickly plunging the tubes into ice water. The contents of the capillaries were analyzed by vpc to determine the ratio of nonane to remaining starting material. A calibration curve prepared from known concentrations of **4** in nonane was used to correct for any nonlinearity in thermal conductivity response. Absence of any pressure or wall effects was established by variations in sample and capillary sizes, none of which affected the linearity of the first-order plots. Nonane had previously been established as an inert carrier in flow experiments. The estimated error limits for evaluation of vpc peak areas are ±3%. Estimated precision of the rate constants obtained at six temperatures is ±8% and error limits of the calculated kinetic parameters were assigned accordingly.[27] All needed slopes and intercepts were calculated by the method of least squares.

Acknowledgments. We wish to thank Dr. Philip L. Levins for the mass spectral analyses and interpretations and Dr. John L. Roebber for stimulating discussions concerning the determination and interpretation of kinetic data.

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Steric and Electronic Effects on the Neighboring General Acid Catalyzed Hydrolysis of Methyl Phenyl Acetals of Formaldehyde

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Abstract: The hydrolysis of a series of methoxymethyl ethers of substituted phenols has been studied at several temperatures (μ = 1.0) between pH 0.0 and 6.5. The pH–log k_{obsd} profiles for those acetals not containing *o*-carboxyl groups are characterized by a straight line of slope −1.0, in agreement with the expectation of specific acid catalyzed hydrolysis. The pH–log k_{obsd} profiles for those acetals possessing *o*-carboxyl groups are characterized by a plateau rate in the acid region, followed by a descending leg of slope −1.0 above pH 3–4. The experimental data for the *o*-carboxyl-substituted acetals is in accord with the kinetically equivalent mechanisms of intramolecular carboxyl group general-acid catalyzed hydrolysis (RCOOH) or specific acid catalyzed hydrolysis of the anionic form of the acetal ($RCOO^- + H^+$). Carboxyl group participation in the reaction is verified on the basis that the log k_H values (k_H the calculated specific acid rate constant) for the anionic form of the *o*-carboxyl-substituted acetals exhibit large positive deviations from a Hammett $\rho\sigma$ plot constructed from the log k_H values for acetals not possessing *o*-carboxyl groups and for the acetals possessing undissociated carboxyl groups. Juxtapositioning of a second carboxyl group increases the rate constant for intramolecular general-acid catalyzed hydrolysis by the first carboxyl group. It is established that this enhancement in rate is a steric effect and not one of electrostatic stabilization of the transition state for intramolecular general-acid catalyzed hydrolysis. The relationship of the results of this model enzyme study to the mechanism of lysozyme action is discussed.

An impressive array of techniques has been used to elucidate the structure of lysozyme.[3] A recent review by Jolles has summarized the known data for this enzyme.[4]

On the basis of this information, a reasonable model may be constructed for the enzyme–substrate complex which reveals that the only side chains near the site of catalysis and likely to be involved in the hydrolytic mechanism are the carboxyl groups of Glu-35 and Asp-52.[3c,5,6] It has been suggested that, due to the local environments, one of these carboxyl groups is dissociated and one is undissociated at the pH at which lysozyme is most active.[7] The pH–log k_{obsd} profile for catalysis by lysozyme describes a bell-shaped curve.[8-11] The simplest interpretation of this behavior considers that there are two acid dissociations, the first of which increases activity and the second of which decreases activity.

Given the probable involvement of the carboxyl groups of Glu-35 and Asp-52 located at the active site, and the presence of an acetamido group in the 2 position of the natural substrate a finite listing of plausible mechanisms for enzymic catalysis may be written.[11,12] These mechanisms take into account the known features of hydrolysis of glycosides, which have been reviewed by Vernon,[13] Long,[14] and Cordes.[15] One common

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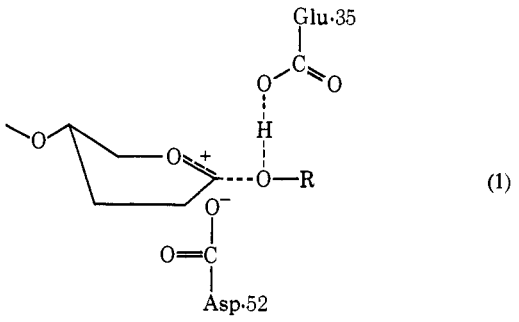
aspect of most of the proposed mechanisms is the suggestion that Glu-35 acts as a general acid catalytic entity. On the basis of X-ray model building, an oxygen of the carboxyl group of Glu-35 has been suggested to be 3 Å from the anomeric oxygen at the site of cleavage thus providing an ideal arrangement for intracomplex protonation.[16] Physical organic studies have established that a neighboring carboxyl group will act as an intramolecular general-acid catalyst for acetal hydrolysis.[17-19] The possibility that the 2-acetamido group of the substrate may act as a nucleophile has been considered. This mechanism has been shown to provide very large rate enhancements in the hydrolysis of 2-deoxy-2-acetamido-β-D-glucosides between pH 2 and 11 in nonenzymatic reactions.[19-21] However, recent evidence by Raftery and Rand-Meir[11] has demonstrated that the neighboring 2-acetamido group is not essential for enzymatic activity.

The ionized carboxyl group of Asp-52 could act either as a nucleophile, to form a glycosyl-enzyme intermediate, or as a gegenion, to stabilize electrostatically the incipient oxocarbonium ion that is presumably formed in the hydrolytic reaction[11,12] (eq 1). Electrostatic stabilization and destabilization



(1)

of the transition state for nucleophilic attack upon the ester bond has recently been established.[22,23] Electrostatic stabilization of incipient carbonium ion formation has been considered in the solvolysis of α-haloacetates[24-26] and the specific acid catalyzed hydrolysis of orthoesters has been shown to be facilitated when the substrate is adsorbed onto the surface of a negative micelle.[27]

The purpose of the present investigation is to seek evidence for electrostatic participation of a carboxylate group in the intramolecular general acid (COOH) catalyzed hydrolysis of acetals. For this purpose the pH dependence of hydrolysis of compounds I–XIII was determined (Table I).

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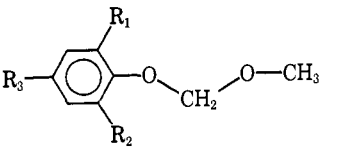
Table I



	R_1	R_2	R_3
I	H	H	H
II	Cl	H	H
III	H	H	Cl
IV	H	H	NO_2
V	$COOCH_3$	H	H
VI	COOH	H	H
VII	H	H	COOH
VIII	$COOCH_3$	$COOCH_3$	H
IX	COOH	COOH	H
X	$COOCH_3$	CH_3	H
XI	COOH	CH_3	H
XII	$COOCH_3$	NO_2	H
XIII	COOH	NO_2	H
XIV 6-CH_3	$COOCH_3$	*t*-Butyl	H

Experimental Section

Synthesis of Substituted Methoxymethoxybenzenes.[28] The method of Reychler[29] has been used to prepare compounds I–XIV. The appropriately substituted phenol (0.1 mol) was dissolved in 100 ml of a suitable solvent (DMSO, benzene, 1,2-dimethoxyethane) and slowly dripped (2 hr with stirring) into a round-bottomed flask containing 0.3 mol of sodium hydride in 200 ml of the same solvent. The resulting suspension was allowed to stir until evolution of hydrogen ceased. The flask was then fitted to the receiving end of a distillation apparatus and 0.2 mol of chloromethylmethyl ether was distilled (59.5°) into the flask. (A small forerun was taken to ensure purity of the haloether.) The flask was then sealed with a drying tube and allowed to stir for a minimum of 12 hr. In many cases a color change is observed in the formation and then destruction of the phenolate ion. The flask was then evacuated for 30 min to remove excess haloether. The solution was then added to 500 ml of 0.1 *N* KOH in a separatory funnel in 20-ml increments. The pH of the aqueous phase was checked after each addition to ensure that the solution was still alkaline. After complete addition, the flask was washed with 200 ml of diethyl ether and this was added to the separatory funnel. The organic phase was then extracted with at least three further 500-ml portions of 0.1 *N* KOH and several 500-ml portions of NaCl solution. Drying of the organic solution ($MgSO_4$) and evaporation to dryness yielded the crude acetals. Compounds I–IV, VIII, X, and XIV were purified by distillation at reduced pressure. Compounds VI, VII, and XII were purified by recrystallization or sublimation.

Thin layer chromatography of most purified products revealed a single spot with an R_f value different from the starting phenol. Ir spectra of all products showed a loss in OH stretching absorption of the parent phenol, a new peak at 2900–2950 cm^{-1} (CH stretch), and new peaks in the region 950–1100 cm^{-1} (CO stretch). All other peaks were consistent with the proposed structures. Proton magnetic resonance spectra for some of the products was recorded in $CDCl_3$ or $(CD_3)CO$ with TMS as internal standard.

Methoxymethoxybenzene (I) had bp 28° (0.01 mm), 53° (3 mm); $n^{22}D$ 1.4980; nmr singlets at δ 3.2 and 5.05, multiplet at 6.9–7.5 ($CDCl_3$, TMS) [lit. bp 63–65° (8 mm), $n^{20}D$ 1.5027;[30] bp 189–190° (760 mm)[29]].

Anal. Calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.29. Found: C, 69.40; H, 6.93.

2-Chloromethoxymethoxybenzene (II) had bp 71.5° (2.75 mm).

Anal. Calcd for $C_8H_9ClO_2$: C, 55.65; H, 5.25; Cl, 20.56. Found: C, 55.80; H, 5.35; Cl, 20.61.

4-Chloromethoxymethoxybenzene (III) had bp 77° (4.25 mm) [lit.[30] bp 90.5–91.0° (7 mm)].

(28) Analyses performed either by Alfred Bernhardt, Max Plank Institute, Mulheim, Germany, or by Elek Microanalytical Laboratories, Torrance, Calif.
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Anal. Cacld for $C_8H_9ClO_2$: C, 55.65; H, 5.25; Cl, 20.56. Found: C, 55.49; H, 5.58; Cl, 20.59.

4-Nitromethoxymethoxybenzene (IV) had bp 72–73° (0.025 mm) [lit. bp 166–167° (14 mm);[31] bp 112° (0.6 mm)[32]].

Methyl 2-methoxymethoxybenzoate (V) had bp 63° (0.05 mm); nmr singlets at δ 3.4, 3.8, and 5.1, multiplet at 6.6–7.9 ($CDCl_3$, TMS) [lit.[31] bp 154–155° (12 mm), 270–273° (760 mm)].

Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.16. Found: C, 61.76; H, 6.33.

2-Methoxymethoxybenzoic Acid (VI). V was stirred in 1.0 *N* NaOH at ambient temperature for 24 hr. 3 *N* HCl was added dropwise at 0° yielding a white precipitate which was recrystallized from petroleum ether (bp 30–60°), mp 63–64° (lit.[31] mp 64–65°).

4-Methoxymethoxybenzoic Acid (VII). Methyl 4-methoxymethoxybenzoate was treated in the same fashion as V in the preparation of VI. Recrystallization from petroleum ether (bp 30–60°) yielded white needles, mp 122–123.2°; nmr singlets at δ 3.4 and 5.2, two doublets centered at 7.0 and 7.87 [$(CD_3)_2CO$, TMS].

Anal. Calcd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.38; H, 5.57.

Dimethyl 2-Methoxymethoxyisophthalate (VIII). **2-Hydroxyisophthalic acid (XV)** was prepared by the method of Todd and Martell,[33] mp 243° (white needles from water). **Dimethyl 2-hydroxyisophthalate (XVI)** was prepared by Fischer esterification of XV, mp 70–71° (lit.[34] mp 70–72°). The acetal VIII was obtained from XVI by the general procedure previously provided: bp 137.5° (0.55 mm); uv λ_{max} 288 mμ (H_2O); nmr singlets at δ 3.5, 3.82, and 5.05, multiplet at 7.0–7.35, doublet centered at 7.85 ($CDCl_3$, TMS).

Anal. Calcd for $C_{12}H_{14}O_6$: C, 56.69; H, 5.55. Found: C, 56.61; H, 5.58.

Methyl 2-Methoxymethoxy-3-methylbenzoate (X). 2-Hydroxy-3-methylbenzoic acid (Eastman Organic) was esterified with diazomethane by the method of Werner[35] to provide **methyl 2-hydroxy-3-methylbenzoate (XVII)**: bp 44.5–45.5° (0.25 mm); $n^{22}D$ 1.5293 [lit.[36] bp 111° (13 mm); $n^{16}D$ 1.5354]. Compound XVII was converted to the acetal X by general procedure previously provided bp 95° (0.90 mm); $n^{24}D$ 1.5095.

Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 63.04; H, 6.78.

Methyl 2-Methoxymethoxy-3-nitrobenzoate (XII). 3-Nitrosalicylic acid (Eastman Organic) was converted to the methyl ester with diazomethane[35] to provide **methyl 2-hydroxy-3-nitrobenzoate (XVIII)**, mp 128–130 (lit.[37] mp 132°). Compound XVIII was converted to the acetal XII by the general procedure previously provided, mp 56–57° (twice recrystallized from ethyl ether then twice sublimated at 65° and 0.5 mm); nmr singlets at δ 3.5, 3.92, and 5.1, triplet centered at 7.28, and a multiplet at 7.75–8.1 ($CDCl_3$, TMS).

Anal. Calcd for $C_{10}H_{11}NO_6$: C, 49.80; H, 4.60; N, 5.81. Found: C, 50.07; H, 4.67; N, 5.76.

Methyl 2-Methoxymethoxy-3-*t*-butyl-6-methylbenzoate (XIV). 2-Hydroxy-3-*t*-butyl-6-methylbenzoic acid (Aldrich) was converted to the methyl ester with diazomethane[35] to yield **methyl 2-hydroxy-3-*t*-butyl-6-methylbenzoate (XIX)**. Recrystallization from ether yielded large rhombic crystals: mp 65°; nmr singlets at δ 1.4, 2.5, and 3.92, two doublets centered at 6.6 and 7.25 ($CDCl_3$, TMS). Compound XIX was converted to the acetal XIV by the general procedure previously provided: bp 107–107.5° (0.5 mm); nmr singlets at δ 1.4, 2.25, 3.5, 3.85, and 5.0, two doublets centered at 6.8 and 7.22 ($CDCl_3$, TMS).

2-Methoxymethoxyisophthalic acid (IX), 2-methoxymethoxy-3-methylbenzoic acid (XI), and 2-methoxymethoxy-3-nitrobenzoic acid (XIII) were obtained by the following procedure. Five ml of a 1.0×10^{-2} *M* solution of the corresponding ester (5.0×10^{-5} mol) in acetonitrile was added to 5 ml of 0.1 *N* KOH (1.0×10^{-4} mol). The solution was mixed and allowed to stand for at least 24 hr. Repetitive scans indicate that reaction is complete within 2 hr. The compounds were not isolated. Product analyses were then performed on the resulting acid hydrolysis products.

Kinetics. All kinetic measurements were done in aqueous buffers at $\mu = 1.0$ with KCl. Buffers employed in this study were hydrochloric acid (pH 0.03–2.40), sodium formate (pH 2.84–4.04), and potassium acetate (pH 4.36–5.71). For the compounds I, II, IV, VII, IX, XI, and XIII buffer dilution experiments were carried out when formate or acetate buffers were employed using three buffer concentrations over at least a fivefold range at constant ionic strength and pH. In some instances this dilution resulted in either an increase or decrease in the observed rate of hydrolysis. In all cases the effect of buffer concentration on the rate was very small, nevertheless, extrapolations to zero buffer concentration were carried out. Rate runs were initiated by introducing a drop of a 10^{-2} *M* solution of the particular acetal in acetonitrile into 3 ml of the buffer solution which had been equilibrated for at least 20 min in the cell holder of the spectrophotometer. The solutions thus contain less than 1% of acetonitrile. Concentration of substrate was between 5×10^{-5} and 10^{-4} *M*. For the more rapid reactions a 20-μl Eppendorf pipet was employed to introduce the acetal solution and mixing was accomplished with a Cal-Biochem "Plumper."

Spectrophotometric rates were obtained by following the formation of substituted phenol. Wavelengths (mμ) utilized are as follows: I 270, II 280, III 280, IV 340, V 302, VI 302.5, VII 275, VIII 314, IX 310, 315, X 308, XI 304, XII 335, XIII 346. The pH's of the solutions were determined before and after reaction to ensure constancy. Reactions were followed to at least four half-lives. The pseudo-first-order rate constants (k_{obsd}) were obtained by calculating the slope of plots of log $(OD_\infty - OD_0)/(OD_\infty - OD_i)$ *vs.* time or by the method of Guggenheim.[38] All actual computations were carried out using an Olivetti-Underwood Programma 101 computer.

Product Analysis. The hydrolysis of each acetal was followed by repetitive scanning at ambient temperature. Acidities were chosen (1.0 *N* HCl to pH 4.8 with buffer) that yielded half-lives of 10–20 min. In all cases the products of hydrolysis were identical in their uv spectra with the phenols from which they had been prepared. For compounds V, VIII, X, and XII the acid hydrolysis yielded the corresponding phenols in which the carbomethoxy group was intact. Adjustment of the pH of these solutions to *ca.* 12 initiated saponification of the esters which was also followed spectrophotometrically.

Spectrophotometric titrations were performed on the products of acid hydrolysis of compounds IX and XIII utilizing an equilibrium cell that will be described elsewhere. A solution of 2.5×10^{-6} mol of IX or XIII in 25 ml of 0.1 *N* HCl was allowed to stir for 1 hr and then titrated. The absorption maxima and isosbestic points in the titration of the products of hydrolysis of IX and XIII were identical with those obtained for genuine samples of **2-hydroxyisophthalic acid (XX)** and **3-nitrosalicylic acid (XXI)**, respectively.

$$\text{(XX)}\quad \underset{314}{Ar(COOH)_2} \overset{324}{\rightleftharpoons} \underset{316.5}{Ar(COOH)COO^-} \overset{310}{\rightleftharpoons} \underset{304}{Ar(COO^-)_2}$$

$$\text{(XXI)}\quad \underset{336}{Ar(COOH)(OH)} \overset{346}{\rightleftharpoons} \underset{348}{Ar(COO^-)(OH)} \overset{\substack{383\\314}}{\rightleftharpoons} \underset{430}{Ar(COO^-)(O^-)}$$

Isosbestic points in mμ are shown above the arrows and absorption maxima in mμ are shown below the species.

The change in absorbance of a solution of XX on titration with base was determined at the wavelengths of 310 and 324 mμ. Between 0.1 *N* HCl and pH 3.2 the absorbance at 310 mμ was found to decrease and become independent of pH above 3.2, while absorbance at 324 mμ was found to be independent of pH below 2.5 but to decrease with pH from pH 2.5 to 6.5. Plots of OD values at 310 and 324 mμ *vs.* pH yield titration curves which when fitted to theoretical curves[39] provided pK_{a1} *ca.* 1.6 and pK_{a2} 4.50.

For XXI a plot of OD at 373 mμ *vs.* pH yielded a titration curve which gave an estimated pK_{a1} of 1.6. A plot of OD at 430 mμ *vs.* pH yielded a titration curve which was used to calculate pK_{a2} as 9.57.

Determination of the p*K*'s of isophthalic acid. A solution of 2.5×10^{-6} mol of isophthalic acid in 25 ml of 0.1 *N* HCl was titrated to determine the acid dissociation constants. A plot of OD at 265

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(39) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley & Sons, Inc., New York, N. Y., 1962, p 73.

mμ *vs.* pH yielded a titration curve and a calculated pK_{a1} of 3.43 ± 0.03. Above *ca.* 5.0 the OD showed no change with pH. A plot of OD at 279 mμ *vs.* pH yielded a titration curve and a calculated pK_{a2} of 4.40 ± 0.05. Below pH 3.0 the OD at 279 mμ showed

$$\underset{\substack{281\\288.5}}{Ar(COOH)_2} \overset{279}{\rightleftharpoons} \underset{\substack{280\\287.5}}{Ar(COOH)(COO^-)} \overset{265}{\rightleftharpoons} \underset{\substack{277\\285}}{Ar(COO^-)_2}$$

no change with pH. Isosbestic points are shown above the arrows and absorption maxima below the species. A search of the literature revealed the values given in Table II.

Table II

T, °C	pK_{a1}	μ	pK_{a2}	Ref
18	3.54		4.60	*a*
25	3.46	0.03	4.46	*b*
25	3.62	0.03	4.60	*c*

[a] H. H. Landolt, R. Bornstein, and W. R. Roth, "Physikalisch-Chemische Tabellen," 2nd Suppl., Julius Springer, Berlin, 1931, p 1090, as quoted by H. C. Brown, D. H. McDaniel, and O. Hafliger in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, New York, N. Y., 1955. [b] W. R. Maxwell and J. R. Partington, *Trans. Faraday Soc.*, **33**, 670 (1937). [c] B. J. Thamer and A. F. Voigt, *J. Phys. Chem.*, **56**, 225 (1952), and references therein.

Apparatus. Infrared spectra were measured using a Perkin Elmer 137 sodium chloride spectrophotometer. Ultraviolet and visible spectra and all repetitive scans were recorded on a Perkin-Elmer 350 or a Cary 15 recording spectrophotometer, each equipped with a repetitive scan accessory. Nuclear magnetic resonance spectra were recorded on a Varian A60 or a Jelco C60 HL.

Rates at 56.0 ± 0.3° were followed using a Gilford Model 2000 recording spectrophotometer equipped with four thermospacers through which water was circulated by a Haake Ultrathermostat NB water bath. Temperature was monitored in the cell block with a Gilford Thermosensor and reactions were not initiated until the system regained thermal equilibrium after the introduction of the cuvettes. Standard taper cuvettes with Teflon stoppers were used to minimize evaporation during the kinetics. pH's of the buffers were determined at 56.2° by means of a Metrohm E type H high-temperature glass electrode, a Radiometer type TTT 1b autotitrator pH meter, and a previously described cell,[40] which was kept at constant temperature by refluxing acetone.

Rates at 30 ± 0.1° were followed with a Gilford 2000 recording spectrophotometer, a Zeiss PM QII spectrophotometer equipped with a Texas Instruments Servo/Riter II recorder and a thermostated brass cuvette holder, or a Zeiss PM QII spectrophotometer equipped with a Honeywell Electronik 15 recorder, a Zieler automatic cell positioner, and a similar thermostated cuvette holder. All pH's at 30.0 ± 0.1° were determined with a Radiometer Model 22 pH meter equipped with a Model 630 scale expander and a combined glass calomel electrode (Radiometer GK 2021 C).

Kinetics runs at 10, 15, 20, and 25° were performed on the Zieler-Zeiss-Honeywell spectrophotometer utilizing the Haake water bath and a Beckman cooling unit. The pH's were measured at the same temperature as the kinetic measurements.

Results

The hydrolysis of acetals not possessing *o*-carboxyl groups were found to be first order in hydrogen ion (eq 2), where k_{obsd} is the pseudo-first-order rate constant at the constant hydrogen ion activity of a_H. The experimental values of k_{obsd} and the derived values of k_H are provided in Table III.

$$k_{obsd} = k_H a_H \quad (2)$$

The hydrolysis of the various acetals possessing *o*-carboxyl functions (VI, IX, XI, and XIII) were generally studied at temperatures which provided convenient time intervals for spectrophotometric measurements. The kinetic results for the hydrolysis of VI, IX, XI, and XIII are provided in Figures 1, 2, and 3. The points of Figures 1–3 are experimental and the curves theoretical, having been generated from eq 3.

$$k_{obsd} = (k_H a_H^2 + k' a_H K_{app})/(K_{app} + a_H) \quad (3)$$

The values of k_H, k', and K_{app} employed to provide the best curves of Figures 1–3 are tabulated in Table IV. Also included in Table IV are the activation parameters associated with k'. The interpretation of the various constants of eq 2 and 3 are provided in the Discussion section.

(40) T. C. Bruice and F. H. Marquardt, *J. Amer. Chem. Soc.*, **84**, 365 (1962).

Table III. The pH Dependence of the Hydrolysis of Acetals without *o*-Carboxyl Groups (Solvent H_2O, 56°, μ = 1.0)

Compd	Log $1/a_H$	k_{obsd} (av), min^{-1}	k_H, M^{-1} min^{-1}
	0.05	1.17 ± 0.06	
I	0.98	(1.39 ± 0.07) × 10^{-1}	
	1.86	(1.36 ± 0.06) × 10^{-1}	
	3.06	(1.15 ± 0.05) × 10^{-1}	1.12
II	0.88	(1.22 ± 0.06) × 10^{-1}	
	1.33	(5.25 ± 0.10) × 10^{-2}	
	1.98	(1.35 ± 0.07) × 10^{-2}	
	2.39	(5.61 ± 0.10) × 10^{-3}	
	2.84	(1.61 ± 0.06) × 10^{-3}	1.06
III	0.92	(1.05 ± 0.04) × 10^{-1}	
	1.35	(4.54 ± 0.15) × 10^{-2}	
	1.94	(1.17 ± 0.05) × 10^{-2}	
	2.36	(4.83 ± 0.15) × 10^{-3}	1.00
IV	0.34	(3.03 ± 0.12) × 10^{-1}	
	0.93	(6.84 ± 0.18) × 10^{-2}	
	1.96	(7.08 ± 0.15) × 10^{-3}	
	3.07	(6.48 ± 0.16) × 10^{-4}	0.708
V	0.88	(3.01 ± 0.06) × 10^{-1}	
	1.33	(1.32 ± 0.03) × 10^{-1}	
	1.94	(3.72 ± 0.05) × 10^{-2}	
	2.37	(1.47 ± 0.02) × 10^{-2}	2.82
VII	0.92	(1.08 ± 0.03) × 10^{-1}	
	1.34	(4.20 ± 0.05) × 10^{-2}	
	1.93	(1.10 ± 0.03) × 10^{-2}	
	2.36	(5.37 ± 0.10) × 10^{-3}	
	3.17	(6.42 ± 0.08) × 10^{-4}	1.00
VIII	0.93	1.02 ± 0.05	
	1.36	(4.21 ± 0.08) × 10^{-1}	
	1.94	(1.18 ± 0.04) × 10^{-1}	
	2.38	(4.98 ± 0.10) × 10^{-2}	
	2.96	(1.24 ± 0.05) × 10^{-2}	10.0
X	0.00	10.44 ± 0.40	
	1.00	1.24 ± 0.02	11.64
XII	0.00	7.93 ± 0.15	
	1.00	(9.38 ± 0.10) × 10^{-1}	8.76

Table IV. Derived Values of Rate Constants and Activation Parameters (for Intramolecular Carboxyl Participation) for the Hydrolysis of Acetals Possessing *o*-Carboxyl Groups

Compd	*T*, °C	k_H, M^{-1} min^{-1}	k', M^{-1} min^{-1}	pK_{app}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S_{298}^{\ddagger}$, kcal mol^{-1}
VI	30	0.56	150	3.75		
	56	4.00	2,000	3.80	19.2	2.3
IX	30	5.00	6,000	2.80		
	25	4.00	3,000	2.80		
	20	2.90	1,600	2.75		
	15	1.80	800	2.75		
	10	0.65	400	2.75	22.6	7.4
XI	30	2.00	470	3.50		
	56	25.60	8,100	3.60	21.2	5.0
XIII	30	40.0	10,000	2.60		

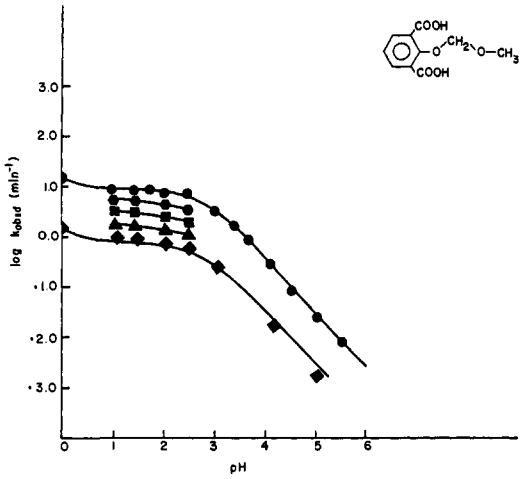


Figure 1. Spectrophotometrically determined pH–log k_{obsd} profiles for the hydrolysis of 2-methoxymethoxyisophthalic acid (IX) at 30° (●), 25° (◉), 20° (■), 15° (▲), and 10° (◆). Points are experimental and the curves are calculated from eq 3 and the values of k_H, k', and pK_{app} provided in Table IV (solvent H_2O, $\mu = 1.0$).

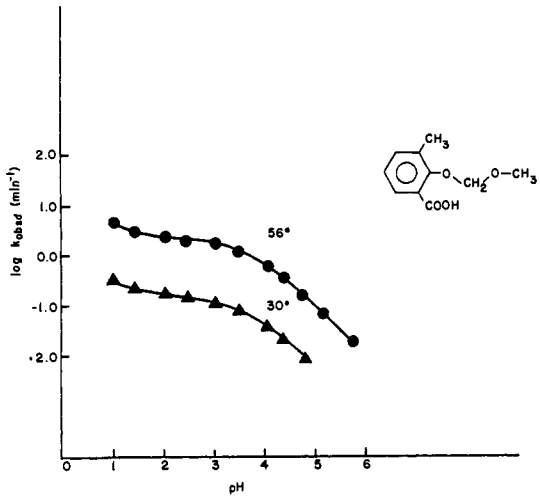


Figure 2. Spectrophotometrically determined pH–log k_{obsd} profiles for the hydrolysis of 2-methoxymethoxy-3-methylbenzoic acid (XI) at 56° (●) and 30° (▲). Points are experimental and the curves are calculated from eq 3 and the values of k_H, k', and pK_{app} provided in Table IV (solvent H_2O, $\mu = 1.0$).

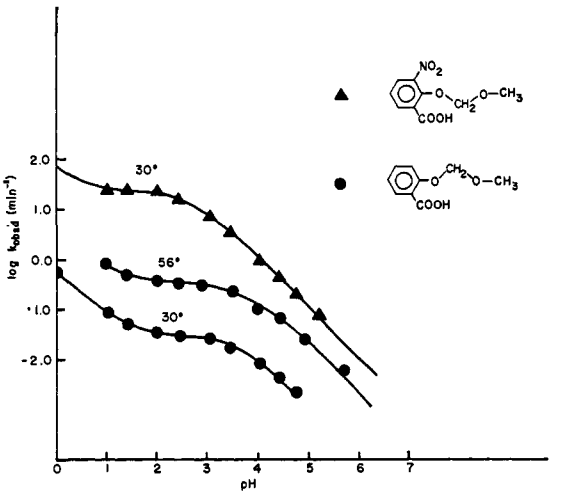


Figure 3. Spectrophotometrically determined pH–log k_{obsd} profiles for the hydrolysis of 2-methoxymethoxy-3-nitrobenzoic acid (XIII) at 30° (▲) and 2-methoxymethoxybenzoic acid (VI) at 56 and 30° (●). Points are experimental and the curves are calculated from eq 3 and the values of k_H, k', and pK_{app} provided in Table IV (solvent H_2O, $\mu = 1.0$).

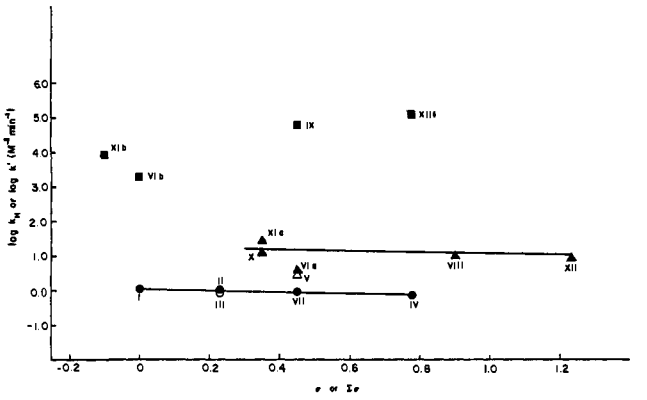
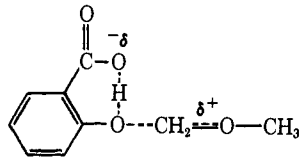


Figure 4. Hammett plot for the specific acid catalyzed hydrolyses (k_H or k') of methyl phenyl acetals of formaldehyde. Values of the calculated rate constants (56°) are provided in Tables III and IV and the values of σ employed are from ref 42. The values of k' for compounds IX and XIII have been estimated by extrapolation from lower temperatures (see Discussion). Points VIa and XIa refer to k_H and points VIb and XIb refer to k'. The Roman numerals refer to the legend of Table I.

Discussion

The catalytic coefficient k_H in eq 2 undoubtedly pertains to specific acid catalysis of acetal hydrolysis.[15] For those acetals possessing *o*-carboxyl groups, k_H (eq 3) is the rate constant for specific acid catalyzed hydrolysis of the undissociated species [*i.e.*, since $a_H \gg K_{app}$ then $a_H^2/(K_{app} + a_H) = a_H$]. The rate constant k' in (3) may pertain to specific acid catalyzed hydrolysis of the acetal with a dissociated carboxyl group or alternatively to the kinetically equivalent intramolecular general acid catalyzed (k_{ga}) mechanism of eq 4. Comparison of (5) and (3) leads to (6). In these expressions the constant K_{app} pertains to the kinetically apparent dissociation constant of the carboxyl group.



(4)

$$k_{obsd} = (k_H a_H^2 + k_{ga} a_H)/(K_{app} + a_H) \quad (5)$$

$$k' K_{app} = k_{ga} \quad (6)$$

In Figure 4 a plot is provided of log k_H and log k' *vs.* the Hammett[41] σ values. The σ values employed have been the *para*-σ values of McDaniel and Brown.[42] The use of *para* substituent constants for *ortho* substitution poses no problem in the present study since the values of ρ are so small. The value of k' for compound IX has been determined by an extrapolation

(41) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p 184.
(42) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, 23, 420 (1958).

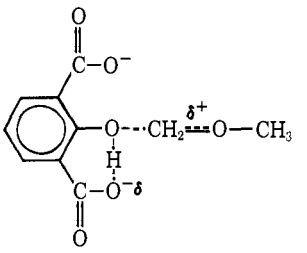
from a five-point Arrhenius plot constructed at four pH's in the plateau region (Figure 1). The plateau rate at 56° thus obtained was related to k' by eq 6. The value of k' for XIII has been estimated by assuming an average activation energy based on the data for compounds VI and XI and using the value of k' for XIII determined at 30°. For the purposes of Figure 4 this approximation is quite suitable.

Examination of Figure 4 reveals little sensitivity of the calculated specific acid rate constants to electronic effects of substituent groups. Thus, the best line drawn through the points for *para*-substituted acetals (I, III, IV, and VII) is of slope (ρ) *ca.* −0.25. *ortho* substitution in general results in larger specific acid rate constants. A comparison of the rate constants (k_H) for the mono-*ortho*-substituted acetals (II, V, and VIa) to the *para*-substituted acetals reveals that *ortho* substitution increases k_H and that the increase in rate is dependent upon the steric requirements of the substituent groups ($CO_2H \geq CO_2Et > Cl$). For the acetals possessing two *ortho* substituents a further steric enhancement of k_H is noted (X, XIa, VIII, and XII). The slope of the best line passing through the points for the 2,6-disubstituted acetals is essentially parallel to that for the *para*-substituted acetals with a positive displacement of 17-fold. The rate constants for specific acid catalyzed hydrolysis of phenyl glycosides have previously been shown to be enhanced by *ortho* substitution[43] as has the intramolecular nucleophilic attack of the 2-acetamido group in the hydrolysis of phenyl 2-acetamido-2-deoxy-β-D-glucopyranosides.[19,20]

The calculated specific acid rate constants for the acetals possessing an *ortho* carboxyl anion (k') show large positive rate enhancements when compared to the values of the calculated specific acid catalyzed rate constant for the undissociated form of the same acetal (k_H). Thus the k' value for the acetal possessing one *ortho* carboxyl (VIb) is 600 times larger than the k_H value (VIa) for the same compound. Since this rate enhancement is too large to be explained on the basis of a steric effect, the constant k' must then pertain to the kinetically equivalent intramolecular general acid catalyzed mechanism (4) as suggested by Capon.[17] The values of k' for those acetals possessing a second *ortho* substituent in addition to an *ortho* carboxyl group may be compared to the least squares line drawn through the points for those acetals possessing two *ortho* substituents. Thus k' for compound XI (XIb in Figure 4) shows a 350-fold rate enhancement, k' for compound IX shows a 3000-fold rate enhancement, and k' for compound XIII shows a 10,000-fold rate enhancement. For these examples the intramolecular mechanism (eq 4) must also apply. The relative rate enhancements for XI:IX:XIII of about 1:8.6:28 follows the order of the steric requirements of the second *ortho* substituent ($NO_2 > CO_2H > CH_3$).

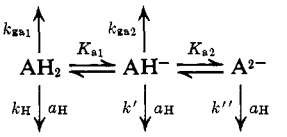
Of the acetals whose hydrolysis has been investigated, IX is of paramount importance in determining if the general acid catalysis of hydrolysis (eq 4) is enhanced *via* electrostatic stabilization of the incipient oxocarbonium ion by a neighboring carboxyl anion (compare eq 1 and 7). A more complete discussion of the kinetics of hydrolysis of IX is, therefore, warranted.

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



(7)

From Figure 1, it is seen that only one plateau exists in the pH region investigated for the hydrolysis of IX. The kinetics of hydrolysis of IX resembles, therefore, that for a mono-*ortho*-carboxyl substituted acetal. A complete understanding of the mechanism of hydrolysis of IX requires an explanation of why the observed pH–log rate profile is insensitive to the ionization of the second carboxyl group. In eq 8, AH_2, AH^-, and



(8)

A^{2-} represent the three ionic species of the dicarboxylic acid expected to exist in the pH region investigated, with K_{a1} and K_{a2} representing the first and second acid dissociation constants. The rate constants k_H, k', k'' represent specific acid catalyzed hydrolysis of AH_2, AH^-, and A^{2-}, respectively, and k_{ga_1} and k_{ga_2} represent first-order rate constants for intramolecular general acid catalyzed hydrolysis of the species AH_2 and AH^-, respectively. The kinetic expression for the observed pseudo-first-order rate constant for hydrolysis based on (8) is provided in eq 9. From (9) it is readily seen

$$k_{obsd} = \frac{k_H a_H^3 + k_{ga_1} a_H^2 + k' a_H^2 K_{a_1} + k_{ga_2} a_H K_{a_1} + k'' a_H K_{a_1} K_{a_2}}{a_H^2 + K_{a_1} a_H + K_{a_1} K_{a_2}} \quad (9)$$

that the following kinetic equivalencies hold

$$k_{ga_1} a_H^2 = k' a_H^2 K_{a_1}$$
$$k_{ga_2} a_H K_{a_1} = k'' a_H K_{a_1} K_{a_2} \quad (10)$$

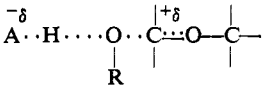
For calculational simplicity one may then employ only the kinetically equivalent route of specific acid catalysis so that eq 9 simplifies to eq 11. We may make

$$k_{obsd} = (a_H)\left[(k_H)\frac{a_H}{K_{a_1}} + k' + (k'')\frac{K_{a_2}}{a_H}\right]\left[\frac{1}{a_H/K_{a_1} + 1 + K_{a_2}/a_H}\right] \quad (11)$$

the assumption that $pK_{app} = pK_{a_1}$ for IX. This constant falls in the region anticipated based on a plot of the pK_{app} *vs.* σ for the mono-*ortho*-carboxyl-substituted acetals. Titration of isophthalic acid yielded pK_a's separated by about 1 pH unit (Experimental Section). The second acid dissociation constant for IX may then be estimated as 3.80, which is one unit higher than pK_{a_1}. The remaining constants in eq 11 may be estimated by computation from the data of Figure 1. From the invariant value of k_H and the estimated values of pK_{a_1} and pK_{a_2} a series of theoretical curves were then computer generated by assuming

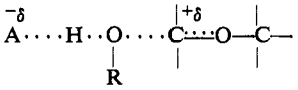
various values for the constants k' and k''. On the basis of the values of k' and k'' required to give the best fit of the experimental points (30°) to eq 11 the best values of k_{ga_1} and k_{ga_2} are calculated to be 9.5 and 0.5 min^{-1}, respectively. Since k_{ga_2} is more than a factor of 10 less than k_{ga_1} it is apparent that electrostatic or nucleophilic participation by the carboxyl anion in the intramolecular general acid catalyzed hydrolysis of IX is not important.

Since the pK_a of Asp-52 in lysozyme is comparable to a normal carboxylic acid in water (see ref 7 and citations therein), it has been suggested that this carboxyl group is located in an ionic environment on the enzyme. This environment should then be similar to that experienced by the carboxyl anion of IX in water. The distance between the carboxyl anion and the incipient carbonium ion for IX is similar to the comparable distance suggested to exist in the lysozyme–substrate complex. On this basis, the effect of the carboxyl groups should be comparable. Since no electrostatic effects are noted in the hydrolysis of compound IX, one might conclude that it is not likely for the carboxyl anion to participate electrostatically in the hydrolytic mechanism of lysozyme. Alternatively, our finding of a lack of an electrostatic stabilization, by a neighboring carboxyl anion, in the general acid catalyzed hydrolysis of a methoxy methyl phenyl ether would be in accord with a transition state possessing little oxocarbonium ion character (eq 12).



(12)

This is not likely, however, on the basis that the minimum difference in k_{ga_1} and k_{ga_2} for IX is about tenfold which suggests a Brønsted α value of *ca.* 1.0 based on the assumed values of pK_{a_1} and pK_{a_2}. Though the exact values of pK_{a_1} and pK_{a_2} cannot be directly determined the assumed values are not likely to be far in error and a value of α of about 1.0 is in accord with a great deal of proton transfer in the transition state and considerable incipient oxocarbonium ion formation (eq 13). If the transition state of (13) has any validity



(13)

then the mechanism for neighboring undissociated carboxyl group participation in the mono-*ortho*-carboxyl-substituted acetals may well be considered to be one of carboxyl anion stabilization of a specific acid catalyzed (A1) mechanism. Thus, (13) could pertain to a slow proton transfer mechanism or to an A1 mechanism, the transition state of which receives stabilization through hydrogen bonding. If the Brønsted value approximated from the pK's and k_{ga}'s for the hydrolysis of IX does approach 1.0 then for the transition state associated with lysozyme-catalyzed hydrolysis there can be no more oxocarbonium ion character than in the transition state for the hydrolysis of IX. The proposed[3c,d] electrostatic role (eq 1) for Asp-52 must, therefore, be considered suspect.

Bulky *ortho* substituents increase not only the values of k_{ga} but also those of the specific acid catalytic coefficient (k_H). Thus, the values of k_H increase in the order *o*-H, *o*-CH_3, *o*-COOH, *o*-NO_2. For both the general acid and specific acid mechanisms a relief of strain occurs in going from the ground state to the transition state due to the lengthening of the PhO---C bond. Similar explanations of steric acceleration have been provided for SN1 and elimination reactions (for a discussion and pertinent references see ref 44). Inspection of the value of $\Delta S_{298}^{\ddagger}$ of Table IV reveals that they become more positive with increasing steric demand of the substituent groups.

Acknowledgments. This work was supported by a grant from the National Institutes of Health.

(44) A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 92.