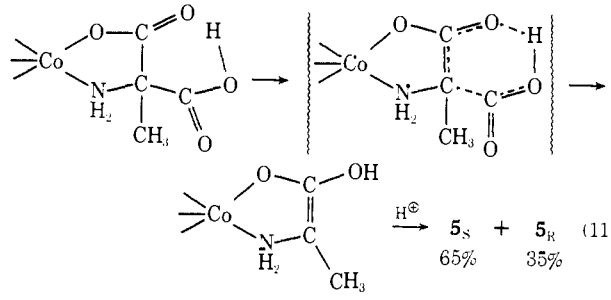
The evidence (CD, X-ray) strongly implies that the dimethyltrien-Co-aminomethylmalonate complex (**3**) contains the N–O bound malonate moiety exclusively in the *R* configuration. This signifies that the dimethyltrien-Co moiety coordinates one carboxylate group to the complete exclusion of the other, identical, carboxylate group. Unless excess base is added, no other $\Lambda(-)_{436}$-β-malonate complex is isolated. *These results represent the first instance of absolute*[31] *chiral recognition of a prochiral center by a small molecule* (a characteristic common in enzymic catalysis).

The structure of **3** (Scheme I) reveals a hydrogen bond between one carboxylate group and the proton on a secondary nitrogen of the dimethyltrien ligand. It is evident that in actuality there exists three-point binding of the malonate moiety; *i.e.*, the metal system has provided a template, which can accept only one configuration for substrate binding, as suggested by Ogston[32] in his three-point hypothesis for enzyme systems. In this light it is not surprising that such a high degree of chiral recognition should exist.

Decarboxylation of **3** in acidic solution (81.6°) yields 65% $\mathbf{5}_S$ and 35% $\mathbf{5}_R$. The reaction has been established to be first order in the species of **3** in which the carboxyl group not coordinated to the Co^{III} moiety is protonated. In analogy to the decarboxylation of other undissociated carboxylic acids possessing β,γ unsaturation[33] it is reasoned that decarboxylation of **3** involves a cyclic transition state to yield, as a metastable intermediate, an "enol"-like product in which the asymmetric carbon of **3** has become sp^2 (eq 11). That $\mathbf{5}_S$ is



observed in 30% excess over $\mathbf{5}_R$ must then require considerable asymmetric induction on protonation of the sp^2 intermediate by the dissymmetric cobalt center. The experiments aimed at ascertaining the effects of bulkier substituents, both on the malonate moiety and on the tetramine ligand, proved discouraging (Table III) in that the extent of asymmetric induction does not become much greater with increase in steric demand. In the case of the isopropyl malonate complex the order of asymmetry was actually reversed (Table III) indicating that bulkiness in the malonate substituent is perhaps of more significance than on the tetramine ligand.

In conclusion, the most important aspect of this study is the establishment that a small chiral metal complex may recognize, in an absolute sense, a prochiral center. Of less importance but considerable interest is our finding that the stereospecifically formed malonate complex decarboxylates with appreciable asymmetric induction from the dissymmetric cobalt moiety.

Acknowledgment. This research was supported by a grant from the National Institutes of Health.

(31) Within experimental error. We estimate that our chromatographic technique could easily have detected 0.5% of $\mathbf{3}_S$.
(32) A. G. Ogston, *Nature (London)*, **162**, 963 (1948).
(33) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill, New York, N. Y., 1962, p 72.

Ring Strain and General Acid Catalysis of Acetal Hydrolysis. Lysozyme Catalysis

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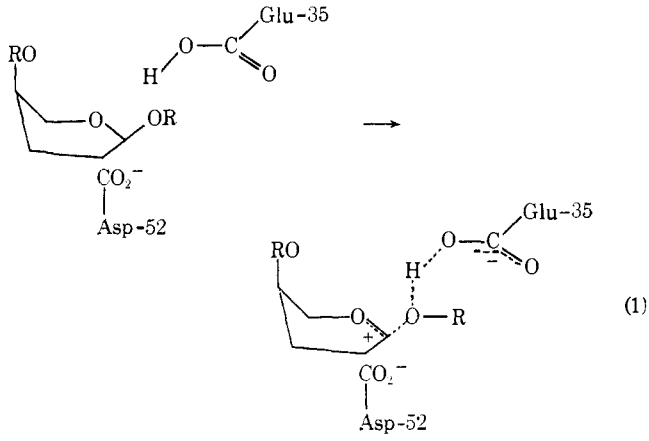
Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106. *Received September 22, 1973*

Abstract: The hydrolysis of 2-methoxy-3,3-dimethyloxetane (**7**) occurs with general acid catalysis as attested to by: (1) dependence of observed rate constant upon the concentrations of acid species in solution (Brønsted $\beta = -0.65$); (2) the near identity of catalytic coefficients for dihydrogen phosphate anion and imidazolium ion; and (3) the values of the deuterium solvent kinetic isotope effects for H_3O^+ and $H_2PO_4^-$ ($k_D/k_H = 1.8 \pm 0.9$ and 0.50 ± 0.10, respectively). These results establish that the proposed ring strain in the Phillips mechanism for lysozyme would allow general acid catalyzed hydrolysis of the glycosidic bond.

In 1965, Phillips[2] announced the elucidation of the three-dimensional structure of lysozyme, the first enzyme whose tertiary structure had been reported in detail. Lysozyme catalyzes hydrolysis of β (1–4) glycosidic bonds in polysaccharides made up of *N*-acetylglucosamine or alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues. A model of an enzyme–substrate complex showed[3] that the only functional groups in the active site which might reasonably be catalytically active are the carboxyl groups Glu-35 and

(1) Work carried out by R. F. Atkinson while on sabbatical leave from The Department of Chemistry, Grand Valley State College, College Landing, Allendale, Mich. 49401.
(2) (a) C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Nature (London)*, **206**, 757 (1965); (b) L. N. Johnson and D. C. Phillips, *ibid.*, **206**, 761 (1965).
(3) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967).

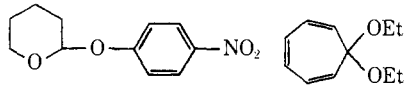
Asp-52 and that the sugar ring whose anomeric C_1–O bond undergoes scission is held in a strained half-chair conformation. Based on these observations a mechanism was proposed to account for the catalytic efficiency of lysozyme (eq 1). In the mechanism of eq 1



(1)

relief of strain in going to the transition state is proposed to facilitate bond breaking, and planarity of the C_5–O and C_1–C_2 bonds ensures maximum overlap of electrons on oxygen with the developing positive charge on C_1. Glu-35 acts as a general acid catalyst, and the positively charged intermediate is stabilized electrostatically by the ionized carboxyl group of Asp-52.

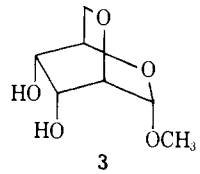
Since general acid catalysis is central to the proposed mechanism for lysozyme, physical organic studies of model compounds were undertaken to delineate the requirements for general acid catalysis in the hydrolysis of acetals.[4] The hydrolysis of acetals normally occurs *via* an A-1 process. General acid catalysis has only been observed when C–O bond breaking is facilitated by a good leaving group as in **1**[5] or by formation of an especially stable oxocarbonium ion as in **2**.[6] Acetals



1 2

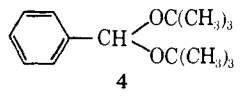
with carboxyl groups capable of intramolecular proton donation are especially good models for the enzyme–substrate complex in which the reactive linkage and the enzyme functional groups are held in close proximity. In fact, for acetals which show buffer acid catalysis, substitution of a suitably placed carboxyl function leads to intramolecular general acid catalysis and large rate enhancements.[7] Dunn and Bruice[8] have concluded that, with the exception of borderline cases, acetals not subject to intermolecular general acid catalysis will not be subject to intramolecular general acid catalysis. No evidence has been offered for bifunctional catalysis (as suggested for lysozyme, eq 1) in molecules with two carboxyl functions.

Glycosides are not subject to intermolecular general acid catalysis since the leaving group is poor and the oxocarbonium ion is relatively unstable. If glutamic acid-35 functions as a general acid in lysozyme, bond breaking must be facilitated in the substrate presumably by distortion of the pyranose ring into a half-chair. However, planarity of the C_5, O, C_1, and C_2 atoms in this conformation is insufficient in itself to give general acid catalysis. The strain-free molecule **3** which incorporates such planarity shows only a modest rate acceleration and reacts by an A-1 mechanism.[9] Relief of strain in the half-chair conformation then appears to be the key element in the lysozyme mechanism.



3

Rate enhancement and general acid catalysis due to relief of strain in the ground state have been demonstrated in the hydrolysis of benzaldehyde di-*tert*-butyl acetal (**4**),[10] but the carbonium ion in this case is already much more stable than a glucosyl carbonium ion. Schaleger and coworkers[11,12] studied the hydrolysis of compounds **5** and **6**. Considerable rate enhancement was observed with both compounds but buffer catalysis was studied at only one pH in the hydrolysis of **6** and not at all with **5**. We report herein our studies of the hydrolysis of **7** which establishes that ring strain (as suggested for lysozyme) does allow general acid catalysis of acetal hydrolysis when the oxocarbonium ion is not stabilized by extraresonance forces and for which the leaving group is poor.



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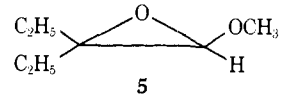

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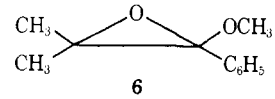

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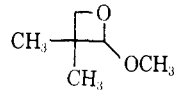


7

Experimental Section

2-Methoxy-3,3-dimethyloxetane (7) was synthesized by the method of Nerdel[13] and was purified by injection directly onto an 8-ft glass vpc column packed with 20% Carbowax 20M on Chromosorb W at 90° with a flow rate of 100 ml/min. Injection of samples into a hot injection port resulted in complete destruction of the product.

Product Analysis. To 10 ml of a pH 6.78 phosphate buffer in 10% dioxane at 30° was added 36 mg of **7**. The solution was maintained at 30° for 1 hr, then saturated with sodium chloride and extracted with two 5-ml portions of ether. The extracts were dried with magnesium sulfate and the solvent was distilled off leaving 17 mg of a colorless oil. A small peak with the same vpc retention time as isobutyraldehyde was observed in the distillate, but there

(4) For reviews, see (a) B. M. Dunn and T. C. Bruice, *Advan. Enzymol.*, **37**, 1 (1973); (b) T. H. Fife, *Accounts Chem. Res.*, **5**, 264 (1972).
(5) T. H. Fife and L. H. Brod, *J. Amer. Chem. Soc.*, **92**, 1681 (1970).
(6) E. Anderson and T. H. Fife, *J. Amer. Chem. Soc.*, **91**, 7163 (1969).
(7) T. H. Fife and E. Anderson, *J. Amer. Chem. Soc.*, **93**, 6610 (1971).
(8) B. M. Dunn and T. C. Bruice, *J. Amer. Chem. Soc.*, **93**, 5725 (1971).
(9) T. A. Giudici and T. C. Bruice, *Chem. Commun.*, 690 (1970).
(10) E. Anderson and T. H. Fife, *J. Amer. Chem. Soc.*, **93**, 1701 (1971).
(11) A. L. Mori, M. A. Porzio, and L. L. Schaleger, *J. Amer. Chem. Soc.*, **94**, 5034 (1972).
(12) A. L. Mori and L. L. Schaleger, *ibid.*, **94**, 5039 (1972).
(13) F. Nerdel, D. Frank, H. J. Lengert, and P. Weyerstahl, *Chem. Ber.*, **101**, 1850 (1968).

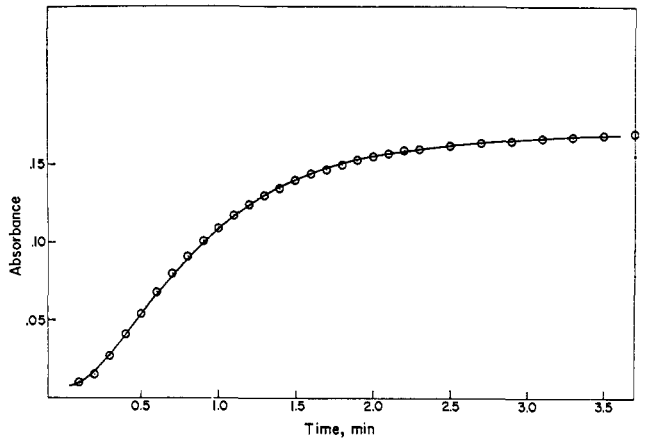


Figure 1. Change in absorbance at 288 nm for the hydrolysis of **7**. Points are experimental and the curve generated on the analog computer employing the program of Scheme I.

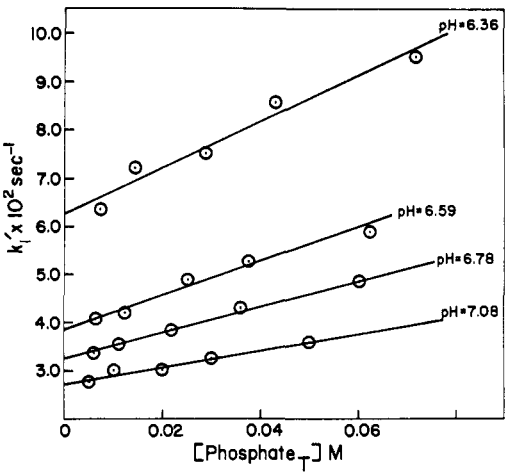


Figure 2. Observed first-order rate constants for ring opening of **7** as a function of total phosphate buffer concentration at constant pH.

was too little to isolate. Upon standing, the oil solidified giving material melting 88–91° (lit.[14] mp 89–90°). After one recrystallization from benzene–petroleum ether, 13 mg of white crystals was obtained, mp 91–92°, whose infrared spectrum was identical with that of the dimer of 2-methyl-2-hydroxymethylpropanal.

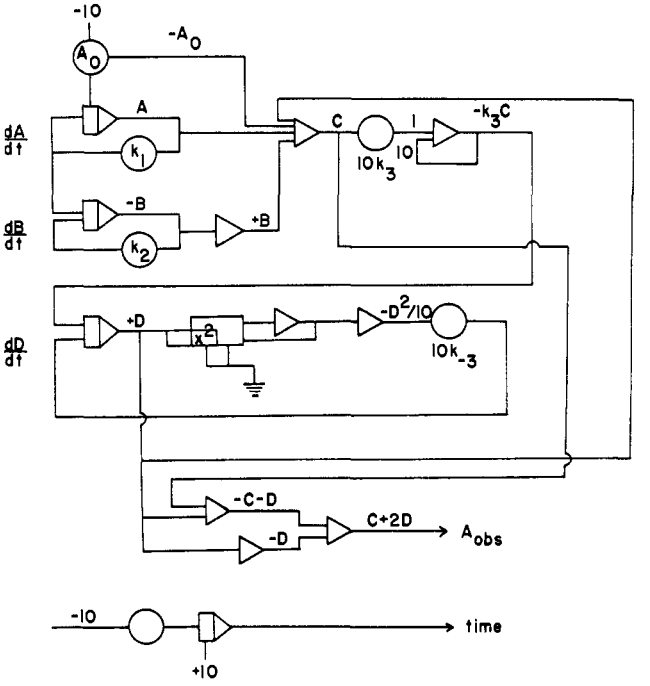
Apparatus. All spectrophotometric kinetic measurements were made on a Cary Model 16 spectrophotometer with a sample compartment thermostated at 29.9 ± 0.1°. pH readings were made with a Radiometer pH meter. The gas chromatograph employed was a Hewlett-Packard Model 5750B. The multiphasic kinetic curves were fitted using an EAI TR-20 analog computer equipped with an EAI 1133 Variplotter XY recorder.

Kinetics. All kinetic measurements were carried out by monitoring formation of aldehyde product at 288 nm (ϵ 17). The solvent used throughout was 10% dioxane–water (v/v) with μ maintained at 0.10 with KCl. Buffers employed were acetate (pH 4.22–5.12), phosphate (pH 6.12–7.08), and imidazole (pH 6.64–7.93).

Buffer solutions were allowed to equilibrate for at least 20 min in the cell compartment of the spectrophotometer, then rate runs were initiated by addition of *ca.* 3 μ of **7** and rapid mixing with a Cal-Biochem "Plumper." Substrate concentrations were about 0.01 *M*.

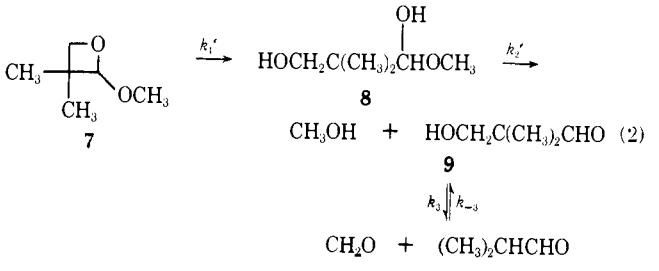
In those cases where first-order behavior was observed, rate constants were calculated by a linear least-squares program or by the Guggenheim method using a Hewlett-Packard Model 9820A calculator. In those runs exhibiting multiphasic kinetics, rate constants were obtained with an analog computer using the program in Scheme I.

Scheme I



Results

Hydrolysis of **7** exhibited good first-order kinetics below about pH 6.1 and above pH 7.9. Between these limits the reaction showed an induction period typical of consecutive first-order reactions.[15] The observed kinetics for appearance of aldehyde are in accord with the mechanism of eq 2 where k_1' is rate determining at



high pH, k_1' and k_2' are comparable around pH 7, and k_2' rate determining at low pH. In runs where multiphasic kinetics were observed, rate constants were obtained by curve fitting with an analog computer using the program in Scheme I. Figure 1 shows the fit to one set of data. When k_1' and k_2' are comparable either constant could be varied only about ±5% without noticeably affecting the fit of the curve. When one constant is much greater than the other the smaller constant could be, in some instances, varied (in the pH range computer fitted) by as much as ±20% without affecting the fit of the curve. Thus it was not possible to obtain the same sort of precision that can be obtained with clean first-order kinetics, and as a result the standard deviations are fairly high for some of the derived second-order rate constants. Nevertheless, the uncertainty in the data is much smaller than the effects being observed and does not invalidate the conclusions drawn below.

In Figures 2 and 3 are plotted the determined values of k_1' *vs.* the concentration of phosphate and imidazole buffers employed. The increase in slope with decreasing pH is evidence for general acid catalysis. A

(14) E. Späth and I. v. Szilagyi, *Chem. Ber.*, **76**, 949 (1943).
(15) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," Wiley, New York, N. Y., 1961, p 166.

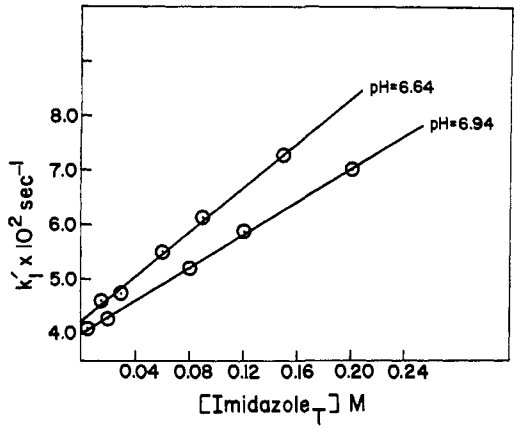


Figure 3. Observed first-order rate constants for ring opening of 7 as a function of total imidazole buffer concentration at constant pH.

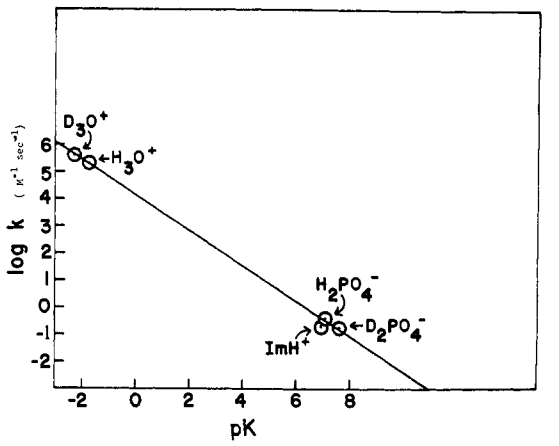


Figure 5. Brønsted plot for the general acid catalyzed ring opening of 7.

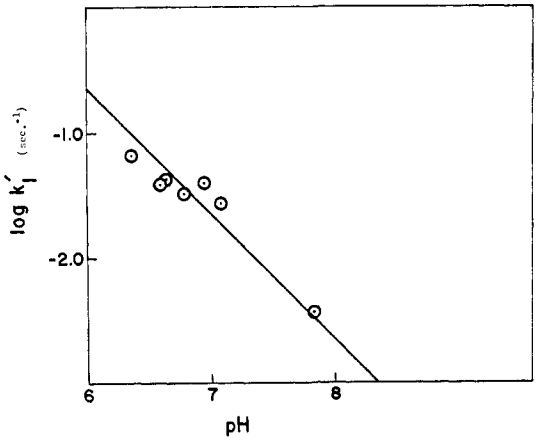


Figure 4. The pH–log k_1' profile for ring opening of 7 at zero buffer concentration.

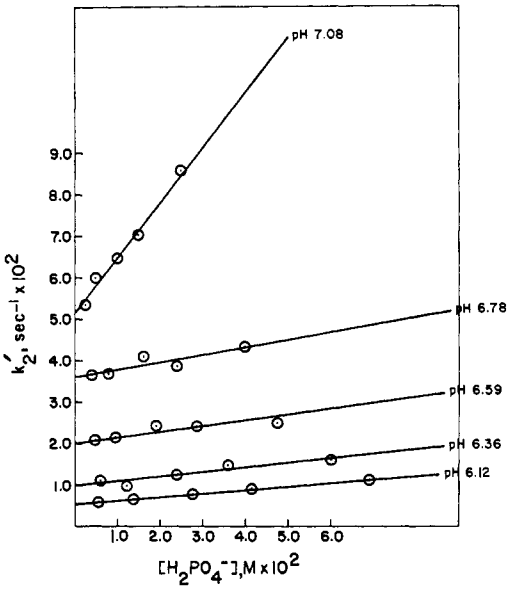


Figure 6. Observed first-order rate constants for hydrolysis of 8 as a function of [$H_2PO_4^-$] and pH.

specific salt effect[16] was ruled out by a duplicate run at pH 6.78 with phosphate buffer using $KClO_4$ instead of KCl to maintain ionic strength. No significant change in rate was observed (Table I). The second-order rate

Table I. Observed Rate Constants for Hydrolysis of 7 (pH 6.78, 30°, and μ = 0.10)

Solvent	Added salt	$k_H a_H$, sec^{-1}	$k_{H_2PO_4^-}$, M^{-1} sec^{-1}
10% dioxane–H_2O	KCl	0.0320 (±0.0004)	0.421 (±0.015
10% dioxane–H_2O	$KClO_4$	0.0327 (±0.0010)	0.405 (±0.036

constants for catalysis by dihydrogen phosphate and imidazolium ion were derived from plots of observed rate constant (k_1') *vs.* [HA] (Figures 2 and 3). Secondary plots of the slopes thus obtained *vs.* K_a/a_H give k_{HA} as intercept and k_A as slope. The close similarity of the second-order rate constants for dihydrogen phosphate and imidazolium ion (Table II) is in accord with general acid catalysis by acids of similar pK, but is contrary to the expectations of nucleophilic catalysis. The second-order rate constant for hydronium ion catalysis, k_H, was determined from a plot of log k_1' (extrapolated to zero buffer concentration) *vs.* pH.

(16) P. Salomaa, A. Kankaanperä, and M. Lahti, *J. Amer. Chem. Soc.*, 93, 2084 (1971).

Table II. Rate Constants for Acetal Hydrolysis (30°, 10% dioxane–H_2O (v/v), μ = 0.10 with KCl)

	Catalyst	k_1, M^{-1} sec^{-1}
7 → **8** (μ = 0.10)	H_3O^+	2.24 (±0.9)[a] × 10^5
	$H_2PO_4^-$	0.418 (±0.054)
	ImH^+	0.207 (±0.182)
	D_3O^+	4.08 (±0.31) × 10^5
	$D_2PO_4^-$	0.207 (±0.016)
5 → **10** (μ = 0.11)[b]	H_3O^+	1.90 × 10^6 [c]
	H_2O	2.60 × 10^{-3} [c]

[a] Standard deviation. [b] Reference 12. [c] Extraporated to 30°.

The best line of slope −1.0 was constructed and the y intercept gave log k_H (Figure 4). No k_{H_2O} term was evident in the pH range of 6.36–7.93; thus eq 3 de-

$$k_1' = k_H a_H + k_{HA}[HA] \quad (3)$$

scribes k_1' in this region. Values of the second-order rate constants are compiled in Table II. A Brønsted plot using these values (Figure 5) has a slope of −0.65 which is in accord with general acid catalysis.

The solvent deuterium isotope effects for hydrolysis

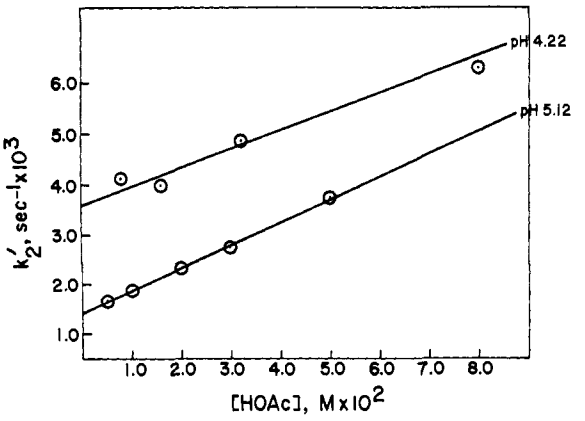


Figure 7. Observed first-order rate constants for hydrolysis of **8** as a function of [CH_3COOH] and pH.

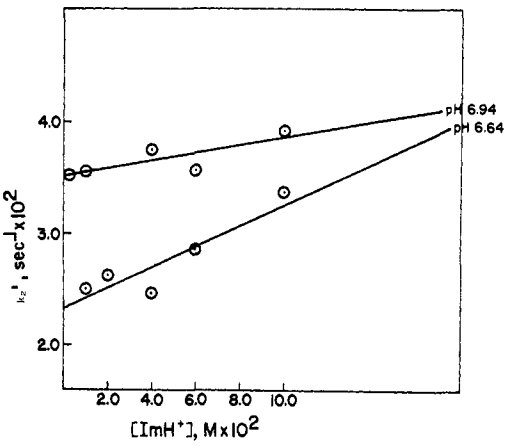


Figure 8. Observed first-order rate constants for hydrolysis of **8** as a function of [ImH^+] and pH.

of **7** are $k_{D_3O^+}/k_{H_3O^+} = 1.8 \pm 0.9$, and $k_{D_2PO_4^-}/k_{H_2PO_4^-} = 0.50 \pm 0.10$. The predicted values for general acid catalysis are 0.7–1.4 for $k_{D_3O^+}/k_{H_3O^+}$ and 0.2–0.35 for k_{HD}/k_{HA} for weak acids.[17] Values of $k_{D_3O^+}/k_{H_3O^+}$ are generally in excess of 2.7 for an A-1 mechanism.[4b]

Hydrolysis of hemiacetal **8** is subject to buffer catalysis as is evident in Figures 6–8. The observed rate constant, k_2', contains terms for acidic as well as basic catalysis (eq 4). The hydroxide ion term, k_{OH}, was ob-

$$k_2' = k_H a_H + k_{OH}[OH^-] + k_{HA}[HA] + k_A[A^-] \quad (4)$$

tained above pH 6 where the contribution of the k_H term is negligible by plotting log k_2' (extrapolated to zero buffer concentration) *vs.* log [OH^-] and taking the intercept of the best line of slope +1.0. The hydronium ion term, k_H, was calculated from the observed rates at pH 4.22 and 5.12. The pH–rate profile of k_2' at zero buffer concentration is shown in Figure 9. The rate constants k_{HA} and k_A were evaluated from the slopes and intercepts, respectively, of plots of k_2' minus the rate for lyate species catalysis *vs.* [HA]. Values of these second-order rate constants are presented in Table III

Table III. Rate Constants for Hemiacetal Hydrolysis (30°, 10% dioxane–H_2O (v/v), μ = 0.10)

	Catalyst	k_2, M^{-1} sec^{-1}
8 → 9		
(μ = 0.10, 30°)	H_3O^+	80 ± 20[a]
	OH^-	3.24 ± 0.56 × 10^6
	$H_2PO_4^-$	6.20 ± 1.80 × 10^{-2}
	HPO_4^{-2}	2.38 ± 0.63 × 10^{-1}
	HOAC	3.07 ± 0.20 × 10^{-2}
	OAC^-	7.71 ± 1.38 × 10^{-3}
10 → 11		
(μ = 0.11, 25°)[b]	H_3O^+	10.1[c]
	OH^-	4.63 × 10^4
	HOAC	1.49 × 10^{-2}
	OAC^-	6.20 × 10^{-3}

[a] Standard deviation. [b] Reference 12. [c] Extrapolated to 30°.

together with comparable data for hemiacetal **10**. The aldehyde product, **9**, undergoes a slow reaction, probably a reverse aldol condensation to formaldehyde and isobutyraldehyde. Since formaldehyde is hydrated rapidly and essentially completely,[18] it shows little absorbance. Isobutyraldehyde is also hydrated, but to a lesser extent than **9** since electron withdrawal favors hydration.[19] Thus formation of isobutyraldehyde from **9** results in an increase in absorbance. Due to the sparing solubility of isobutyraldehyde in 10% dioxane hazing was frequently encountered which led to a drift in absorbance at infinite time. Values of k_3' ranged from 1 to 20% of k_2'. However, these values are probably accurate to only about an order of magnitude since they were determined from small absorbance changes, and light scattering effects made these unreliable. In view of this feature k_3 is not reported and no attempt was made to evaluate k_{-3}.

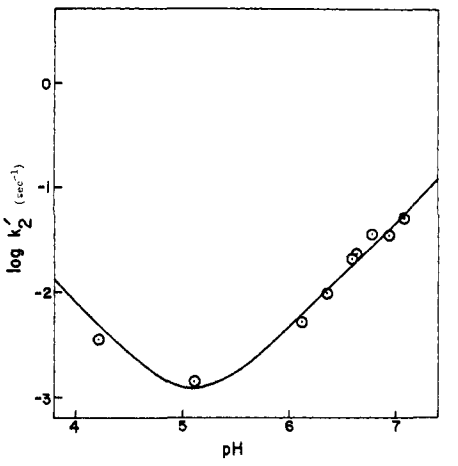
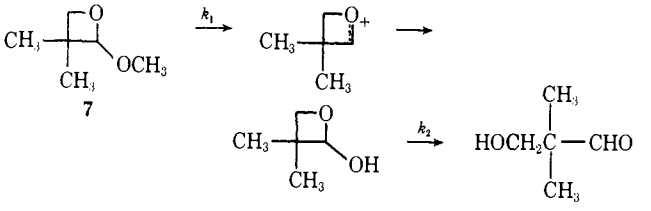


Figure 9. pH *vs.* the log of the pseudo-first-order rate constant (k_2') for hydrolysis of **8** at zero buffer concentration.

Discussion

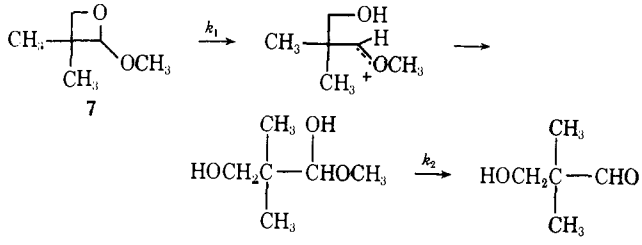
Two pathways may be considered for the hydrolysis of **7** (Schemes II and III). In Scheme II initial C–O

Scheme II



(17) F. A. Long, *Ann. N. Y. Acad. Sci.*, **84**, 596 (1960).
(18) R. P. Bell and P. G. Evans, *Proc. Roy. Soc., Ser. A*, **291**, 297 (1966).
(19) P. Greenzaid, Z. Luz, and D. Samuel, *J. Amer. Chem. Soc.*, **89**, 749 (1967).

Scheme III



bond cleavage is exocyclic and ring cleavage occurs in the final step. In Scheme III, on the other hand, the initial bond cleavage is endocyclic giving an open oxocarbonium ion. Although no direct evidence is available to prove the structure of the intermediate hemiacetal, nevertheless, it is still possible to deduce the course of the reaction in a reasonable fashion.

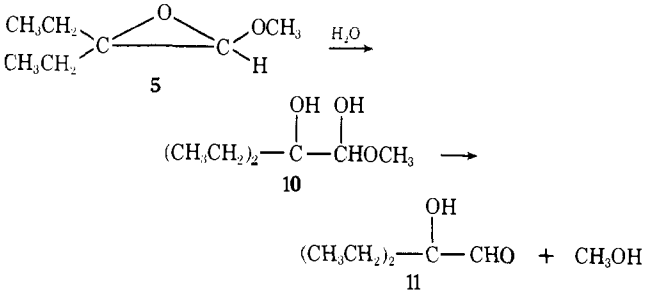
The two schemes differ significantly in their predicted rates. Since the four-membered ring is highly strained,[20] relief of strain in the transition state for ring opening should be reflected in a large rate enhancement. Scheme II thus predicts a rate enhancement for conversion of hemiacetal to aldehyde (k_2), while Scheme III predicts enhancement in the initial C–O bond cleavage (k_1). Furthermore, in Scheme II the oxocarbonium ion intermediate is even more strained than the starting material since it contains double bond character;[21] its formation should thus be retarded. It was found that initial bond cleavage was accelerated by a factor of at least 5×10^5 compared to hydrolysis of simple acyclic acetals by an A-1 mechanism (Table IV).

Table IV. Rates of Acid-Catalyzed Acetal Hydrolysis

Compound	Solvent	Temp, °C	k, M^{-1} sec^{-1}
$CH_3CH(OEt)_2$	50% dioxane	25	0.248[a]
$(CH_3)_2CHCH(OEt)_2$	50% dioxane	25	0.164[a]
$CH_3CH(OEt)_2$	H_2O	30	0.490[b]
7	10% dioxane	30	2.24×10^5
5	10% dioxane	30	1.90×10^6 [c]

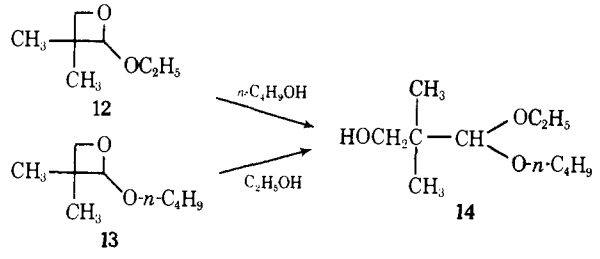
[a] M. Kreevoy and R. W. Taft, Jr., *J. Amer. Chem. Soc.*, **77**, 5590 (1955). [b] J. Koskikallio and E. Whalley, *Trans. Faraday Soc.*, **55**, 809 (1959). [c] Reference 12.

The hydrolysis of **7** follows the same course as **5**,



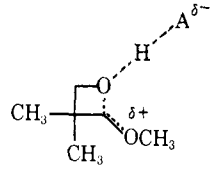
another strained cyclic acetal.[11,12] Hydrolysis of **5** shows a change in mechanism at about pH 8 with **5** → **10** rate determining at high pH and **10** → **11** rate determining at low pH. Comparison of **7** and **5** shows that the four-membered ring reacts about one-tenth as fast as the more highly strained three-membered ring. The intermediate hemiacetal, on the other hand, reacts at about the same rate as **10**. Thus the observed rates support Scheme III.

Further evidence favoring an open oxocarbonium ion comes from the reactions of acetals **12** and **13**.[22] Heating **12** in *n*-butyl alcohol or **13** in ethyl alcohol gives the mixed acetal **14** exclusively and in high yield. This



product could not have been formed from a cyclic oxocarbonium ion, and since the cyclic acetals are stable toward alcoholic potassium hydroxide,[23] a direct SN2 reaction is unlikely.

It is clear, therefore, that relief of strain in acetal hydrolysis facilitates bond breaking which can lead to a sizable rate enhancement and, most importantly, to general acid catalysis even though the intermediate oxocarbonium ion is not stabilized and the leaving group is poor. The transition state for hydrolysis of **7** thus involves proton donation concerted with bond breaking.



Comparison of this transition state with that proposed for lysozyme (eq 1) reveals that the major difference between the two is endocyclic bond cleavage for the former and exocyclic bond cleavage for the latter. In the case of the four-membered ring, the bond angles are smaller than normal for tetrahedral carbon and the resulting strain can be relieved only by breaking the endocyclic C–O bond. With a six-membered ring in the half-chair conformation, on the other hand, the distorted bond angles are greater than normal, and the resulting strain can be relieved either by breaking a bond in the open chain, or by going to a strain-free cyclohexene conformation. The latter pathway is preferred in the lysozyme-catalyzed hydrolysis of glycosides, and undoubtedly one factor which favors it is planarity in the ground state which maximizes overlap between the electrons on the ring oxygen and the developing charge on C-1 in the transition state.

These results establish that, if the acetal possesses considerable strain in the ground state, general acid catalysis of acetal hydrolysis is possible when the leaving group is poor and the oxocarbonium ion does not receive extra resonance stabilization. It is this

(20) (a) Reported values of the strain energy of cyclobutane vary from 26 to 28 kcal/mol: P. v. R. Schleyer, J. E. Williams, and K. R. Blanchard, *J. Amer. Chem. Soc.*, **92**, 2377 (1970); (b) S. W. Benson, *J. Chem. Phys.*, **34**, 521, (1961); (c) S. Kaarsemaker and J. Coops, *Recl. Trav. Chim. Pays-Bas*, 71, 261 (1952).

(21) The strain energy of cyclobutene is reported as 30.6 (ref 18a) and 56.31 kcal/mol: I. J. Miller, *Tetrahedron*, **25**, 1349 (1969).

(22) S. H. Schroeter, *J. Org. Chem.*, **34**, 1188 (1969).

(23) **7** is synthesized in ethanolic potassium hydroxide and other analogs are prepared similarly; see ref 13.

feature of strain which makes the Phillips mechanism for lysozyme plausible. Perhaps the most pleasing mechanism[4a] now stands as general acid catalyzed, by Glu-35, formation of an oxocarbonium ion–alkoxide ion stereoretained ion pair followed by oxocarbonium ion capture by Asp-52 to form an acylal which can spontaneously regenerate[24] oxocarbonium ion.

Acknowledgment. Supported by a grant from the National Institutes of Health.

(24) A. Brown and T. C. Bruice, *J. Amer. Chem. Soc.*, **95**, 1593 (1973).

Direct Determination of C-Protonation and Hydrolysis Rates in Enamines. Application to Ethyl β-Cyanomethylaminocrotonate[1]

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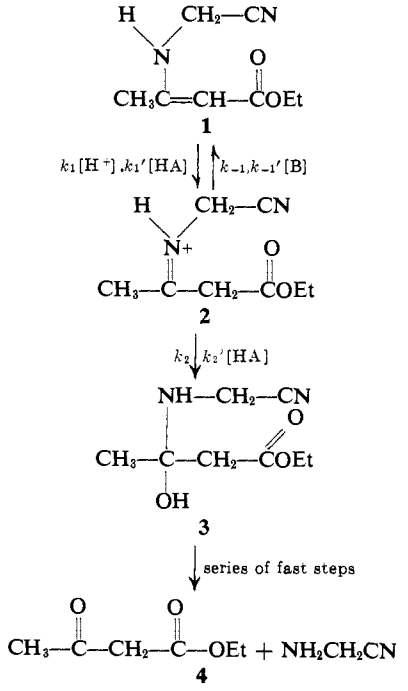
Received May 24, 1973

Abstract: Nmr studies were performed on the rates of hydrolysis of ethyl β-cyanomethylaminocrotonate (**1**), the enamine of ethyl acetoacetate, and the primary amine, cyanomethylamine. Simultaneous observation of the rates of C-protonation and overall hydrolysis enables unequivocal assignment of the steps in the mechanism involving a change in rate-limiting step with changing buffer concentration. As previously suggested, in the limit of zero buffer concentration C-protonation of the enamine is found to be rate limiting.

Recently, the detailed mechanism of the hydrolysis of ethyl β-cyanomethylaminocrotonate (**1**) was presented[3] and the intermediacy of the closely related enamine of acetoacetic acid in the amine catalyzed decarboxylation of acetoacetic acid was substantiated.[4] For the hydrolysis of **1** the mechanism in Scheme I was proposed.

Scheme I. Mechanism of Enamine Hydrolysis



At pH 4, 5, and 6 the rate of hydrolysis of the enamine was found to be subject to both specific acid and general acid–base catalysis. The perceptably curved k_{obsd} *vs.* buffer_{total} relationship found at these pH values was interpreted to mean a change in rate-limiting step with changes in buffer concentration as discussed by Jencks for such behavior.[5] Based on precedent, isotope effects, etc. the buffer behavior was interpreted as a change from rate-limiting C-protonation at low buffer concentration to rate-limiting hydration of Schiff base (or Schiff base salt) at high buffer concentration in the pH range studied.[3] C-Protonation at or near zero buffer concentration was suggested because of the sensitivity of the rate to buffer catalyst concentration and the observed isotope effect ($k^0_{H^+}/k^0_{D^+} = 2.3$) found in this buffer range.

Based on extensive indirect evidence such rate-determining C-protonation had also been proposed for two related enamines (nonconjugated), the hydrolysis of 1-*N*-morpholino-1-isobutene[6] above pH 4 (measured isotope effect $k_{H_3O^+}/k_{D_3O^+} = 2.5 \pm 0.7$, rate subject to general acid catalysis) and for the hydrolysis of the morpholine enamine of propiophenone[7] above pH 5 (this reaction also sensitive to general acid catalysis).

The present results support the mechanism proposed for the hydrolysis of the enamine **1**, prove that C-protonation can in fact be rate limiting under certain conditions (in this study at low buffer concentrations) and suggest a general method for identifying rate-limiting C-protonation steps in analogous mechanisms.

The rate of the tautomerization of the enamine **1** to Schiff base (or Schiff base salt) **2** can be followed by nmr

(1) Supported by GM04712 from the Institute of General Medical Sciences of the National Institutes of Health.
(2) NIH Postdoctoral Fellow. Address correspondence to the Department of Chemistry, Newark College of Arts and Sciences, Rutgers University, Newark, N. J. 07102.
(3) J. P. Guthrie and F. Jordan, *J. Amer. Chem. Soc.*, **94**, 9132 (1972).
(4) J. P. Guthrie and F. Jordan, *J. Amer. Chem. Soc.*, **94**, 9136 (1972).
(5) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 477–480; E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 4319 (1962).
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