MECHANISMS OF CATALYSIS OF NUCLEOPHILIC REACTIONS OF CARBOXYLIC ACID DERIVATIVES

MYRON L. BENDER

Department of Chemistry, Illinois Institute of Technology, Chicago 16, Illinois

Received September 28, 1959

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I. Introduction

A. Scope and importance of these reactions

The reactions of carboxylic acid derivatives such as esters, amides, acid halides, and anhydrides have long been the subject of investigation. The catalytic action of dilute acids and alkali in esterification and hydrolysis was studied by Scheele as early as 1792 (10). When kinetics was intensively applied to the study of organic reactions, a favorite subject for investigation was the saponification of an ester. Continued studies have resulted in the accumulation of a large body of data regarding the effect of structure on reactivity in these systems. This information has been collected and analyzed (190, 304) and has been of importance in the elucidation of the mechanism of the reactions of carboxylic acid derivatives, as has information from tracer studies and exchange studies (212, 219).

Studies of the catalysis of these reactions, although not entirely neglected, have tended until recently to lag behind the investigation of the other facets of the mechanisms of the reactions of carboxylic acid derivatives. It is toward the catalytic aspects of the mechanisms of reaction of carboxylic acid derivatives that this review is pointed. In certain specific instances it will be advantageous to compare the catalysis of the reaction of a carboxylic acid derivative with catalysis in other reaction systems, but only reactions of carboxylic acid derivatives will be treated here in an exhaustive manner.

The rapid growth of the theories of catalysis of the reactions of carboxylic acid derivatives in recent years makes feasible a review at this time. This is due in part to the impetus given to this field by the rapid advances in the elucidation of the mechanisms of enzymatic catalysis of these reactions. This article will, therefore, attempt to cover a portion of the borderline between physical organic chemistry and biochemistry. The concepts to be outlined here are of importance to the fundamental theories of catalysis as well as to the more specific problem of the unraveling of the mysteries of enzymatic catalysis. Progress in the borderline field embracing physical organic chemistry and biochemistry has been enhanced by results from both sides of the artificial boundary; furthermore, concepts arising from this borderline field are of use to disciplines on either side of the boundary.

B. Limitations of coverage of this review

The mechanisms of esterification and hydrolysis of esters were classified in an elegant manner by Day and Ingold (127). They utilized three bases in their scheme: (*1*) kind of catalysis; (*2*) kind of fission; (*3*) the molecularity of the reaction. One might add a fourth subdivision, denoting a concerted or stepwise mechanism, a more recent development. On the basis of the three categories, Day and Ingold erected a mental scaffolding for the classification of esterification and hydrolysis reactions shown in table 1. It is seen that the tabulation defines eight mechanistic divisions, of which six have been experimentally identified as of this time (219). However useful this scheme has been, and its utility cannot be denied, it is desirable for present purposes to change the emphasis given in the above scheme as shown in table 2. Perhaps the second scheme, which puts the primary emphasis on the type of fission and not on the type of catalysis, may appear to defeat the emphasis necessary to this article. But in fact it does just the opposite. The latter classification points out

TABLE 1

A classification of ester hydrolysis reactions

Basic Catalysis	Acidic Catalysis
Acyl fission	Acyl fission
Monomolecular	Monomolecular
Bimolecular	Bimolecular
Alkyl fission	Alkyl fission
Monomolecular	Monomolecular
Bimolecular	Bimolecular

TABLE 2

A new classification of ester hydrolysis reactions

Acyl Fission	Alkyl Fission
Bimolecular	Bimolecular
Basic catalysis	Basic catalysis
Acidic catalysis	
	Monomolecular
Monomolecular	Basic catalysis
Acidic catalysis	Acidic catalysis

that of the six real cases listed, the three falling under the heading of alkyl fission are nothing but reactions at a saturated carbon atom. These reactions are simply S_N2 and S_N1 reactions, involving RC(=O)O— as a leaving group, and are profitably treated with other reactions at a saturated carbon atom. They, therefore, have no place in a discussion of the mechanisms of reaction of carboxylic acid derivatives in which it is desired to emphasize the reactions involving the carbonyl portion of the grouping RC(=O)X. This review article will be limited to those reactions of carboxylic acid derivatives involving acyl–oxygen fission. Furthermore, since the one kind of monomolecular acyl–oxygen fission involves a special case (this reaction is an analog of a reaction involving a carbonium-ion intermediate), it will be ignored at the present time. Finally, the limits of the present discussion can be set as the bimolecular substitution reactions of carboxylic acid derivatives, with acyl–oxygen fission.

The apparently limited area defined above is not an adequate expression of the diversity of the catalytic mechanisms that will be discussed in this article. Whereas only basic and acidic catalysis constituted a comprehensive account of the catalytic possibilities in the bimolecular substitution reactions of carboxylic acid derivatives a few years ago, a large number of catalytic mechanisms must now be considered.

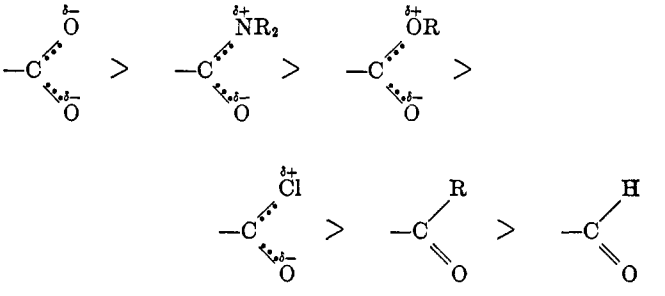
II. Theoretical Concepts of Reactions at a Carbonyl Carbon Atom

Before discussing the various kinds of catalysis which comprise the main subject of this article, it is desirable to discuss certain general aspects of the mechanism of reactions of carboxylic acid derivatives as a framework on which the catalytic processes can be presented.

A. Structure of the Reactants

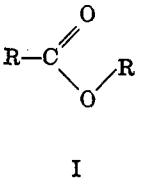
The structural feature which is common to all of the molecules whose reactions are to be discussed is the carbonyl group. The carbon atom of the carbonyl group is approximately hybridized in its sp^2 state with three planar σ-bonding orbitals, the interbond angle being 120°. The fourth orbital is a p orbital at right angles to the sp^2 hybrids. The carbonyl bond is formed by overlapping one of the sp^2 hybrids of the carbon atom with a p orbital of the oxygen atom to form the σ bond, the other p orbital of the oxygen atom forming the π bond with the carbon p orbital. Because of the greater electronegativity of the oxygen atom the π electrons will not be equally shared. Equally important to the structure of the carbonyl group are the nonbonding electrons of the oxygen atom, the $2s^2$ and $2p^2$ electrons. These electrons belong to two lone-pair orbitals (each doubly filled and nonbonding) in a plane at right angles to the π bond, with a probable angle of 120° between the lone-pair orbitals and the carbon–oxygen σ bond (113). Indirect evidence for this arrangement comes from the structure of compounds containing groups capable of hydrogen bonding to the carbonyl group (338). The lone-pair electrons of the carbonyl group are those responsible for hydrogen bonding and thus are of importance in a discussion of catalysis.

The structure of the carbonyl group is, of course, perturbed by the rest of the molecule in which it resides. Both resonance and inductive effects alter the electronic distribution of the carbonyl group. A double bond conjugated with the carbonyl group is a resonance-stabilized system in which the carbonyl group is less reactive. Conjugation with an aromatic ring also leads to resonance stabilization and lowered reactivity, especially when the carbonyl group and the ring are constrained in a planar configuration as in phthalide. The various derivatives of carboxylic acids form a series with varying degrees of resonance stabilization, decreasing in the following order (219):

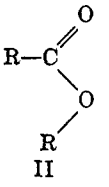


Qualitatively this order seems reasonable, from consideration of the relative ability of the top groups to donate electrons and to stabilize a positive charge. Quantitatively it is difficult to specify the order given above. For example, the resonance energies calculated from heats of combustion for acetic acid, ethyl acetate, acetamide, and acetic anhydride vary from 13 to 16 kcal./mole (244) and from 14 to 18 kcal./mole (163) for two sets of calculations, whereas the error involved in these calculations is such that the differences in resonance energies between the various molecules cannot be said to be real nor are the absolute values of great significance (401).

The dipole moments of esters are of interest as evidence for the lack of free rotation of the alkoxyl group. The dipole moment of an ester of a saturated monohydric alcohol and a saturated monocarboxylic acid is approximately 1.7–1.9 D. These moments do not vary appreciably with the temperature over ranges of as much as 190°. The temperature independence of the moment can be explained in two ways: (*1*) rotation about the carbon–oxygen bond is completely free at all temperatures or (*2*) rotation is inhibited and the molecule can assume only one of the following two forms:



I



II

Predicted moments for I and II are 1.53 and 3.53 D, respectively. The observed moment is in satisfactory agreement with the value calculated for configuration I but seems much too small to be any sort of average of the values for I and II. Therefore, I is assumed to be the correct structure of simple esters. This conclusion is further supported by the fact that the moment of γ-butyrolactone, in which the configuration is fixed in a position analogous to II by the five-membered ring, is 4.12 D in fair agreement with the value calculated for configuration II (276, 401). One explanation for the planar configuration I in normal esters is that the ester is a resonance hybrid in which the carbon–oxygen bond of the alkoxyl group has considerable double-bond character (401). Another explanation states that the hindrance to rotation is due to repulsive forces between the lone-pair electrons of the ether oxygen and either the sp^2 lone-pair electrons or the π electrons of the carbonyl group (113).

The amide group is resonance stabilized to a greater extent than the ester group. This is manifested in the geometrical structure of the peptide bond, which has been investigated intensively by x-ray analysis (313). It is further shown by the lower reactivity of the amide linkage, as well as the shorter carbon–nitrogen bond distance in amides (401).

Inductive effects have also been shown to influence the electronic distribution of the carbonyl group. They have been clearly demonstrated in the infrared frequency shifts of a number of substituted carbonyl compounds, including ketones and carboxylic acids. A number of correlations (157, 158) have been demonstrated such as (*1*) a relationship between the carbonyl stretching frequency of substituted acetophenones and the Hammett sigma constants for the substituents (169); (*2*) a relationship between the carbonyl stretching frequency of methyl ketones and the Taft polar substituent constants (235); and (*3*) the intensity of the carbonyl stretching frequency of substituted ethyl acetates and the Taft polar substituent constants (371). Dipolar field effects have also been observed by analyzing the spectra of various carbonyl compounds. α-Halogen substituents in keto steroids and cyclohexanones in an equatorial position raise the frequency of the carbonyl stretching band by about 25 cm.$^{-1}$, but in the axial position do not perturb the band at all (114, 115). This phenomenon has been explained by a field effect in which there is a mutual induction of opposite charges, resulting in the negative character of both the halogen and the carbonyl group becoming less polar. In compounds such as ethyl difluoroacetate one observes two carbonyl stretching frequencies, which presumably arise from two rotational isomers. The high-frequency peak is due to the cis (to the carbonyl) isomer and the low-frequency peak is due to the *gauche* form (30, 31, 88).

B. TYPE OF FISSION

It was specified earlier that this review is limited to those reactions of carboxylic acid derivatives which occur with acyl–oxygen fission. Both structural and isotopic tracer evidence has been utilized to indicate the type of fission in these reactions. Tracer experiments provide the most direct evidence. Polanyi and Szabo (319) studied the saponification of *n*-amyl acetate in H_2O^{18}. They observed that the amyl alcohol produced was isotopically normal and concluded that acyl–oxygen fission had occurred.

$$R-\overset{O}{\overset{\|}{C}}-O-R + H_2O^{18} \rightarrow R-\overset{O}{\overset{\|}{C}}-O^{18}-H + ROH \quad (1)$$

This conclusion is undoubtedly correct, but it has been pointed out recently that the original evidence is probably meaningless, because the method of isotopic analysis involved a step where oxygen isotopes could be lost (255). An unequivocal demonstration of acyl–oxygen fission was provided by the alkaline hydrolysis of ethyl propionate-*ether*-O^{18} (255). Analogous observations have been made in the acid hydrolysis of methyl hydrogen succinate (121) and in the basic and acidic hydrolysis of γ-butyrolactone (271).

Structural evidence for acyl–oxygen fission includes studies with esters containing optically active alcohol moieties (215), α,β-unsaturated alcohols (220), and neopentyl alcohols (306). In each case the alcohol in the product was identical with the alcohol in the reactant, indicating that no scission of the carbon–oxygen bond of the alcohol had occurred.

The possibility of two modes of fission can only arise in reactions of esters. In other carboxylic acid derivatives such as acid chlorides and amides, the mere fact of reaction is proof of acyl fission.

C. KINETIC CONSIDERATIONS

This review is limited to bimolecular reactions of carboxylic acid derivatives. Molecularity and kinetic order are not necessarily the same, nor are kinetic order and stoichiometry. In reactions involving general basic catalysis, a third component, the general base, enters into the reaction, although the overall stoichiometry indicates a bimolecular reaction. In a concerted catalytic process which involves the substrate, an electrophilic catalyst, and a nucleophilic agent, third-order kinetics can result, although again the overall stoichiometry indicates a bimolecular reaction. Finally, in solvolytic reactions the kinetic order with respect to solvent cannot be determined.

Kinetic salt effects have been observed in a number of reactions of carboxylic acid derivatives. For example, the alkaline hydrolysis of ethyl acetate exhibits a slight negative salt effect (320), as does the acidic hydrolysis of ethyl acetate (324). The salt effect on alkaline hydrolysis was found to conform to the predictions of the ion–dipolar molecule rate theory (2).

More striking salt effects in ester hydrolysis have been observed in reactions involving charged esters. In the alkaline hydrolysis of half-esters of dicarboxylic acids with alkali metal salts there is a negative specific salt effect whose magnitude depends on the distance of the charge from the reaction center (216). In reactions of esters containing a quaternary ammonium group, specific salt effects were found to be negative for acid hydrolysis and positive for alkaline hydrolysis, and again to increase as the distance increased between the positively charged quaternary nitrogen atom and the reaction center (1). These salt effects are consistent with the transition state of the reaction to be discussed later.

Ingold has discussed the effect of solvent in the S_N2 reaction of isopropyl bromide with hydroxide ion (219). He concluded that since the transition state is less polar than the ground state, the former will be stabilized less than the latter by increasing the polarity of the solvent medium. This will result in a higher energy of activation and a lower rate of reaction, as has been observed. On the basis of this argument one would expect that the rate of the saponification of an ester would also decrease as the polarity of the solvent is increased. In fact the opposite is found experimentally (42, 383). This result implies that the transition state of the base-catalyzed ester hydrolysis is more polar than the ground state, in contrast to the reaction of hydroxide ion and isopropyl bromide. The results of ester hydrolysis can be explained by considering that the activity of the polar ester molecule as well as the activities of the charged hydroxide ion and transition state must be important in determining the overall solvent effect for this reaction.

Although this review is primarily concerned with catalysis, mention should be made of the inhibition of reactions of carboxylic acid derivatives through the formation of complexes. The rate of hydrolysis of benzocaine in aqueous solution can be substantially inhibited by the addition of caffeine, a known complexing agent for benzocaine. The complexed form of benzocaine undergoes no perceptible cleavage whatever at the ester linkage (208). The rate of hydrolysis of procaine ion is also reduced in the presence of caffeine; probably this inhibition is also due to formation of a molecular complex between the drug and caffeine (258).

D. THERMODYNAMIC CONSIDERATIONS

It is of interest in a discussion of the mechanisms of catalysis to consider the equilibrium constants and standard free energy and enthalpy changes for various reactions of carboxylic acid derivatives. The information in this field is rather meager. Because a number of enzymatic processes and some of the catalyses to be discussed occur near neutrality, it is convenient to tabulate the available information in terms of $\Delta F'_0$, and $\Delta H'_0$, the standard free energy and enthalpy changes of hydrolysis at pH 7.0. This is a convention that has been used by biochemists and will permit direct comparison of this data with other hydrolytic data compiled by biochemists. A tabulation of available data is given in table 3.

The meager data in table 3 can be broken down into reactions of amides, esters, and anhydrides. The amides appear to possess relatively low standard enthalpies and free energies of hydrolysis, with the exception of *N*-acetylimidazole. It has been pointed out that the difference in free energies of hydrolysis between A,1 and A,7 is due to differences in the pK's of the ammonium group in amino acids and amino acid amides (168).

The free energies of hydrolysis of esters are somewhat higher than those of amides. In the biochemical literature there has been speculation that the free energies of hydrolysis of thiol esters are considerably higher than those of their oxygen analogs. The available data do not tend to bear out this speculation. Comparison of the free energies of hydrolysis of the oxygen ester B,1 and the thiolesters B,3 to B,5 indicates little difference. Comparison of the enthalpies of hydrolysis of a thiol ester (B,6) and of oxygen esters indicates little difference between the two (388). The free energies of hydrolysis of the anhydrides in table 3 appear to be the highest of the compounds listed.

Other thermodynamic data exist for the esterification reactions of various carboxylic acids in alcohol solutions carried out at high temperatures. In general these studies indicate equilibrium constants not far from unity for a number of systems. These studies have not been included in table 3, since it is desired to restrict the thermodynamic comparisons to reactions in aqueous solution at room temperature (155, 183).

E. SUBSTITUTION REACTIONS AT A CARBONYL CARBON ATOM

Substitution reactions occurring at a carbonyl carbon atom belong to a wide group of substitution reactions at unsaturated centers, including substitution at olefinic and aromatic carbon atoms. Nucleophilic substitution reactions have been studied in great detail in aromatic systems (96, 98) and have been investigated in a cursory way in olefinic systems (233, 292, 296); they appear to occur mainly via an addition-elimination mechanism. Theoretically, substitution reactions at an unsaturated center may proceed through one of three mechanisms: (*1*) a direct displacement mechanism, (*2*) an addition-elimination mechanism, and (*3*) an elimination-addition

TABLE 3

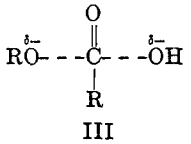
Thermodynamic quantities of some reactions of carboxylic acid derivatives in aqueous solution

Reaction	pH	Temperature	$-\Delta H'_0$	$-\Delta F'_0$	References
		°C.	kcal./mole		
A. Amides:					
1. Benzoyl-L-tyrosylglycinamide + $H_2O \rightleftarrows$ benzoyl-L-tyrosine$^-$ + glycinamide$^+$	7.9	25	1.55	0.4	(136, 167)
2. Carbobenzoxyglycyl-L-phenylalanine + $H_2O \rightleftarrows$ carbobenzoxyglycine$^-$ + L-phenylalanine			2.55		(137)
3. Carbobenzoxyglycyl-L-leucine + $H_2O \rightleftarrows$ carbobenzoxyglycine$^-$ + L-leucine	5.65		2.11		(369)
4. Poly-L-lysine + $H_2O \rightleftarrows$ *n*-lysine	7.6		1.24		(370)
5. Benzoyl-L-tyrosinamide + $H_2O \rightleftarrows$ benzoyl-L-tyrosine$^-$ + NH_4^+		25	5.84		(137)
6. Hippurate$^-$ (1 *M*) + H_2O(l) $\rightleftarrows$ benzoate$^-$ (1 *M*) + glycine	7	25		2.64	(74)
7. Alanylglycine (1 *M*) + H_2O(l) $\rightleftarrows$ alanine + glycine	7	25		4.00	(74)
8. Glutamine + $H_2O \rightleftarrows$ glutamate$^-$ + NH_4^+	7	25		3.4	(103)
9. *N*-Acetylimidazole + $H_2O \rightleftarrows$ acetate$^-$ + imidazole	7	25		12.7–15.0	(355)*
B. Esters:					
1. Acetyl-L-phenylalanine methyl ester + $H_2O \rightleftarrows$ acetyl-L-phenylalanine + methanol	7	25		6.03	(47)
2. Acetylcholine + $H_2O \rightleftarrows$ acetate$^-$ + choline$^+$	5.1	25		3.2	(207)
3. AcetylSCoA + $H_2O \rightleftarrows$ acetate$^-$ + CoASH	7.5			7.3–9.6	(205, 355)*†
4. Acetylglutathione + $H_2O \rightleftarrows$ acetate$^-$ + glutathione	7			7.1–9.4	(355)‡
5. AcetoacetylSCoA + $H_2O \rightleftarrows$ acetoacetate$^-$ + CoASH				12.0	(362)
6. Ethyl thiolacetate(l) + H_2O(l) $\rightleftarrows$ acetic acid(l) + ethanethiol(l)	7	25	0.95		(388)
C. Anhydrides:					
1. Acetyl phosphate + $H_2O \rightleftarrows$ acetate$^-$ + phosphate^{--}	7.3	29		9.9–12.2	(270, 328)†
2. Luciferyl adenylate + $H_2O \rightleftarrows$ luciferate$^-$ + adenylate^{--}	7.1			12.4–14.7	(323)
3. Acetyladenylate + $H_2O \rightleftarrows$ acetate$^-$ + adenylate^{--}	7			12 –14	(225)

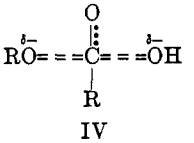
* Based on acetyl phosphate. † Based on ATP $\Delta F'_0 \approx 7.0$–9.3. ‡ Based on *N*-acetylimidazole.

mechanism. In aromatic systems the addition-elimination mechanism is the most commonly encountered pathway, although the elimination-addition path through a benzyne intermediate is also known in special cases. In olefinic systems, both the addition-elimination and the elimination-addition pathways are known (118). A direct displacement mechanism has not been observed in either aromatic or olefinic systems.

In reactions at a carbonyl carbon atom substitution could occur by a direct displacement, or through an addition-elimination or an elimination-addition reaction. The transition state in a hypothetical direct displacement mechanism for the saponification of an ester has been depicted in the following two ways:



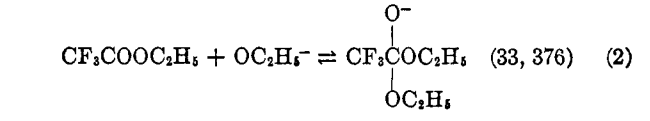
III



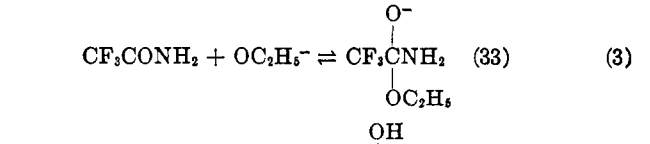
IV

III (219) is a direct analog of the S_N2 reaction applied to an ester hydrolysis. It is difficult to perceive why this transition state should form so much more readily than the corresponding transition state of the displacement of an alkoxyl group in an ether molecule. IV (128) attempts to explain the more facile reaction of an ester by involving the π orbital of the carbonyl group in a resonance-stabilized transition state.

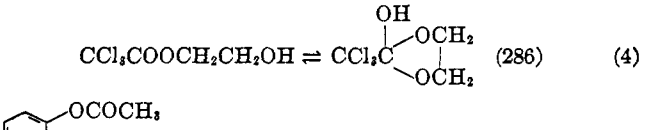
Although it is possible to produce pictorial representations for a transition state of a direct displacement reaction at a carbonyl carbon atom, these hypotheses totally ignore the most important chemical property of carbonyl groups: namely, addition. Numerous addition reactions of aldehydes and ketones are well known. In many instances stable adducts have been isolated; in other instances physical properties indicate the formation of adducts. Such addition compounds have been demonstrated for formaldehyde in aqueous solution (70), acetaldehyde in aqueous solution (26), chloral hydrate, various ketones in methanol solution (400), and various carbonyl compounds with nitrogen bases such as hydroxylamine and semicarbazide (228), to name a few. There are also a number of well-documented examples of stable addition compounds of carboxylic acid derivatives. Each of the following equilibria has been shown to be on the side of the addition compound:



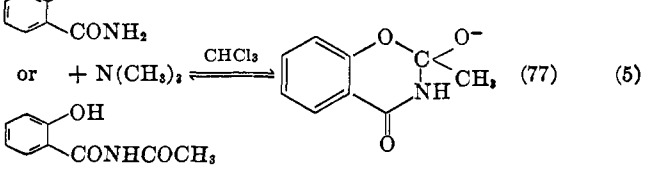
(33, 376) (2)



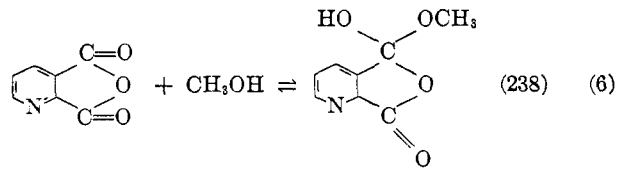
(33) (3)



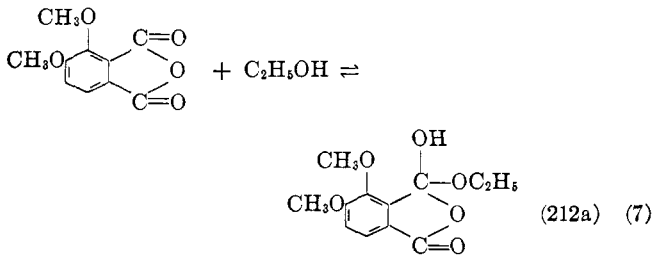
(286) (4)



(77) (5)



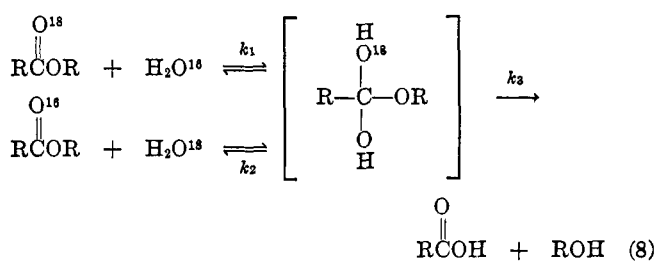
(238) (6)



(212a) (7)

Although these examples of stable addition compounds of anhydrides, amides, and esters are special cases, it is comforting to know that they do exist. They can be utilized to extrapolate to reactive addition intermediates which cannot be isolated or detected in any direct manner.

Evidence for the formation of addition intermediates in the bimolecular substitution reactions of carboxylic acid derivatives rests mainly on the finding of concurrent hydrolysis and isotopic oxygen-exchange reactions. The alkaline hydrolysis of ethyl benzoate is a second-order reaction and exhibits acyl–oxygen fission. Two possible mechanisms can be postulated on the basis of this information: one, an S_N2 displacement of the alkoxyl group by the hydroxyl ion, according to transition states III or IV; and the other, a mechanism involving the formation of an unstable addition intermediate as shown in equation 8.



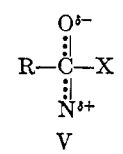
(8)

Concurrent isotopic oxygen exchange and hydrolysis of an ester (equation 8) are consistent with the mechanism involving an addition intermediate (32). On the other hand, lack of isotopic oxygen exchange is expected if a nucleophilic displacement (S_N2) reaction is operative, for then the labelled carbonyl oxygen atom is not a participant in any reversible step. Concurrent carbonyl oxygen exchange and hydrolysis have been demonstrated for the hydrolyses of a number of benzoate esters (32), benzamide (39, 99), benzoic anhydride (99), and certain substituted benzoyl chlorides (99). Many of the rate constants of concurrent hydrolysis (k_h) and exchange (k_{ex}) do not differ from one another by more than a factor of 10, although in no case are the rate constants of isotopic exchange and hydrolysis equal. The values of k_h/k_{ex} for acidic and basic catalysis of the hydrolysis of ethyl benzoate differ by a factor of approximately 2 (41). Since the rates of acidic and basic hydrolysis differ by a factor of roughly 10^4, a common intermediate, the unionized hydrate of the ester, must be formed in these instances.

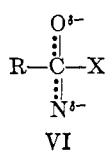
While concurrent oxygen exchange and hydrolysis have been found in a large variety of hydrolytic substitution reactions of carboxylic acid derivatives, not all such reactions which involve bimolecular substitutions do exhibit concurrent oxygen exchange and hydrolysis. In alkaline hydrolysis there is a gradation of k_h/k_{ex} from amides, which exhibit the largest oxygen exchange, to phenyl benzoate (100), benzyl benzoate, and phthalide (46), which do not exhibit detectable oxygen exchange. The latter result is probably due to the experimental difficulty of detection of the isotopic oxygen species. In general, isotopic analysis can detect only ratios of k_3/k_2 (equation 8) which are equal to or less than about 100. It is postulated that reactions in which oxygen exchange is not detected still conform to the general mechanism involving an addition intermediate but involve ratios of k_3/k_2 greater than 100. There is no reason to postulate any qualitative difference between benzyl benzoate, which shows no oxygen exchange, and methyl benzoate, which shows oxygen exchange; the only difference that is reasonable is a quantitative difference in the ratio of k_3/k_2. All evidence given above is consistent with the postulate of an addition intermediate in the bimolecular substitution reactions of carboxylic acid derivatives. Considerable additional evidence from correlations of structure with reactivity is also consistent with this hypothesis (see Section II,H) (304, 378).

F. THE TRANSITION STATE IN THE FORMATION OF THE ADDITION INTERMEDIATE

In general the formation of the addition intermediate is the slow step in the overall reaction of carboxylic acid derivatives (see Section II,G). It is of interest, therefore, to consider the structure of the transition state of this reaction. The transition state can be represented as V



V

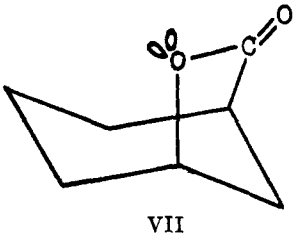


VI

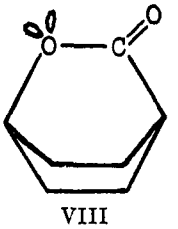
for the attack of a neutral nucleophilic agent at the carbonyl carbon atom, and as VI for the attack of a negatively charged nucleophilic agent. Of course there are many variants of these transition states, depending on the specific kinds of catalysis to be discussed later. The degree of polarization of the carbonyl group in the transition state will depend to some extent on the

identity of the nucleophilic agent. (Further discussion of this point will be deferred to the discussion of nucleophilicity at a carbonyl carbon atom (see Section IV,A).)

It is pertinent here to discuss the geometry of the transition state V or VI. Three geometries can be postulated: one in which the attacking nucleophile approaches the carboxylic acid derivative in a line perpendicular to the plane of the C(O)X group, leading to maximum overlap of the bonding orbital of the nucleophile and the π-electron cloud of the carbonyl carbon atom, and two others in which the attacking nucleophile approaches the carbonyl carbon atom from the backside with respect to the eventual leaving group or to the carbonyl group. The suggestion of backside attack with respect to the carbonyl group was made originally on the basis of the effect of structure on the reactivity of some substituted *N*-carboxyanhydrides with substituted glycine dimethylamides (11). The experimental evidence indicated a diminution in the rate of reaction when both the attacking nucleophile and the nitrogen atom of the ring (in the 3-position to the carbonyl carbon atom) contained a bulky substituent. This phenomenon was interpreted in terms of the approach of the attacking base to the carbonyl carbon atom along a line slightly inclined to the plane of the ring passing over the ring nitrogen atom. This suggestion has been utilized (189) to explain the extraordinary reactivity of certain lactones with respect to their open-chain analogs. It is postulated (189) that when hydroxide ion attacks the *cis*-lactone it avoids unfavorable electrostatic repulsion from the lone-pair atom dipole of oxygen by a backside attack with respect to the carbonyl group; in open-chain trans esters this electrostatic repulsion cannot be avoided, so esters react slowly. The kinetics of hydrolysis of 6-oxabicyclo[3.3.1]octan-7-one (VII) and 2-oxabicyclo[2.2.2]octan-3-one (VIII) are germane to these arguments. The conformational formulas show that rearward attack as described above



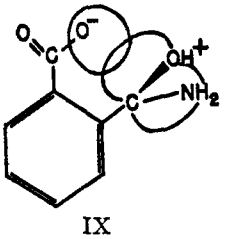
VII



VIII

is possible for VII over the five-membered ring, and the rate constant approximates those for other five-membered lactones which undergo saponification about 10^3 faster than acyclic esters. Rearward attack is impossible for VIII, however, and the rate constant is lowered to a value ten times above that for an aliphatic ester. Since rearward attack cannot occur in this instance, it was postulated that attack at the maximum electron density of the *p* orbital of the carbonyl group takes place (perpendicular attack) (189).

The author disagrees with the views expressed above and believes that the best approximation to the transition state is a system in which the attacking nucleophile is approximately perpendicular to the plane of the carboxylic acid grouping. Such a transition state would be favored on theoretical grounds on the basis that this configuration gives maximum overlap of the nucleophile and the π-electron cloud of the carboxylic acid groups. Such a transition state also follows the Hammond postulate of the resemblance of a transition state and an unstable intermediate (193). The structure–reactivity arguments involving *N*-carboxyanhydrides can be explained on this basis, since the bulky groups involved in that study lead to steric repulsions in models of the "perpendicular transition state." The reactivity of lactones can also be explained in terms of this transition state, again invoking a difference in electrostatic repulsion from the "lone-pair atom dipole" of oxygen in the lactone and open-chain ester. The reactivity of VIII is of great significance; this compound reacts ten times faster than an ordinary ester, even with backside approach completely blocked. This result means that π-electron approach must be very important. Finally, the intramolecular step of phthalamic acid hydrolysis, the conversion of phthalamic acid to phthalic anhydride (35, 38) (see Section V,A), offers convincing stereochemical evidence for a transition state involving perpendicular π-electron approach. In this case the nucleophilic attack of *o*-carboxylate ion on the protonated amide is impossible, since the two groups cannot lie in the same plane (of the benzene ring). On the other hand, π-orbital attack is very easy, as shown in IX, and the reaction is extremely facile. The present experimental evidence is consistent with and is explained most adequately by a transition state for bimolecular substitution reactions of carboxylic acid derivatives involving perpendicular approach to the carbonyl carbon atom by the attacking nucleophile.



IX

G. STEPWISE NATURE OF THE NUCLEOPHILIC REACTIONS OF CARBOXYLIC ACID DERIVATIVES

The existence of an intermediate in the reactions of carboxylic acid derivatives requires that these reactions be stepwise in nature. The reaction sequence

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_3} C \qquad (9)$$

represents a simplified stepwise reaction. It is possible to treat the kinetics of this process by the steady-state

approximation: $dB/dt = k_1A - k_2B - k_3B = 0$. It can then be shown that

$$-dA/dt = dC/dt = [k_1k_3/(k_2 + k_3)]A = [k_1/((k_2/k_3) + 1)]A \quad (10)$$

If k_3 and k_2 are of comparable magnitude (case A), equation 10 cannot be simplified; the overall rate is affected both by the rate of formation of the addition intermediate (k_1) and by the partitioning of the intermediate (k_2/k_3). If k_2 is large compared to k_3, a preequilibrium occurs followed by a rate-determining step (case B). This situation leads to $-dA/dt = dC/dt = k_3KA$, where $K = k_1/k_2$.

Case A is presumed to be the general situation in bimolecular substitution reactions of carboxylic acid derivatives. Case B does apparently hold in a limited number of special cases, including the reaction of amines with *N*-carboxysarcosine anhydride (13) and the lactamization of phenyl γ-(4-imidazolyl)butyrates (95). Both of these special examples involve the addition of a nitrogen nucleophile to the carbonyl group. Nitrogen nucleophiles such as hydroxylamine and semicarbazide add to aldehydes and ketones in neutral solution in a fast preëquilibrium step (followed by a slow dehydration to form the oxime or semicarbazone) (228). From this scanty evidence there appears to be a tendency for nitrogen nucleophiles to add in a fast preëquilibrium to carbonyl compounds. It is conceivable that unstable addition intermediates can be detected in the carboxylic acid family, as they have been detected in the carbonyl family, by the use of nitrogen nucleophiles (228).

H. THE EFFECT OF STRUCTURE ON REACTIVITY

Since the overall rate constant for the reaction of a carboxylic acid derivative is a function of two parameters, the rate constant for the formation of the addition intermediate (k_1) and the partitioning of the intermediate (α or k_2/k_3), any discussion of the effect of structure on reactivity must be couched in those terms. One must further consider structural changes in both the attacking nucleophile and the carboxylic acid de-

$$\mathrm{R\overset{O}{\overset{\|}{C}}X + Y^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R\overset{O^-}{\overset{|}{C}}X\overset{|}{Y} \xrightarrow{k_3} R\overset{O}{\overset{\|}{C}}Y + X^-} \quad (11)$$

rivative, both of which will intimately affect both k_1 and α.

1. The carboxylic acid derivative

Let us first consider structural changes in the carboxylic acid derivative, RCOX, and their effect on k_1 and α. The effects of structural changes in R are relatively easy to assess. An increase in the electron-attracting character of R will result in an increase in k_1. This prediction assumes a relationship between the rates and equilibria of addition reactions of carbonyl compounds and appears to hold well for those systems for which a comparison can be made. Table 4 gives a comparison of relative reactivities and equilibria of halogenated esters. Resonance interactions involving R and the carbonyl group tend to stabilize the ground state with respect to the transition state, which must be quite similar electronically to the tetrahedral addition intermediate. Taft has been able to separate resonance and inductive effects in ester hydrolysis and related reactions (378). He has also considered steric effects which are omitted from discussion here.

TABLE 4

Relative reactivities and addition equilibria of halogenated esters (33)

Ester	Relative k_o^{OH} of Ethyl Chloroacetates	Addition of Methoxide Ion to Ethyl Fluoroacetates
		per cent
Acetate	1	0
Monohaloacetate	761	26
Dihaloacetate	16,000	77
Trihaloacetate	100,000	99

It is assumed in the discussion above that a structural change in R will not affect α, and thus k_1 and k_{obs} will be affected to an equal extent. This hypothesis is being tested at present by the determination of the oxygen exchange accompanying the hydrolysis of a series of substituted ethyl benzoates.

The effects of structural changes in X of RCOX are more difficult to ascertain. An imperfect generalization can be made that structural changes that increase the electron-withdrawing power of X will increase k_1 and decrease α, both effects tending to increase k_{obs}. The effect of electron-withdrawing groups in X on k_1 follows by analogy, from the effect of electron-withdrawing groups in R on k_1. In esters the effect of an inductive change in X is usually less pronounced than a comparable change in R because of the greater distance of the substituent in X from the reactive center. Resonance effects of the group X must also be considered. Increased resonance interaction between X and the carbonyl group will in general increase the stability of the ground state relative to the transition state and will, therefore, decrease k_1. Examples of inductive effects on k_1 (and α) can be seen in the rates of hydrolysis of substituted phenyl acetates. Examples of resonance effects on k_1 can be seen in the relative rates of hydrolysis of acid chlorides, anhydrides, esters, and amides. Resonance interaction increases and reactivity decreases in the above order.

The effect of structural changes in X on the partitioning of the addition intermediate (α) can be seen from the oxygen-exchange data in table 5. Structural changes which decrease k_1 lead to an increase in α, both effects tending to reduce the overall rate of the reaction.

TABLE 5

Oxygen exchange during the hydrolysis of carboxylic acid derivatives

Reaction	Temperature	Leaving Group	$k_{hydrolysis}/k_{exchange}$	$10^4 k_1$	$k_2/k_3 = \alpha$	Reference
	°C.			*l./mole sec.*		
Benzamide + OH^-	40	NH_2^-	0.53	2.09	4.22	(41)
Ethyl benzoate + OH^-*	25	$OC_2H_5^-$	11.3	97	0.18	(41)
Benzoic anhydride + H_2O‡	25	$C_6H_5COO^-$	20	0.17†	0.10	(99)
Benzoyl chloride + H_2O*	25	Cl^-	25	9.3†	0.08	(99)

* 33 per cent dioxane–water. † Per second. ‡ 75 per cent dioxane–water.

The effect on k_1 is seen to be much greater than the effect on α, indicating that the important factor in these cases is the effect on k_1. It is postulated that the effect of structural changes on α may be equal to or perhaps greater than the effect on k_1 in a series of para-substituted acetanilides. In this system structural changes in X are remote from the carbonyl group and no resonance interaction is possible.

2. *The nucleophile*

While structural effects of the carboxylic acid derivative have long been considered, the structural effects of the nucleophile have not been considered in detail heretofore. This aspect of the reactions of carboxylic acid derivatives is of direct and great importance to a consideration of nucleophilic catalysis (Section IV).

There are a whole host of reactions in which one carboxylic acid derivative is converted to another by the action of a nucleophile. Many nucleophiles containing a negative oxygen atom such as hydroxide, alkoxide, phenoxide, carboxylate, phosphate (89), arsenate, nitrite (181), oxime, chloralate (173), hypochlorite (229a), and hydroperoxide ions will react with a carboxylic acid derivative. In some cases a nucleophile containing an uncharged oxygen atom such as water or alcohol will react with carboxylic acid derivatives. Likewise, most nucleophiles containing a nitrogen atom such as primary, secondary, and tertiary amines, both aliphatic and heterocyclic, react with carboxylic acid derivatives. In addition, a number of other nucleophiles such as cyanide, sulfite, fluoride, azide, and thiolate ions have been shown to react with carboxylic acid derivatives.

Not all carboxylic acid derivatives are susceptible to nucleophilic attack by all the substances listed above. It is pointed out in Section II,H,4 that the more reactive carboxylic acid derivatives are less selective in their reactions with nucleophiles. Thus it is the more reactive carboxylic acid derivatives which exhibit reactivity with a wide range of nucleophiles, while the less reactive carboxylic acid derivatives react with only a limited number of nucleophiles. This fact has led to a number of important conclusions, including the observation of catalysis by nucleophiles other than hydroxide ion with only a limited number of relatively highly reactive carboxylic acid derivatives. Furthermore, the most extensive comparative studies of nucleophilicity have been carried out with carboxylic acid derivatives of high reactivity.

As pointed out above, the overall rate constant of the forward reaction of RCOX + Y will depend on k_1 and the partitioning of the intermediate, k_3/k_2 (405). k_1 depends on the nucleophilicity of the attacking agent, the parameter to be discussed here; k_2/k_3, on the other hand, depends on the relative stabilities of the reactants and products, including the relative stabilities of the nucleophiles X and Y as well as the carboxylic acid derivatives RCOX and RCOY. This kinetic dependency complicates the discussion of nucleophilicity. In the following discussion, where restricted structural changes are made, the overall rate constant will be assumed to be a function of the nucleophilicity. In some cases this oversimplification may be justified; in the general case it is not.

From data on the kinetics of the simultaneous alkaline hydrolysis and alcoholysis of esters in aqueous alcohol solutions it is possible to determine the relative nucleophilicities of hydroxide ion, methoxide ion, and ethoxide ion, as shown in table 6. It is seen from this table that the relative nucleophilicities, E_n, calculated from the equation of Edwards (145), follow the order ethoxide ion > methoxide ion > hydroxide ion, which parallels the experimentally determined rate constants. On the other hand, the relative basicities do not fall in this order. Although there is a parallelism between the relative rate constants and the calculated nucleophilicities, the nucleophilicities calculated from the equation $\log k/k_0 = AP + BH$ (145) contain contributions from the basicity term which in all cases amount to over 90 per cent of the total rate of the reaction, indicating that the basicity of the attacking nucleophile is the main factor in the relative reactivity of this group of nucleophiles toward the carbonyl carbon atom of esters.

A relationship between the relative basicities and the relative nucleophilicities of a family of nucleophiles implies that linear free-energy relationships similar to

TABLE 6

Nucleophilicities, polarizabilities, and basicities of some oxygen bases

Donor	pK_a	E_n (calculated)	Nucleophilicity to C=O†
H_2O	−1.74	0.00	—
HO^-	15.8	1.60	1.0
CH_3O^-	15.0	2.74*	1.6
$C_2H_5O^-$	16.6	3.28*	4.2

* Calculated from $E_n = AP + BH$.

† Calculated from the ratio k_{OR}/k_{OH} for the two substrates used in the investigation.

TABLE 7

The effect of structure on reactivity in some reactions of carboxylic acid derivatives with nucleophiles

I. Reaction series in which a relationship is found between log k_N and pK_N

Reaction	Solvent	Temperature	Number of Points	$-\log k_0$	Slope	Reference
		°C.				
1. Pyridines + phenyl acetate	Water	109	3	2.92	1.31	(305)
2. Pyridines + *p*-nitrophenyl acetate	Water	25	3	2.75	1.075	(305)
3. Imidazoles + *p*-nitrophenyl acetate	28.5% ethanol–water	30	5	1.3	0.8	(92)
4. Pyridines + acetic anhydride	Water	0	4	2.18	1.015	(176)
5. Pyridines + 2,4-dinitrophenyl acetate	Water	25	3	−0.3	0.914	(305)
6. Pyridines + acetic formic anhydride	Toluene	39.92	2	2.7	0.513	(177)
7. Phenoxides + *p*-nitrophenyl acetate	28.5% ethanol–water	30	3	−1.3	0.8	(89)
8. Amino acids + acetic anhydride	Water	0	9	−3.2	0.375	(83)
9. Amino acids + 2,4-dinitrofluorobenzene	Water	25	9	0.7	0.513	(83)
10. Amino acids + anhydro-*N*-carboxyglycine	Water	0	3	4.2	0.46	(22)
11. Amino acids + anhydro-*N*-carboxyalanine	Water	0	6	3.7	0.554	(22)
12. Anilines + benzoyl chloride	Nitrobenzene	25	8		1.2	(75)
13. Anilines + *p*-nitrophenyl acetate	28.5% ethanol–water	30	3		0.8	(89)

II. Reaction series in which a relationship is found between log k/k_0 and sigma

1. Hydroxide ion + phenyl acetates	60% acetone–water	25	7	0.3	1.00	(91)
2. Imidazole + phenyl acetates	28.5% ethanol–water	25	5	2.7	1.90	(91)
3. Intra-imidazole + phenyl acetates			3			(95)

Brønsted plots should be found in these cases. In fact, a number of such relationships have been demonstrated in the nucleophilic reactions of carboxylic acid derivatives, as shown in figure 1 and table 7. Brønsted-type relationships have been found to hold in reactions involving families of substituted phenoxide ions, pyridines, imidazoles, amino acids, and anilines. Some of the Brønsted-type plots contain insufficient experimental data. Taken together, however, there is no question that a relationship does exist between basicity and nucleophilicity in reactions of restricted families of nucleophiles.

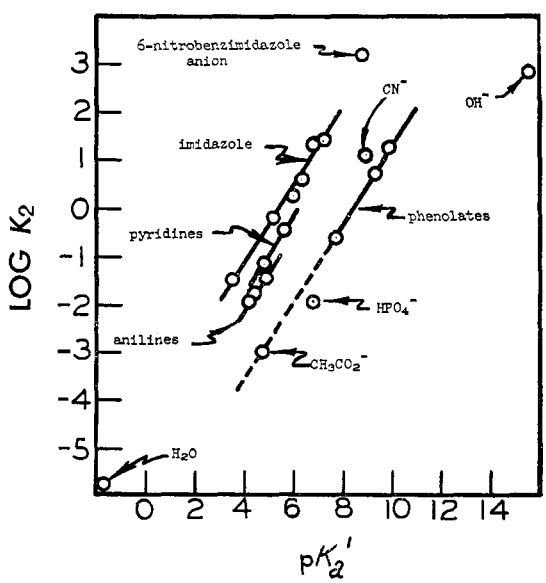


FIG. 1. Brønsted type plot of log k_2 vs. pK_a' for the reaction of nucleophiles with *p*-nitrophenyl acetate (89).

From these studies it is evident that there is a considerable disparity between the nucleophilicity of substances containing a nitrogen atom as the attacking nucleophile and substances of the same basicity having an oxygen anion as the attacking nucleophile. For example, while acetate ion and pyridine are of comparable basicity, pyridine exhibits a nucleophilicity about one hundredfold greater (figure 1). The same disparity is found to a somewhat lesser extent when comparing anilines and phenoxide ions of comparable basicity. An extreme example of this phenomenon is the greater nucleophilicity of aniline toward benzoyl chloride than either hydroxide ion or acetate ion (375a). These differences result in the generalization that separate Brønsted-type plots are necessary to characterize nitrogen and oxygen bases.

Two pieces of evidence indicate that thiolate anions are better than the corresponding alkoxide anions in nucleophilic reactions at the carbonyl carbon atom. A rough comparison of the reactivities of the sulfhydryl ion and the hydroxide ion toward benzonitrile indicates that the former is a better nucleophile than the latter by one or two powers of 10 (404). Furthermore, the reactions of *p*-nitrophenyl acetate with the dianion of *o*-mercaptobenzoic acid (341) and with a phenoxide ion of equivalent acidity (89) again indicate that the former is a better nucleophile by one or two powers of 10. Thiosulfate and thiocyanate, however, are extremely unreactive toward ethyl chloroformate (182). It is not clear why this apparent discrepancy between thiolate ions and other sulfur anions exists.

When the requirement of restricted families of nucleophiles is lifted, the comparison of nucleophilicity

TABLE 8

Reactions of nucleophiles with ethyl chloroformate and p-nitrophenyl acetate (226, 229a)

Nucleophile	pK_a	Ethyl Chloroformate (182)* k_2	*p*-Nitrophenyl Acetate† k_2
		l./mole minute	*l./mole minute*
H_2O	−1.7		6×10^{-7}
$S_2O_3^{--}$	1.9		0.0011
F^-	3.1	0.24	0.001
NO_2^-	3.4	32.2	0.0013
NCO^-	3.5		0.006
4-Aminopyridine *N*-oxide	3.7		0.57
N_3^-	4.0	17.5	2.2
Aniline	4.6		0.015
Acetate ion	4.8		0.00051
$(CH_3)_2NOH$	5.2		10.7
Pyridine	5.4		0.10
N-Hydroxyphthalimide	6.1		28.9
Hydroxylamine	6.2		110.
Carnosine	6.8		10.4
Histidylhistidine	6.8		5.6
$HAsO_4^{--}$	6.8		0.041
HPO_4^{--}	6.9		0.0074
Imidazole	7.0		29
Ethylenediamine	7.0		2.0
$(CH_3)_2NNH_2$	7.2		0.73
N-Hydroxypiperidine	7.7		7.5
OCl^-	7.1		1600
SO_3^{--}	7.1		46
Hydrazine	8.1		~350
Tris(hydroxymethyl)-aminomethane	8.1		0.070
Isonitrosoacetone	8.3		2000
Salicylaldoxime	9.2		3200
CN^-	9.3		10.8
Mercaptoethanol	9.5		700
Ethylenediamine	10.0		420
Sodium mercaptoacetate	10.3		2500
Phenol	10.0	90.6	105
CO_3^{--}	10.4		1.06
CH_3COO^-	11.5		100,000
HOO^-	11.7		270,000
$(CH_3)_2C{=}NO^-$	12.4	500	1670
OH^-	15.7	169	890

* At 25°C. in 85.15 per cent water–acetone.
† At 25°C. in aqueous solution.

toward a carbonyl carbon atom becomes more difficult. Table 8 illustrates the reactions of ethyl chloroformate and *p*-nitrophenyl acetate with a number of widely varying nucleophiles. In addition, chloride, bromide, iodide, thiocyanate, and nitrate ions had no effect on the rate of reaction of either the ethyl chloroformate or the *p*-nitrophenyl acetate. This result is in wide disagreement with the reactivity order typical of a bimolecular (S_N2) displacement reaction at a saturated carbon atom in which such nucleophiles as thiosulfate, thiocyanate, and iodide are among the most reactive. Since the transition state of the carboxylic acid reactions resembles a tetrahedral intermediate, the energy of bond formation may be more important in the reactions of nucleophiles at a carbonyl carbon atom than at a saturated carbon atom, particularly with those elements (such as first row elements) which possess a high heat of formation, with carbon exhibiting the highest nucleophilicity (182). The anions of hydrogen peroxide, methyl hydroperoxide, and hydrazoic acid react abnormally rapidly (with respect to their basicities). This abnormal reactivity is not due to a concerted acid-base attack (227) with hydrogen bonding, but may be correlated with the high polarizability of peroxide and azide ions (229a). Hydroxylamine and hydrazine, which have normal polarizabilities, *N*,*N*-dimethylhydroxylamine, *N*-hydroxypiperidine, pyridine *N*-oxides and anions of hydroxamic acids, relatively acidic oximes, and hypochlorous acid also react abnormally rapidly. While there is a very rough correlation evident in table 8 between basicity and nucleophilicity, there appear to be many additional factors which must be considered to explain the nucleophilicity values, including polarizability, steric factors, electronic overlap, hydrogen bonding, and proton transfer (229a).

3. Summary of the effect of structure on reactivity

Summarizing the various effects of structure on the reaction of RCOX + Y a rather complex picture emerges, as shown in table 9. In changes involving X and Y, the situation is fairly straightforward, since a structural change tends to affect k_{obs} in the same direction through both k_1 and α. A structural change in R, however, results in opposing effects in k_1 and α, but apparently in all known cases it is the effect on k_1 which is dominant.

At the present time quantitative calculations involving these six effects (structural changes of R, X, and Y on k_1 and α) are not possible and one must be content with qualitative arguments such as those given above except in restricted series of reactions where only one or two parameters are changed at any one time. Table 10 lists such quantitative correlations. In summary, then, the effects of structural changes on nucleophilic reactions of carboxylic derivatives are reflected in both the rate constant of the addition step (k_1) and the partitioning of the intermediate ($\alpha = k_2/k_3$). The effects of structural changes on k_1 are more important than and usually are parallel to effects on α. For a complete analysis, however, effects on both parameters must be considered.

If one considers the general equilibrium case, the more complete scheme can be written:

$$\text{RCOX} + \text{Y} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{I} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{RCOY} + \text{X} \qquad (12)$$

TABLE 9

Effect of structural changes on the rate of reaction of RCOX + Y

Increase in Electron-attracting Power of a Substituent in	Effect on k_1	Effect on α	Effect on k_{obs}
R	Increase	Increase	Increase
X	Increase	Decrease	Increase
Y	Decrease	Increase	Decrease

TABLE 10

Quantitative correlations in reactions of carboxylic acid derivatives

Type of Correlation	Reference
1. Hammett relationships involving changes in R on k_{obs}	(224)
2. Taft relationships involving changes in R on k_{obs}	(378)
3. Hammett relationships involving changes in X on k_{obs}	(224)
4. Taft relationships involving changes in X on k_{obs}	(378)
5. Bronsted relationships involving changes in N on k_{obs}*	Section II,H,2
6. Hammett relationships involving changes in X on α (and k_1)	In progress
7. Hammett relationships involving changes in R on α (and k_1)	In progress
8. Hammett relationships involving changes in N on k_1 and α	No known method at present

* These can alternatively be expressed as Hammett relationships.

The equilibrium constant can be expressed as

$$K = \frac{(RCOY)(X)}{(RCOX)(Y)} = \frac{k_1k_3}{k_2k_4} \quad (13)$$

In this most general formulation it is seen that a catalysis can be manifested in any one of four steps: two steps, k_1 and k_4, which express addition to the carbonyl group, and two steps, k_2 and k_3, which denote the partitioning of the intermediate.

4. Selectivity–reactivity relationships in nucleophilic reactions

In many organic reactions it has been observed that a family of related reactions will show larger differences in the rates of reaction of the individual members if the inherent "reactivity" of these reactions is smaller. This concept has been expressed by H. C. Brown in a quantitative relationship between the *reactivity* of a system and its *selectivity*, and has been enunciated and exemplified in great detail in a large number of aromatic substitution reactions (84, 85, 86, 87). This relationship is a general phenomenon in chemical reactions. It is instructive to consider the Brønsted and Hammett relationships of the nucleophilic reactions of carboxylic acid derivatives in this regard, as shown in table 7. In the various linear relationships a slope, sometimes referred to as a Brønsted α and sometimes a Hammett ρ, can be calculated. In either of these cases *the slope is a function of the sensitivity of the rate constant to the structural changes in the family of reactions.* Under consideration now is the relationship of the various slopes to one another. The limiting values of the magnitude of the slopes are illustrative of the selectivity–reactivity relationship: when the slope is zero, the rate of reaction is insensitive to structural change of the members of the family and follows a statistically determined course; when the slope is infinity, the rate of reaction is infinitely sensitive to structural change of the members of the family and indicates reactions with infinitely high activation energies. Between these two extremes lie the real cases cited in table 7. It is postulated that in a restricted set of reaction families (which occur in a common solvent and at a common temperature and which are structurally related to one another), that family with the highest slope will be the most selective and least reactive and that family with the lowest slope will be the least selective and most reactive. Theoretically a relationship between selectivity (slope) and reactivity (the intercept) should exist if it can be shown that both the variation in the leaving group and the variation in the attacking agent follow Hammett relationships between structure and reactivity. In such a situation it can be rigorously shown that a linear relationship must exist between the slopes of the various Brønsted lines and the intercepts of the various lines. This conclusion follows from a study of multiple structure–reactivity correlations (291). If y is a function of x and z and if a multiple structure–reactivity correlation is considered such that two independent sets of lines are involved, such as

$$y_{ij} = a_ix_i + b_j \quad (14)$$

where the parameter z is held constant and

$$y_{ij} = c_iz_j + d_i \quad (15)$$

where the parameter x is held constant, then a four-parameter equation can be written such as

$$y = px + qxz + rz + s \quad (16)$$

Equation 14 represents the Brønsted plots; the slopes, a_i, and the intercepts, b_i, of these plots are then related to one another by equation 17 (305).

$$r/q(a_n - a_m) = b_n - b_m \quad (17)$$

Equation 17 indicates a linear relationship between the Brønsted slope, a, defined as the selectivity of the compound, and the intercept, b, defined as the inherent reactivity of the compound. Families 1, 2, 4, and 5 of table 7 conform to the conditions set forth above for a quantitative relationship between selectivity and reactivity. The data for these three families of reactions have been plotted in figure 2, which indicates that log

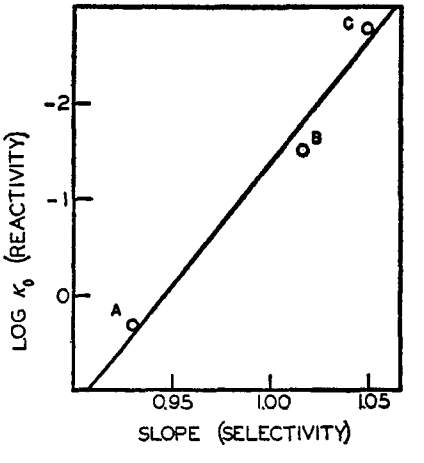


FIG. 2. Relationship between reactivity and selectivity in the nucleophilic reactions of carboxylic acid derivatives. A, pyridines and 2,4-dinitrophenyl acetate; B, pyridines and acetic anhydride; C, pyridines and *p*-nitrophenyl acetate.

k_0 (a_i) is a linear function of the slope (b_i). While the data are not very extensive in this case, a quantitative relationship has been demonstrated between reactivity and selectivity in one restricted set of carboxylic acid reactions.

With families other than 2, 4, and 5 it cannot be demonstrated that the variations in both the leaving group and the attacking agent follow Hammett relationships. Therefore with these families a rigorous argument for selectivity–reactivity relationships cannot be made. However, empirically such relationships do appear to hold, such as with families I,8 and I,9, families I,2 and I,3, families I,10 and I,11, and families II,1 and II,2. In each of these cases the family of reactions which is less reactive (smaller log k_0) is more sensitive to structural change, as indicated by a larger slope. Although these correlations are not rigorous, as is the correlation shown in figure 2, the weight of their empirical evidence strongly favors a selectivity–reactivity relationship.

III. Acidic Catalysis

A. Specific hydronium-ion catalysis

Catalysts have been defined as "substances which change the velocity of a given reaction without modification of the (equilibrium) energy factors of the reaction" (309), or "substances whose concentration occurs in the velocity expression to a higher power than in the stoichiometric equation" (24). Some of the earliest physicochemical investigations of catalysis involved the reactions of carboxylic acid derivatives, including the effect of acids on the hydrolysis of esters (310) and the formation of esters (178). In aqueous solutions which, of course, contain hydronium ions, hydroxide ions, and water, the general expression for the observed velocity constant of a hydrolytic reaction may be written as

$$k = k_0 + k_{H^+}(H^+) + k_{OH^-}(OH^-) \qquad (18)$$

The variation of velocity over the whole range of hydrogen-ion concentrations can be represented by plotting log k against pH. The most general curves observed in hydrolytic reactions of carboxylic acid derivatives are shown in figure 3. Minima and maxima are observed in some curves. The velocity at a minimum point depends on the "spontaneous" reaction, k_0,

$$k_{min} = k_0 + 2\sqrt{(K_w k_{H^+} k_{OH^-})} \qquad (19)$$

as well as the acid- and base-catalyzed reactions. The maximum will be discussed in more detail later. The lines with slopes of 45° correspond to catalyses by hydronium ion or hydroxide ion. The horizontal lines correspond to the spontaneous reaction k_0, ordinarily a water reaction. Catalyses by hydronium ion and hydroxide ion were for many years thought to represent the sole forms of catalysis of carboxylic acid derivatives. The possibilities have now increased to embrace many new forms of catalysis, of which catalysis by hydronium and hydroxide ions is only a small part.

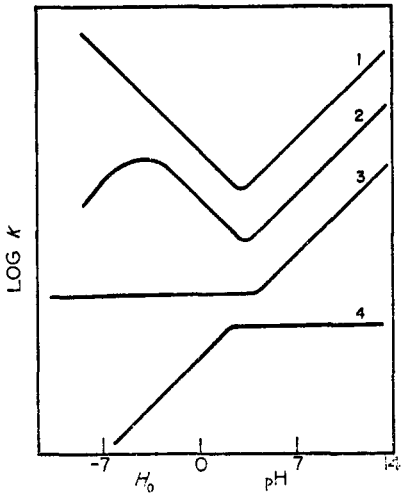


Fig. 3. Some relationships between pH and reaction velocity in the hydrolytic reactions of carboxylic acid derivatives. Curve 1, ethyl acetate; curve 2, acetamide; curve 3, β-lactone; curve 4, methyl benzimidate.

Most carboxylic acid derivatives are weak bases which in acidic solution are protonated to some extent. Methyl benzimidate is a moderately strong base ($pK_a' = 5.67$) (365) and below about pH 3 its protonation approaches completion. Amides are reasonably strong bases with pK's of the order of -1 to -3 (141). pK_a's of this order indicate that in mineral acid solutions of moderate concentration (3–6 M) amides are appreciably protonated. Carboxylic acids are somewhat less basic than amides; the pK_a's of substituted benzoic acids are of the order of -6 to -8 (363). The basicity of esters is not known but is presumably similar to that of acids.

There are two possibilities for the position of the proton in a protonated carboxylic acid derivative, a protonated carbonyl group and a protonated "X" group. From the effect of substituents on the pK_a's of substituted benzoic acids in comparison to the effect of substituents on the pK_a's of acetophenones, it appears that the protonated benzoic acid must be written as $RC(OH)_2^+$ (364). The argument is particularly convincing with regard to those substituents which can stabilize a charge on a benzylic carbon atom which would be formed if the carbonyl group is protonated. The mode of protonation of amides was investigated by means of nuclear magnetic resonance (162). The fact that the methyl doublet in N,N-dimethylformamide is retained in solutions of high acidity indicates that the carbon–nitrogen bond still has considerable double-bond character under those conditions, indicating protonation on the oxygen atom of the amide. From these two cases it appears that the position of protonation of carboxylic acid derivatives is on the oxygen atom of the carbonyl group. This protonated species is

not necessarily the kinetically important one, but for a first approximation it will be assumed that this is the case.

Ethyl acetate, benzamide, and methyl benzimidate all exhibit hydrolysis catalyzed by hydronium ion. It is interesting to note, however, that these three hydrolyses exhibit entirely different pH–rate profiles, as shown in figure 3. The kinetics of the hydrolysis of ethyl acetate in acidic solutions as concentrated as 10 M hydrochloric acid and 7 M sulfuric acid exhibit a direct proportionality between the reaction rate and the concentration of the acid (27). This behavior is exemplified by curve 1 of figure 3 and is strong evidence for the acid catalysis of the hydrolysis of esters.

A more complicated case embodies the effect of acidity on the hydrolysis of amides. The kinetics of these systems have been extensively studied. In dilute acid solution the rate of hydrolysis is proportional to the hydronium-ion concentration. The most significant feature of the kinetics, however, is that a rate maximum, as illustrated in curve 2 of figure 3, has been found to occur in the hydrolysis of a number of aliphatic and aromatic amides in strong acid (153, 252, 321, 380). The concentration of sulfuric acid for maximum rate varies from about 2.5 to 5 M, depending on the structure of the amide, where amides would be expected to be appreciably protonated. The hypothesis of a protonated amide intermediate effectively accounts for all the experimental facts, as shown in equation 20.

$$RCONH_2 + H^+ \rightleftharpoons RC(OH)=NH_2^+ \xrightarrow{H_2O} \text{hydrolysis products} \quad (20)$$

Below the concentration for maximum rate, the effect of increasing acid strength is chiefly to increase the concentration of the protonated intermediate; above the concentration for maximum rate, the effect is chiefly to decrease the concentration or activity of the water (252). This hypothesis can account quantitatively for the changes in the rate of hydrolysis with concentration of the acid (141). The experimental rate constant, k_e, can be related to the second-order rate constant, k_2, by the expression

$$k_e = k_2K(H_3O^+)/(K + h_0) \quad (21)$$

where K is the equilibrium constant for the protonation of the amide and h_0 is the Hammett acidity function. For very weak bases such as esters, $K \gg h_0$ in the range of acid concentration of 2–5 M sulfuric acid and equation 21 simplifies to

$$k_e = k_2(H_3O^+) \quad (22)$$

Equation 22 predicts a linear dependence of the experimental first-order constant on the concentration of hydronium ion which has been found for the hydrolysis of esters in strong acid solution (27). However, when $K \ll h_0$, equation 21 simplifies to

$$k_e = k_2K(H_3O^+)/h_0 \quad (23)$$

Since h_0 increases with concentration much more rapidly than (H_3O^+) when concentrations of the mineral acid exceed about 2 M, k_e will decrease in high concentrations of acid. When $K \ll h_0$, ionization to the protonated amide will be substantially complete; the decrease in rate of hydrolysis can then be regarded as due to the decreasing availability of water, since $(H_2O) = K_A(H_3O^+)/h_0$, where K_A is the equilibrium constant for the reaction $H_3O^+ \rightleftharpoons H_2O + H^+$.

A further corroboration of the mechanism of the acid-catalyzed hydrolysis of an amide stems from the effect of structure on the hydrolysis of amides. In the hydrolysis of substituted benzamides the effect of a substituent on the overall rate constant could be manifested in either the preëquilibrium step, in the subsequent rate-determining step, or in both steps. The Hammett ρ constants for the various steps bear the following relationship to one another: $\rho_{overall} = \rho_1 + \rho_2$, where 1 and 2 refer to the preëquilibrium and rate-determining steps, respectively. In this system it is possible to determine each of the three ρ constants independently of one another. It was found that the theoretical equation given above for the relationship of the various ρ constants was closely approximated by the experimental data (266).

In the acid-catalyzed hydrolysis of methyl benzimidate (142) below pH 1, the effect of increasing acidity can best be described by curve 4 of figure 3, a decrease in the rate constant with increasing acidity. This unusual phenomenon can be easily explained in terms of the analysis given above for amide hydrolysis. The much greater basicity of methyl benzimidate simply exaggerates the effect found in the amide case, where the decrease in the rate of hydrolysis with increasing acidity can be regarded as due to the decreasing availability of water.

The kinetic requirement of a molecule of water in the transition state of the acid-catalyzed hydrolysis of methyl acetate was demonstrated by carrying out the reaction in acetone solution with a limiting amount of water (164). In acid-catalyzed ester hydrolysis, the dependence of the rate on acid may, however, be somewhat greater than that predicted from dependence on the concentration of hydronium ion, and can be better fitted above 3 M acid by a dependence on a hydrogen ion which is solvated by four water molecules (265).

A special acid-catalyzed hydrolysis is that of benzoyl fluoride by dilute solutions of hydrochloric acid in aqueous acetone (69). This example constitutes the only catalysis of the hydrolysis of an acid halide by hydronium ion, and is presumably due to the special hydrogen-bonding properties of the fluorine atom.

The effect of deuterium oxide on the rates of a number of acid-catalyzed hydrolyses of carboxylic acid derivatives has been determined. Since the deuteronium ion in deuterium oxide is a stronger acid by a factor of

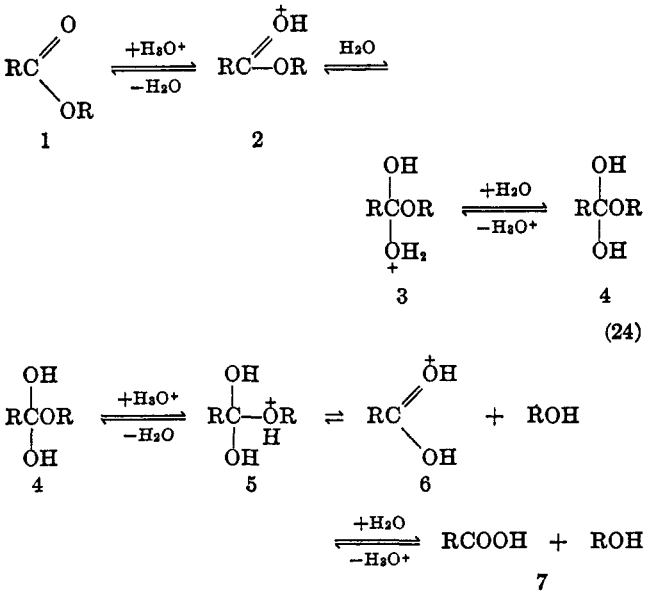
TABLE 11

Deuterium isotope effect in the acid-catalyzed hydrolysis of some carboxylic acid derivatives (406)

Reaction	k_D/k_H
Ethyl formate	1.37
Methyl acetate	1.60 (1.86)
Acetonitrile	1.36
Acetamide (0.1 *N*)	1.45
Acetamide (4.0 *N*)	0.86

3 than is the hydronium ion in water, the concentration of a protonated substrate should be greater in deuterium oxide than in water (406). This should result in a greater hydrolytic rate constant in deuterium oxide than in water, as is demonstrated by the data of table 11. In general the ratio of k_D/k_H for a hydronium-ion-catalyzed hydrolysis of a carboxylic acid derivative lies around 1.5. One obvious exception to this generalization is the isotope effect in the hydrolysis of acetamide under conditions of high acidity. This exception can be satisfactorily explained in terms of the complete protonation of the amide under these conditions. The lower rate constant in deuterium oxide is, therefore, only a function of the difference of the nucleophilicities of deuterium oxide and water and not of the concentrations of the protonated species. The lower nucleophilicity of deuterium oxide in deuterium oxide than that of water in water also explains why the k_D/k_H ratio is only 1.5 and not the factor of 3 found for the difference in the preëquilibria.

On the basis of the evidence given above in conjunction with the isotopic oxygen-exchange evidence given earlier, it is possible to postulate the following mechanism, illustrated in equation 24 and figure 4, for a typical hydronium-ion-catalyzed reaction of a carboxylic acid derivative.



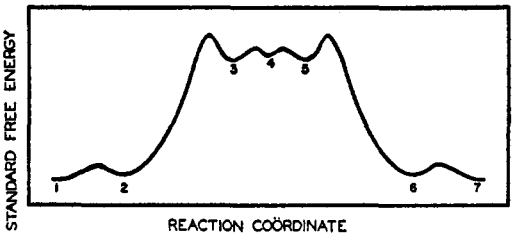
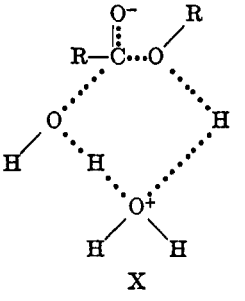


FIG. 4. Standard free energy vs. reaction coördinate for the acid-catalyzed hydrolysis of an ester (35a).

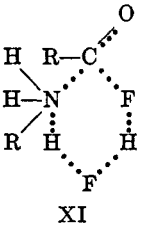
The numbers in figure 4 correspond to the various reactants, intermediates, and products in this process.

A cyclic transition state of the solvolysis of carboxylic acid derivatives has been pointed out as a stereochemically attractive possibility. Studies of the effect of solvent on the hydrolysis of esters, amides, and anilides under acidic conditions indicate that the rate increases with an increase in the dielectric constant (260, 383). It was therefore postulated that the activated complex is more polar than the reactants, as indicated by the transition state X.



X

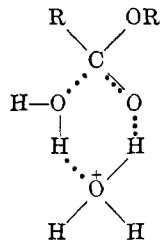
This transition state predicts a reduced entropy of activation due to the electrostriction of water, which has been found in these reactions. The synthesis of polyamino acids from aminoacyl fluorides in anhydrous hydrogen fluoride was explained in terms of a similar cyclic transition state to obviate the unreasonable postulate of free amino groups in anhydrous hydrogen fluoride. The cyclic form (XI) facilitates the process through the hydrogen-bond stabilization (245).



XI

The cyclic transition states above involve one molecule of general acid and one molecule of nucleophile (either the solvent or an added nucleophile) in addition to the substrate. The above formulations can be criticized, since they ignore the formation of a tetrahedral addition intermediate in the first step of the process. This objection can be met, however, by modifying the

transition state in a relatively minor way to include the carbonyl oxygen atom, and not the leaving group in the cyclic process.



XII

This proposal has been made in a recent review article by Syrkin and Moiseev (377). These authors have proposed that the intermediacy of unstable cationic species is not consistent with experimental data and further that the role of cyclic transition states in hydrolysis reactions is more important than has heretofore been realized. They base their criticism of the intermediacy of the protonated species on the assumption that substrates such as acids or esters are not sufficiently basic to form appreciable quantities of kinetically important protonated species in dilute aqueous solutions. While there is no question that the concentrations of certain protonated species will be extremely low, this fact does in no way exclude their participation in kinetic processes. The data presented in Section III,A, especially those concerning amide hydrolysis, are fully consistent with the formation of protonated species as unstable intermediates in certain acid-catalyzed reactions.

While the author is critical of the exclusion of protonated substrates as intermediates in acid-catalyzed reactions, the proposal of cyclic transition states such as XII is welcomed as an ingenious and possibly important suggestion. Certainly such a mechanism can accommodate specific hydronium-ion catalysis as well as general acid catalysis (Section III,B). Furthermore, cyclic transition states can account for the data on solvent effects and the peculiar properties of anhydrous hydrogen fluoride. The cyclic transition state is an attempt to specify the role of the solvent molecules in a more exact fashion than is immediately obvious from the kinetics; this hypothesis may lead to future experimental tests of interesting consequence.

B. GENERAL ACIDIC CATALYSIS

General acidic catalysis, that is, catalysis by any proton donor as opposed to specific hydronium-ion catalysis, can be described by a number of mechanisms (166). The following three cases will be discussed.

Case 1: A rate-determining proton transfer from the general (Brønsted) acid to the substrate, giving either a final product or an intermediate which rapidly gives the final product

$$\begin{aligned} S + HA &\rightarrow SH + A \quad \text{(slow)} \\ SH &\rightarrow \text{products} \quad \text{(fast)} \end{aligned} \tag{25}$$

Case 2: An equilibrium involving hydrogen bonding of the general acid with the substrate, followed by a slow step not solely involving a proton transfer.

$$\begin{aligned} S + HA &\rightleftharpoons S\cdot HA \quad \text{(fast)} \\ S\cdot HA + R &\rightarrow \text{products} \quad \text{(slow)} \end{aligned} \tag{26}$$

Case 3: A prototropic equilibrium between the general acid and the substrate, followed by a rate-determining step involving the conjugate base of the general acid.

$$\begin{aligned} S + H^+B &\rightleftharpoons SH^+ + B \quad \text{(fast)} \\ SH^+ + B &\rightleftharpoons \text{products} \quad \text{(slow)} \end{aligned} \tag{27}$$

In all cases the rates of the general acid-catalyzed reactions may be expressed as a function of the substrate concentration times the summation of the catalytic rate coefficients of the general acids times their concentrations.

$$\text{Rate} = (\text{substrate}) \sum^{i} k_i (HA_i) \tag{28}$$

It is very difficult to conceive of a rate-determining proton transfer from the oxygen atom of a general acid to the oxygen atom of a carboxylic acid derivative as required by case 1 (equation 25). Those prototropic transfers from oxygen to oxygen which have been measured indicate that they are very fast indeed (152).

The formation of a hydrogen-bonded complex between a general acid and the carboxylic acid derivative followed by a rate-determining step of some kind (equation 26) is a classical view of the catalytic process in which a catalyst changes the electronic distribution in the substrate molecule, thereby making it more susceptible to attack by another species. It is this mechanism that will be defined as general acid catalysis for the present purposes.

Although the reaction involving proton transfer in a fast preëquilibrium followed by reaction of the conjugate base of the general acid with the protonated substrate in the rate-determining step of the process (equation 27) is kinetically a general acid catalysis, it is mechanistically not a general acid catalysis at all but rather one variant of a nucleophilic-electrophilic catalysis. In acid-base catalysis involving only proton transfers, this mechanism fits easily with other mechanisms of general acid catalysis. In the reactions of carboxylic acid derivatives, however, the conjugate base can serve as a nucleophile, leading to important chemical consequences. Since this review emphasizes mechanisms of catalysis, examples known to conform to equation 27 will be discussed in the section concerned with nucleophilic-electrophilic catalysis (Section V). The catalyses discussed in the present section kinetically follow equa-

tion 28 and mechanistically follow equation 26 or are mechanistically unspecified at present.

1. Intermolecular general acidic catalysis

Although general acid catalysis is a well-recognized phenomenon in a number of organic processes, the proof of its occurrence in reactions of carboxylic acid derivatives has had a stormy history. The hydrolysis of ethyl acetate in chloroacetate buffers was interpreted in terms of a catalysis by the undissociated monochloroacetic acid (123). Primary and secondary salt effects were taken into account, but the salt concentrations employed were so high that both the primary and the secondary salt effects probably depended on the nature of the ions present. This consideration may account for the 35 per cent change in velocity noted at 1 *N* sodium acetate, and it thus seems doubtful whether there is any catalysis by an undissociated (general) acid. Bisulfate ion has also been postulated to have an intrinsic catalytic activity in the hydrolysis of ethyl acetate (125). However, the catalysis by bisulfate ion has been subsequently interpreted in terms of catalysis by hydronium ion (20).

An investigation of the esterification of acetic acid in methanol provides the only reasonably straightforward evidence for general acid catalysis of a reaction involving a carboxylic acid (325). The kinetics of the esterification reaction in methanol solution can be expressed as

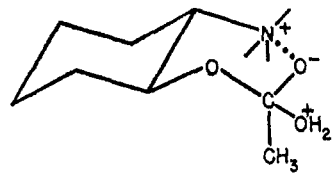
$$-\mathrm{d(HAc)}/\mathrm{d}t = (\mathrm{CH_3OH})[(k_0)(\mathrm{HAc})(\mathrm{H^+}) + k_1(\mathrm{HAc})^2 + k_2(\mathrm{HAc})(\mathrm{X})] \quad (29)$$

where X is a substance whose concentration is inversely proportional to the acetic acid concentration. In the presence of acetate ion as a buffer, conditions obtain in which the k_1 term is the most important term in equation 29. This term involves two acetic acid molecules, one of which is the reactant in the esterification reaction and the other of which is a general catalyst.

2. Intramolecular general acidic catalysis

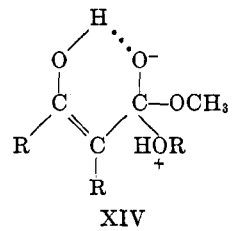
The carbonyl stretching frequencies of esters illustrate interactions of the carbonyl group with internal general acids. Whereas normal saturated esters exhibit a carbonyl band in the region of 1750 to 1735 cm.$^{-1}$, salicylates and anthranilates in which intramolecular hydrogen bonding to the carbonyl group is possible exhibit carbonyl bands in the region of 1670 to 1690 cm.$^{-1}$ (29). Internal hydrogen bonding in compounds with peptide linkages is also prevalent; both simple peptides (293) and proteins (313) show this phenomenon.

It is postulated that such interactions can lead to intramolecular general acid catalysis. There is experimental evidence that such catalysis is important. In certain steroids, the monoester of a 1,3-diol is held in such a configuration that hydrogen bonding of the alcoholic group to the alcohol oxygen of the ester occurs. In these systems hydrolysis of ester by hydroxide ion is facilitated and the usual rule whereby equatorial esters are more easily hydrolyzed than axial esters is reversed by this phenomenon (206, 254a). Hydrogen bonding also accounts for the greater reactivity of *cis*- than of *trans*-2-hydroxycyclohexanecarboxylic acid with *p*-toluidine (312), and for the fact that *cis*-acetoxy-*p*-toluidide is hydrolyzed more rapidly than the corresponding trans isomer. The labilization of ester bonds has also been observed in aminocyclitol derivatives (214). In this reaction a quaternary ammonium ion of the proper configuration is postulated to stabilize the transition state of a neighboring ester hydrolysis through hydrogen bonding or general acid catalysis.



XIII

Nucleophilic reactions of β-keto esters exhibit exceptional reactivity which can be attributed to their enolic character. For example, the transesterification of methyl and ethyl β-keto esters has been carried out under relatively mild conditions in the absence of external catalysts (6, 7). The enhanced reactivity does not parallel the acidity of the corresponding acid, and therefore the unusual reactivity cannot be attributed to an inductive effect. Furthermore, when ethyl dimethylacetoacetate, which cannot enolize, was used, the uncatalyzed transesterification no longer occurred. While no direct proof of the intramolecular character of the reaction exists, it is reasonable to postulate the following transition state to account for the enhanced reactivity of β-keto esters in the transesterification reaction.



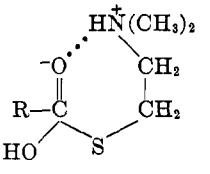
XIV

The hydrolysis of ethyl acetoacetate has been investigated kinetically (179). The rate constant for hydrolysis at 90°C. in water is of the order of 0.2×10^{-4} min.$^{-1}$, a value appreciably larger than that expected for ethyl acetate under similar conditions. This enhanced reactivity may be attributed to the stabilization of the hydrolysis transition state in a similar fashion to that shown in formula XIV. An intramolecular proton donor

TABLE 12
Relative rates of hydrolysis of some thiol esters (193a)

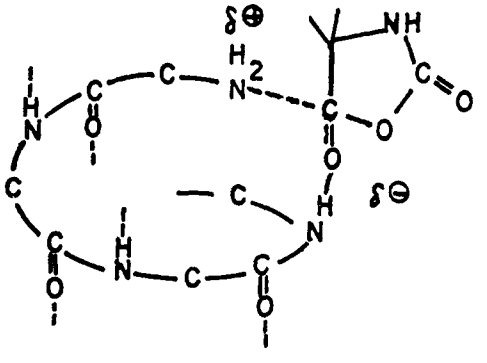
Ester	Relative k_2
$CH_3CH_2C(O)SCH_2CH_2NH^+(CH_3)_2$	240
$CH_3CH_2C(O)SCH_2CH_2N^+(CH_3)_3$	1

appears to play an important role in the alkaline (pH 7 to 10) hydrolysis of the two thiol esters shown in table 12. The important difference between these two esters is that one contains a β-quaternary ammonium ion, while the other contains a β-tertiary ammonium ion which can contribute a proton to the carbonyl group in the transition state of the hydrolysis, as shown in formula XV.



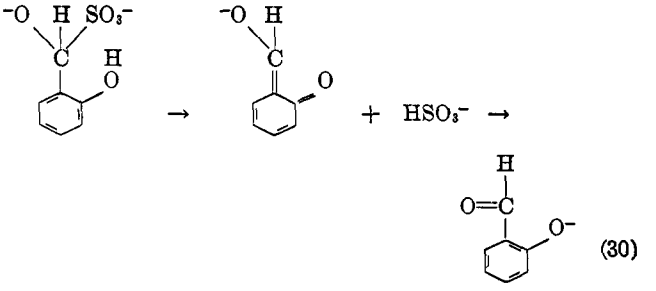
XV

A further example of the role of an intramolecular proton donor in the reaction of a carboxylic acid derivative involves the polymerization of an *N*-carboxy-α-amino acid anhydride. It has been postulated that the anhydride can form a hydrogen bond to the peptide, leading to the hypothetical transition state XVI (392).



XVI

The examples of acid catalysis given above involve catalysis of the formation of the tetrahedral addition intermediate by a specific or general acid. An acidic substance may catalyze the overall reaction by aiding one of the steps involved in the partitioning of the intermediate. While no example of this catalysis is known for carboxylic acid derivatives, it does occur in the reactions of aldehydes. The decomposition of the addition compound of sodium bisulfite and benzaldehyde is greatly aided by an *o*-hydroxy group, while an *o*-methoxy group has a slight retarding effect (72). This intramolecular general acid catalysis can be explained by proton donation from the *o*-hydroxy group to the departing sulfite group.



(30)

C. CATALYSIS BY CATIONIC EXCHANGE RESINS

In recent years ion-exchange resins such as polystyrenesulfonic acid (67) and polyvinylsulfonic acid (239) have been shown to catalyze the hydrolysis of esters and peptides and the esterification of carboxylic acids (290). Investigations have been carried out to ascertain whether catalysis by these resins is more efficient than the equivalent concentration of hydronium ions in aqueous solution. While small differences have indeed been found, no striking enhanced reactivities of the resin catalysts have emerged from these studies. In ester hydrolysis the resin catalysts impose a loss in entropy on the transition state whose magnitude differs from ester to ester (199). In the hydrolysis of peptides by cationic exchange resins, however, the entropy of activation was found to be significantly less than hydrolysis of the same compounds by hydrochloric acid, while the enthalpies of activation for the two cases were practically indistinguishable (401a). While the entropy changes associated with catalysis by cationic exchange resins remain obscure, presumably the mechanism of the catalysis follows that outlined earlier for homogeneous acids.

D. CATALYSIS BY METAL IONS

Acid catalysis may be considered to function through the introduction of a positive charge into a substrate, distorting the electronic distribution in the molecule and thus making reaction feasible. It follows from this hypothesis that a metal ion which can complex with a substrate and introduce several units of positive charge into a molecule will function as a superacid catalyst.

Various metal ions have been shown to form complexes with carboxylic acid derivatives, in particular with amides. For example, the carbonyl stretching frequencies of δ-caprolactam and acetamide are lowered about 15 $cm.^{-1}$ by the addition of zinc chloride (268). This result suggests a direct interaction between the carbonyl group and the metal ion and agrees with results found for the interaction of a number of other Lewis acid salts with urea (314). Presumably a specific interaction between the carbonyl group of a carboxylic acid derivative and a metal ion should lead to catalysis of reaction.

Metal ions have been shown to catalyze a large number of organic and enzymatic reactions, including

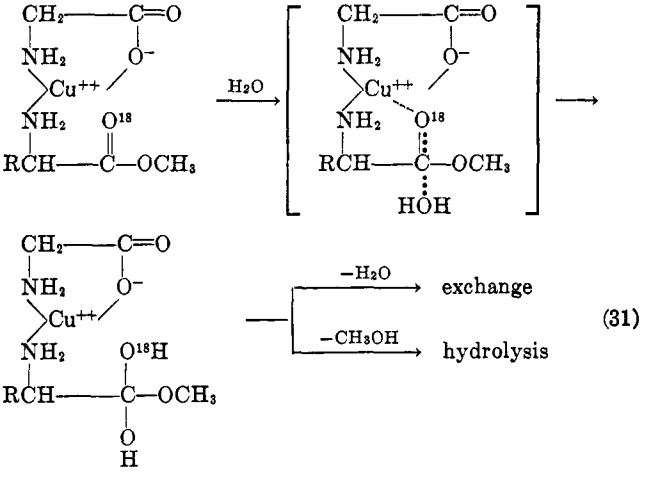
the hydrolysis of Schiff bases (148), the decarboxylation of β-keto acids (360), and the carboxylation of primary nitro compounds and methyl ketones (366, 367). The role of metal ions in the catalysis of organic reactions has been reviewed (398). Metal ions have also been shown to be essential to the catalytic activity of a number of enzyme systems, such as reactions catalyzed by pyridoxal phosphate-containing enzymes, certain exo-peptidases, and many oxidation-reduction enzymes (285).

The hydrolysis of a number of esters has been shown to be subject to catalysis by metal ions. Structurally these esters may be divided into two categories: (*1*) those esters which contain a secondary functional group which can serve as a ligand for the metal ion and (*2*) those esters which do not contain such a group. Cupric ion, cobaltous ion, manganous ion, and calcium ion have been shown to catalyze the hydrolysis of amino acid esters in solutions of pH from 7.5 to 8.5 (253). It was postulated that a 1:1 complex is formed between the metal ion and the amino acid ester in which the metal ion chelates with the amino group and the carbonyl oxygen of the ester, and further that this chelate is attacked by hydroxide ion to give the products of reaction. With glycine ethyl ester and cysteine methyl ester in the presence of nickelous and cupric ions, a small increase in the (alkaline) bimolecular rate constant was shown to parallel an increase in the stability of the metal-ion complex (403).

Detailed kinetic studies revealed that glycine methyl ester and phenylalanine methyl ester in glycine buffer at pH 7.3 undergo a facile hydrolysis catalyzed by cupric ion (54a). Under these conditions the reactions closely follow a first-order rate law in the substrate. Using this kinetic data it is possible to compare the rates of hydrolysis of DL-phenylalanine ethyl ester as catalyzed by hydronium ion, hydroxide ion, and cupric ion (see table 13).

The enhanced reactivity in the cupric-ion-catalyzed hydrolysis cannot be due solely to an attack of hydroxyl ion on a positively charged α-amino ester, since it has been shown that the introduction of a positive charge, two atoms from the carbonyl group of an ester, increases the rate constant of alkaline hydrolysis by a factor of 10^3 (52), whereas there is a difference of approximately 10^6 between the cupric-ion-catalyzed and the alkaline hydrolyses of DL-phenylalanine ethyl ester. The effective charge on the cupric ion–glycine(buffer)–ester complex is +1, so that the factor of 10^6 cannot be explained by an increase in charge over that present in the case of betaine. Furthermore, the reaction cannot be due to attack by a water molecule on a positively charged α-amino acid ester, since it has been shown that the rate constant of the acidic hydrolysis of phenylalanine ethyl ester is very small. It thus seems reasonable to postulate that the rapid hydrolysis of α-amino esters at pH 7.3, as catalyzed by cupric ion, is due to a direct interaction of the metal ion with the reaction center, the ester group.

Carbonyl oxygen exchange was found during the cupric-ion-catalyzed hydrolysis of DL-phenylalanine ethyl ester-*carbonyl*-O^{18} at pH 7.3. This result indicates that an addition intermediate is formed in this reaction. A mechanism (54a) which is consistent with both the kinetic evidence and the oxygen-exchange evidence is given in equation 31.



(31)

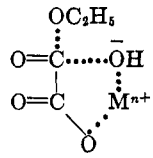
TABLE 13

Acidic, basic, and cupric-ion-catalyzed hydrolysis of DL-*phenylalanine ethyl ester at pH 7.3 and 25°C.*

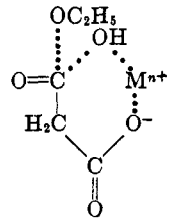
Catalyst	Solvent	k_2	k_1	Reference
		l./mole sec.	*sec.*$^{-1}$	
H^+	70% dioxane		1.46×10^{-14}	(399)
OH^-	85% ethanol	2.97×10^{-2}	5.8×10^{-9}	(52)
Cu^{++}	Water		2.67×10^{-3}*	(54a)

* The rate constant for the cupric-ion catalysis equals k_3K((glycine)Cu^+). To obtain k_3, the true catalytic constant for cupric-ion catalysis, approximations must be made for K and for ((glycine)Cu^+). It appears reasonable that K/((glycine)Cu^+) is greater than 1, so that the tabulated rate constant is a lower limit for the catalytic constant of the cupric ion.

Catalysis by metal ions has also been demonstrated in the hydrolysis of esters containing an α- or β-carboxylate ion. The alkaline hydrolysis of potassium ethyl oxalate and potassium ethyl malonate is catalyzed by calcium ion, barium ion, hexaamminocobalt(III), and thallous ion in that order (216). The oxalate ester is catalyzed to a greater extent than the malonate ester, which in turn is more susceptible to catalysis by metal ion than is the corresponding adipate ester. Alkali metal ions, on the other hand, have only a small negative salt effect on the hydrolysis of potassium ethyl malonate. On the basis of the structural and metal-ion effects, it is postulated that the transition states of the hydrolyses which are catalyzed by calcium, barium, cobaltic, and thallous ions can be represented by the chelate structures XVII and XVIII.



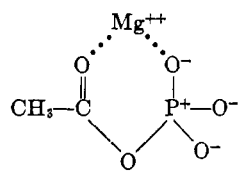
XVII



XVIII

These chelates are structurally similar to that postulated above for the metal-ion-catalyzed hydrolysis of α-amino esters; the position of the protons in the transition state is different, but this is a completely arbitrary distinction. A similar explanation will account for the large effect of calcium ions on the alkaline hydrolysis of acetylcitric and benzoylcitric acids (347).

Magnesium and calcium ions catalyze the hydrolysis of acetyl phosphate (247) in neutral and acid solutions. At pH 7.7, magnesium ion catalyzes the reaction markedly and the catalyzed reaction is first order in magnesium ion as well as first order in acetyl phosphate. The catalysis by metal ion is greater at pH 7.7, where acetyl phosphate exists as a dinegative ion, than at pH 0.63, where it exists as a neutral molecule. It was therefore postulated that the chelate XIX may be active in the catalyzed reaction (247).



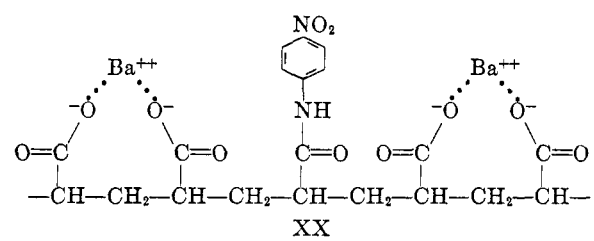
XIX

Calcium ions also catalyze the hydrolysis of benzoylglycine methyl ester and acetyl-L-valine methyl ester at pH's from 7.9 to 8.4 (278). It was possible to analyze the kinetics of this metal-ion-catalyzed hydrolysis in terms of the reversible formation of a complex between calcium ion and substrate, followed by attack of hydroxide ion on this complex to form the products of the reaction. The interaction between calcium ion and the ester involves either a complex of the calcium ion with the carbonyl group of the ester or a chelate involving the carbonyl group and the secondary amide group of these esters.

Metal ions have been shown to promote the hydrolysis of glycine amide and of phenylalanylglycine amide (288). Cupric ion, nickelous ion, and cobaltous ion catalyze the hydrolysis of glycinamide in slightly alkaline solution. In the absence of metal ions, phenylalanylglycine amide undergoes ring closure to 3-benzyl-2,5-diketopiperazine; in the presence of cupric ion, however, at pH 5, hydrolysis of both the amide and peptide bonds is competitive with ring closure. The effect of catalysis by metal ions in the hydrolysis of amides is not nearly as striking as in the hydrolysis of esters. This result is surprising, since the most direct evidence for the interaction of metal ions and carboxylic acid derivatives has involved amides (*vide infra*).

Thiol esters appear to be particularly susceptible to cleavage by heavy metal ions such as mercuric ion. This reaction was first noted in a nonaqueous system when an alcoholic mercuric acetate solution was treated with a thiol ester (331). Thiol esters such as acetyl coenzyme A (274a), acetoacetyl coenzyme A (361), and thioaspirin (341) have been demonstrated to undergo hydrolysis almost instantaneously in aqueous solution at about neutrality in the presence of stoichiometric amounts of mercuric ion salts. In each case a mercuric mercaptide is formed. In the hydrolysis of acetoacetyl-S-coenzyme A, a mercuric chelate involving two oxygen atoms of the acetoacetyl group and two oxygen atoms of the coenzyme A portion of the molecule is postulated as an intermediate. Since mercuric ion will cleave simple esters containing no secondary ligand groups, it is not clear that such chelation is essential. Presumably the coördination of the sulfur atom with a mercuric ion is the principal driving force of this reaction. Rigorously the reaction should not be called a metal-ion-catalyzed reaction but rather a metal-ion-promoted reaction, since mercuric ion is consumed stoichiometrically in the process.

Small concentrations of barium hydroxide accelerate the rate of alkaline hydrolysis of *p*-nitroanilide residues attached to polyacrylic acid (397). The acceleration is almost proportional to the square of the barium-ion concentration. The results are consistent with the assumption that the initial low reactivity of nitroanilide residues is due to hydrogen bonding with neighboring carboxylate ions. Barium ion has been shown to form complexes with polycarboxylic acids, and such complex formation of two barium ions with the carboxylate groups flanking the nitroanilide residue would eliminate the stabilizing effect of hydrogen bonds and increase the nitroanilide reactivity as in XX.



XX

In addition to catalysis of hydrolytic reactions by metal ions, metal ions facilitate the esterification of acetic acid in methanol (262). This catalysis, however, is not attributable to the direct action of metal ions but rather to an acid catalysis brought about by the action of strong complex acids. This result demonstrates one of the possible pitfalls when dealing with the effects of metal ions on kinetic processes. Effects of metal ions can also be confused with ionic strength effects.

Various Lewis acids have been postulated to catalyze reactions of carboxylic acid derivatives. For example, it is claimed that titanium tetrafluoride and neodymium chloride catalyze the alcoholysis of terephthalic esters with glycols at elevated temperatures (371). Also, magnesium iodide has been shown to cleave esters in neutral ether solution (403a).

It has been postulated that a number of organometallic compounds catalyze the reactions of carboxylic acid derivatives, including the alcoholysis of esters and isocyanates. Organotitanium, organotin, organozirconium, organoaluminum, organolead, and organomagnesium compounds have been utilized as catalysts in the alcoholysis of esters (104, 105, 106, 107, 108, 393). Organotin compounds such as dimethyldiacetoxytin have also been shown to catalyze the methanolysis of phenyl isocyanate in di-*n*-butyl ether solution. It is postulated that a weak complex between the organotin compound and the isocyanate occurs in this catalytic process (116).

IV. Nucleophilic Catalysis

A Brønsted base, defined as a substance which is a proton acceptor, can carry out two functions in organic reactions: (*1*) accept protons and thus serve in its classical function as a base and (*2*) act as a nucleophile toward a carbon atom. The two separate functions of a Brønsted base lead to two possibilities for its catalytic action. Both of these possibilities have been realized in the reactions of carboxylic acid derivatives, leading to general basic catalysis (Section VI) through the first function and to nucleophilic catalysis through the second function.

A. Hydroxide-ion catalysis

It was pointed out in Section III that the pH dependence of ester hydrolysis can be explained by a hydronium-ion-catalyzed reaction in acidic solution and a hydroxide-ion-catalyzed reaction in basic solution. In general the minimum in the pH–rate profile for the hydrolysis of simple esters is rather sharp, and occurs not at neutrality but usually between pH 4 and 6 (73). The sharpness of the minimum is a function of a possible uncatalyzed reaction by a water molecule (24); a sharp minimum means no water reaction and a broad minimum indicates a considerable water reaction. The fact that the minimum occurs on the acid side of neutrality indicates that catalysis of ester hydrolysis by hydroxide ion is ordinarily more powerful than hydronium-ion catalysis. This inequality is not true for all carboxylic acid derivatives; in amide hydrolysis, for example, hydronium-ion catalysis is equivalent to or greater than hydroxide-ion catalysis.

The pH–rate profiles for the hydroxide-ion-catalyzed hydrolysis of all carboxylic acid derivatives are not as simple as those shown for ethyl acetate in line 1 of figure 3. The rates of hydrolysis of diacetylamine, succinimide (144), and phthalimide (382) in alkaline solution, for example, are proportional not to the concentration of hydroxide ion but rather to the fraction of the imide ionized, leading to a sigmoid relationship between the pH and log k_{obs}. These results are explicable on the basis of a mechanism in which the rate-determining step is the reaction of a hydroxide ion with an unionized molecule of imide. The same dependence of the rate on pH was found in the hydroxide-ion-catalyzed hydrolysis of 2-thiohydantoins, in which the rate-determining step is again the attack of a hydroxide ion on the unionized substrate (143). These examples are cited to emphasize that without full consideration of the mechanistic possibilities of the reaction a cursory examination of the pH–rate profile may be misleading.

The hydroxide-ion-catalyzed hydrolysis (or saponification) of esters has been one of the most intensively studied of all chemical reactions. The effect of structure on reactivity, tracer and structural data on the position of fission, and isotopic exchange data complement one another, pointing to a mechanism which is akin to nucleophilic catalysis. Nucleophilic catalysis may be defined as the reaction of a nucleophilic substance with a substrate, leading to the formation of an unstable intermediate which in subsequent reaction yields the products of the reaction and regenerates the catalyst (10). Hydroxide ion fulfills all the functions enumerated above except the last—a final equilibrium consumes the hydroxide ion. The reaction, therefore, should rigorously be called a nucleophilic-promoted hydrolysis. The mechanism for this process is parallel to that previously given for the hydronium-ion-catalyzed hydrolysis of esters (equation 32a). A number of similar reactions can be related in a formal way to this process, such as the base-catalyzed alcoholysis of esters (equation 32b). The alcoholysis reaction is a true catalytic process (42).

$$\mathrm{RC(=O)OR + OH^- \rightleftarrows RC(O^-)(OH)OR \rightarrow RCOO^- + ROH} \qquad (32a)$$

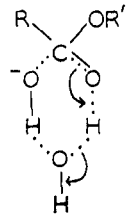
XXI

$$\mathrm{RC(=O)OR + OR'^- \rightleftarrows RC(O^-)(OR')OR \rightleftarrows RC(=O)OR'^- + OR^-} \qquad (32b)$$

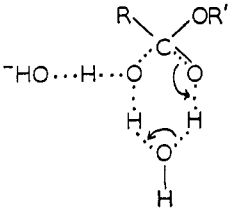
The intermediate in equation 32b is analogous to the stable compound $CF_3C(O^-)(OC_2H_5)_2Na^+$ (*vide infra*). Catalysis of the alcoholysis reaction by cyanide ion (316), alkali or alkaline earth metals, oxides, hydroxides, or hydrides (71, 287, 198) operates via equation 32b.

The Hammond postulate (193) predicts that the transition state for the formation of the tetrahedral intermediate XXI resembles that intermediate closely. As mentioned in Section III, it has been postulated

that the transition state in ester hydrolysis is more complicated than XXI, involving possibly a cycle containing, in addition to the nucleophile hydroxide ion, a molecule of water or alternatively two molecules of water. These latter formulations, while pictorially attractive, are based on little more than speculation and artistic elegance (260, 377).



XXII



XXIII

It is possible to catalyze the hydrolysis of esters by ion-exchange resins of the quaternary ammonium hydroxide type (332) and by alumina impregnated with potassium hydroxide (109). Reactions catalyzed by these solids show no fundamental differences from homogeneous hydroxide-ion catalysis.

B. OTHER NUCLEOPHILIC CATALYSES

While the hydroxide ion discussed above is the nucleophile most familiar to aqueously oriented chemists, it should be evident that there is nothing chemically unique about its ability to function as a nucleophilic catalyst (or promoter). Therefore it might be expected that there would exist other nucleophilic catalysts for the reactions of carboxylic acid derivatives. Development of this idea was retarded by the fixation that what must be sought was a catalysis analogous to the elegant examples of general basic catalysis illustrated in profusion for other organic reactions by the Brønsted school.

Nucleophilic catalysis of organic reactions is not restricted to the reactions under consideration here but is a general phenomenon. The nucleophilic catalysis of the hydrolysis of methyl bromide by iodide ion has long been known (190, 294, 295).

$$\begin{aligned} CH_3Br + I^- &\rightarrow CH_3I + Br^- \\ CH_3I + H_2O &\rightarrow CH_3OH + I^- + H^+ \end{aligned} \qquad (33)$$

In Section II an extensive number of reactions of nucleophiles with carboxylic acid derivatives was described. The products of these reactions were not described, however; some of the products are stable while others are unstable under the conditions of their preparation. The first group of reactions is uninteresting from the point of view of nucleophilic catalysis, while the second group of reactions is precisely that which falls under the definition of nucleophilic catalysis as defined above. Nucleophilic catalysis is of great importance in the reactions of carboxylic acid derivatives, since a large number of these compounds can serve as reactive intermediates.

The mechanism of nucleophilic catalysis, as pointed out before, must involve the reaction of a nucleophile with a substrate to produce an unstable entity of some kind that will react either spontaneously or with another molecule to give the reaction products and regenerate the catalytic entity. This description of nucleophilic catalysis implies the presence of at least two consecutive reactions. It will be profitable to consider some of the characteristics of such consecutive processes. The discussion will be limited to consideration of a mechanism involving two consecutive reactions for the sake of simplicity, although it should be clear that more extensive sequences are possible.

Two kinds of consecutive reactions can be delineated. In the first type, the intermediate reaches only an infinitesimally small concentration in comparison with the concentrations of reactants or products. The kinetics of such a system can be treated by the steady-state approximation. This system is exemplified by the nucleophilic catalysis of ester hydrolysis brought about by hydroxide ion. The progress of the reaction may be described by a plot of standard free energy vs. reaction coördinate shown in figure 5. In this instance the intermediate is quite unstable, lying at an energy level considerably above that of the reactant or product. While the overall kinetics are determined by both the formation of the unstable intermediate (k_1) and the partitioning of the intermediate (k_2/k_3), the slow step of the reaction is the k_1 step.

In the second type of consecutive process, the concentration of the intermediate reaches a finite value with respect to the concentrations of reactants and products. In this process, the standard free energy of the intermediate connot be appreciably higher than that of the reactant. Such a system is shown in figure 6.

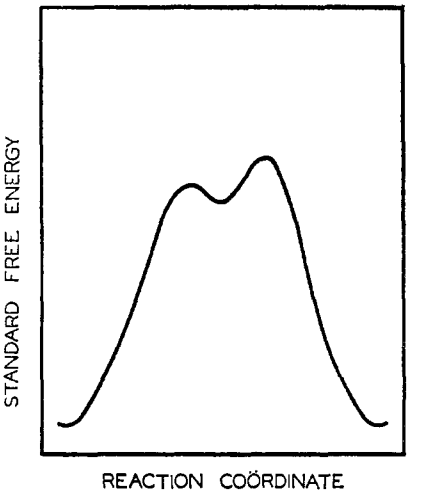


FIG. 5. A consecutive catalytic process involving an unstable intermediate which is formed in infinitesimal amount.

It is difficult to specify where the dividing line between these two cases will lie. It might be stated that when the intermediate is no more than 5 kcal. greater than the reactants in standard free energy, it will be formed in finite amounts. In such a case it is theoretically possible to detect the intermediate directly through physical observations, while in the first case proof of intermediate formation must rest on indirect evidence. The relative rate constants of the two steps will also be a factor in determining whether an intermediate will actually become finite in concentration. If the second step of the overall reaction is faster than the first step, no amount of thermodynamic stability will enable the intermediate to accumulate in a finite amount (figure 6, solid line). If the rate of the first step of the overall reaction is equal to or greater than that of the second step, and if the standard free energy of the intermediate is not appreciably higher than that of the reactant as described above, the intermediate will be formed in finite amount (figure 6, dotted line).

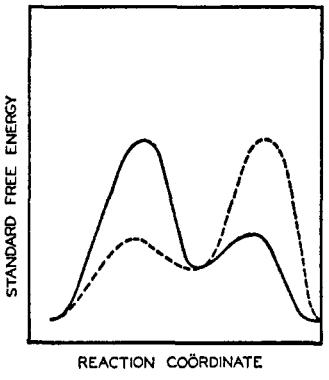


FIG. 6. A consecutive catalytic process involving a relatively stable intermediate which may (– – –) or may not (——) be formed in finite amount.

Nucleophilic catalysis to be discussed here will include examples of each of the above possibilities. Substances that can participate in nucleophilic catalysis of the reactions of carboxylic acid derivatives include carboxylate ions, tertiary amines, phosphate ion, nitrite ion, and to a lesser extent alcohols and phenoxide ions. These catalytic processes involve anhydride, acylammonium, acyl phosphate, acyl nitrite, ester, and lactone intermediates, respectively. Nucleophilic catalyses involving carboxylate ions, tertiary amines, and phosphate ion have been demonstrated in both intermolecular and intramolecular cases. Catalyses involving alcohols and phenoxide ions have been demonstrated in the more favorable intramolecular cases.

1. Intermolecular nucleophilic catalysis

It has been common knowledge that many preparative acylations are facilitated by the presence of alkali, carboxylate ions, and tertiary amines such as pyridine. These facts are superficially suggestive of general basic catalysis. A number of attempts were made to ascertain the importance of general basic catalysis of ester hydrolysis. The catalytic effects of acetate ion on the hydrolysis of ethyl acetate were studied (122, 123, 124). However, the reported catalytic rate constant for acetate ion is minute (0.25×10^{-7} l./mole second, while that for hydroxide ion is 1.08 l./mole second) and its reality is therefore questionable. The data have been critically discussed from the viewpoint of the primary salt effect and the effect of the nature of the buffer on the rate (24). The hydrolysis of methyl glycerate in sodium glycerate buffers was interpreted in terms of general basic (and acidic) catalysis (184), but careful analysis of the data indicates that the reaction can be completely explained on the basis of hydronium-ion and hydroxide-ion catalysis.

(a) Carboxylate and phosphate ions

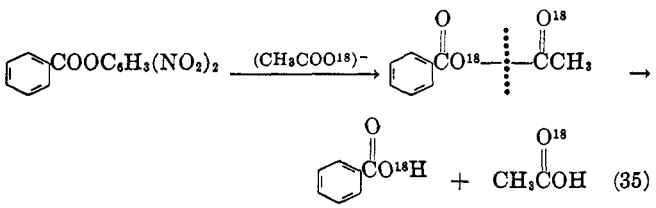
Although initial attempts to find general basic (in reality, nucleophilic) catalysis of ester hydrolysis were fruitless, nucleophilic catalysis was observed early in the carboxylate-ion-catalyzed hydrolysis of anhydrides. In the hydrolysis of acetic anhydride, formate ion is a remarkably good catalyst (240). Likewise, acetate ion and formate ion catalyze the hydrolysis of propionic anhydride, the latter being more effective than the former (241). Finally, in the hydrolysis of acetic propionic anhydride, acetate and formate ions catalyze the reaction, formate again being the more efficient catalyst (242). These accelerations can be explained on the basis of the nucleophilic catalysis shown in equation 34. A catalysis is expected only if the intermediate anhydride is more susceptible to attack by water than the original anhydride. This hypothesis is verified experimentally, since the order of reactivity of anhydrides is formic > acetic > propionic. This catalytic mechanism further predicts that the formation of an intermediate anhydride which is less susceptible to attack by water than the original will lead to inhibition, which is observed in the hydrolysis of acetic anhydride in propionate and butyrate ion buffers (242). One further point should be noted about these catalyses: namely, acetate ion catalyzes the hydrolysis of acetic anhydride. This catalysis cannot be explained by mechanism 34; it is truly a general basic catalysis to be discussed in Section VI.

$$\mathrm{RCOCR} + \mathrm{R'COO^-} \rightarrow \mathrm{RCOO^-} + \mathrm{RCOCR'} \xrightarrow{\mathrm{H_2O}} \mathrm{RCOOH} + \mathrm{R'COOH} \quad (34)$$

Catalysis by acetate ion has also been observed in the hydrolysis of aspirin anhydride (172). Three nucleophiles were found to catalyze the hydrolysis of this anhydride—water, hydroxide ion, and acetate ion. The mechanism of the catalysis by acetate ion presumably follows equation 34.

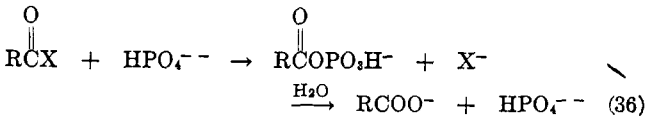
Acetate ion also catalyzes the hydrolysis of 2,4-dinitrophenyl acetate (54), *p*-nitrophenyl acetate (48, 89), phenyl acetate (48), 2,4-dinitrophenyl benzoate (48), methyl pyrrolidylacetylsalicylate hydrochloride (170), and diethylaminoethyl acetylsalicylate hydrochloride (171). In each of these cases the rate of hydrolysis in acetate buffers was shown to be dependent on the acetate-ion concentration at constant pH and constant ionic strength. These phenyl esters are particularly susceptible to acetate-ion catalysis whereas alkyl esters are not, probably because the tetrahedral addition intermediates formed from phenyl esters are favorably partitioned to give anhydrides whereas those from alkyl esters are not.

In no case has an anhydride intermediate of the type postulated in equation 34 been isolated or observed directly in a catalytic process involving acetate ion. This failure is to be expected, since hydrolysis of the intermediate anhydride is ordinarily faster than its formation. The isotopic tracer experiment illustrated in equation 35 provides indirect evidence for the formation of anhydride intermediates in the acetate-ion-catalyzed hydrolysis of phenyl esters. The majority of the cleavage of the unsymmetrical anhydride acetyl benzoyl anhydride should occur at the bond denoted by the dotted line if the cleavage follows the relative reactivity of the two acyl groups. This experiment therefore predicts that the benzoic acid resulting from the acetate-O^{18} ion-catalyzed hydrolysis of 2,4-dinitrophenyl benzoate should contain a majority of the oxygen-18 atoms from one of the two labelled oxygen atoms of the original acetate ion. The benzoic acid isolated from this hydrolysis was found to contain about 75 per cent of the oxygen-18 derived from one of the oxygen atoms of the acetate-O^{18} ion (48).



(35)

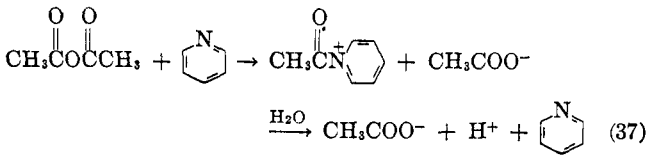
Phosphate ion has been shown to be involved in the hydrolysis of chloramphenicol (209, 210, 384), *p*-nitrophenyl acetate (49, 89), esters of thiocholine (203), *N*-acetylimidazole (355), and possibly acetic anhydride (5). The kinetics of these reactions indicate that phosphate ion (probably in all cases in the form of monohydrogen phosphate ion) participates in the reaction. From the studies of the hydrolysis of acetyl phosphate (247), it can be postulated that the conversion of the acyl compound into an acyl phosphate is followed by the spontaneous hydrolysis of the acyl phosphate. However, the complete catalytic process has not as yet been demonstrated.



(36)

(b) Tertiary amines

The most important and extensive studies of nucleophilic catalysis have involved tertiary amines such as imidazole and pyridine. As noted earlier, the preparative utility of pyridine in acylation reactions has been widely recognized. The acylation of amines, alcohols, and phenols with an acyl chloride or acid anhydride in pyridine solution is a well-known synthetic procedure. However, only recently has the mechanism of the catalytic action of tertiary amines been specified.



(37)

The hydrolysis of acetic anhydride in acetone–water is strongly catalyzed by small amounts of pyridine (8, 176). A series of substituted pyridines of constant steric requirement catalyzes the hydrolysis of acetic anhydride, with relative rates conforming to a Brønsted-type relationship (Section II) (177). Pyridine also catalyzes the acetylation of *o*-chloroaniline and of ethanol by acetic anhydride. Although attempts to detect an intermediate were unsuccessful, the data are consistent with the postulate that the catalysis proceeds as shown in equation 37, involving an acetylpyridinium intermediate. The intermediate acetylpyridinium ion was shown to be long-lived enough to react with hydroxylamine in the presence of water; the partitioning of this postulated intermediate is good evidence for its existence (247). It was further possible to isolate the postulated intermediate, acetylpyridinium chloride, from the reaction of acetyl chloride and pyridine under anhydrous conditions. Pyridine and substituted pyridines also catalyze the hydrolysis of *p*-nitrophenyl acetate and 2,4-dinitrophenyl acetate (54, 89). Presumably these reactions of esters proceed similarly to the reactions of acid anhydrides catalyzed by pyridine, with the intermediacy of an acylpyridinium ion.

Aliphatic tertiary amines also catalyze the nucleophilic reactions of carboxylic acid derivatives. Trimethylamine catalyzes the hydrolysis of *p*-nitrophenyl acetate, presumably through the formation of an acetyltrimethylammonium-ion intermediate (54). Its catalytic rate constant is small compared to its basicity, because of its large steric requirement. Triethylamine catalyzes the reaction of diisocyanates with 1-butanol in toluene solution (101); a quaternary ammonium intermediate has also been postulated for this reaction (9).

TABLE 14

*Catalysis by imidazole of the hydrolysis of p-nitrophenyl acetate**

Catalyst	pK_a	k_2'	References
		l./mole min.	
Imidazoles:			
1. Imidazole	6.95	20.2	(92)
2. 2-Methylimidazole	7.75	2.7	(92)
3. 4-Methylimidazole	7.45	25.1	(92)
4. *N*-Methylimidazole‡	7.05	0.5	(54)
5. 4-Bromoimidazole	3.7	0.28	(92)
6. 4-Hydroxymethylimidazole	6.45	5.6	(92)
7. 4-Nitroimidazole	1.5 (9.1)	35.5	(92)
Histidines:			
1. Histidine	6.0	§	(273a)
2. *N*-Acetylhistidine	7.05	11.2	(92)
3. Histidine methyl ester	5.2	5.6	(92)
4. *N*-Benzoyl-L-histidine methyl ester			(76, 196)
5. 1-Methylhistidine	6.5	§	(273a)
6. β-Aspartylhistidine	6.9	§	(273a)
7. Histidylhistidine	6.8	§	(273a)
8. Histamine	6.0	7.0	(92)
9. Carnosine	6.8	§	(273a)
10. Anserine	7.0	§	(273a)
11. Carbobenzoxy-L-histidyl-L-tyrosine ethyl ester	6.25	8.9	(92)
12. 8:1 copolymer of alanine + histidine†		6.0	(245a)
13. Poly-L-histidine		¶	(288a)
Benzimidazoles:			
1. Benzimidazole	5.4	0.96	(92)
2. 2-Methylbenzimidazole	6.1	0.0375	(92)
3. 6-Aminobenzimidazole	6.0	2.95	(92)
4. 6-Nitrobenzimidazole	3.05	4.8	(92)
5. 4-Hydroxybenzimidazole	5.3	2.8	(92)
6. 4-Methoxybenzimidazole	5.1	0.31	(92)
7. 4-Hydroxy-6-nitrobenzimidazole	3.05	3.75	(92)
8. 4-Hydroxy-6-aminobenzimidazole	5.9	6.15	(92)
9. 2-Methyl-4-hydroxy-6-nitrobenzimidazole	3.9	1.1	(92)
10. 2-Methyl-4-hydroxy-6-aminobenzimidazole	6.65	1.5	(92)
11. 4-(2′,4′-Dihydroxyphenyl)imidazole	6.45	9.4	(92)

* In 28.5 per cent ethanol–water, at pH 8.0, ionic strength = 0.55 *M*, and 30°C. unless otherwise noted.

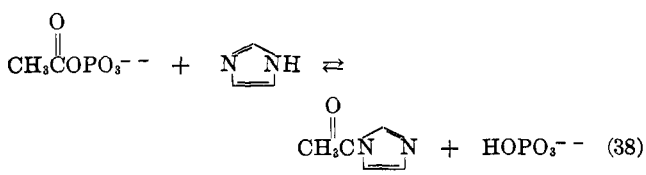
† In 5 per cent aqueous ethanol, pH 7.0.

‡ In 5 per cent dioxane–water, pH 7.0, 25°C.

§ Zero-order rates reported. The relative rates of these six compounds varied from histidine (relative rate = 1) to anserine (relative rate = 3.6).

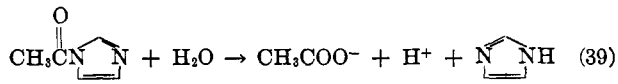
¶ Reported to be five to ten times more effective than histidine (per residue.)

Imidazole and its derivatives have been studied most extensively as nucleophilic catalysts for the hydrolytic and other nucleophilic reactions of carboxylic acid derivatives. Stadtman demonstrated the equilibrium between acetyl phosphate and imidazole as shown in equation 38 (355, 356). This process implies a facile reaction of imidazole with a carboxylic acid derivative.



(38)

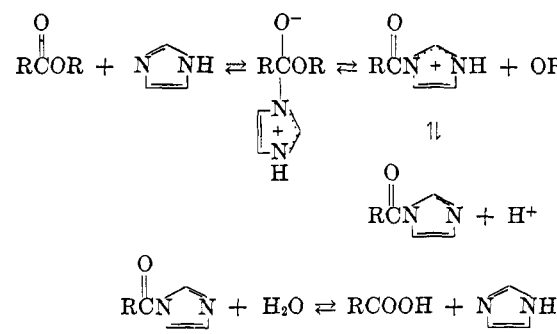
N-Acetylimidazole was further shown to undergo hydrolysis in water at an appreciable rate even in neutral solution, a highly unusual phenomenon for a molecule which is formally an amide (equation 39).



(39)

The sum of equations 38 and 39 constitutes a hydrolysis of acetyl phosphate, catalyzed by imidazole, when the rates of reaction are suitable. *N*-Acylimidazoles were further shown to react with a host of nucleophiles other than water, such as ammonia, hydroxylamine, and amines (406a).

The hydrolysis of *p*-nitrophenyl acetate by imidazole and its derivatives has subsequently been studied quantitatively and in great detail (34, 53, 54, 66, 76, 82, 90, 91, 92, 196, 245a, 273a). The hydrolysis of *p*-nitrophenyl acetate has been shown to be catalyzed by the imidazole derivatives in table 14. The kinetics of the imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate in the region of neutrality has been determined spectrophotometrically by measurement of the disappearance of ester, the appearance of *p*-nitrophenoxide ion, and the change in *N*-acetylimidazole. The rate of disappearance of ester or of appearance of *p*-nitrophenoxide ion is proportional to both the ester concentration and the imidazole concentration at constant hydrogen-ion concentration and constant ionic strength. By variation of the pH, it was shown that the rate is proportional to the free imidazole concentration and is independent of the imidazolium concentration. The kinetics of the formation and decomposition of the intermediate *N*-acetylimidazole were quantitatively correlated with the disappearance of the reactants and the appearance of the products (66, 81). The mechanism proposed for this reaction is given in equation 40 and is similar to the combination of equations 38 and 39. It involves attack of the ester by imidazole to form *N*-acetylimidazole, which is subsequently hydrolyzed by water, yielding acetate ion and regenerating the catalyst.



(40)

The concentration of the intermediate in this reaction, *N*-acetylimidazole, reaches a high enough level to account for the entire catalytic process (82). In the reaction of benzimidazole with *p*-acetoxybenzoic acid, the pathway through *N*-acetylbenzimidazole accounts for at least 90 per cent of the conversion of this phenyl ester to hydrolytic products (338). Furthermore, the acetylimidazole intermediate has been isolated in the reaction of imidazole with *p*-nitrophenyl acetate (261) and in the reaction of *N*-benzoyl-L-histidine methyl ester with *p*-nitrophenyl acetate (60, 76). It is therefore possible to rule out a general basic

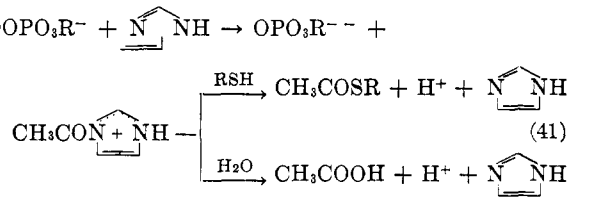
catalysis mechanism for these imidazole reactions. However, imidazole sometimes functions as a general basic catalyst, as discussed in Section VI.

One important facet of these investigations revealed that *N*-methylimidazole, which cannot form a stable (neutral) intermediate, is an efficient catalyst for the hydrolysis of *p*-nitrophenyl acetate, indicating that the conversion of *N*-acetylimidazolium ion to *N*-acetylimidazole in equation 40 is not a necessary part of the catalytic process. The *N*-acetyl-*N*-methylimidazolium intermediate is only one of several quaternary ammonium intermediates postulated in nucleophilic catalyses, others being the acylpyridinium and acyltrimethylammonium ions.

As pointed out previously, the catalytic rate constants of a series of imidazoles (or a series of pyridines) of constant steric requirement are related to the basicity of the catalyzing species in a manner similar to the Brønsted catalysis law (54, 89). Data for six imidazoles are given in the Brønsted plot illustrated in figure 1. The imidazoles in table 14 do not obey the Brønsted catalysis law because of changing steric requirements and because both the neutral and the anionic species can exhibit catalytic properties (92).

Imidazole also catalyzes the hydrolysis of ethyl thiolacetate (54) and acetylthiocholine (57), but does not catalyze the hydrolysis of ethyl acetate or acetylcholine (54). Data on the catalysis by imidazole of the hydrolysis of substituted phenyl acetates indicate that the rate of catalysis by imidazole depends quantitatively on the nature of the alcoholic portion of the ester (54, 91). Furthermore, it appears that the catalysis of ester hydrolysis by imidazole is of importance only for esters containing an alcohol that is a reasonably strong acid ($pK_a < 11$). Catalysis of thiol ester hydrolysis by imidazole is, of course, feasible under such a restriction. Cleavage of the thiol ester linkage is extraordinarily sensitive to imidazole catalysis, since the second-order rate constants for the reaction of imidazole and hydroxide ion with ethyl thiolacetate are of the same order of magnitude (0.996 (54) and 1.54 (330) l./mole minute, respectively), whereas the K_a' of imidazole is about 10^7 smaller than that of hydroxide ion. The greater nucleophilicity of imidazole than hydroxide ion toward thiol esters (with respect to its basicity) may account for the important role of thiol esters in biochemical reactions.

Imidazole also catalyzes the transfer of activated acyl groups from acyl phosphates to a number of nucleophiles. For example, imidazole catalyzes the reaction of acetyl phenyl phosphate and acetyl ethyl phosphate with thiols. In the absence of imidazole, reaction is negligible. The rate of the reaction is dependent on the imidazole and substrate concentrations but independent of the thiol concentration, although the products of the reaction are dependent on the thiol concentration. In the presence of 0.002 *M* mercaptoacetic acid, the product is entirely the thiol ester, indicating that the nucleophilicity of this thiol is about 10^4 greater than that of water. In reactions with thiol nucleophiles, no accumulation of the *N*-acetylimidazole was observed, but mechanism 41 may be postulated with confidence (230).

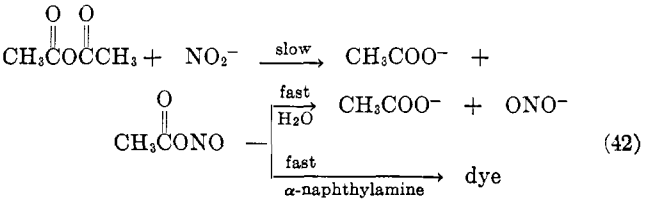


(41)

Nucleophiles that will react with *N*-acetylimidazole include not only water and thiols but also phosphate ion, arsenate ion, ammonia, hydroxylamine, and primary amines.

(c) Other nucleophilic catalysts

The hydrolysis of acetic anhydride is markedly catalyzed by nitrite ion. The rate-determining step in the reaction is the nucleophilic attack of nitrite ion on acetic anhydride. Evidence for the postulated intermediate, acetyl nitrite, has been obtained by the addition of α-naphthylamine to the system which diverts the acetyl nitrite intermediate from its usual hydrolytic path to form 4-amino-1,1′-azonaphthalene according to equation 42 (264a).



(42)

Reaction of the hypochlorite ion with carboxylic acid derivatives may also lead to catalysis of hydrolysis in a manner similar to that observed for the reaction of hypochlorite ion with phosphoric acid derivatives (149).

An unusual manifestation of nucleophilic catalysis involves the use of carbon dioxide as catalyst in the polymerization of *N*-carboxy-α-amino acid anhydrides (12, 13, 218). The kinetics of this process and the requirement of a strong amine indicate that the reaction proceeds through the formation of a carbamic acid intermediate from carbon dioxide and the free amine. This carbamic acid presumably reacts more readily as a nucleophile than the original amine, possibly because of the driving force caused by the loss of carbon dioxide in the reaction.

2. Intramolecular nucleophilic catalysis

It is well known that sterically favorable intramolecular reactions proceed more rapidly than the corre-

sponding intermolecular processes. The data reported above indicate that nucleophilic catalysis of the reactions of carboxylic acid derivatives can occur. In intramolecular cases nucleophilic catalysis should be favored and should therefore be of special importance. Much work has been done on anchimeric or synartetic assistance (neighboring group participation) in solvolysis at saturated carbon atoms, principally by Winstein and by Ingold (212, 219). The work described here constitutes studies of neighboring group participation in the reactions of carboxylic acid derivatives, principally in hydrolysis reactions. The effects in intramolecular catalysis are often quite large. Such studies are therefore desirable, since they show the possibilities of nucleophilic catalysis that might not be observed in less favorable intermolecular cases.

(a) Carboxylate ion

A classical example of intramolecular catalysis, although unrecognized at first, is the hydrolysis of aspirin in the region of pH 4 to 8. Edwards obtained a complete pH–rate profile of the hydrolysis of aspirin from pH 0 to 13 (146, 147) as shown in figure 7. Garrett has ex-

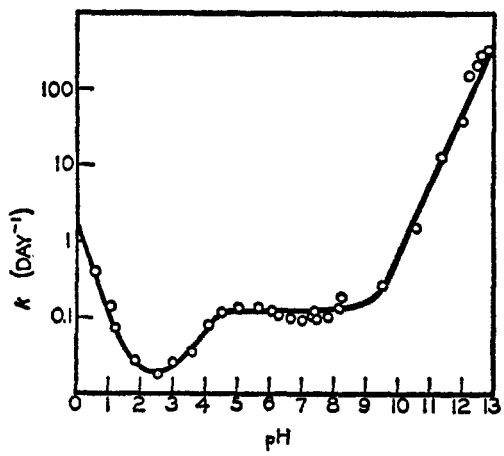
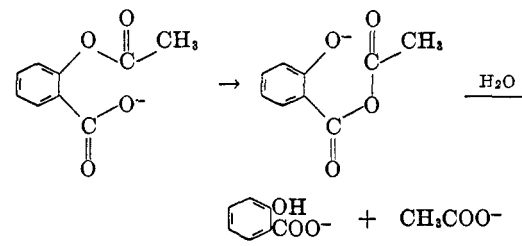


FIG. 7. The hydrolysis of aspirin at 25°C. (146).

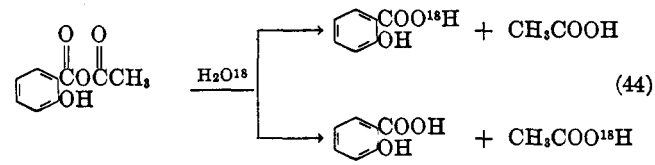
tended this work to include a number of acyl salicylates in a very complete investigation (169a). In the hydrolysis of acyl salicylates there exists catalysis by external hydrogen ion at low pH values (pH 1–3) and catalysis by external hydroxide ion at high pH values (pH 8–14). However, from pH 4 to 8 the rate constant is independent of the pH. This region of spontaneous hydrolysis is usually described (24) as due to catalysis by a water molecule. However, if such a reaction were a water reaction, it would be reasonable that external acetate ion, a more powerful nucleophile than water, would have an effect on the rate of hydrolysis, which is not the case (146). Hydrolysis of aspirin at pH 4 to 8 is most reasonably interpreted as a spontaneous reaction of the aspirin anion which occurs by an intramolecular attack of carboxylate ion on the carbonyl carbon atom of the ester, producing an anhydride intermediate which subsequently is hydrolyzed rapidly to the products of the hydrolysis, salicylate and acetate (equation 43).



Proposals in essentially this form have been made by a number of workers (111, 120, 170). Stereochemically such a pathway looks attractive. It gains added support from the fact that acetate ion is indeed a catalyst for the hydrolysis of a number of phenyl acetates (48, 54, 89).

As an alternative to the intermediacy of acetyl salicoyl anhydride in the hydrolysis of aspirin, it has been proposed that the addition of the carboxylate ion to the carbonyl group of the ester is directly followed by a reaction of the tetrahedral intermediate with water, leading to the products (111). No precedent for this reaction exists.

The hydrolysis of aspirin anion in H_2O^{18} should yield the isotopic species shown in equation 44 if acetyl salicoyl anhydride is the intermediate. The cleavage of



acetyl benzoyl anhydride with hydroxylamine (406b) leads to the prediction that in equation 44 the reaction producing salicylic acid-O^{18} should occur to the extent of 6 per cent. The hydrolysis of aspirin at pH 6 in water containing 4.3 atom per cent of oxygen-18 produced salicylic acid containing 6 per cent of the excess oxygen-18 in the water (36). The agreement between the theoretical prediction and the experimental result is fortuitously good and is consistent with the mechanism postulated in equation 43.

Morawetz has investigated the participation of carboxylate ion in the hydrolysis of ester groups in acrylic acid copolymers (297, 299, 300, 415). The pH dependence of the hydrolysis of an acrylic acid polymer containing 9 mole per cent of *p*-nitrophenyl methacrylate is shown in figure 8. For comparison the hydrolysis of *p*-nitrophenyl pivalate and of mono-*p*-nitrophenyl glutarate is also shown. The rate constant of the hydrolysis of the copolymer increases with increasing pH, a variation which cannot be explained by electrostatic considerations. The rate constant for the hydrolysis of the ester

linkages in the polymer molecule behaves in a strikingly different fashion from that of the pivalic acid derivative, which can be expressed in the usual form:

$$k_1 = k_{H^+}(H^+) + k_{OH^-}(OH^-)$$

The hydrolytic rate constant of the copolymer is constant between pH 8 and 11, but falls off at lower pH. The reaction is proportional to the degree of ionization of the carboxyl groups attached to the polymeric chain. The hydrolysis rate of mono-*p*-nitrophenyl glutarate, an aliphatic analog of the copolymer, depends in a similar fashion on the degree of ionization of the free carboxyl group, and not on catalysis by hydronium or hydroxide ion.

The acceleration of the ester hydrolysis in the copolymer and in the monoester amounts to a factor of about 10^5, around pH 6, over the reactions catalyzed by hydronium ion or hydroxide ion. These rapid solvolyses have been explained by the intermediate formation of substituted glutaric anhydrides (containing six-membered rings) through the attack of a neighboring carboxylate ion on the ester linkage. The facile intramolecular catalysis in the copolymer is due in part to the rigidity of the system and also to the multiple substitution of the chain. A careful kinetic analysis of the solvolysis of copolymers of methacrylic acid containing 1–2 per cent of *p*-nitrophenyl methacrylate indicates that a fraction of the ester groups are very much more reactive than the remainder. It has been postulated that this behavior reflects differences in the stereochemical arrangements in the immediate neighborhood of the ester group, the "slow" ester groups being fixed in a mixed (atactic) sequence in the chain and the "fast" ester groups being bound in an isotactic or syndiotactic sequence in the polymer chain, leading to a more favorable configuration for intramolecular catalysis (297).

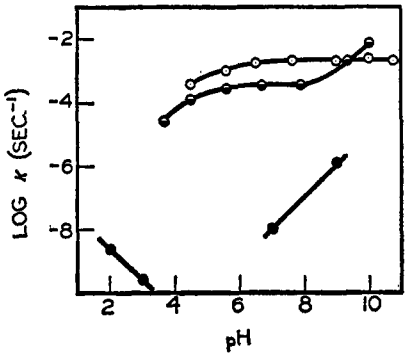
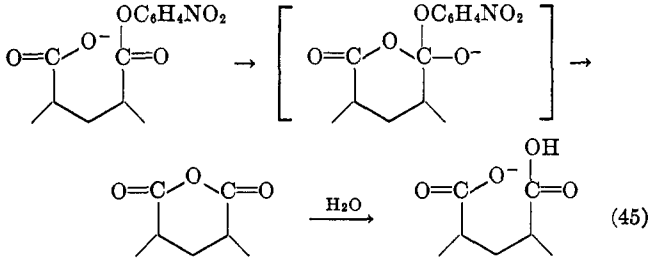


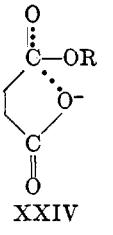
FIG. 8. Intramolecular hydrolysis of *p*-nitrophenyl esters (415). ●, *p*-nitrophenyl; ◓, mono-*p*-nitrophenyl glutarate; ⊙, copolymer of acrylic acid (91 mole per cent) and *p*-nitrophenyl methacrylate (9 mole per cent).



(45)

The intramolecular catalysis in the aliphatic system mono-*p*-nitrophenyl glutarate is especially interesting, since no configurational help is given by the backbone of this freely rotating molecule. A kinetic study of the hydrolysis of substituted phenyl succinates and glutarates reveals more information about intramolecular carboxylate-ion catalysis (297a). The intramolecular reaction seems to be much more sensitive to substituent effects than intermolecular carboxylate-ion catalysis, mono-*p*-nitrophenyl glutarate being hydrolyzed approximately 600 times faster than monophenyl glutarate. The substituent effects are largely due to changes in the entropy of activation (the *p*-methoxy- and the *p*-nitrophenyl glutarates have the same $\Delta H^{\ddagger}$, although the rate differs by a factor of over 10,000). This substituent effect is in sharp contrast to hydroxide-ion-catalyzed hydrolyses of phenyl esters.

The alkaline hydrolysis of succinylcholine could not be stopped at the half-hydrolyzed product in which only one of the choline groups was removed. The reason given for this negative result is that the half-ester could subsequently be hydrolyzed in a fast step involving an intramolecular catalysis by neighboring carboxylate ion, whose transition state is shown in formula XXIV (317).

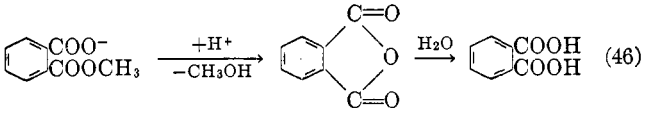


XXIV

Succinyl-S-coenzyme A has been reported to have a half-life of 1 to 2 hr. at pH 7–7.7 at room temperature, which is not the expected behavior for a thiol ester (346). This phenomenon can also be explained by an intramolecular attack of the free succinyl carboxylate ion on the thiol ester, leading to an unstable anhydride intermediate through a transition state analogous to XXIV.

A feature common to all intermolecular and intramolecular catalyses by carboxylate ion described above is that the group displaced from the ester molecule consists of a stabilized ion of some sort. It was thought that an ordinary ester such as ethyl acetate could not be hydrolyzed by nucleophilic catalysis since imidazole, the best of the intermolecular catalysts, failed with ethyl acetate. However, it is conceivable that in a sterically favored intramolecular hydrolysis, nucleophilic catalysis of a simple alkyl ester might be feasible.

That is, in such a case, the Brønsted relationship between catalytic rate constant and basicity of the nucleophile for intermolecular catalysis would not hold, so that intramolecular carboxylate ion could compete with catalysis by the solvent. The kinetics of hydrolysis of methyl hydrogen phthalate at 109°C. exhibits a pH–rate profile from pH 1 to 7 which is similar in shape to that for aspirin, containing a plateau from pH 4 to 7. If the hydrolysis in the aspirin plateau can be explained by an intramolecular catalysis by *o*-carboxylate ion, then certainly the hydrolysis in the plateau of the methyl hydrogen phthalate curve may be explained by the similar mechanism in equation 46, although no evidence exists for the phthalic anhydride intermediate. The catalysis of hydrolysis of a methyl ester by intramolecular carboxylate ion appears significant because of the failure to effect hydrolysis of simple alkyl esters by all intermolecular nucleophilic catalysts, including imidazole, a catalyst more powerful than carboxylate ion by about six powers of 10. This result demonstrates the powerful nature of intramolecular catalysis.

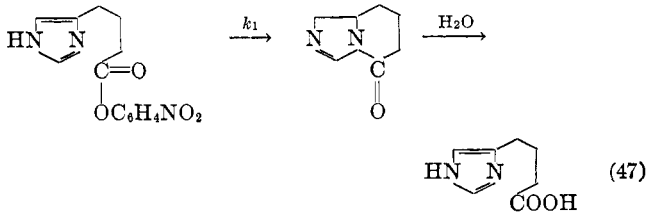


(46)

(b) Imidazole

Intramolecular catalysis by imidazole, investigated by Bruice and coworkers, has proved to be an important phenomenon. The solvolysis of 4-(2′-acetoxyphenyl)-imidazole was found to be first order with respect to the substrate. The pH–rate profile can be readily explained on the assumption that the rate of solvolysis of the substrate depends upon the state of ionization of a single group of pK_a' approximately 5.5; it was independently shown that the pK_a' of the imidazolyl group of the substrate is 5.6. These results indicate that the solvolysis of the substrate occurs with participation of the imidazolyl group (337). It was not possible to detect an acylimidazole intermediate in this reaction. The rate of ester hydrolysis with maximum participation of the imidazolyl group was found to be about 1000 times that without participation.

In the solvolysis of *p*-nitrophenyl γ-(4-imidazolyl)-butyrate (XXV) the lactamization step in the reaction sequence 47 follows first-order kinetics with a half-time in 50 per cent aqueous ethanol at 25°C. and pH 7.5 of 0.2 sec.

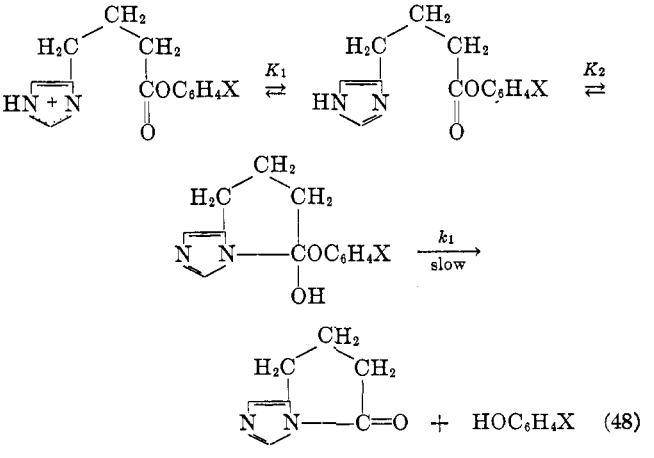


(47)

The rate was found to vary with pH in a manner which establishes the participation of a group with a pK_a' of 6.3 (94). The participation of the imidazole group in the reaction of XXV can be rationalized by postulating either a nucleophilic attack of an amidine nitrogen on the ester bond (equation 47) or a general basic catalysis of ester hydrolysis. These two possibilities cannot be differentiated kinetically. The spectrophotometric observation of the lactam, however, proves the validity of the nucleophilic catalysis mechanism in equation 47 (88).

Although the methyl ester of γ-(4-imidazolyl)-butyric acid does not undergo hydrolysis with imidazole participation (its hydrolysis is catalyzed by hydroxide ion), 4-(2′-acetoxyethyl)imidazole does exhibit imidazole participation at an elevated temperature. This reaction is another example of an intramolecular catalysis of the hydrolysis of a simple alkyl ester which has no intermolecular analog.

The participation of the imidazole group in the hydrolysis of the substituted phenyl esters related to XXV possesses an unusual characteristic. The apparent dissociation constant of the imidazolyl group—as determined kinetically—depends markedly on the meta or para substituent of the carbophenoxy group, being much lower for the *p*-nitrophenyl ester (95). These results are in contrast to that found in the hydrolysis of 2-(4′-imidazolyl)phenyl acetate (337), where the value of pK_a' was found to correspond to that of the imidazolyl group. They suggest that another substituent-dependent equilibrium—in addition to the dissociation of the imidazolium ion—occurs prior to the rate-limiting step. The following mechanism has been postulated (95):



(48)

On the basis of the results of this kinetic analysis it appears that the intramolecular reaction of imidazole, leading to lactam formation, proceeds through the equilibrium formation of a tetrahedral intermediate followed by a slow collapse of this intermediate, whereas the intermolecular reaction apparently proceeds in more usual manner with the slow formation of the tetrahedral intermediate followed by a rapid collapse. This

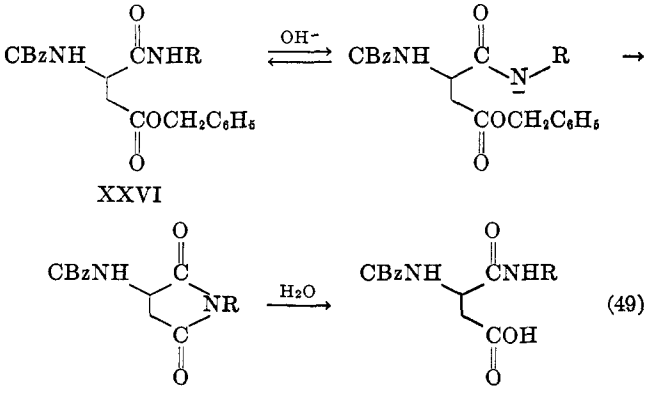
result is in conformity with other differences between inter- and intramolecular reactions in organic chemistry.

In the hydrolysis of the thiol ester *n*-propyl γ-(4-imidazolyl)thiobutyrate, the imidazole group also participates in the solvolysis, leading to the same intermediate lactam shown in equation 47. The thiol ester hydrolyzes at pH's near neutrality between 10^6 and 10^7 times as fast as a normal thiol ester would react under those conditions with hydroxide ion (88a). This large rate enhancement is due to two factors: (*1*) the usual advantage of an intramolecular process over the corresponding intermolecular one and (*2*) the fact that thiol esters are much more susceptible to nucleophilic attack by nitrogen bases such as imidazole than by oxygen bases such as hydroxide ion. Whereas oxygen esters are either not susceptible (methyl ester) or difficultly susceptible (ethyl ester) to an intramolecular imidazole catalyst, a thiol ester is easily hydrolyzed in this manner. These experiments, as well as the corresponding ones with an intermolecular imidazole catalyst, point up an important difference between oxygen esters and thiol esters, which is not apparent when comparing their reactivities toward catalysis by hydroxide ion or hydrogen ion (301, 330).

The rate of hydrolysis of the lactam shown in equation 47 or 48 has been measured. For the intramolecular catalyzed hydrolysis of the phenyl and thiol esters around neutrality, the hydrolysis of the lactam is the slow step of the overall reaction (88).

(c) Other nucleophilic catalysts

The treatment of asparagine and glutamine esters with alkali results in imide formation (352). An imide has been postulated as an intermediate in the hydrolysis of β-esters of aspartyl peptides, via intramolecular catalysis by the conjugate base of the amide group (63). The primary α-amide of *N*-carbobenzoxyaspartic acid β-benzyl ester (XXVI), when subjected to the conditions for basic hydrolysis, eliminates benzyl alcohol to form the corresponding imide. However, if instead of a primary α-amide, a substituted amide of XXVI is subjected to basic conditions, a facile overall hydrolysis results, according to equation 49. The relative rate data



(49)

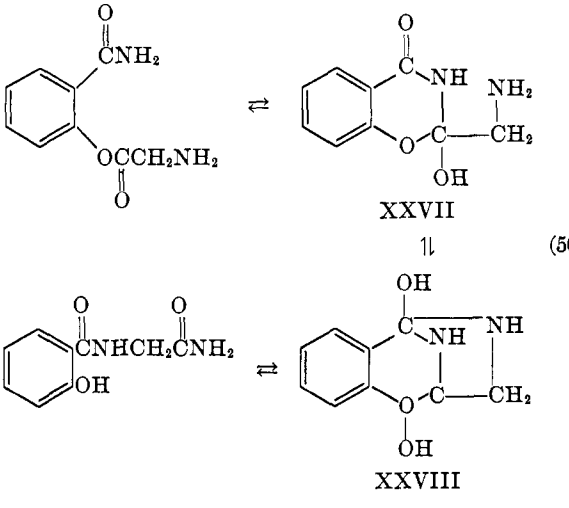
TABLE 15

Relative rates of hydrolysis of benzyl esters of $CBzNHCH(CH_2COOC_7H_7)X$

X	Relative Rate
1. H	3
2. C(O)OBz	1
3. C(O)NHCH₃	10^3
4. C(O)NHCH₂CH₂OH	10^3
5. C(O)NHCH₂CONH₂	10^3

shown in table 15 are pertinent to this point, indicating that the presence of an amide group increases the rate constant for basic hydrolysis about 10^3 over that of the corresponding molecule without an amide group (63). Since the imides of this series have high negative specific optical rotations, it was possible to follow polarimetrically the appearance and disappearance of the imide intermediate in the reaction of compound 3 of table 15 (63).

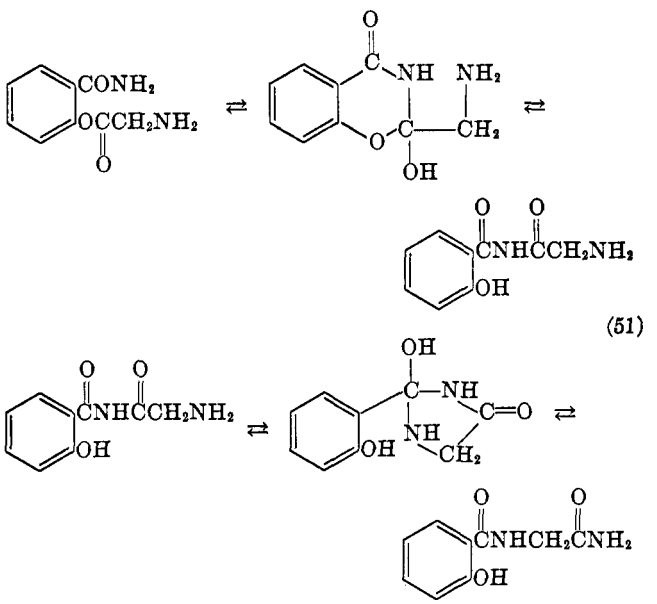
A similar phenomenon involving the conjugate base of an amide has been shown to occur in the "amino acid insertion" reaction (80). Under mildly basic conditions aminoacetylsalicylamide undergoes rearrangement, transferring its side chain from the phenolic oxygen to the amide carbonyl group. This reaction has been postulated by Brenner to proceed first through a six-membered cyclic intermediate formed by a nucleophilic attack of a neighboring group of the acyl moiety (XXVII) and secondly through a bicyclic intermediate in which both original carbonyl carbon atoms are in a tetrahedral configuration (XXVIII). The reaction can alternatively be explained by a series of transformations



(50)

involving an imide intermediate. Mechanism 51 is questionable, since benzoyl glycyl *N*-methylimide does not undergo rearrangement to the corresponding peptide (77) but it is attractive, since it has been shown that *O*-acetylsalicylamide can be converted to the corresponding imide under basic conditions (282). Corresponding "amino acid insertion" reactions have been carried out in the aliphatic series with derivatives of

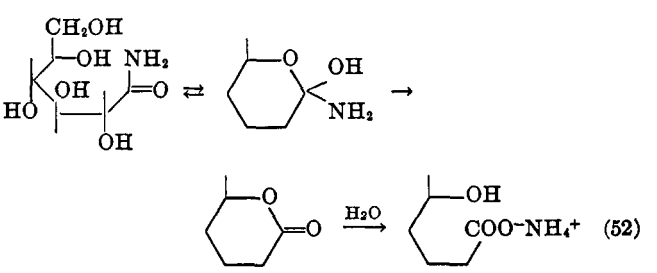
serine, threonine, and cysteine, although more strenuous basic conditions are necessary (78, 79).



(51)

Hydroxyl groups have been implicated in the intramolecular catalysis of amide hydrolysis. Treatment with hydrogen chloride can result in the rearrangement of ethanolamides into aminoethyl esters (318) and in acyl migration from nitrogen to oxygen in various epimeric acetylinosamines (281). When an acetylamino group and a hydroxyl group bear a cis relationship to one another, an overall hydrolysis of the acetyl group by dilute hydrochloric acid is fast, presumably because of the intermediate formation of an acetoxy group (281). The hydrolysis of the amide *N*-benzoyl-ψ-ephedrine under acidic conditions has also been reported to proceed through the intermediate formation of *O*-benzoyl-ψ-ephedrine and thence to the products, ψ-ephedrine and benzoic acid (394, 395).

More interesting examples of intramolecular catalysis by hydroxyl groups involve the intermediate formation not of ordinary esters but rather of lactones, which are hydrolyzed with great ease compared to ordinary esters. The hydrolytic instability of aldonamides in dilute aqueous solutions at room temperature has been attributed to intramolecular catalysis by a hydroxyl group (412). This hydrolysis is catalyzed by acids or bases, especially the latter, which apparently facilitate the formation of the cyclic lactone intermediate shown in equation 52.



(52)

The intermediate formation of a lactone has also been postulated to account for the fact that 1-carbomethoxy-2-hydroxytriptycene is saponified much faster than 1-carbomethoxytriptycene itself (21).

Finally, an intramolecular catalysis by phosphate ion may be noted. It has been reported that the phenolic group of the phenyl ester of salicyl phosphate is liberated in 10 min. at pH 5 to 9 (4, 289). This observation has been interpreted in terms of the nucleophilic attack of the neighboring phosphate ion, presumably followed by hydrolysis of the intermediate mixed anhydride (111).

An effective intramolecular catalysis depends on a number of factors. Proper steric orientation is, of course, one of the prime requisites. In addition the intramolecular nucleophile must be a powerful one, as compared to external hydroxide ion or other nucleophiles. And finally the intermediate formed in the initial reaction must be subject to ready attack by the nucleophile (usually the solvent) which is to be eventually part of the product.

Differentiation between nucleophilic catalysis and general basic catalysis is not always easy. In the last analysis, this differentiation depends on the observation of an acyl–catalyst intermediate. Nucleophilic catalysis of a carboxylic acid derivative predicts such an intermediate, while general basic catalysis does not. In some of the preceding cases, formation of an intermediate has been demonstrated and there is no question that nucleophilic catalysis occurs. In other cases formation of an intermediate has not been demonstrated and no unequivocal statement can be made as to the mechanism of the reaction, although present opinion indicates that the mechanism involves nucleophilic catalysis.

V. Electrophilic-Nucleophilic Catalysis

The concept that catalysis solely by a nucleophile or by an electrophile can be surpassed in efficiency by catalysis by some combination of a nucleophile and an electrophile has received much attention in recent years. This interest has taken several forms: (*1*) investigations of reactions in which a substrate, a nucleophile, and an electrophile constitute the rate-determining transition state and (*2*) studies of possible bifunctional catalysts which in themselves contain both an electrophilic and a nucleophilic function.

In studies of the mutarotation of tetramethylglucose, Lowry originally suggested the possibility of "simultaneous contact with proton donor and acceptor" (272). On the basis of studies of the mutarotation of tetramethylglucose in benzene solution in the presence of phenol–pyridine mixtures (373) and in studies of the enolization of ketones (372), Swain suggested that third-order processes generally occur, involving one molecule of substrate, one molecule of electrophile, and

one molecule of nucleophile, with the solvent water serving as a possible electrophile or nucleophile. Recent studies on the effect of deuterium oxide solvent on the rates of the enolization of ketones have indicated that this general statement cannot hold (375). For example, in the acetic acid catalysis of the enolization of a ketone, the catalysis proceeds via a preëquilibrium involving protonation of the ketone, followed by a slow removal of a proton by acetate ion. The one instance where it has been shown (28, 126) that a third-order term involving both acetic acid and acetate ion does exist, the enolization of acetone, has now been treated as a special case (375).

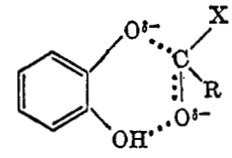
The mutarotation of glucose catalyzed by acetic acid is still believed to be a concerted process, involving the bifunctional molecule, acetic acid, which simultaneously donates a proton to the glucose oxygen and accepts a proton from the glucose hydroxyl group in a slow cyclic process following a preëquilibrium hydrogen bonding (375). This process is an example of a number of processes which have been postulated to proceed with bifunctional catalysts. The most widely recognized example of a bifunctional catalyst is 2-hydroxypyridine, which has been shown to be an exceptionally efficient catalyst for the mutarotation of tetramethylglucose in benzene solution (374). Electrophilic-nucleophilic catalyses will be discussed in these terms in the following discussion of the reactions of carboxylic acid derivatives.

A. INTERMOLECULAR ELECTROPHILIC-NUCLEOPHILIC CATALYSIS

1. Bifunctional reagents

A number of nucleophilic agents which react with carboxylic acid derivatives have also been shown to possess electrophilic character, making them bifunctional reagents. These bifunctional reagents have in most cases not involved catalytic processes, although in a few cases a catalytic phenomenon has occurred. It is of interest, however, to consider possible bifunctional reactions as a guide to future catalytic possibilities.

The rates of reaction of catechol with several acyl halides in 95 per cent acetone indicate a first-order dependence on the monocatecholate ion (112). Furthermore resorcinol, hydroquinone, and phenol are relatively unreactive toward acyl halides. A concerted bifunctional attack was postulated on this basis and on the basis that it had been previously shown that catecholate ion reacts readily with isopropylmethylphosphonofluoridate, presumably via a concerted process. The transition state of the acyl halide reaction can be depicted as XXIX and that of the phosphorus derivative in a similar manner (151). Presumably other bifunctional derivatives of benzene such as *o*-phenylenediamine monohydrochloride could function in an analogous fashion in concerted reactions with carboxylic acid derivatives.



XXIX

The reactions of *N*-acetylimidazole with amines, sulfhydryl compounds, carboxylic acids, phosphate ion, and arsenate ion proceed via the attack of the conjugate base of the nucleophile on the conjugate acid of *N*-acetylimidazole (230, 231). The pH dependence of the uncatalyzed reactions of *N*-acetylimidazole with thiols or amines indicates that the rates of these reactions are proportional to the concentrations of the species RSH or RNH_3^+ (231). Reactions of thiols, however, almost always involve the anion, RS^-, as nucleophilic reagent, and the species RNH_3^+ has no free electrons available for nucleophilic attack and therefore almost certainly cannot react with *N*-acetylimidazole. These reactions are therefore best formulated as attacks of free amine or thiol anion on the conjugate acid of *N*-acetylimidazole, processes which are kinetically indistinguishable from reactions of the thiol or ammonium ion on the neutral substrate.

2. Bifunctional catalysts

The hydrolytic cleavage of the amide linkage in chloramphenicol is accelerated by a partly neutralized form of citric acid to a greater extent than by either free citric acid or the trinegative citrate ion (209, 210). It was suggested in this instance that the presence of both electrophilic and nucleophilic centers in the same molecule is necessary for a high degree of reactivity, presumably through a bifunctional attack.

The hydrolysis of *N*-butylacetamide in nearly neutral, buffered solutions of acetic acid at 220°C. shows kinetic dependence upon the concentration of undissociated acetic acid (413). Since the deuterium solvent isotope effect led to the result $k_H/k_D = 1.8$, it was postulated that the mechanism of the reaction is related to electrophilic-nucleophilic catalysis involving the nucleophilic attack of acetate ion on the protonated amide in the rate-determining step. Although kinetically this reaction is an example of general acid catalysis, it is seen to be mechanistically an electrophilic-nucleophilic catalysis.

$$CH_3COOH + R'CONHR \rightleftarrows R'CONH_2R^+ + CH_3COO^- \quad \text{(fast)}$$
$$R'CONH_2R^+ + CH_3COO^- \rightarrow R'COOOCCH_3 + RNH_2 \quad \text{(slow)}$$
$$R'COOOCCH_3 + H_2O \rightarrow R'COOH + CH_3COOH \quad \text{(fast)}$$
$$(53)$$

In some of the above reactions of bifunctional reagents and catalysts, the reaction has been described

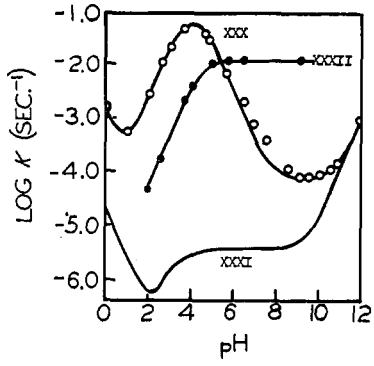


FIG. 9. Hydrolysis of aspirin and derivatives at 25°C. (298).

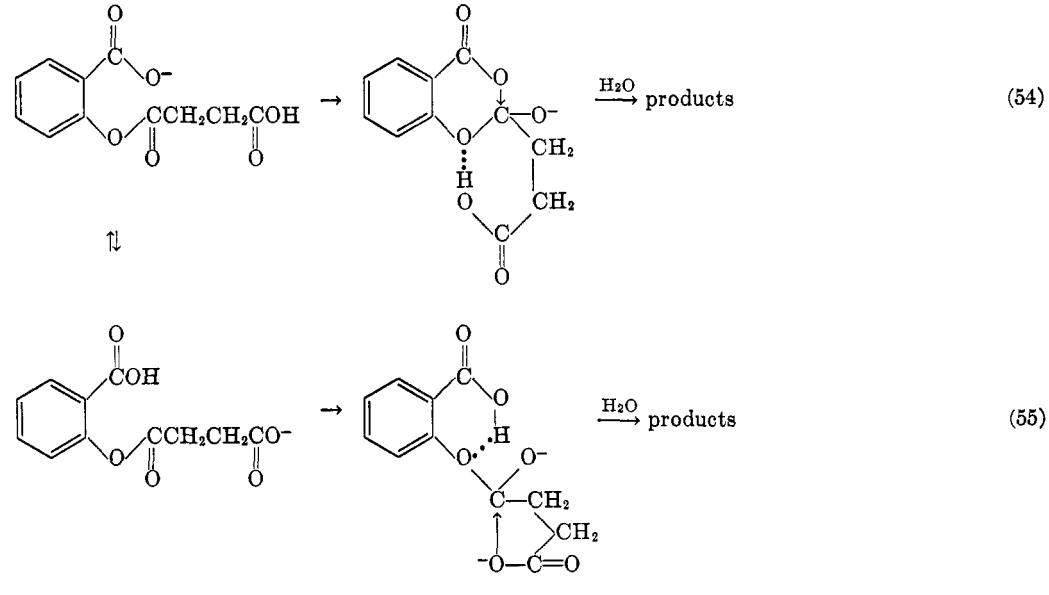
as a one-stage (concerted) process and in others the reaction has been described as a two- or three-stage (stepwise) process. In fact, no clear experimental evidence, except perhaps the evidence of the deuterium solvent effect, exists for differentiation between these mechanistic possibilities. The proposals that have been made above, therefore, are mainly arbitrary representations which may need modification when more experimental evidence is available.

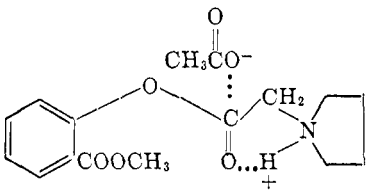
B. INTRAMOLECULAR ELECTROPHILIC-NUCLEOPHILIC CATALYSIS

One of the few bifunctional catalyses of ester hydrolysis in which the two functionalities are unambiguously separated occurs in the solvolysis of the aspirin derivative XXX. Whereas aspirin (XXXI) and the monomethyl ester (XXXII) are hydrolyzed in the pH range of 3 to 9 at rates proportional to the ionized carboxylate ion, XXX is hydrolyzed at a rate which is proportional to the concentration of a species containing both a carboxylate ion and a free carboxylic acid, assuming pK's of 3.62 and 4.5 for the salicylic and succinic carboxylic acid groups, respectively (figure 9) (298). Two kinetically indistinguishable pathways are possible, each involving a nucleophilic attack by carboxylate ion and an electrophilic assistance from an unionized carboxylic acid group. If the reaction proceeds through equation 54, the compound would hydrolyze 24,000 times as fast as the aspirin anion, which lacks only the second, unionized carboxylic acid group. If the reaction proceeds through equation 55, the compound would hydrolyze 66 times as fast as XXXII.

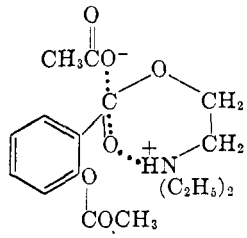
In the hydrolysis of methyl pyrrolidylacetylsalicylate hydrochloride, nucleophilic catalysis by acetate ion is accompanied by intramolecular protonic catalysis from the pyrrolidyl proton. The electrophilic interaction facilitates attack of the negative nucleophile at the carbonyl carbon atom (170) (XXXIII). In the hydrolysis of diethylaminoethyl acetylsalicylate hydrochloride it is claimed that acetate ion catalyzes the hydrolysis of both the phenyl ester and the diethylammoniumethyl ester. The hydrolysis of the latter ester is the only known case of intermolecular acetate-ion catalysis of an alkyl ester. This special case is probably due to facilitation of acetate-ion attack by intramolecular protonic catalysis from the β-ammonium group (171) (XXXIV).

XXX XXXI XXXII





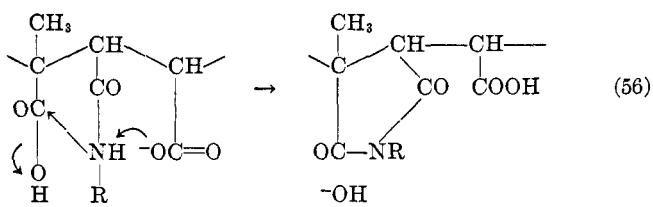
XXXIII



XXXIV

The hydrolysis of an ester by both electrophilic and nucleophilic catalysis is an important phenomenon. Esters, however, are much more susceptible to nucleophilic attack than electrophilic attack, whereas amides are known to be subject to nucleophilic and electrophilic attack to approximately equal extents. Therefore it would be expected that amide reactions would exhibit a greater susceptibility to catalysis by both electrophilic and nucleophilic agents. This hypothesis has been verified experimentally. Some of the amide reactions are possibly concerted attacks of the two catalytic entities; some are probably stepwise reactions involving prototropic equilibria followed by a rate-determining attack of the nucleophile. In most cases, the overall kinetic form conforms to the definition of general acid catalysis given earlier.

The electrophilic and nucleophilic groups are clearly differentiated in the imidization of a copolymer of methacrylic acid and maleic anhydride which had been treated with *p*-nitroaniline to give a number of anilide groups surrounded in the polymer chain by β-carboxylic acid groups. In the region from pH 2 to 8, this copolymer is not hydrolyzed but is rather converted in a pseudo-first-order process to a polyimide. The pH dependence of the imidization rate shows a maximum velocity around pH 5, indicating that the reaction involves the nitroanilide group together with one ionized and one unionized carboxyl group. The mechanism of the reaction may be represented by equation 56 (397, 415).

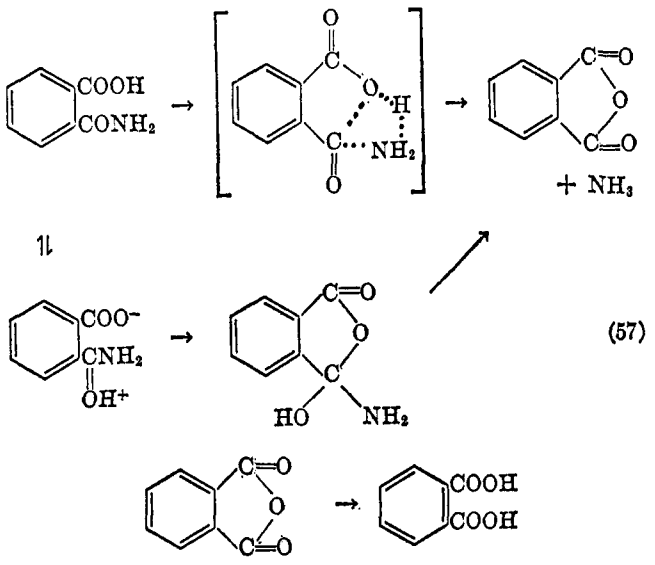


(56)

Several examples of intramolecular electrophilic-nucleophilic catalysis involve the hydrolysis of "amic acids." These molecules contain both an amide linkage and a carboxylic acid group appropriately situated with respect to one another for possible interaction. The hydrolyses of the amide bonds of glycyl-L-asparagine and L-leucyl-L-asparagine in aqueous solution from pH 1.2 to 3.5 are first order in the organic reactant containing an undissociated carboxyl group and are independent of the external hydrogen-ion concentration over this pH range (263). On the basis of the first-order character of the reactions, the independence of the external hydrogen-ion concentration and the small, negative entropies of activation, it was postulated that the hydrolyses proceed through an internal mechanism involving an (internal) proton transfer from the unionized carboxyl group at one end of the molecule to the amide group at the other end. It was postulated that the peptides exist in solution in a six-membered cyclic hydrogen-bonded structure which is close to the postulated activated complex of a protonated amide group. At pH 3 the intramolecular process is approximately 10^3 faster than the hydrolysis of asparagine, which is catalyzed by external hydrogen ion. It is possible that the reason why the glycyl and L-leucyl peptides exhibit intramolecular catalysis, while asparagine, the parent compound, does not, depends on restriction of rotation in the former compounds because of the bulky substituents.

Phthalamic acid is a molecule closely analogous to the asparagine derivatives except for an additional rigidity imposed by the benzene ring. The hydrolysis of phthalamic acid in aqueous solution exhibits kinetic dependence on the undissociated phthalamic acid and independence of external hydrogen ion from pH 1 to 5. At pH 3 the hydrolysis of phthalamic acid is about 10^5 faster than the hydrolysis of benzamide and about 10^6 faster than the hydrolysis of *o*-nitrobenzamide. On the other hand, the hydrolysis of terephthalamic acid is somewhat slower than that of benzamide. The large rate enhancements in the former case suggest that the *o*-carboxylic acid group does not exert a substituent effect but rather catalyzes the amide hydrolysis by a direct intramolecular process (35, 38). It is suggested that this process (as well as the cases of asparagine) is a concerted electrophilic-nucleophilic catalyzed reaction involving the intermediate formation of an anhydride. The concerted character of the reaction cannot be proven unambiguously, since zwitter-ion formation followed by a nucleophilic attack is also consistent with the kinetic form. However, the fact that the intramolecular hydrolysis of phthalamic acid is so much faster than the intermolecular hydrolysis of *N*-butylacetamide by acetic acid (the former reaction takes place conveniently at 47.3°C., while the latter takes place conveniently at 220°C.) indicates that the concerted action of the *o*-carboxylic acid group may be operative here (38) (equation 57). A tracer experiment involving the hydrolysis of phthalamic acid-*carboxamide*-C^{13} in H_2O^{18} provides indirect evidence for the formation of a symmetrical phthalic anhydride intermediate (38).

The rate of hydrolysis of an acrylic acid copolymer containing 3 mole per cent of *p*-nitroacrylanilide between pH 2 and 8 indicates that the reaction rate is governed by the reaction of the unionized neighboring

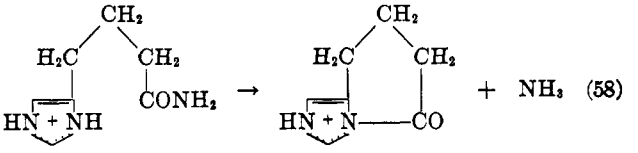


(57)

γ-carboxylic acid groups with the nitroanilide. This behavior is not found for *p*-nitro-α-trimethylacetanilide or for the mono-*p*-nitroanilide of glutaric acid. The behavior of the copolymer is similar mechanistically to that described above for the asparagine derivatives and phthalamic acid. However, the absolute rate of reaction is low, the reaction taking place conveniently at 135.4°C. (397, 415).

With dilute hydrochloric acid, partial hydrolysates were prepared from four different proteins. Total *N*-terminal amino acid content was twice that of the aspartic acid released, and only trace amounts of other amino acids were released, indicating a high degree of specificity for the aspartic acid residue (344). This phenomenon can be readily interpreted in terms of the mechanism given above for the intramolecular catalysis of an amide bond by an intramolecular carboxylic acid group. The fission of peptide bonds in insulin with hydrogen chloride in anhydrous media consisted mainly of cleavage of bonds adjacent to aspartyl, aspariginyl, glutamyl, and glutaminyl residues, together with cleavage of some bonds involving serine and threonine amino groups (385a). Certainly some of the specificity shown above again can be explained in terms of intramolecular catalysis by a carboxylic acid group.

The amide of γ-(4-imidazolyl)butyric acid is hydrolyzed at a rate which indicates that the protonated imidazolyl group participates in the reaction (95). This result is in contrast to that found in the hydrolyses of the corresponding esters in which the free imidazolyl group participates as an intramolecular catalyst. In this set of reactions, as well as in the phthalic acid series and in the reactions of the copolymers discussed previously, it has been consistently found that the intramolecular catalysis of an ester group involves a nucleophile and the intramolecular catalysis of an amide involves a general acid (mechanistically a combination of an electrophile and a nucleophile). The specific rate associated with the carboxylic acid participation in the hydrolysis of phthalamic acid is much greater than that for the participation of carboxylate ion in the hydrolysis of monomethyl phthalate. Likewise, the specific rate constant for the participation of protonated imidazolyl in the hydrolysis of the amide above is greater than that of the participation of imidazolyl in the hydrolysis of the corresponding ester. These results lend credence to the concerted nucleophilic-electrophilic mechanisms proposed above for the intramolecular catalysis of amide hydrolysis. The mechanism of the protonated imidazolyl participation in the hydrolysis of an amide may be written in an analogous fashion, as in equation 58.



(58)

C. A KINETIC COMPARISON OF INTERMOLECULAR AND INTRAMOLECULAR CATALYSIS

In table 16 is shown a summary of the kinetic results of a number of intermolecular and intramolecular reactions of the hydrolysis of carboxylic acid derivatives, brought about by nucleophilic and by electrophilic-nucleophilic catalysis. In addition the important case of the intramolecular and intermolecular hydrolysis of phosphate esters is listed. It is seen that within each grouping, showing a direct comparison of corresponding intermolecular and intramolecular processes, the intramolecular process is much more powerful, as has been noted before (48, 111).

A quantitative comparison of inter- and intramolecular catalyses, which correspond to first- and second-order kinetic processes, can be made by assuming an equal concentration of the two substrates and determining what concentration of the intermolecular catalyst is necessary for equivalent *rates* of hydrolysis of any concentration of substrate. In this way, for example, the hydrolysis of phenyl acetate with acetate ion is seen to require about 8 *M* acetate ion in order that the rate of hydrolysis of this intermolecular process be equivalent to the rate of the intramolecular hydrolysis of a corresponding concentration of aspirin. The differences between the intermolecular hydrolysis of *p*-nitrophenyl acetate by acetate ion and the intramolecular hydrolysis of mono-*p*-nitrophenyl glutarate or of the related copolymer are even more striking, requiring concentrations of acetate ion of the order of 600 *M* for equivalent rates.

The intramolecular hydrolyses of methyl hydrogen phthalate and phthalamic acid are even more striking. In the former case the corresponding intermolecular process has not as yet been found. In the latter case there is a huge difference in the rates which is difficult

TABLE 16

A kinetic comparison of intermolecular and intramolecular catalysis of hydrolysis

Substrate	Hydrogen Ion	Temperature	Catalysis		$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	References
			Intramolecular k_1	Intermolecular k_2			
	$M \times 10^6$	°C.	*sec.*$^{-1}$	*l./mole sec.*	*kcal./mole*	*e.u.*	
Aspirin anion	1	60.3	83.8×10^{-6}		17.6	−24.7	(169a)
Phenyl acetate + acetate ion	3	63.0		10.2×10^{-6}	16.6	−31.2	(48)
p-Acetoxybenzoic acid	1	60.3	1.9×10^{-6}§				(337)
Mono-*p*-nitrophenyl glutarate	3	0	3×10^{-4}		19.4	−3.5	(414)
Copolymer of acrylic acid and *p*-nitrophenyl methacrylate	3	0	2×10^{-3}				(414)
p-Nitrophenyl acetate + acetate ion	3	22.6		5.7×10^{-6}	15.7	−28.7	(48)
Methyl hydrogen phthalate	1	109	5.9×10^{-5}		33.7	+7.5	(36)
Methyl benzoate + acetate ion	1	109		$0\text{-}1.2 \times 10^{-6}$			(36)
Phthalamic acid	1000	47.3	2.35×10^{-4}		20.7	−12.4	(35, 37)
Benzamide + acetic acid	1000	47.3					(35, 37)
Benzamide + hydrogen ion	1000	48.7	3.1×10^{-9}§	3.1×10^{-6}	22.8	−13.9	(321)
o-Nitrobenzamide + hydrogen ion	1000	48.7	1×10^{-10}§	1×10^{-7}			(322)
N-Butylacetamide + acetic acid	10	220		12.3×10^{-5}			(413)
Copolymer of acrylic acid and *p*-nitromethacrylanilide		135		1.6×10^{-5}			(415)
Glycyl-L-asparagine	1000	90	1.92×10^{-4}		24.0	−9.4	(263)
L-Leucyl-L-asparagine	1000	90	2.48×10^{-4}		22.2	−14.1	(263)
L-Asparagine + hydrogen ion	1000	90		3.97×10^{-4}	19.8	−19.5	(263)
o-Carboxyphenyl phosphate†	150	80	1.3×10^{-2}		23.5	−1.2	(111)
Phenyl phosphate*	15	80	3.2×10^{-3}		28.4	+0.9	(110)
m-Carboxyphenyl phosphate†	15	80	3.3×10^{-5}		27.1	−2.6	(110)
p-Carboxyphenyl phosphate†	15	80	4.1×10^{-5}		26.8	−3.1	(110)
4-(2′-Acetoxyphenyl)imidazole	0.1	30	2×10^{-3}				(337)
p-Nitrophenyl acetate + substituted imidazole	0.1	30		1			(337)
Phenyl γ-(4-imidazolyl)butyrate	0.1		0.043				(95)
Phenyl acetate + imidazole	0.1	25		0.18×10^{-2}			(91)
n-Propyl γ-(4-imidazolyl)thiobutyrate	0.01	15	8.84×10^{-2}				(88)
Ethyl thiolacetate + imidazole	0.1	26.2		1.66×10^{-4}			(54)

* Rate constant of the monoanion. † Rate constant of the dianion. § A pseudo-first-order rate constant of an intermolecular catalysis.

to assess because of the large difference in the temperatures of the corresponding intra- and intermolecular processes.

From a general consideration of table 16, the kinetic differences between inter- and intramolecular catalyses cannot be ascribed to either the enthalpy or the entropy of activation. Only a few cases exist for comparison of intra- and intermolecular catalyses of identical mechanistic type, such as catalysis by carboxylate ion in both instances. These are comparisons of aspirin anion with phenyl acetate and acetate ion; comparison of mono-*p*-nitrophenyl glutarate (or the related copolymer) with *p*-nitrophenyl acetate and acetate ion; comparison of phthalamic acid with *N*-butylacetamide and acetic acid; and comparison of intra- and intermolecular catalyses by imidazole. In all other cases the intramolecular reaction, has, of necessity, been compared with an intermolecular reaction brought about by a different catalytic species. A comparison of the two equivalent intramolecular-intermolecular cases involving carboxylate-ion catalysis reveals that although the rates of a given set are quite different, the activation enthalpies of a given set are not far separated from one another. In fact, in both sets the activation enthalpy of the intramolecular catalysis is somewhat higher than that of the corresponding intermolecular catalysis. However, in both sets the entropy of activation of the intramolecular reaction is significantly more positive than that of the corresponding intermolecular reaction. This result is what one might expect from the qualitative argument that the probability of reaction should be greater for an intramolecular reaction than for the corresponding intermolecular reaction, while the enthalpy of activation should remain constant. It is particularly interesting that the difference in $\Delta S^{\ddagger}$ in intramolecular and intermolecular catalysis can be as high as 25 e.u. (in the *p*-nitrophenyl set).

VI. General Basic Catalysis

As mentioned in Section IV, several abortive attempts to find general basic catalysis in the nucleophilic reactions of carboxylic acid derivatives were made some years ago. Very recently interest has been renewed in the general basic catalysis of these reactions. It is well to reëmphasize the difference between general basic catalysis and nucleophilic catalysis at this time. In both cases the catalyst is involved in the transition state of the slow step of the reaction, but not in the products of the reaction. In general basic catalysis the catalyst reacts by means of a nucleophilic attack on hydrogen, whereas in nucleophilic catalysis the catalyst is involved in a nucleophilic reaction at a carbon atom.

Acetate ion catalyzes the hydrolysis of acetic anhydride (175, 240). Likewise propionate ion catalyzes the hydrolysis of propionic anhydride (241). These catalyses, which are small but real, obviously cannot be

attributed to nucleophilic catalysis, for the nucleophilic reaction of acetate ion with acetic anhydride, while certainly possible, leads to no kinetically observable result. These results must be interpreted in terms of general basic catalysis.

The rates of the alkaline hydrolysis of *N*-methylanilides (23) and anilides (50, 256) are proportional to both the first and the second powers of the hydroxide-ion concentration. The term which is second order in hydroxide ion can be considered to be a hydroxide-ion catalysis of the reaction of the nucleophile hydroxide ion with an anilide. An analogous case involves the general basic catalysis of the carbonyl addition reaction, the hydration of acetaldehyde (25).

The reactions of *N*-acetylimidazole with water, amines, and thiols are catalyzed by imidazole. The pH dependence of this reaction indicates that the catalytic form of imidazole is the free base. In the present instance direct reaction of imidazole with *N*-acetylimidazole can again have no catalytic effect, and therefore imidazole must serve as a general basic catalyst. These results indicate that imidazole and acetate ion can serve either as nucleophilic catalysts or as general basic catalysts. In the hydrolysis of *N*-acetylimidazole, its reaction with mercaptoethanol, and its reaction with various amines such as ammonia and glycylglycine in the presence of imidazole, the rate laws take the form of equations 59, 60, and 61, respectively (231). Equation 61 indicates that the amino compounds can catalyze

$$v = k_2(\mathrm{AcIm})(\mathrm{Im}) \tag{59}$$

$$v = k_1(\mathrm{AcIm})(\mathrm{RSH}) + k_2(\mathrm{AcIm})(\mathrm{RSH})(\mathrm{Im}) \tag{60}$$

$$v = k_1(\mathrm{AcIm})(\mathrm{RNH_3^+}) + k_2(\mathrm{AcIm})(\mathrm{RNH_3^+})(\mathrm{Im}) + k_3(\mathrm{AcIm})(\mathrm{RNH_2})(\mathrm{Im}) + k_4(\mathrm{AcIm})(\mathrm{RNH_2})^2 \tag{61}$$

their own reaction with *N*-acetylimidazole and that the free base is again the catalytically active species.

The aminolysis of esters has shed considerable light on the problem of general basic catalysis. The first detailed experiments of Betts and Hammett (68) were concerned with the reactions of ammonia with methyl esters of phenylacetic acid and some of its ring-substituted derivatives in unbuffered methanol solution. The reactions are approximately 3/2 order in ammonia as well as first order in ester; the reactions are accelerated by added sodium methoxide and retarded by added RNH_3Cl in such a way that a plot of the apparent second-order coefficient against $1/(RNH_3^+)$ is linear. These observations were explained on the basis of the rate equation

$$\text{Rate} = k_a(\mathrm{E})(\mathrm{RNH_2}) + (k_b K_{\mathrm{Am}}/K_B^{1/2})(\mathrm{E})(\mathrm{RNH_2})^{3/2} \tag{62}$$

where k_a is the rate constant of the reaction of ammonia and ester, k_a is the rate constant of the reaction of amide ion and ester, K_{Am} is the autoprotolysis constant of ammonia, and K_B is the equilibrium constant between ammonia and the alcohol solvent. This kinetic analysis implies a basic catalysis to be sure, but one which involves the catalyst in a preëquilibrium which is characteristic of specific hydroxide-ion catalysis in aqueous solution. Alternatively it was pointed out that a ternary complex of amine, ester, and basic catalyst could be involved in the transition state.

Catalysis by methoxide ion and inhibition by amine salts were also observed in the reactions of piperidine, morpholine, and butylamine with methyl acetate in methanol (19). The kinetics of the simultaneous aminolysis and hydrolysis of ethyl thiolacetate and β-acetaminoethyl thiolacetate contains a term in the rate expression which involves both amine and hydroxide ion (202). Similarly, the kinetics of the aminolysis and hydrolysis of α-naphthyl acetate indicates a term in the rate expression involving both amine and hydroxide ion (or its kinetic equivalent, amide ion) (201). The form of the rate law for the aminolysis of ethyl thiolacetate by glycine is a complicated one, involving a term which is second order in glycine and another term which is first order in glycine and first order in hydroxide ion (311). Likewise, the rate law for the amine-initiated polymerization of a series of *N*-carboxy-α-amino acid anhydrides contains a term which is second order in amine (392).

Watanabe and De Fonso (391) intensively studied the kinetics of reaction of *n*-butylamine with ethyl formate. The rate law showed a first-order dependence on ester and an approximately 3/2-order dependence on amine. However, the overall 5/2-order rate coefficients were not constant throughout any run. When *n*-butylamine hydrochloride was present in constant amount, the kinetics in any run was accurately third order overall. Strong catalysis by added ethoxide ion to an ethanol solution was also found. These observations are similar to those of Betts and Hammett (68) and were interpreted in the same fashion.

Recently two studies have shed considerable light on the problem of general basic catalysis of the aminolysis of esters. Bunnett and Davis (97) reinvestigated the reaction of ethyl formate with *n*-butylamine in ethanol solution, showing it to be subject to general basic catalysis. The rate law contains terms second order in amine (representing amine catalysis) and 3/2 order in amine (representing alkoxide-ion catalysis) but no detectable term first order in amine. The kinetic resolution into terms 3/2 order and second order in amine stands in contrast to earlier studies (68, 391) in which reaction orders could only be approximated. At 0.1 *M* *n*-butylamine the reaction is 33 per cent catalyzed by amine and at 1.0 *M* amine it is 61 per cent amine catalyzed, the remainder in either case representing catalysis by alkoxide. The addition of *n*-butylammonium chloride sharply reduces the rate. Furthermore, the addition of sodium ethoxide markedly accelerates the reaction, as found before. The mechanism favored by

the earlier workers exemplified by equation 62 predicts that the term 3/2 order in amine will be eliminated by the addition of RNH_3Cl, and also predicts that the remainder be an uncatalyzed reaction which is first order in amine. Instead, the addition of *n*-butylammonium chloride eliminates the term 3/2 order in amine but not the term second order in amine. This result is consistent only with a mechanism which involves a true general basic catalysis (that is, a transition state containing ester, amine, and base). Unfortunately these experiments were not carried out in water, so that the usual criterion for general basic catalysis could not be carried out (determination of an increase in rate with increase in buffer concentration at constant pH and constant ionic strength).

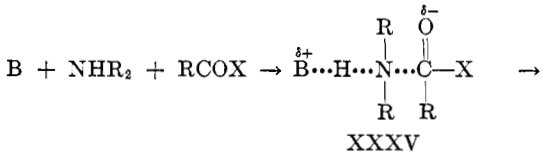
The rates of aminolysis and ammonolysis of phenyl acetate have been determined by Jencks and Carriuolo under conditions of controlled pH and ionic strength in aqueous solution (229). Uncatalyzed reactions of the various amines were found. The reactions of all amines examined except imidazole are subject to catalysis. Glycine, ammonia, glycylglycine, and glycine ethyl ester are subject to general basic catalysis by a second molecule of amine. Piperidine and morpholine are subject to hydroxide-ion catalysis. Dimethylamine and butylamine are subject to both general basic and hydroxide-ion catalysis. Methoxyamine is subject to general acid catalysis by methoxyammonium ion and hydroxylamine to both general basic and general acidic catalysis.

Of the many conceivable mechanisms which are consistent with general basic catalysis of the form

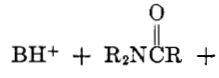
$$\text{Rate} = (\text{ester})(\text{nucleophile}) \sum^{i} k_i(\text{base})_i \qquad (63)$$

three are sufficiently in accord with existing chemical theory to warrant close attention (97, 229). It is assumed in the following discussion that proton transfers between nitrogen and oxygen are not, in themselves, slow steps. In the following equations RCOX is the carboxylic acid derivative, NHR_2 a generalized nucleophile, and B a general base including hydroxide ion.

1. General basic catalysis of nucleophilic attack by proton removal by the base from the nucleophile in the activated complex XXXV. The kinetic data do not of

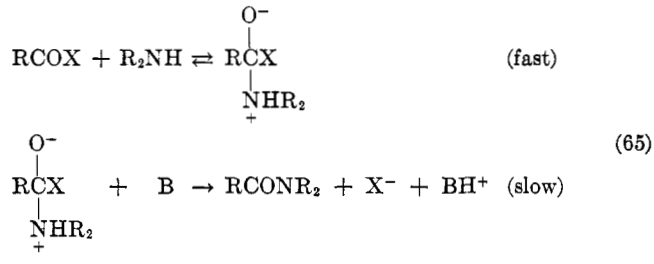


XXXV



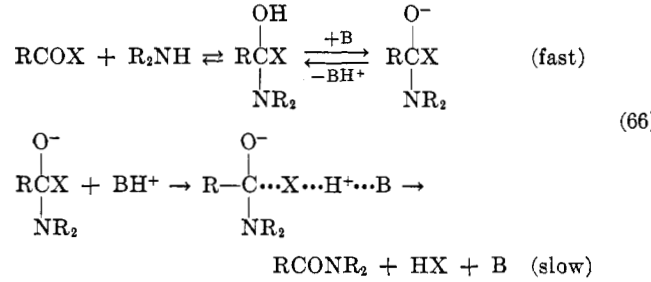
course permit conclusions as to the order of combination of reactants to form the transition state. Suffice it to say that the termolecular transition state does not imply a termolecular collision.

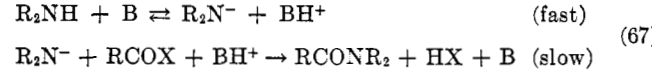
2. General basic catalysis of the breakdown of a tetrahedral addition intermediate to give products rather than starting materials. This can involve removal of a proton from one of the electronegative atoms of the intermediate either after or before initial proton transfer has occurred as in equation 65.



The action of the base on the tetrahedral intermediate might be thought of as an *E*2 elimination, the removal of a proton from the nucleophile being concerted with the departure of the group X as a negative ion. This mechanism has been suggested by a number of workers (202, 390).

3. General basic catalysis via a combination of prototropic preëquilibria and general acid catalysis. This possibility is kinetically indistinguishable from general basic catalysis because of the equilibrium relationship between B and BH^+ (97). This mechanism can be represented by equations 66 or 67.





It is a difficult task indeed to distinguish between these three mechanisms for they contain, as they must, identical transition states except for the distribution of atoms. The differences, however, are important mechanistically and their relative merits will be discussed.

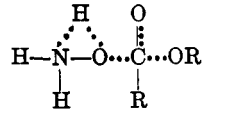
Bunnett and Davis (97) favor path 3 because it explains the presence of general basic catalysis in ester aminolysis and its absence in the reactions of amines with 2,4-dinitrochlorobenzene. The equilibrium formation of the addition intermediate in path 3 finds analogy in the formation of oximes and semicarbazones from aldehydes (228). Furthermore, path 3 explains the surprisingly low reactivity of carboxylic acid esters with

alkali metal amides in liquid ammonia (because of the absence of suitable general acids in that medium). The mechanism of path 3 implies, by the principle of microscopic reversibility, that the reverse of ester aminolysis, the alcoholysis (or hydrolysis) of amides, should be general base catalyzed via path 1. The alcoholysis reaction is base catalyzed but the question of general basic catalysis vs. specific lyate-ion catalysis has not been resolved (387, 396). The hydrolysis of anilides is also base catalyzed, but there is no information about its general basic nature. This argument is of course not sufficient, since the aminolysis may proceed via path 1 and the reverse by path 3, both conforming to the kinetic rule of general basic catalysis.

Jencks and Carriuolo (229) favor path 1 or 2 for their system. They point out that path 3 would require that the reverse reaction must involve path 1. Since catalysis by glycine causes a fourfold increase in rate at pH 10 in the forward direction, it should cause a fourfold catalysis in the reverse direction also, but at most could cause a twofold increase, since phenol is already half-ionized at pH 10. This argument assumes an ideal equilibrium system which has in no way been perturbed. According to their interpretation, the aminolysis reaction is general base catalyzed (path 1 or 2) and the reverse reaction of alcoholysis or hydrolysis of amides is general acid catalyzed (path 3). Such general acid catalysis has not been detected experimentally, since under the usual conditions for such experiments the predominant acid present is water or alcohol. One piece of evidence that substantiates this picture is the peculiar solvent dependence in the oxygen exchange of amides which, in contrast to that of esters, exhibits a smaller ratio of hydrolysis (loss of NH_2 from the tetrahedral intermediate) to exchange (loss of OH from the tetrahedral intermediate) with increasing amounts of dioxane in dioxane–water mixtures (39a).

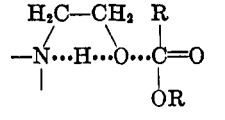
The specific mechanism of general basic catalysis probably depends on the substrate and the nucleophile. If the structure of the carboxylic acid derivative provides a good leaving group, the need for acid catalysis in its departure in path 3 would decrease. In such a case, path 1 or 2 might prevail. If the nucleophile is acidic, path 1 might be more important. With a distinctly acidic nucleophile and a very good leaving group, the mechanism involving a preëquilibrium might be expected, possibly in the reaction of a phenol with an acid chloride. Thus a spectrum of mechanisms is conceivable for general basic catalysis, differing in the relative kinetic significance of the various reaction steps and/or the timing of the proton transfers.

The abnormally high nucleophilicity of the neutral hydroxylamine molecule toward *p*-nitrophenyl acetate has been commented on earlier (table 8). If the uncharged molecule reacts, attack of the hydroxyl group leading to oxygen acylation may be aided by an intramolecular proton transfer to the nitrogen atom as in XXXVI (227).



XXXVI

The measurable reactivity of tris(hydroxymethyl)-aminomethane toward *p*-nitrophenyl acetate (table 8) is surprising, since amines of comparable steric hindrance are unreactive. The product of this reaction is an ester and not an amide, however, indicating that an oxygen rather than a nitrogen atom is the nucleophilic entity. Since this hydroxyl group is 10^5 times as reactive as the hydroxyl group of water, this reaction may proceed with intramolecular general basic catalysis in a manner similar to that proposed for the reaction of hydroxylamine (229).



XXXVII

It is difficult to assess the importance of general basic catalysis at present, since serious investigation of its potentialities is just beginning. It may be wise to consider carboxylate-ion catalysis in the hydrolysis of methyl hydrogen phthalate (36) and imidazole catalysis in the hydrolysis of diethyl oxalate (81) as general basic catalysis rather than nucleophilic catalysis. The latter requires the preferential partitioning of alkoxide ions versus a very weak base from the tetrahedral intermediate, whereas the former requires only proton transfer to explain the catalysis. Similar arguments may apply to enzymatic catalysis (*vide infra*).

VII. Enzymatic Catalysis

Catalysis of the nucleophilic reactions of carboxylic acid derivatives by various enzymes is widespread and varied. It is beyond the scope of this review to attempt to make a complete survey of the enzymatic catalysis of these reactions. Attention will be restricted to the reactions catalyzed by proteolytic enzymes which parallel the hydrolyses discussed previously and which offer the most detailed mechanistic evidence at this time. Although enzymatic catalysts are characterized by both extremely high catalytic efficiency and extremely high specificity, the present discussion will be restricted to the catalytic aspects of enzymatic action. Koshland has pointed out that a number of enzymes of diverse function have a similar active site, that part of the protein chain which is associated with catalytic action (250). He has suggested that while there must be an intimate steric relation between the amino acids responsible for the catalytic action and those responsible for the speci-

ficity, the above results indicate that the two functions may be separable. The following discussion of the catalytic aspects of hydrolytic enzyme action will be based on this hypothesis.

A. THE RELATIONSHIP OF PROTEIN STRUCTURE TO ENZYMATIC ACTIVITY

The enzymes whose catalytic action will be discussed in this section include the endopeptidases chymotrypsin, trypsin, pepsin, papain, and ficin, and the enzyme acetylcholinesterase. All of these substances except acetylcholinesterase have been crystallized and demonstrated to be protein in nature. The presence of a prosthetic group has never been demonstrated with any of these enzymes (248). This indicates that the catalytic action of these molecules (of molecular weight from 25,000 to 1,000,000) is associated with the amino acid moieties of the polypeptide chain. Furthermore, the available evidence, except for acetylcholinesterase, indicates that there is only one catalytic site per molecule of enzyme (248).

It is difficult to define the "active site" of one of these enzymes, for none has ever been unambiguously identified. If this were the case, the riddle of enzymatic catalysis would have been solved and this section would revert into a dull recitation of established facts. In reality enzymatic catalysis is the most powerful and least understood catalysis of the reactions of carboxylic acid derivatives.

Since there is usually one active site per enzyme molecule, and since this one active site is probably small compared to the entire enzyme, it is reasonable to assume that this active site can ultimately be described in chemical terms, hopefully in terms of the catalytic concepts that have been described earlier. Although there is no way of predicting how many constituents make up the active catalytic site of these enzymes, the dictates of scientific simplicity force one to postulate at any given time the least number of groups necessary to explain all properties of the enzyme. It is encouraging to think that the number of groups necessary for catalytic action may be as small as two or three. This hypothesis is consistent with the fact that several enzymes such as papain and pepsin have been cleaved of appreciable fractions of their total bulk with little or no loss of catalytic activity (211, 315). If the active site is indeed small, it may well lie within the province of the chemist to elucidate the active site and the mechanism of its catalysis in the near future.

1. Chemical studies

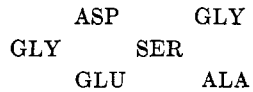
An ultimate solution of the relation of protein structure to enzymatic activity requires a precise assignment of position for each amino acid of the total enzyme in three-dimensional space (269). However, a solution to this problem may be obtained more simply by determination of the active site which represents the key to enzyme action (135). Four main methods of study of the active site have been profitable. The first employs inhibition by a group-specific reagent, protection against this inhibition by substrate being used as evidence that the observed reaction occurs at the active site. The second involves the pH dependence of catalytic activity as evidence for the pK of the groups at the active site. The third utilizes a reagent which forms a stable compound at the active site which on degradation can lead to identification of the amino acids in the vicinity of the active site. The fourth involves reactions in which an observable or stable enzyme–substrate intermediate is obtained whose properties can be explored.

Evidence is mounting that covalent enzyme–substrate intermediates are formed in the course of reactions of carboxylic acid derivatives. Most of these intermediates are too unstable, however, to survive the degradation conditions required for the identification of amino acid derivatives. It was, therefore, a major breakthrough when it was found that the nerve gas diisopropyl phosphofluoridate (DFP) reacted stoichiometrically with esterases to give a stable covalent phosphorylated enzyme (15, 222, 223). The parallelism between the stoichiometry of the DFP reaction (equation 68) and the enzyme inactivation, and also the ability of substrates to protect against inhibition by DFP,

$$(C_3H_7O)_2POF + EH \rightarrow E{-}PO(OC_3H_7)_2 + HF \qquad (68)$$

indicate strongly that phosphorylation occurs at the active site. Chymotrypsin, trypsin, papain, acetylcholinesterase, and phosphoglucomutase among others are subject to inhibition by DFP (204, 237). When DFP–chymotrypsin is degraded by acid hydrolysis, serine phosphate is obtained (335). Serine phosphate has also been isolated by similar treatment of other enzymes, including trypsin and acetylcholinesterase.

Treatment of chymotrypsin with radioactive DFP or Sarin and degradation of the labelled protein gives a series of phosphopeptides from which amino acid sequences have been obtained. The amino acid sequence at the active site of chymotrypsin (334, 336, 385), trypsin (130, 131, 132, 307, 333), liver aliesterase (224a), pseudocholinesterase, thrombin (174), and phosphoglucomutase (250) can be represented by the common structure



where GLY is glycine, ASP is aspartic acid, GLU is glutamic acid, SER is serine and ALA is alanine, and where ASP or GLU and GLY or ALA are alternatives in the sequence. The common sequence of amino acids around serine for many hydrolytic enzymes is rather

surprising. Even more surprising is the fact that the same sequence is indicated for the active site of phosphoglucomutase, a catalyst of considerably different specificity requirements from those of the hydrolytic enzymes.

A possible conclusion from the above data is that the hydroxyl group of serine is the nucleophilic entity of the active site. The side chains of glycine or alanine certainly have no functional behavior. The side chains of glutamic or aspartic acids possess a carboxylate ion which could conceivably participate in nucleophilic or general basic catalysis (*vide infra*), but the primary site to be considered must be the serine hydroxyl group.

Evidence, mostly pertaining to chymotrypsin, implicates histidine in the catalytic process. It should be noted that histidine appears neither adjacent to serine in the amino acid sequence above nor does it appear at the turn of a helix away from serine. It is conceivable that histidine could be brought into juxtaposition with serine by proper folding of the helix, although it is found that proline, an amino acid which prohibits helical configuration, appears fairly close to serine in both chymotrypsin and trypsin. The experiments supporting histidine as a constituent of the active site include (*1*) photoöxidation experiments showing a parallelism between the loss of histidine and the loss of enzyme activity (392a); (*2*) pH–activity curves suggesting a group having the pK of imidazole (histidine) but not that of serine; (*3*) model experiments in nonenzymic systems showing strong catalysis of phosphate and carboxylic ester hydrolyses by imidazole (histidine), but very weak catalysis by alcohol (serine). Arguments 2 and 3, both of which are ambiguous, will be discussed in detail later. It has been reported, however, that a trypsin fragment containing no histidine still retains a considerable portion of its enzymatic activity (387a).

Further chemical studies with 2,4-dinitrofluorobenzene and the fluorescent dye 1-dimethylaminonaphthalene-5-sulfonyl chloride have not given clear-cut results with respect to the active site. 2,4-Dinitrofluorobenzene was reported to react with at least 0.6 mole of histidine per mole of enzyme without decreasing the maximum velocity (280), but under mild conditions to lead to a decrease in enzymatic activity which parallels the fraction of one mole of histidine bound by the reagent (402). Dinitrophenylation of chymotrypsinogen was reported to involve groups other than histidine; since on activation the resulting enzyme has the same specific activity as normal chymotrypsin, the possible involvement of histidine in the active site can be suggested (402). Furthermore, it has been reported (81) that in chymotrypsin one histidine reacts readily with 2,4-dinitrofluorobenzene under mild conditions, while one is sluggish; in DIP–chymotrypsin both are reactive, indicating that the breakage of a hydrogen bond between serine and imidazole occurs on phosphorylation, thus making the imidazole nitrogen which was previously unreactive available for reaction. The fluorescent dye forms a fluorescent conjugate with chymotrypsinogen and DIP–chymotrypsin as well as the active enzyme, with a spectrum similar to that of a dye–imidazole compound. Substrate protection experiments demonstrate that both DFP and the dye react at the active site, but the reactivity of the latter with chymotrypsinogen and DIP–chymotrypsin indicates that the amino acid involved is different from that of the DFP reaction (197, 279).

There seems to be no definitive chemical evidence for the involvement of histidine (or really the imidazole group of histidine) in the active site. In summary, in the active site of a number of hydrolytic enzymes, the chemical evidence points to the involvement of serine, the possible involvement of histidine, and the conceivable involvement of aspartate or glutamate.

Another group of hydrolytic enzymes includes papain and ficin. In this group of enzymes, chemical studies demonstrate that a sulfhydryl group is a necessary part of the active site. One mole of mercuric ion combines with one mole of papain, leading to stoichiometric inhibition of enzymatic activity (350). Similar studies with methylmercury indicate that ficin is also stoichiometrically inactivated (65). Studies to be described later concerning the relationship between pH and enzymatic activity indicate that a carboxylate ion and an ammonium ion may be involved in the active site of these enzymes.

It thus appears that two different catalytic units will account for the action of perhaps a dozen different hydrolytic enzymes. Of course, there are a number of hydrolytic enzymes about which nothing is known. However, it is encouraging to think that intensive effort on only a few enzymes may unlock the structural secrets behind the catalytic action of a large number of enzymes which catalyze the nucleophilic reactions of carboxylic acid derivatives.

2. Inhibition studies

Inhibition studies have provided information concerning the active site of several hydrolytic enzymes, in particular chymotrypsin and acetylcholinesterase. α-Amino acids or simple functional derivatives of these compounds that are specific substrates or competitive inhibitors of α-chymotrypsin may be described by the general formula RCHR′R″, where R, R′, and R″ are the three prominent structural features of these molecules, the α-amino or acylamido group, the α-amino acid side chain, and the carboxyl group or carboxylic acid derivative. It has been suggested (217) that the above specific substrates and competitive inhibitors may combine with the enzyme via an interaction involving the three groups R, R′, and R″, and a set of centers, ρ_1, ρ_2, and ρ_3, which are assumed to be a char-

acteristic feature of the catalytically active site of the enzyme and which present a complementary aspect to the substrate or inhibitor molecules. The extent to which any given compound will be bonded to the active site of the enzyme is assumed to depend upon the degree to which the molecule and the asymmetric catalytic surface complement each other, and by the ability of both the combining molecule and the active site to alter their respective surfaces to improve the closeness of fit. The results of kinetic studies on specific substrates and competitive inhibitors of α-chymotrypsin appear to be consistent with the above view. Unfortunately many of the results cannot specify structural features of the active site.

The β-aryl and α-acylamido groups have been postulated as necessary for the stereospecificity in the hydrolysis by chymotrypsin (217). However, the stereospecific hydrolysis of diethyl α-acetamidomalonate by chymotrypsin (112a) indicates that the presumed three-point contact postulated above is not necessary for stereospecificity. The β-aryl and α-acylamido groups lead to high reactivity if the stereochemistry is correct. However, the association of the two groups with the enzyme is not apparently required for stereospecificity.

The development of a negative charge in the environment of the catalytically active site of the enzyme results in lesser affinity in the case of chymotrypsin under certain conditions, indicating a repulsive interaction with a negative group at the catalytic site, presumably the aspartate or glutamate carboxylate ions (161, 213).

With acetylcholinesterase, studies with reversible inhibitors such as diamines and stereospecific amino-alcohols have been used to reveal features of the surface structure of the active site, indicating at least one anionic site in the region of the active site (56, 165). This picture also explains acceptance by acetylcholinesterase of certain tailored cationic substrates besides acetylcholine (303).

B. THE EFFECT OF STRUCTURE OF THE SUBSTRATE ON REACTIVITY

The hydrolytic enzymes discussed above will catalyze the reactions of a large number of substrates including peptides, amides, hydrazides, hydroxamides, esters, acid chlorides, anhydrides, acids, and β-keto esters (at the β-keto group) with a large number of nucleophiles including water, alcohols, and amines such as amino acids, hydroxylamine, and phenylhydrazine (44, 45, 55, 59, 134, 139, 159, 160, 200, 221, 232, 236, 267, 274, 354, 358, 379, 386, 389). Reactions of various combinations of the substrates and nucleophiles given above lead to a large number of reactions which run the gamut of the nucleophilic reactions of carboxylic acid derivatives. The examples include hydrolysis, transpeptidation, transesterification (or alcoholysis), oxygen exchange of acids, conversion of an acid to a phenylhydrazide, hydroxylaminolysis of esters, aminolysis of amides, and other reactions. Most of the above examples involve chymotrypsin as catalyst, although some involve other enzymes such as acetylcholinesterase or papain. It should not be inferred that every hydrolytic enzyme will catalyze every one of the reactions, or to an equal extent; for example, papain will catalyze transpeptidations to a greater extent than chymotrypsin (168).

TABLE 17

Limiting velocities of trypsin- and ficin-catalyzed hydrolysis of benzoyl-L-arginine ethyl ester and benzoyl-L-argininamide

Enzyme	V_{max}		Reference
	Ester	Amide	
Trypsin	250	1	(61)
Ficin	1	1	(65)

Although no attempt will be made to discuss the specificity patterns of the various hydrolytic enzymes, it is of interest to consider those effects of structure on reactivity which bear on catalytic action. For this purpose it is of interest to consider the simple Michaelis–Menten treatment of enzymic catalysis

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \tag{69}$$

where E, S, ES, and P represent the enzyme, the substrate, the enzyme–substrate complex, and the product, respectively. The Michaelis–Menten kinetic equation is usually written in the form

$$v = V(S)_0/(K_m + (S)_0) \tag{70}$$

where $K_m = (k_2 + k_3)/k_1$ and $V = V_{max} = k_3(E)_0$.

Of primary interest to a consideration of catalytic action is not the formation of the enzyme–substrate complex, which in many cases is a fast preëquilibrium ($k_2 \gg k_3$), but rather the reactivity associated with the catalytic step k_3 (or V). One of the most clear-cut reactivity relationships is expressed in table 17. The rate of trypsin catalysis (like that of chymotrypsin) is dependent on the nature of the substrate, either ester or amide, while the catalysis by ficin (like that of papain (242a)) is not. The former behavior is easily explained by the relative ease of nucleophilic attack on an ester and an amide. The latter behavior of identical reaction rates with two widely different substrates is reminiscent of a number of cases in organic chemistry in which a common intermediate is formed, the decomposition of which is rate-determining. In other words, the identity of the relative rates of ficin (and papain) catalysis are strongly suggestive of intermediate formation.

Relative values of V_{max} also parallel nucleophilic attack in the hydrolysis of a series of butyrate and propionate esters by horse liver esterase. In this case there

is a precise parallelism between the limiting velocities (V_{max}) and the catalytic constants of hydroxide-ion-catalyzed hydrolysis of the various esters, and no correlation with the hydronium-ion-catalyzed rate constants (117).

A number of investigators have studied the hydrolysis of phenyl esters with hydrolytic enzymes. With hydrolysis catalyzed by hydroxide ion, the relative rates can be correlated by means of a Hammett ρ–σ relationship, explained on the basis that electron withdrawal from the site of reaction increases the ease of nucleophilic attack. In studies with eel esterase, cobra venom cholinesterase, and serum cholinesterase, a linear Hammett relationship is not observed. Both electron-withdrawing and electron-donating substituents in the para position of the phenyl ester decrease the rate of reaction (57, 302). These results may indicate that the relative rates of reaction are not determined solely by nucleophilic attack but by some combination of nucleophilic and electrophilic catalysts which leads to the concave Hammett relationship observed in these cases. This analysis is clouded by the fact that it is not clear what steps the rate constants signify in any given case, for the detailed stepwise kinetic analysis, worked out for chymotrypsin (*vide infra*), has not been applied to these systems.

In addition to changes in the leaving group, the effect of changes in the acyl moiety of the carboxylic acid derivative on the relative rate of hydrolysis has been probed. A comparison of the relative k_3 values in the α-chymotrypsin-catalyzed hydrolysis of a series of ethyl α-substituted-β-phenylpropionates with the relative rate constants of these compounds in acidic and basic hydrolysis indicates that at least as far as the breakdown of the enzyme–substrate complex is concerned there is no correlation possible between enzymatic and nonenzymatic hydrolysis (52). These studies are of course complicated by the intervention of steric factors. On the other hand, the relative rates of deacylation of a number of acyl–chymotrypsins parallel the relative rates of alkaline hydrolysis of the corresponding esters fairly closely, indicating that in the deacylation of the acyl–enzyme intermediate (Section VII,E) the reaction follows a nucleophilic reactivity pattern (table 18).

However, the enhanced reactivity in the deacylation of acetyl-L-tyrosine ethyl ester (table 18) indicates that structural specificity, in addition to nucleophilic reactivity, must be considered in the final explanation of enzymatic reactivity (303a, 353).

TABLE 18

Rates of deacylation of acyl–chymotrypsins and alkaline hydrolysis of ethyl acylates

Acyl Group	Relative Rate of Deacylation (134, 284)	Relative Rate of Alkaline Hydrolysis of Ethyl Esters* (243)
Acetyl	1	1
Propionyl	1.64	0.584
n-Butyryl	0.91	0.276
n-Valeryl	1.52	0.308
Isobutyryl	0.37	0.129
Trimethylacetyl	0.032	0.041
Hydrocinnamyl	16.0	0.81
Hippuryl	100	
Acetyl-L-tyrosyl	6000†	

* In 87.83 per cent ethanol at 30°C. or 85 per cent ethanol at 25°C.

† Assuming that k_3 for the overall reaction is controlled by the deacylation (353).

C. THE EFFECT OF STRUCTURE OF THE COSUBSTRATE ON REACTIVITY

Not only is the substrate (the carboxylic acid derivative) bound to the enzyme surface but also the cosubstrate (usually water) is bound to the enzyme surface as well, forming an overall ternary complex. This conclusion has been reached on the basis of studies utilizing nucleophiles other than water. The idea of using a "water analog" to indicate whether water is adsorbed on the enzyme surface was first enunciated by Koshland (251). He reasoned that if the enzyme myosin can catalyze the reaction between phosphates and water, one should expect a similar catalysis of the reaction between phosphates and methanol, in water as the solvent, unless specific binding of water on the enzymatic surface were involved. The experimental results indicated that water is much more reactive than methanol in this enzymatic process, in direct contrast to the nonenzymatic reaction. The conclusion was therefore reached that myosin contains a specific site for water on its surface.

A study of the kinetics of simultaneous hydrolysis and hydroxylaminolysis of a number of esters in the presence of chymotrypsin indicates that hydroxylamine (and by analogy water) is bound independently of the substrate to the active site of the enzyme (62, 64). A similar study of the kinetics of the simultaneous hydrolysis and methanolysis of acetyl-L-phenylalanine methyl ester also indicates the independent binding of both the ester and water (or methanol) on the enzyme surface (43). Hydrogen bonding of the water molecule to a basic group on the enzyme surface is an attractive possibility for binding. All the cosubstrates which have been shown to be bound to the enzyme surface, e.g., water, methanol, and hydroxylamine, possess hydroxyl groups which are capable of hydrogen bonding. Data on the rate enhancement of the α-chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl acetate in alcohol–water solutions can be interpreted in terms of the apparent binding strength of the various alcohols (283). It was observed that the rate enhancement increased with increasing chain length and decreased with branching in the chain. Methanol was anomalous, producing a larger rate enhancement than ethanol; this fact can be explained by the greater hydrogen-bonding ability of

methanol due to its greater acidity. The general increase with chain length can be attributed to increased van der Waals attraction (283). A possible site for hydrogen bonding of the cosubstrates could be any basic group on the enzyme surface, such as the imidazole group of histidine or the carboxylate group of aspartate, both previously postulated as being associated with the active site of the enzyme. It is conceivable that imidazole hydrogen bonded to a nucleophile such as water could serve as a general basic catalyst in removing a proton from the water molecule as it attacks the acyl group of the substrate or an acyl–enzyme compound.

D. TRACER STUDIES

The α-chymotrypsin-catalyzed hydrolysis of esters was found to differ from nonenzymatic (alkaline) hydrolysis with respect to carbonyl oxygen exchange during the hydrolytic process. Oxygen exchange was found to occur during the alkaline hydrolysis of methyl β-phenylpropionate-*carbonyl*-O^{18} and benzoyl-L-phenylalanine ethyl ester-*carbonyl*-O^{18} (*cf.* Section II), but not during the α-chymotrypsin-catalyzed hydrolysis of these esters (45). However, both the α-chymotrypsin-catalyzed and the base-catalyzed hydrolytic reactions occur by means of acyl–oxygen fission (45). Acyl–oxygen fission has also been noted in ester hydrolysis catalyzed by acetylcholinesterase (359). The lack of carbonyl oxygen exchange during enzymatic hydrolysis rules out a symmetrical addition intermediate in the enzymatic hydrolysis of esters. The lack of exchange can be explained by a number of mechanisms including the formation of an acyl–enzyme intermediate, the nonequivalence of the carbonyl oxygen atom with other oxygen atoms because of an interaction of the former with the enzyme surface at the active site, or the occurrence of a concerted process.

E. DETAILED KINETIC ANALYSIS AND INTERMEDIATE FORMATION

1. Chymotrypsin

Although the gross kinetic features of many chymotrypsin reactions have long been investigated, detailed kinetic analyses, leading to mechanistic conclusions in the chymotrypsin-catalyzed hydrolysis of esters, are of recent origin. A number of investigations of the effect of pH on the kinetic parameters of the chymotrypsin reaction have been carried out. Hammond and Gutfreund (191) determined the effect of pH on k_3, the catalytic rate constant, and K_m, the Michaelis constant, in the chymotrypsin-catalyzed hydrolysis of acetyl-L-phenylalanine ethyl ester. A different pH behavior was found for each constant. K_m is a true dissociation constant in this system; information concerning the catalytic action can therefore be gained by focusing attention on the variation of k_3 with pH. The catalytic rate constant, k_3, can be expressed as being dependent on a group with an apparent pK of 6.85. Earlier, it was shown that the k_3 in the trypsin-catalyzed hydrolysis of benzoyl-L-arginine ethyl ester is dependent on the ionization of a group with an apparent pK of 6.25 (186). Subsequent investigations showed that k_3 for the chymotrypsin-catalyzed hydrolysis of acetyl-L-tyrosine ethyl ester, acetyl-L-tryptophan ethyl ester, and acetyl-L-tyrosine amide depends on the ionization of a single group of apparent pK_a of 6.7 (119, 188). These results may be interpreted in terms of the participation of the imidazole group of a histidine residue in the catalytic process. This approach involving the pH effect on the catalytic step constituted an important advance in consideration of the active site in hydrolytic enzymes, since, earlier, bell-shaped curves relating overall rates of enzyme reactions to pH were usually only considered.

It has been pointed out that bell-shaped activity curves can be due to three causes: (*1*) a variation of k_3 with pH; (*2*) a variation in K_m with pH; or (*3*) an irreversible inactivation of the enzyme at extreme pH (186a). With chymotrypsin at least, a bell-shaped pH–rate profile of the catalytic step does not exist.

Further delineation of the catalytic process has been made possible by the use of *p*-nitrophenyl acetate as a substrate for chymotrypsin. In this reaction Hartley and Kilby observed a rapid liberation of one mole of *p*-nitrophenol per mole of chymotrypsin and then a slow reaction of the remaining substrate (194, 195). They described the reaction in terms of a catalytic sequence involving two distinct steps in addition to the primary adsorption, a total of three steps (equation 71).

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{ES'} \xrightarrow{k_3} \mathrm{E} + \mathrm{P_2} \qquad (71)$$
$$\mathrm{ES'} \rightarrow \mathrm{P_1}$$

The initial rapid reaction can be followed by a stopped-flow method; using this method to follow the pre-steady-state portion of the reaction, as well as the steady-state portion of the reaction, it was found that the kinetics are consistent with the scheme shown above (187). The first step, which involves the rapid adsorption of the substrate on the enzyme, is assumed to have a rate constant (k_1) greater than 10^6 sec.$^{-1}$ molar.$^{-1}$ The second step, assumed to involve the acylation of the enzyme and the simultaneous liberation of *p*-nitrophenol (P_1), has a rate constant of 3 sec.$^{-1}$ The third step is postulated to involve the liberation of acetate (P_2) and reactivation of the enzyme, the rate constant being 0.0254 sec.$^{-1}$ (187). Using both *p*-nitrophenyl acetate and 2,4-dinitrophenyl acetate as substrates, it was found that both steps k_2 and k_3 were pH-dependent, the k_2 step being dependent on a group with an apparent ionization constant of 6.7 and the k_3 step being dependent on a group with an apparent ionization constant of 7.3 (188) or 7.4 (353). At pH 6.6

there is a net uptake of 0.5 proton by the enzyme from the buffer upon acetylation of chymotrypsin with *p*-nitrophenyl acetate (188). At this pH, protons would be expected to be liberated by the enzyme if the imidazolyl group became appreciably acetylated.

Dixon and Neurath developed methods by which the rates of the formation and decomposition of the acyl–enzyme (ES′) can be studied separately (129, 134). The pH dependencies determined by their methods are in reasonable agreement with those determined by the stopped-flow method, corresponding in each case to the deprotonation of a basic group, the pK's being 6.2 for the acetylation reaction and 7.0 for the deacetylation reaction. The further important observation was made that the deacetylation of acetyl–chymotrypsin, as measured by reaction with hydroxylamine, is dependent on the structural integrity of the protein, since it is entirely abolished in 8 *M* urea. This result indicates that at least two groups are required for catalytic activity: one to which the acetyl group is bound (perhaps a serine hydroxyl group), and another not immediately adjacent on the chain to the first, which is necessary for the deacylation reaction (for example, an imidazole group of an adjacent helix).

The basis on which these pH dependencies have been related to the ionization of specific groups on the enzyme has been recently criticized (93). This criticism is based on the fact that the apparent dissociation constants depend on the intricacies of mechanism. Specifically, the constant of any equilibrium occurring prior to the rate-determining step must be a part of the kinetically determined pK_a' value.

Paralleling these kinetic studies, significant preparative investigations showed that it is possible to isolate a stable monoacetyl derivative of chymotrypsin at low pH (14, 18). The monoacetyl derivative is an intermediate (ES′ in equation 71) in the catalytic decomposition of *p*-nitrophenyl acetate by chymotrypsin. This derivative is inactive as an enzyme, but highly reactive with respect to the acetyl group. Acetyl–chymotrypsin reacts with water, forming acetic acid, and reacts preferentially with primary alcohols, even in dilute alcohol–water solutions, forming the corresponding acetate esters (16). In both cases the enzymatic activity is quantitatively recovered. A number of analogs of acetyl–chymotrypsin have been prepared, some less stable and some more stable than acetyl–chymotrypsin. Both nitrophenyl esters and acid chlorides have been used for their preparation (284). Their relative rates of deacylation have been described earlier. The most stable acyl–chymotrypsin, trimethylacetyl–chymotrypsin, was successfully crystallized (17).

Since both histidine and serine have been implicated in the active site, it has been of interest to determine whether the intermediates, followed kinetically and isolated as stable species, involve the acyl group either on the serine hydroxyl group or on the imidazole nitrogen atom of the enzyme. The decomposition of acetyl-C^{14}–chymotrypsin was shown to give a peptide with a composition which is compatible with the sequence demonstrated for the peptide isolated from DIP–chymotrypsin. This evidence indicates that the acyl–chymotrypsins described above are indeed derivatives of the active site as defined by stoichiometric inhibition by diisopropyl phosphofluoridate, and further that the point of attachment of the acyl group to the enzyme is the serine hydroxyl group (308). On the basis of competition experiments between *p*-nitrophenyl acetate and a specific substrate, acetyl-L-tyrosine ethyl ester, hydrolysis of the latter certainly involves the same active site and probably involves the same three-step mechanism as the hydrolysis of the former (353). This demonstration is an argument against the criticism that the nitrophenyl substrates are special cases and that the hydrolysis of a specific substrate by chymotrypsin does not involve an acyl–enzyme intermediate (62).

Acetylation on serine is further confirmed by spectral examination of acetyl–chymotrypsin at pH 3, in which it was impossible to observe the characteristic absorption maximum of an *N*-acetylimidazole at 245 mμ (133). A transient spectrum corresponding to *N*-acetylimidazole during the deacetylation of acetyl–chymotrypsin at pH 8 was reported (133); however, this finding has been disproved as being the result of an artifact of the enzyme (353).

Although the above spectral evidence has failed to indicate two intermediates involving both an acyl–serine and an acyl–imidazole, recent kinetic evidence indicates that two acyl–enzyme intermediates may indeed exist (275). The two different intermediates are distinguished by the pH at which they are formed; they are observed by differences in the time necessary for achievement of steady-state liberation of *p*-nitrophenol from *p*-nitrophenyl acetate.

The direct spectrophotometric detection of an acyl–enzyme intermediate in a reaction catalyzed by chymotrypsin has been achieved by the use of the substrate *o*-nitrophenyl cinnamate (figure 10). Since cinnamic acid is not appreciably formed by the time *o*-nitrophenol formation is complete, the decrease in absorption at 250 mμ must correspond to the formation of a cinnamoyl–chymotrypsin intermediate. The acylation step is dependent on an assumed single group with a pK of 6.3 and the deacylation step is dependent on a group with a pK of 7.4, results similar to those reported above. Cinnamoyl–chymotrypsin has a single peak at 293 mμ, while cinnamoyl esters have an absorption maximum at about 285 mμ and cinnamoylimidazole has an absorption maximum at 307 mμ. This result tentatively indicates that the cinnamoyl–enzyme intermediate is one in which the cinnamoyl group is attached to serine. The same intermediate is formed regardless of the pH

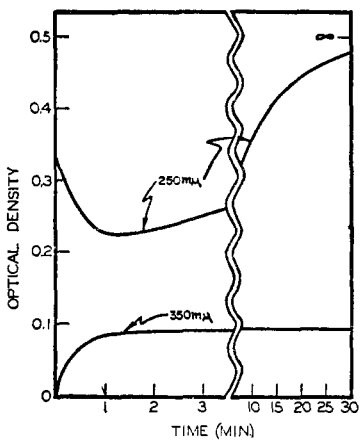


FIG. 10. The α-chymotrypsin-catalyzed hydrolysis of *o*-nitrophenyl cinnamate at pH 6.2 at 25°C. in phosphate buffers containing 10 per cent acetonitrile. E = S = $0.42 \times 10^{-4}M$ (342).

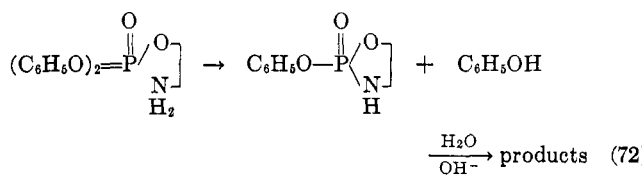
employed from 5.48 to 8.24, and the intermediate isolated according to the procedure of Balls has the same spectrum and the same kinetic behavior in the region of pH 7 to 8. This method of detection of an acyl–enzyme intermediate may enable the direct observation of many facets of the acylation and deacylation of a large number of enzymes, including most hydrolytic enzymes (342).

Substrates such as *p*-nitrophenyl acetate and *o*-nitrophenyl cinnamate do not differ greatly from diisopropyl phosphofluoridate, which is classified as an inhibitor of chymotrypsin. Various phenyl acylates can be considered as a graded series of inhibitors of chymotrypsin, from carbobenzoxy-L-tyrosine *p*-nitrophenyl ester at one extreme, a good substrate for chymotrypsin (277), which follows the same stepwise kinetics as in equation 71 (185), to *p*-nitrophenyl trimethylacetate at the other extreme, which produces an inactive enzyme derivative stable enough to be crystallized.

The diisopropylphosphoryl derivatives of chymotrypsin and other enzymes can be converted to the active enzyme on treatment with an efficient nucleophile such as hydroxylamine or one of its derivatives (408). In the reactivation of acetylcholinesterase, hydroxylamine reagents containing a positive charge, such as quaternary ammonium hydroxamic acids, which can interact with the anionic site on the enzyme, lead to efficient reactivation (411).

In the reactivation of phosphorylated chymotrypsin by such reagents as hydroxyiminoacetone and picolinohydroxamic acid, the rate of the reaction is proportional to the concentration of reactivator at constant pH (181). Studies involving variations in pH indicate that the anion of the activator and the protonated form of the inhibited enzyme are the kinetically active species. Furthermore, chymotrypsin inhibited by Sarin slowly recovers activity spontaneously at low pH. Faster dephosphorylation at lower pH is in direct contrast to the deacylation reactions in which greater reactivity occurs at higher pH. In either case, it is postulated that a serine derivative is transformed to an imidazole derivative, which then reacts with water to give the product. In the deacylation reaction, the conversion of the serine derivative to the imidazole derivative is postulated as the slow step (accounting for the fact that the imidazole derivative has never been unequivocally demonstrated), whereas in the dephosphorylation the reaction of the imidazole derivative with water is postulated as the slow step (leading to the prediction that the phosphoryl–imidazole might then become an observable intermediate).

The treatment of diphenylphosphoryl–chymotrypsin with dilute base (pH 12) immediately liberates one mole of phenol per mole of enzyme. This reaction has been postulated as being due to an intramolecular attack on phosphorus by a nitrogen nucleophile in the vicinity of the phosphorylated active site, possibly an imidazole group (264). A reaction similar to this enzymatic process has been observed in a model system shown in equation 72 (140).



(72)

2. *Papain and ficin*

The fact that the catalytic rate constants (k_3) of ester and amide hydrolyses by papain (and ficin) are exactly the same may be explained most easily by a stepwise catalytic process in which an acyl–enzyme is formed in a fast step and is decomposed to form products in a slow step. An investigation of the initial phase of the hydrolysis of benzoyl-L-arginine ethyl ester by ficin (by following the rate of formation of hydrogen ions) shows a typical pre-steady-state period, again suggesting the formation of an acyl–enzyme intermediate in the catalytic process (65). The effect of pH on the catalytic rate constant (k_3) of papain indicates that a group, possibly carboxylate ion, with an ionization constant of 3.5 is involved in the catalytic step (351). The effect of pH on the catalytic rate constant for ficin indicates that two groups with pK_a's of 4.40 and 8.46 are involved in the catalytic process.

These two pK_a's have been assigned to the carboxylate ion and an ammonium ion (192). These results lead to the postulation of two (or three) groups in the active site, the sulfhydryl group from chemical studies and the carboxylate ion (and ammonium ion) from pH studies (192, 349, 350, 368). Presumably the sulfhydryl group participates in some (fast) step which is kinetically unimportant, while the carboxylate ion and ammonium ion participate in the slow step of the reaction.

3. Esterases

The effect of pH on the hydrolysis of methyl *n*-butyrate and ethyl *n*-butyrate by horse liver esterase indicates that the catalytic process involves a group with an ionization constant of 2×10^{-5}, possibly a carboxylate ion (117).

The effects of pH on the hydrolysis of acetylcholine and uncharged esters by eel acetylcholinesterase and pseudocholinesterase have been explained by postulating that two groups with pK's of 6.5 and 9.3 are involved in catalytic action (56, 57, 58). The bell-shaped curves describing the pH dependence of the catalytic process of these enzymatic hydrolyses probably contain the pH dependence of the binding step (K_m) as well as the pH dependence of the catalytic step, k_3. However, it has been proposed on the basis of these data that a concerted process by both nucleophilic (imidazole) and electrophilic (ammonium ion) groups occurs in the enzymatic catalysis.

In a study of the activation parameters of the hydrolysis of acetylcholine by acetylcholinesterase, nonlinear Arrhenius plots were observed (410). These plots were interpreted as resulting from a summation of two steps involving the formation and decomposition of an acyl–enzyme intermediate. It was postulated that one step is rate-determining at one end of the 30° interval over which measurements were taken, whereas the second step is rate-determining at the other extreme, leading to an overall curvature of the Arrhenius plots. Alternative hypotheses explaining these data should not be excluded.

F. MODEL SYSTEMS OF ENZYMATIC HYDROLYSIS

All scientific investigations utilize models in conceptual approaches to real systems. When dealing with very complicated catalytic systems such as enzymes, it is difficult to perform a direct assault such as synthesis of the complete entity. On the basis that the mechanism of enzyme action does not involve any special chemistry but rather a combination of ordinary mechanisms, it is conceivable that enzyme models may be created, simple substances which will catalyze the same reactions as enzymes, and by the same fundamental mechanisms. All the models to be described here function with much lower efficiency than the corresponding enzymes. However, it is possible that the model studies reported here together with structural investigations and physicochemical investigations discussed in previous sections will ultimately lead to an elucidation of the mechanism of enzyme action.

Sections II through VI encompass the fundamental model systems on which enzymatic catalysis may be based. In this section, some of the specific implications of model systems for enzymatic catalysis will be discussed.

1. Chymotrypsin models

The implications of nucleophilic attack, and in particular the involvement of the imidazole group of histidine in the catalytic action of chymotrypsin, has led to studies of model systems involving imidazole and its derivatives. These studies have been extensively described above in Section IV, where it was pointed out that imidazole and other nucleophiles could serve as nucleophilic catalysts for the hydrolysis of esters, and in Section VI where it was pointed out that imidazole and other general bases could serve as general basic catalysts for the reactions of carboxylic acid derivatives.

It is possible to compare the chymotrypsin-catalyzed and imidazole-catalyzed hydrolyses of *p*-nitrophenyl acetate, although the former reaction is complicated by the preliminary adsorption of substrate on the enzyme (equations 73 and 74).

$$CH_3COC_6H_4NO_2 + En \overset{0}{\rightleftarrows} CH_3COC_6H_4NO_2 \cdot En \xrightarrow{1} CH_3C(=O)-En + OC_6H_4NO_2^- \xrightarrow[H_2O]{2} CH_3COO^- + En \quad (73)$$

$$CH_3COC_6H_4NO_2 + Im \xrightarrow{1} CH_3C(=O)-Im + OC_6H_4NO_2^- \xrightarrow{2} CH_3COO^- + Im \quad (74)$$

If this equilibrium is taken into account, by converting the first-order acylation rate constant into a pseudo-second-order rate constant, it is possible to calculate that the acylation in catalysis by chymotrypsin is about 10^3 faster than the acylation by imidazole (54). The deacylation of acetyl–chymotrypsin is only about one hundredfold faster than that for *N*-acetylimidazole (54). The unfavorable comparison between imidazole and chymotrypsin, together with the fact that imidazole will not cleave simple alkyl esters or amides whereas chymotrypsin will, indicates that imidazole alone as a

TABLE 19

Rates of acylation and deacylation of imidazole and chymotrypsin with p-nitrophenyl acetate

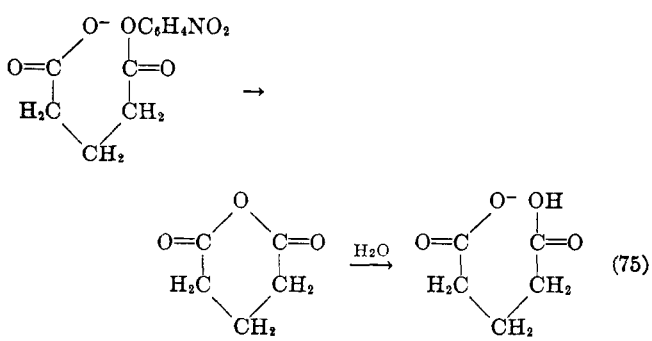
Catalyst	Acylation (1)		Deacylation (2)	References
	k_1	k_2	k_1	
	sec.$^{-1}$	*l./mole sec.*	*sec.*$^{-1}$	
Imidazole		0.47	1.5×10^{-4}*	(54, 355)
Chymotrypsin	3		2.5×10^{-2}	(353)

*pH 7.

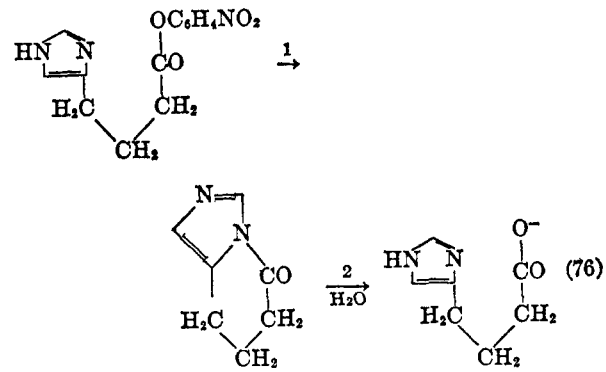
nucleophilic catalyst is not a suitable model for chymotrypsin catalysis.

Imidazole catalysis by the removal of a proton from the attacking reagent (general basic catalysis) has been demonstrated in Section VI but is quantitatively much less significant than catalysis by direct attack of imidazole on the acyl group to form an *N*-acylimidazole (nucleophilic catalysis), in reactions involving reactive compounds such as *p*-nitrophenyl acetate. The nucleophilic catalytic efficacy of imidazole is largely due to the peculiar ease of breakdown or acyl transfer by *N*-acylimidazole. In some cases, however, proton abstraction by imidazole may be of importance. In particular, general basic catalysis by imidazole could operate as efficiently on simple alkyl esters or amides as with *p*-nitrophenyl acetate if the function of the imidazole is only to remove a proton from the attacking nucleophile. This mechanism may then be operative in the chymotrypsin-catalyzed hydrolysis of simple alkyl esters and peptides. The rigid steric requirements of the enzymatic reaction may prohibit direct attack of imidazole on the substrate and rather favor (imidazole) general basic catalysis of an attack by the serine hydroxyl group and/or water. With enzymes such as papain or ficin the same type of catalysis may promote the attack of a sulfhydryl group on the substrate. The experiments in Section VI on general basic catalysis may be regarded as model systems of this kind. Their low efficiency and only indirect relevance to enzyme systems require that other factors must also be involved in enzymatic reactions.

It has been postulated that enzymatic processes proceed through the formation of an adsorptive complex between substrate and enzyme, followed by a catalytic process during which the substrate is constrained with respect to the reactive site by means of hydrogen bonding, van der Waals forces, and electrostatic attraction. Such constraint in the catalytic process likens enzymatic action to intramolecular catalysis, and like many intramolecular organic chemical reactions, enzymatic catalysis should proceed at a greater rate than the corresponding intermolecular process. This hypothesis has been utilized to construct a number of models of enzymatic hydrolysis (35, 94, 299), most of which have been discussed in Section IV. Of those intramolecular catalyses discussed in Section IV, two systems appear to be of particular interest since they show surprising kinetic similarity to the solvolysis of the enzyme–ester complex. One is the monoglutarate ester of *p*-nitrophenol (415), which has been shown to proceed with anchimeric participation of the carboxylate ion, the rate constant being in the range of esteratic rates. The other, the most successful model from the point of view of the relative efficiency of the model system compared to that of enzymatic catalysis, is the intramolecular catalysis of the hydrolysis of *p*-nitrophenyl γ-(4-imidazolyl)butyrate (95). For the model system employing intramolecular catalysis, the rate and its pH dependence



(75)



(76)

curve for acylation are almost identical to those for the hydrolysis of the *p*-nitrophenyl acetate–chymotrypsin complex; however, the rates of deacylation are widely divergent. This comparison demonstrates that if a molecule of *p*-nitrophenyl acetate were bound to a protein so as to possess an equivalent steric restraint relative to a histidine residue as the ester bond of the model bears to the imidazolyl ring, then an enzymic-like rate of acylation would be observed, and, furthermore, for the hydrolysis of this substrate, no other group would be required. However, the same steric requirements which allow assistance to such a degree with the *p*-nitrophenyl ester fail to give any assistance with the corresponding methyl ester. This simplified version of intramolecular catalysis therefore still lacks some essential feature as a complete model for enzymatic hydrolysis.

The intramolecular catalysis of the hydrolysis of *n*-propyl γ-(4-imidazolyl)thiobutyrate serves as a model for those enzymes responsible for the specific hydrolysis

TABLE 20

Rates of acylation and deacylation of p-nitrophenyl γ-(4-imidazolyl)butyrate and the p-nitrophenyl acetate–chymotrypsin complex

System	Acylation k_1	Deacylation k_1	References
	sec.$^{-1}$	*sec.*$^{-1}$	
Model	3.3	2×10^{-4}*	(94, 95, 88)
Enzyme	3.0	2.5×10^{-2}	(353)

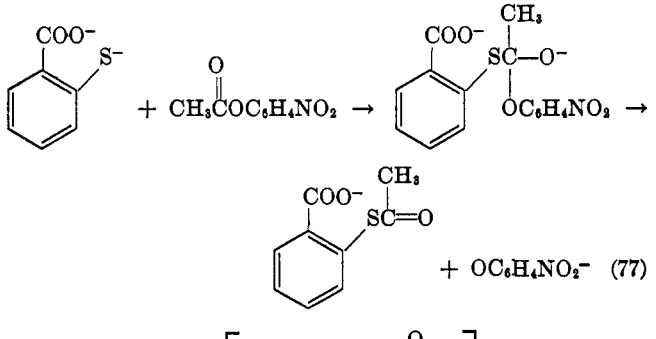
* pH 7.

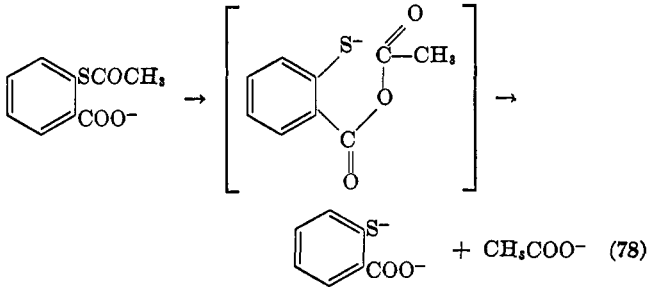
of thiol esters (88a). If a thiol ester were adsorbed on a protein so that the steric relationship of the ester bond to an imidazolyl group of histidine would be analogous to that in the model system, then the protein would undergo acylation at an enzymic rate. The protein involved in such a process would then be a specific thiol ester hydrolase, since neither the methyl ester nor the amide of the model undergoes hydrolysis at room temperatures.

The concerted catalytic process in which nucleophilic and electrophilic catalysts combine to produce a particularly efficient process has been much in vogue as a model for enzymatic catalysis since its exposition, particularly by Swain and Brown (374). In Section V a number of interesting examples of concerted reactions of carboxylic acid derivatives are given, such as the hydrolysis of phthalamic acid and the hydrolysis of succinyl salicylate. Comparisons of the pH dependence and substrate reactivities in these reactions with those found in enzyme catalyses seem to indicate that the analogy between the concerted model system and the enzymatic system cannot be drawn very closely, at least with chymotrypsin. However, the concerted model is an intellectually satisfying one and may be applicable to some enzymatic systems.

2. *Papain and ficin models*

Since the structural and kinetic aspects of catalysis by papain and ficin are completely different from those of most other hydrolytic enzymes, the model systems applicable to these enzymes will be expected to be different from those for chymotrypsin. The reaction of *p*-nitrophenyl acetate with *o*-mercaptobenzoic acid (340) in neutral solution exhibits certain analogies to the papain and ficin systems. This reaction can be expressed by equations 77 and 78, which show an attack





by the dianion of *o*-mercaptobenzoic acid on the ester to give thioaspirin, which hydrolyzes in neutral solution at a rate slower than its rate of formation. The thioaspirin hydrolysis is postulated to proceed via intramolecular catalysis by the *o*-carboxylate ion, analogously to the aspirin hydrolysis.

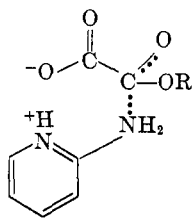
The overall process illustrated in equations 77 and 78 constitutes a catalysis of the hydrolysis of *p*-nitrophenyl acetate by *o*-mercaptobenzoic acid. It is of interest to compare this catalytic process with the catalytic processes exhibited by the enzymes papain and ficin. These two enzymes require a reduced SH group for catalytic activity, since it has been shown that these enzymes are inactivated by a single equivalent of a mercuric compound (65, 350) (as is the model system). Furthermore, papain exhibits a pH–rate profile of the catalytic (k_3) step which indicates the involvement of a group with a pK of 3.5 in the anionic form, presumably a carboxylate ion (351), and ficin exhibits a pH–rate profile indicating the involvement of a carboxylate ion and an ammonium ion (192). In the organic system two functional groups are necessary, a sulfhydryl group and a carboxylate ion; if one were to plot a pH–rate profile of the overall catalytic action of the organic system, this profile would be dependent on the second, slow step and would thus exhibit the same pH behavior as that of papain. Therefore it may be said that the catalyst *o*-mercaptobenzoic acid is a reasonable model for papain and a somewhat poorer model for ficin.

The efficiency of the catalyst *o*-mercaptobenzoic acid does not approach that of the enzyme. However, a comparison of the nucleophilicity of *o*-mercaptobenzoic acid with that of other nucleophiles for *p*-nitrophenyl acetate reveals that it is indeed a powerful nucleophile. It is suggested that one possible reason for enhanced enzymatic activity lies in the use of a coupled series of reactions in which the two groups of the enzyme do not interact simultaneously as in a concerted process, but rather consecutively to produce an overall efficient catalytic process. Such a process has indeed been proposed for the action of a number of hydrolytic enzymes (*vide infra*).

3. *Acetylcholinesterase models*

The hydrolysis of the half-ester *o*-nitrophenyl hydrogen oxalate is catalyzed by heterocyclic bases such as pyridine. This half-ester also reacts with nucleophiles such as aniline. The rate of reaction of *o*-nitrophenyl hydrogen oxalate with 2-aminopyridine and 4-aminopyridine is marked in buffered aqueous solutions in the region pH 3 to 6. The reacting species in these cases include both the free aminopyridine and in addition the corresponding monoprotonated species, the aminopyridinium ion. In this region the substrate ester exists mainly in the form of the anionic species. It was therefore suggested that part of the reaction of the amino-

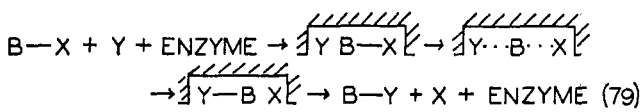
pyridines and *o*-nitrophenyl hydrogen oxalate in the region pH 3 to 6 includes a reaction of a negatively charged substrate and a positively charged nucleophile (37). While the ratio of the basicities of 2-aminopyridine and 2-aminopyridinium ion is about thirteen powers of 10, the ratio of the nucleophilicities of these two substances in this reaction is only a factor of 30. The high reactivity of 2-aminopyridinium ion with respect to its basicity is postulated to be due to a specific electrostatic interaction, as shown in formula XXXVIII. It was concluded that this interaction constitutes a catalytic action through its stabilization of the transition state of this nucleophilic reaction. This system, like the acetylcholine–acetylcholinesterase system, consists of a substrate and a catalyst of opposite charge. It was postulated that the considerable rate enhancement in the model system is formally similar to the rate enhancement found in the acetylcholinesterase system (37).



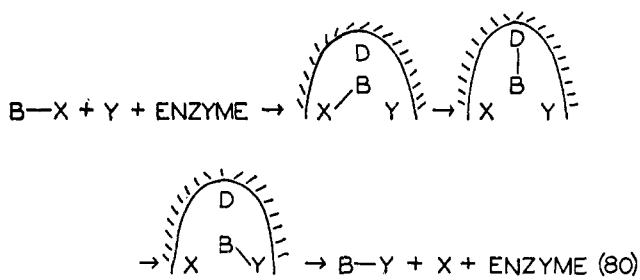
XXXVIII

G. MECHANISMS OF ENZYMATIC CATALYSIS

Many years ago it was proposed that enzymatic catalysis is not magical but rather chemical in nature. However, no completely satisfactory mechanism for an enzymatic catalysis has as yet been put forth. A number of generalizations can be made about enzymatic catalyses, relating them to other chemical processes. Koshland has proposed that enzymes may catalyze by either single displacement mechanisms (equation 79) or double displacement mechanisms (equation 80) (246, 249).



(79)



(80)

The single displacement mechanism may correspond to what Lumry refers to as a propinquity mechanism in which the enzyme acts as a surface on which two substrates may be positioned so as to be held in contact with one another, leading to an accelerated rate solely because of increased collisional frequency over that found in homogeneous solution (273). A single displacement mechanism may also correspond to the "rack" of Eyring (154), in which the substrate is caught in the protein rack and made reactive by distortion. Thus far these suggestions which do not specify a chemical role for the enzyme have not been of great utility. Suggestions involving a chemical role for the enzyme in the single displacement mechanism encompass the possibilities of (*1*) the positioning of a dipolar group on the enzyme surface in such a way as to be oppositely oriented to the dipole formed in the substrate-activated complex (357) and (*2*) various forms of general acidic-general basic catalysis including concerted catalysis to facilitate both the breaking of the B—X bond and the making of the B—Y bond.

In a double displacement mechanism, the enzyme participates directly in the catalytic process by the formation of an enzyme–substrate compound. In this mechanism a specific chemical role is assigned to the enzyme which has been verified by chemical methods in certain cases (Section VII,E).

1. Chymotrypsin and cholinesterases

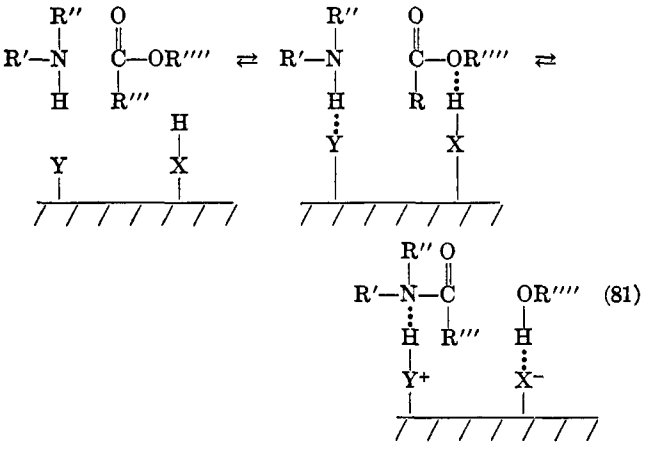
A large number of mechanistic proposals have been put forth to explain the catalytic action of these enzymes. The proposals may be roughly divided into two categories: (*1*) those which involve general acidic and/or general basic catalysis, which are essentially single displacement mechanisms; and (*2*) those proposals which involve nucleophilic catalysis, that is, attack at the carbonyl carbon atom with the formation of an enzyme–substrate compound, which in the reactions of carboxylic acid derivatives may take the form of an acyl–enzyme compound or a tetrahedral addition compound, both involving a nucleophile of the enzyme. The latter proposals are essentially double displacement mechanisms.

(a) General acidic-basic catalysis

General acidic catalysis has been postulated as the sole function of esterase enzymes (381). Since this mode of catalysis has been demonstrated to be relatively unimportant in model systems, it is difficult to conceive of the importance of this mechanism in enzymatic catalysis.

Several proposals of concerted general acidic-basic catalysis as mechanisms of hydrolytic enzyme action have been made (259, 345, 374). In these proposals, a general acid facilitates the reaction by proton donation to the leaving group or to the carbonyl oxygen atom, while a general base facilitates bond formation by proton abstraction from the attacking nucleophile. The proposal of Swain and Brown for the enzymic formation

of amides from esters illustrates a possible general acidic-basic catalysis by an enzyme (equation 81).



(81)

Ronwin has made a proposal based on general acidic-general basic catalysis to produce opposite charges at the two ends of the bond to be hydrolyzed. The postulation of an acylium-ion intermediate in neutral solution makes it difficult to believe that this proposal adds much to the concept of general acidic-general basic catalysis (326, 327). As pointed out previously, while concerted general acidic-general basic catalysis mechanisms are intellectually pleasing, they do not appear to fit the facts, in particular the pH behavior, of enzymatic hydrolysis of carboxylic acid derivatives as set forth above, except perhaps in the cases of acetylcholinesterase and ficin.

(b) Nucleophilic and/or general basic catalysis

Nucleophilic catalysis or some combination of nucleophilic and general basic catalysis is suggested in chymotrypsin-catalyzed hydrolysis by the effect of structure on reactivity (Section VII,B) and the effect of pH on the catalytic steps (Section VII,E), determined both with the overall catalytic rate constants of specific substrates where no intermediate is observable and with the two individual rate constants for nitrophenyl esters where an intermediate is observable.

A number of mechanistic suggestions have been made, postulating (*1*) nucleophilic and/or general basic catalysis and (*2*) formation of an acyl–enzyme intermediate. The hypothesis of an acyl–enzyme intermediate requires that these proposals involve nucleophilic catalysis. These suggestions, involving the hydroxyl group of serine and the imidazolyl group of histidine as components of the reaction system, have been made by Gutfreund and Sturtevant (187), Cunningham (119), Dixon and Neurath (133), Westheimer (398a), Spencer and Sturtevant (353), and Bruice and Schmir (93). The proposals can be summarized succinctly as (*1*) general basic or nucleophilic catalysis by imidazole in the acylation step and (*2*) general basic or nucleophilic catalysis by imidazole in the deacylation step. The possible variations are shown in equations 82, 83, and 84.

The spectrophotometric evidence given before indicates that any nucleophilic catalysis by imidazole is unlikely, since it should be possible to observe the spectrum of the transient intermediate corresponding to an *N*-acylimidazole. If one assumes that the catalysis occurs by means of one of the above possibilities, general basic catalysis of both acylation and deacylation by imidazole (equation 82) is to be preferred.

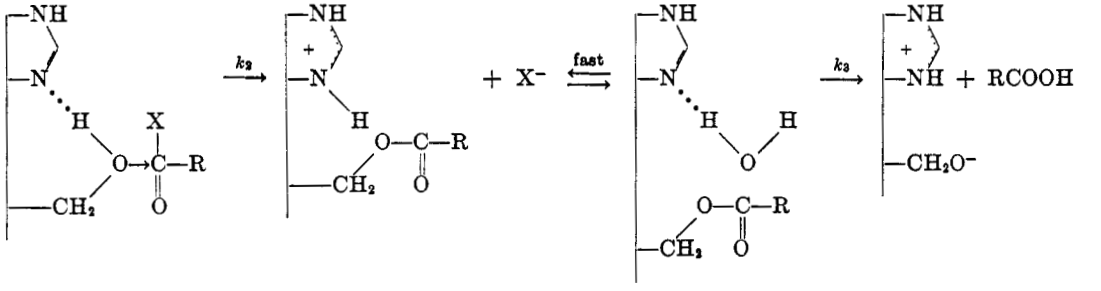
While the proposal of Westheimer (398a) concerning nucleophilic catalysis does not appear to be in accord with all experimental facts, his suggestion of testing the fit of a specific substrate to the active site of an enzyme in its three-dimensional (possibly helical) configuration should be profitable.

Bruice and Schmir (93) have pointed out that while the above possibilities are compatible with the observed pH dependence, there are variants on these mechanisms involving additional equilibria which are also compatible with the sigmoid pH–rate profiles. These additional preëquilibria can cause the actual dissociation constant of a group involved in the catalysis to be different from that determined kinetically. They include the preëquilibrium formation of a tetrahedral intermediate and the preëquilibrium formation of a tetrahedral intermediate and the reversible formation of an acyl–enzyme intermediate. This argument introduces considerable ambiguity into the interpretation of the pH dependency of the acylation and deacylation steps of enzyme catalysis.

Rydon (329) has suggested that catalysis involving serine and imidazole can be alternatively explained by the formation of a Δ^2-oxazoline group through a ring closure involving the serine hydroxyl and the adjacent peptide bond of an aspartyl residue. It is difficult to conceive of this fundamental change in covalency under physiological conditions. If indeed the oxazoline ring were formed, it certainly could attack a carboxylic acid derivative as a nucleophile, although its nucleophilicity (and basicity) is certainly less than that of an imidazole ring. The hydrolysis of the acyl–enzyme in this case is postulated to proceed via nucleophilic catalysis by the aspartate carboxylate ion. This suggestion takes into account neither the low nucleophilicity of carboxylate ion nor the observed pH dependence of deacylation.

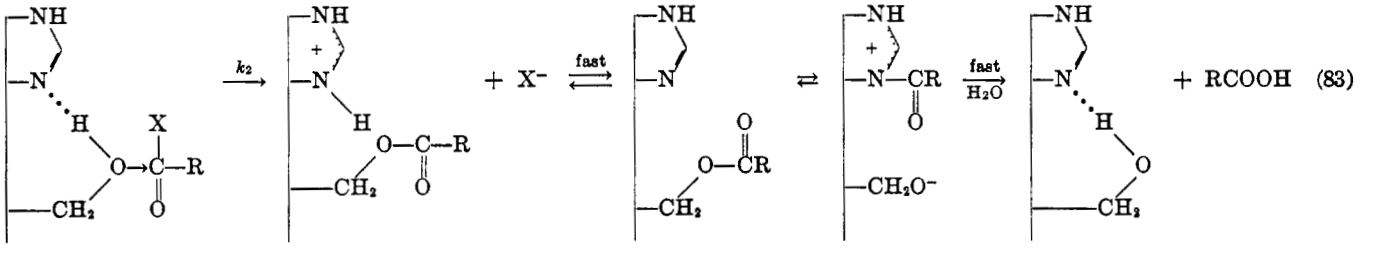
The mechanism proposed for the catalytic action of acetylcholinesterase resembles that discussed above. It is postulated that a group or groups GH which contains both nucleophilic (possibly imidazole) and electrophilic properties (possibly an ammonium ion) reacts with the ester at the carbonyl carbon atom to produce a tetrahedral intermediate (the enzyme–substrate complex), followed by loss of the alcohol to give an acyl–enzyme which then reacts with water to produce another tetrahedral intermediate (the enzyme–product complex)

1. General basic catalysis in acylation and deacylation:



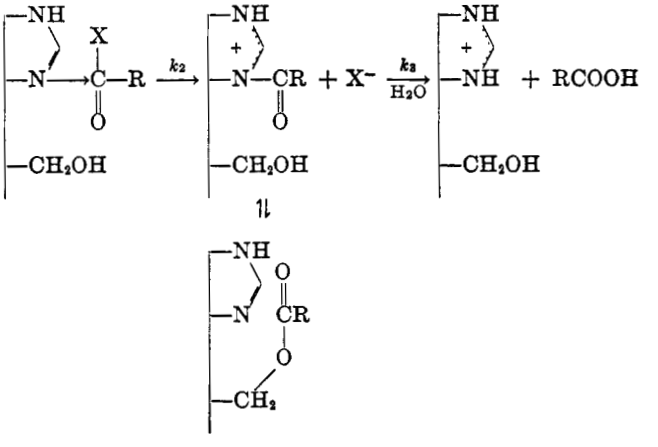
(82)

2. General basic catalysis in acylation and nucleophilic catalysis in deacylation:



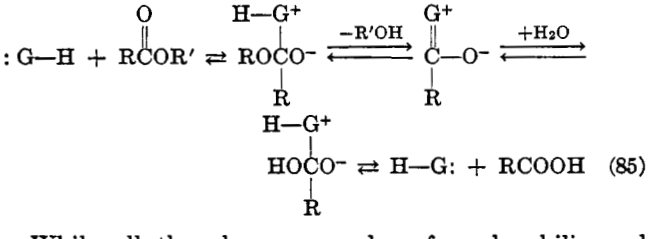
(83)

3. Nucleophilic catalysis (acylation and deacylation):



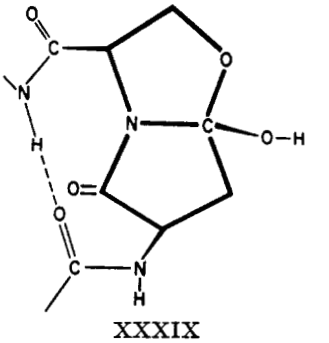
(84)

followed by decomposition to products (equation 85) (407, 409). While the author is in concord with the idea of the formation of tetrahedral intermediates, it is not clear that they would correspond to the enzyme–substrate and enzyme–product complexes which would require them to be in equilibrium with the enzyme and substrate and enzyme and product, respectively. This suggestion is akin to the proposal of the preëquilibrium formation of a tetrahedral intermediate after the formation of the enzyme–substrate complex (93). There is one piece of evidence, involving intramolecular imidazole attack (95), which supports the preëquilibrium formation of a tetrahedral intermediate.



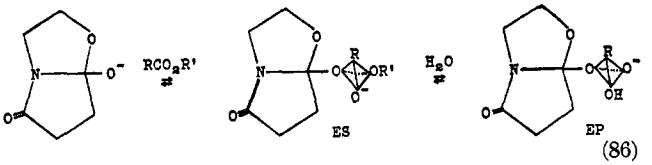
(85)

While all the above examples of nucleophilic and general basic catalysis have involved the intermediate formation of an acyl–enzyme, it is conceivable that instead a tetrahedral intermediate may be formed in enzymatic catalysis (66). The facile hydrolysis of the benzyl ester of the model peptide of aspartylserine, blocked at either end by peptide bonds (Section IV,C) (63), has led to a suggestion that this hydrolysis proceeds through the formation of the tetrahedral intermediate XXXIX, formed from the serine hydroxyl group and the nitrogen atom of the peptide link between

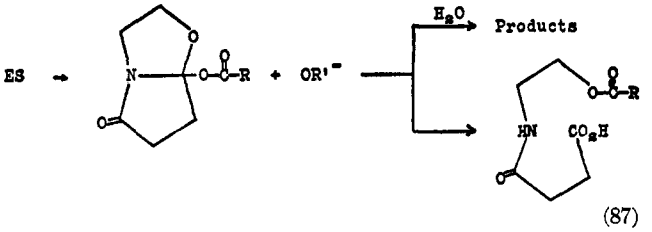


XXXIX

aspartate and serine. It has further been postulated that such an intermediate could function as the active site of a hydrolytic enzyme. It is postulated that the hydroxyl group of XXXIX, which has a pK in the region of neutrality, chemisorbs the ester in the form of a tetrahedral intermediate (ES) (equation 86). The tetrahedral intermediate, ES, is further attacked by a water molecule (on the enzyme surface) in a Walden inversion, leading to the enzyme–product complex, another tetrahedral intermediate, EP. Alternatively, if the leaving group of the carboxylic acid derivative has a great tendency to cleave before attack by a water molecule, an acyl–enzyme would form (equation 87). The acyl group could be subsequently cleaved by water



(86)

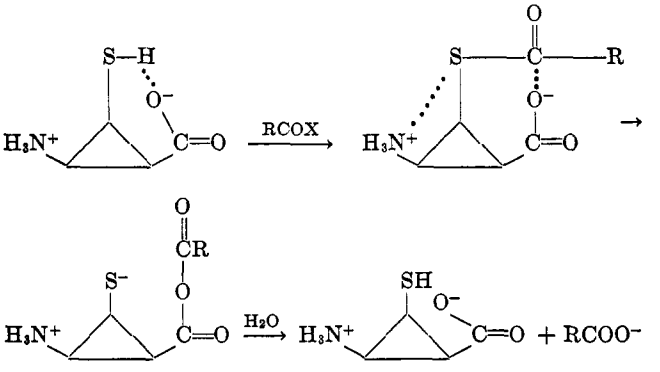


(87)

or transferred to the oxygen of the serine moiety. This mechanism prefers to relegate the acyl–enzyme intermediate observed with nitrophenyl esters to a special category and proposes that for specific substrates only a tetrahedral intermediate is formed. From data on the kinetics of acetyl-L-tyrosine ethyl ester (353) and carbobenzoxy-L-tyrosine p-nitrophenyl ester (185), which are closely allied to specific substrates, it is questionable whether the relegation of the acyl–enzyme to a subsidiary position is correct. Furthermore, from this mechanism it is difficult to conceive of reasons for the extraordinary nucleophilicity of XXXIX or the facile hydrolysis of the first acyl–enzyme intermediate which certainly occurs experimentally. This proposal is stereochemically satisfactory and does fit the structural data, which indicate that aspartylserine (or glutamylserine) is present in the active site of a large number of hydrolytic enzymes.

2. *Papain and ficin*

It has been pointed out previously that the mercapto group is involved in the active site of these enzymes. On the basis of these observations it has been postulated that the mercapto group functions through the formation of an intermediary thiol ester (65, 350). From studies of the pH dependence of the catalytic step, it has been shown that the carboxylate ion and the ammonium ion are involved in the active site of ficin and that the carboxylate ion is involved in the active site of papain. It was postulated that the carboxylate ion catalyzes the rate-determining hydrolysis of the thiol ester (65), through nucleophilic catalysis (36) involving an anhydride intermediate. The catalysis by ficin has been postulated to follow equation 88 (192). Equation 88 accounts for the fact that amide and ester substrates



(88)

of these enzymes are hydrolyzed at exactly the same rate, powerful evidence for the formation of a common intermediate, the thiol ester. This mechanism also accounts for the increased feasibility of transfer reactions with these enzymes in contrast to chymotrypsin, for here it is postulated that the hydrolysis of the acyl–enzyme (thiol ester) is the slow step of the reaction, whereas with chymotrypsin it is postulated that the formation of the acyl–enzyme is the slow step. Although a two-step process analogous to equation 88 was first suggested for papain by Smith (350), it has recently been criticized by him, primarily on the grounds that it is thermodynamically unsound (348). He has instead suggested that the active site in papain is not a sulfhydryl group and a carboxylate ion but rather a combination of these of higher free energy, namely a thiol ester. It is difficult to assess the thermodynamic argument because of the paucity of data available for these substances. It should be pointed out that any mechanism for catalysis of ester hydrolysis by a thiol ester is extremely complicated and probably must involve another nucleophilic grouping which at present is not justified by the experimental evidence. On the basis of the above evidence and of the model system described earlier, it appears that equation 88, involving two nucleophilic catalyses, best describes the mechanism of catalysis by ficin and also perhaps catalysis by papain in a modified form without the ammonium ion.

VIII. Conclusions

Catalysis of the nucleophilic reactions of carboxylic acid derivatives has proliferated in the past decade. An attempt has been made to present the important concepts involved in these catalyses. As in the past, the reactions of carboxylic acid derivatives have provided

a framework whereby important physicochemical principles have been elucidated. While many details of the catalytic processes of hydrolytic enzymes may have to be changed, it now seems clear that the basic framework on which mechanistic arguments will be advanced has been presented. It is hoped that the synthesis involved in this review will hasten the ultimate solution of the problem of enzymatic catalysis.

The author is indebted to many of his colleagues who have provided much constructive criticism and many suggestions and who have provided new material in advance of publication. The work was assisted in part by an Alfred P. Sloan, Jr., Research Fellowship.

IX. References

(1) Aksnes, G., and Prue, J. E.: J. Chem. Soc. **1959**, 103.
(2) Amis, E. S., and Jaffé, G.: J. Chem. Phys. **10**, 598 (1942).
(3) Anderson, G. W., and Paul, R.: J. Am. Chem. Soc. **80**, 4423 (1958).
(4) Arai, J.: J. Biochem. (Japan) **20**, 465 (1934).
(5) Avison, A. W. D.: J. Chem. Soc. **1955**, 732.
(6) Bader, A. R., Cummings, L. O., and Vogel, H. A.: J. Am. Chem. Soc. **73**, 4195 (1951).
(7) Bader, A. R., and Vogel, H. A.: J. Am. Chem. Soc. **74**, 3992 (1952).
(8) Bafna, S. L., and Gold, V.: J. Chem. Soc. **1953**, 1406.
(9) Baker, J. W., and Gaunt, J.: J. Chem. Soc. **1949**, 9, 19.
(10) Baker, J. W., and Rothstein, E.: In *Handbuch der Katalyse*, edited by G.-M. Schwab, Vol. 2, p. 46. J. Springer, Vienna (1940).
(11) Ballard, D. G. H., and Bamford, C. H.: J. Chem. Soc. **1958**, 355.
(12) Ballard, D. G. H., and Bamford, C. H.: Nature **172**, 907 (1953).
(13) Ballard, D. G. H., and Bamford, C. H.: Proc. Roy. Soc. (London) **A223**, 495 (1954).
(14) Balls, A. K., and Aldrich, F. L.: Proc. Natl. Acad. Sci. U.S. **41**, 190 (1955).
(15) Balls, A. K., and Jansen, E. F.: Advances in Enzymol. **13**, 321 (1952).
(16) Balls, A. K., and McDonald, C. E.: J. Biol. Chem. **221**, 993 (1956).
(17) Balls, A. K., McDonald, C. E., and Brecher, A. S.: Proceedings of the International Symposium on Enzyme Chemistry, Tokyo, Maruzen, 1958, p. 392.
(18) Balls, A. K., and Wood, H. N.: J. Biol. Chem. **219**, 245 (1956).
(19) Baltzly, R., Berger, I. M., and Rothstein, A. A.: J. Am. Chem. Soc. **72**, 4149 (1950).
(20) Barnum, D. W., and Gorin, G.: J. Phys. Chem. **58**, 1169 (1954).
(21) Bartlett, P. D., and Greene, F. D.: J. Am. Chem. Soc. **76**, 1088 (1954).
(22) Bartlett, P. D., and Jones, R. H.: J. Am. Chem. Soc. **79**, 2153 (1957).
(23) Beichler, S. S., and Taft, R. W., Jr.: J. Am. Chem. Soc. **79**, 4927 (1957).
(24) Bell, R. P.: *Acid-Base Catalysis*. Oxford University Press, London (1941).
(25) Bell, R. P., and Clunie, J. C.: Proc. Roy. Soc. (London) **A212**, 33 (1952).
(26) Bell, R. P., and Clunie, J. C.: Trans. Faraday Soc. **48**, 439 (1952).
(27) Bell, R. P., Dowding, A. L., and Noble, J. M.: J. Chem. Soc. **1955**, 3106.
(28) Bell, R. P., and Jones, P.: J. Chem. Soc. **1953**, 88.
(29) Bellamy, L. J.: *The Infrared Spectra of Complex Molecules*, 2nd edition, p. 179. John Wiley and Sons, Inc., New York (1958).
(30) Bellamy, L. J., Thomas, L. C., and Williams, R. L.: J. Chem Soc. **1956**, 3704.
(31) Bellamy, L. J., and Williams, R. L.: J. Chem. Soc. **1957**, 4294.
(32) Bender, M. L.: J. Am. Chem. Soc. **73**, 1626 (1951).
(33) Bender, M. L.: J. Am. Chem. Soc. **75**, 5986 (1953).
(34) Bender, M. L.: "Proceedings of the International Congress of Catalysis, 1956," *Advances in Catalysis*, Vol. IX, edited by A. Farkas, p. 374. Academic Press, New York (1957).
(35) Bender, M. L.: J. Am. Chem. Soc. **79**, 1258 (1957).
(35a) Bender, M. L.: In *Techniques of Organic Chemistry*, 2nd edition, Vol. 8, Chap. XXIII. Interscience Publishers, Inc., New York (1960).
(36) Bender, M. L., Chloupek, F., and Neveu, M. C.: J. Am. Chem. Soc. **80**, 5384 (1958).
(37) Bender, M. L., and Chow, Y.-L.: J. Am. Chem. Soc. **81**, 3929 (1959).
(38) Bender, M. L., Chow, Y.-L., and Chloupek, F.: J. Am. Chem. Soc. **80**, 5380 (1958).
(39) Bender, M. L., and Ginger, R. D.: J. Am. Chem. Soc. **77**, 348 (1955).
(39a) Bender, M. L., and Ginger, R. D.: Suomen Kemistelehti **33**, in press (1960).
(40) Bender, M. L., Ginger, R. D., and Kemp, K. C.: J. Am. Chem. Soc. **76**, 3350 (1954).
(41) Bender, M. L., Ginger, R. D., and Unik, J. P.: J. Am. Chem. Soc. **80**, 1044 (1958).
(42) Bender, M. L., and Glasson, W. A.: J. Am. Chem. Soc. **81**, 1590 (1959).
(43) Bender, M. L., and Glasson, W. A.: J. Am. Chem. Soc. **82**, in press (1960).
(44) Bender, M. L., and Kemp, K. C.: J. Am. Chem. Soc. **79**, 111 (1957).
(45) Bender, M. L., and Kemp, K. C.: J. Am. Chem. Soc. **79**, 116 (1957).
(46) Bender, M. L., and Matsui, H.: Unpublished results.
(47) Bender, M. L., and Meyer, P. D.: Unpublished results.
(48) Bender, M. L., and Neveu, M. C.: J. Am. Chem. Soc. **80**, 5388 (1958).
(49) Bender, M. L., and Neveu, M. C.: Unpublished results.
(50) Bender, M. L., and Thomas, R.: Unpublished results.
(51) Bender, M. L., Tobey, S. E., and Thomas, R.: Unpublished results.
(52) Bender, M. L., and Turnquest, B. W.: J. Am. Chem. Soc. **77**, 4271 (1955).
(53) Bender, M. L., and Turnquest, B. W.: J. Am. Chem. Soc. **79**, 1652 (1957).
(54) Bender, M. L., and Turnquest, B. W.: J. Am. Chem. Soc. **79**, 1656 (1957).
(54a) Bender, M. L., and Turnquest, B. W.: J. Am. Chem. Soc. **79**, 1889 (1957).
(55) Bentley, R., and Rittenberg, D.: J. Am. Chem. Soc. **76**, 4883 (1954).
(56) Bergmann, F.: Discussions Faraday Soc. **20**, 126 (1955).
(57) Bergmann, F. Rimon, S., and Segal, R.: Biochem. J. **68**, 493 (1958).
(58) Bergmann, F., Segal, R., Shimoni, A., and Wurzel, M.: Biochem. J. **63**, 684 (1956).
(59) Bergmann, F., Wurzel, M., and Shimoni, E.: Biochem. J. **55**, 888 (1953).

(60) BERGMANN, M., AND ZERVAS, L.: Z. physiol. Chem. **175,** 145 (1928).
(61) BERNHARD, S. A.: Biochem. J. **59,** 506 (1955).
(62) BERNHARD, S. A.: In *The Enzymes,* edited by P. D. Boyer, H. Lardy, and K. Myrbäck, 2nd edition, Vol. 1, p. 126. Academic Press, Inc., New York (1959).
(63) BERNHARD, S. A.: J. Cellular Comp. Physiol., in press (1960).
(64) BERNHARD, S. A., COLES, W. C., AND NOWELL, J. F.: J. Am. Chem. Soc. **82,** in press (1960).
(65) BERNHARD, S. A., AND GUTFREUND, H.: Biochem. J. **63,** 61 (1956).
(66) BERNHARD, S. A., AND GUTFREUND, H.: Proceedings of the International Symposium on Enzyme Chemistry, Tokyo, Maruzen, 1958, p. 124.
(67) BERNHARD, S. A., AND HAMMETT, L. P.: J. Am. Chem. Soc. **75,** 5834 (1953).
(68) BETTS, R. L., AND HAMMETT, L. P.: J. Am. Chem. Soc. **59,** 1568 (1937).
(69) BEVAN, C. W. L., AND HUDSON, R. F.: J. Chem. Soc. **1953,** 2187.
(70) BIEBER, R., AND TRUMPLER, G.: Helv. Chim. Acta **30,** 1860 (1947).
(71) BILLICA, H. R. AND CARRIEL, J. T.: U.S. patent 2,739,957 (March 27, 1956); Chem. Abstracts **50,** 11719 (1956).
(72) Blackadder, D. A., AND HINSHELWOOD, SIR C. N.: J. Chem. Soc. **1958,** 2726.
(73) BOLIN, I.: Z. anorg. allgem. Chem. **177,** 244 (1929).
(74) BORSOOK, H., AND DUBNOFF, J. W.: J. Biol. Chem. **132,** 307 (1940).
(75) BOSE, A. N., AND HINSHELWOOD, SIR C. N.: J. Chem. Soc. **1958,** 4085.
(76) BRECHER, A. S., AND BALLS, A. K.: J. Biol. Chem. **227,** 845 (1957).
(77) BRENNER, M.: Personal communication.
(78) BRENNER, M., AND ZIMMERMAN, J. P.: Helv. Chim. Acta **40,** 1933 (1957).
(79) BRENNER, M., AND WEHRMÜLLER, J.: Helv. Chim. Acta **40,** 2374 (1957).
(80) BRENNER, M., ZIMMERMAN, J. P., WEHRMÜLLER, J., QUITT, P., HARTMAN, A., SCHNEIDER, W., AND BEGLINGER, W.: Helv. Chim. Acta **40,** 1497 (1957).
(81) BROUWER, D. M.: Doctoral Thesis, University of Leiden, 'sGravenhage (1957).
(82) BROUWER, D. M., VLUGT, M. J. VAN DER, AND HAVINGA, E.: Proc. Koninkl. Ned. Akad. Wetenschappen **60B,** 275 (1957).
(83) BROUWER, M., VLUGT, M. J. VAN DER, AND HAVINGA, E.: Proc. Koninkl. Ned. Akad. Wetenschappen **B61,** 141 (1958).
(84) BROWN, H. C., AND MCGARY, C. W., JR.: J. Am. Chem. Soc. **77,** 2300 (1955).
(85) BROWN, H. C., AND MCGARY, C. W., JR.: J. Am. Chem. Soc. **77,** 2306 (1955).
(86) BROWN, H. C., AND NELSON, K. L.: J. Am. Chem. Soc. **75,** 6292 (1953).
(87) BROWN, H. C., AND SMOOT, C. R.: J. Am. Chem. Soc. **78,** 6255 (1956).
(88) BROWN, T. L.: J. Am. Chem. Soc. **80,** 3513 (1958).
(88a) BRUICE, T. C.: J. Am. Chem. Soc. **81,** 5444 (1959).
(89) BRUICE, T. C., AND LAPINSKI, R.: J. Am. Chem. Soc. **80,** 2265 (1958).
(90) BRUICE, T. C., AND SCHMIR, G. L.: Arch. Biochem. Biophys. **63,** 484 (1956).
(91) BRUICE, T. C., AND SCHMIR, G. L.: J. Am. Chem. Soc. **79,** 1663 (1957).
(92) BRUICE, T. C., AND SCHMIR, G. L.: J. Am. Chem. Soc. **80,** 148 (1958).
(93) BRUICE, T. C., AND SCHMIR, G. L.: J. Am. Chem. Soc. **81,** 4522 (1959).
(94) BRUICE, T. C., AND STURTEVANT, J. M.: Biochim. et Biophys. Acta **30,** 208 (1958).
(95) BRUICE, T. C., AND STURTEVANT, J. M.: J. Am. Chem. Soc. **81,** 2860 (1959).
(96) BUNNETT, J. F.: Quart. Revs. (London) **12,** 1 (1958).
(97) BUNNETT, J. F., AND DAVIS, G. T.: J. Am. Chem. Soc. **82,** in press (1960).
(98) BUNNETT, J. F., AND ZAHLER, R. E.: Chem. Revs. **49,** 273 (1951).
(99) BUNTON, C. A., LEWIS, T. A., AND LLEWELLYN, D. H.: Chem. & Ind. (London) **1954,** 1154.
(100) BUNTON, C. A., AND SPATCHER, D. N.: J. Chem. Soc. **1956,** 1079.
(101) BURKUS, J., AND ECKERT, C. F.: J. Am. Chem. Soc. **80,** 5948 (1958).
(102) BURTON, K.: Biochem. J. **59,** 44 (1955).
(103) BURTON, K., BENZIGER, A., *et al.*: Biochem. J. **71,** 400 (1959).
(104) CALDWELL, J. R.: U.S. patent 2,720,502 (October 11, 1955); Chem. Abstracts **50,** 2204 (1956).
(105) CALDWELL, J. R.: U.S. patent 2,720,507 (October 11, 1955); Chem. Abstracts **50,** 2205 (1956).
(106) CALDWELL, J. R., AND WELLMAN, J. W.: U.S. patent 2,720,504 (October 11, 1955); Chem. Abstracts **50,** 2205 (1956).
(107) CALDWELL, J. R., AND WELLMAN, J. W.: U.S. patent 2,720,505 (October 11, 1955); Chem. Abstracts **50,** 2205 (1956).
(108) CALDWELL, J. R., AND WELLMAN, J. W.: U.S. patent 2,727,881 (December 20, 1955); Chem. Abstracts **50,** 15126 (1956).
(109) CASTELLS, J., AND FLETCHER, G. A.: J. Chem. Soc. **1956,** 3245.
(110) CHANLEY, J. D., AND FEAGESON, E.: J. Am. Chem. Soc. **77,** 4002 (1955).
(111) CHANLEY, J. D., GINDLER, E. M., AND SOBOTKA, H.: J. Am. Chem. Soc. **74,** 4347 (1952).
(112) CHURCHILL, J. W., LAPKIN, M., MARTINEZ, F., AND ZASLOWSKY, J. A.: J. Am. Chem. Soc. **80,** 1944 (1958).
(112a) COHEN, S. G., AND ALTSCHUL, L.: Nature **183,** 1678 (1959).
(113) COOK, D.: J. Am. Chem. Soc. **80,** 49 (1958).
(114) COREY, E. J., AND BURKE, H. J.: J. Am. Chem. Soc. **77,** 5418 (1955).
(115) COREY, E. J., TOPIE, T. H., AND WOZNIAK, W. A.: J. Am. Chem. Soc. **77,** 5415 (1955).
(116) COX, E. F., AND HOSTETTLER, F.: Abstracts of Papers Presented at the 135th meeting of the American Chemical Society, Boston, Massachusetts, April, 1959, p. 1120.
(117) CRAIG, N. C., AND KISTIAKOWSKY, G. B.: J. Am. Chem. Soc. **80,** 1574 (1958).
(118) CRISTOL, S. J., AND NORRIS, W. P.: J. Am. Chem. Soc. **76,** 3005 (1954).
(119) CUNNINGHAM, L. W., AND BROWN, C. S.: J. Biol. Chem. **221,** 287 (1956).
(120) DAVIDSON, D., AND AUERBACH, L.: J. Am. Chem. Soc. **75,** 5984 (1953).
(121) DATTA, S. C., DAY, J. N. E., AND INGOLD, C. K.: J. Chem. Soc. **1939,** 838.
(122) DAWSON, H. M., AND LOWSON, W.: J. Chem. Soc. **1927,** 2444.
(123) DAWSON, H. M., AND LOWSON, W.: J. Chem. Soc. **1929,** 393.

(124) DAWSON, H. M., PYCOCK, E. R., AND SPIVEY, E.: J. Chem. Soc. **1933,** 291.
(125) DAWSON, H. M., AND SMITH, J. E.: J. Chem. Soc. **1930,** 79.
(126) DAWSON, H. M., AND SPIVEY, E.: J. Chem. Soc. **1930,** 2180.
(127) DAY, J. N. E., AND INGOLD, C. K.: Trans. Faraday Soc. **37,** 686 (1941).
(128) DEWAR, M. J. S.: *The Electronic Theory of Organic Chemistry,* p. 117. Oxford University Press, New York (1948).
(129) DIXON, G. H., DREYER, W. J., AND NEURATH, H.: J. Am. Chem. Soc. **78,** 4810 (1956).
(130) DIXON, G. H., GO, S., AND NEURATH, H.: Biochim. et Biophys. Acta **19,** 193 (1956).
(131) DIXON, G. H., KAUFFMAN, D. L., AND NEURATH, H.: J. Am. Chem. Soc. **80,** 1260 (1958).
(132) DIXON, G. H., KAUFFMAN, D. L., AND NEURATH, H.: J. Biol. Chem. **233,** 1373 (1958).
(133) DIXON, G. H., AND NEURATH, H.: J. Am. Chem. Soc. **79,** 4558 (1957).
(134) DIXON, G. H., AND NEURATH, H.: J. Biol. Chem. **225,** 1049 (1957).
(135) DIXON, G. H., NEURATH, H., AND PECHÈRE, J.-F.: Ann. Rev. Biochem. **27,** 489 (1958).
(136) DOBRY, A., FRUTON, J. S., AND STURTEVANT, J. M.: J. Biol. Chem. **195,** 149 (1952).
(137) DOBRY, A., AND STURTEVANT, J. M.: J. Biol. Chem. **195,** 141 (1952).
(138) DOHERTY, D. G.: J. Am. Chem. Soc. **77,** 4887 (1955).
(139) DOHERTY, D. G., AND VASLOW, F.: J. Am. Chem. Soc. **74,** 931 (1952).
(140) DURANT, G. J., TURNBULL, J. H., AND WILSON, W.: Chem. & Ind. (London) **1958,** 157.
(141) EDWARD, J. T., AND MEACOCK, S. C. R.: J. Chem. Soc. **1957,** 2000.
(142) EDWARD, J. T., AND MEACOCK, S. C. R.: J. Chem. Soc. **1957,** 2009.
(143) EDWARD, J. T., AND NIELSEN, S.: J. Chem. Soc. **1957,** 5080.
(144) EDWARD, J. T., AND TERRY, K. A.: J. Chem. Soc. **1957,** 3527.
(145) EDWARDS, J. O.: J. Am. Chem. Soc. **78,** 1819 (1956).
(146) EDWARDS, L. J.: Trans. Faraday Soc. **46,** 723 (1950).
(147) EDWARDS, L. J.: Trans. Faraday Soc. **48,** 696 (1952).
(148) EICHHORN, G. L., AND BAILAR, J. C., JR.: J. Am. Chem. Soc. **75,** 2905 (1953).
(149) EPSTEIN, J., BAUER, V. E., SAXE, M., AND DEMEK, M. M.: J. Am. Chem. Soc. **78,** 4068 (1956).
(150) EPSTEIN, J., DEMEK, M. M., AND ROSENBLATT, D. H.: J. Org. Chem. **21,** 796 (1956).
(151) EPSTEIN, J., ROSENBLATT, D. H., AND DEMEK, M. M.: J. Am. Chem. Soc. **78,** 341 (1956).
(152) EIGEN, M.: Discussions Faraday Soc. **17,** 194 (1955).
(153) EULER, H. VON, AND OLANDER, A.: Z. physik. Chem. **131,** 107 (1927).
(154) EYRING, H., LUMRY, R., AND SPIKES, J. D.: In *The Mechanism of Enzyme Action,* edited by W. D. McElroy and B. Glass, p. 123. Johns Hopkins Press, Baltimore (1954).
(155) FABER, E. M., AND REID, E. E.: J. Am. Chem. Soc. **39,** 1930 (1917).
(156) FERREN, R. A., MILLER, J. G., AND DAY, A. R.: J. Am. Chem. Soc. **79,** 70 (1957).
(157) FLETT, M. ST. C.: Trans. Faraday Soc. **44,** 767 (1948).
(158) FLETT, M. ST. C.: J. Chem. Soc. **1951,** 962.
(159) FOSTER, R. J., JENNINGS, R. R., AND NIEMANN, C.: J. Am. Chem. Soc. **76,** 3142 (1954).
(160) FOSTER, R. J., AND NIEMANN, C.: J. Am. Chem. Soc. **77,** 1886 (1955).
(161) FOSTER, R. J., AND NIEMANN, C.: J. Am. Chem. Soc. **77,** 3365 (1955).
(162) FRAENKEL, G., AND NIEMANN, C.: Proc. Natl. Acad. Sci. U.S. **44,** 688 (1958).
(163) FRANKLIN, J. L.: Ind. Eng. Chem. **41,** 1070 (1949).
(164) FRIEDMAN, H. B., AND ELMORE, G. V.: J. Am. Chem. Soc. **63,** 864 (1941).
(165) FRIESS, S. L.: In *The Enzymes,* edited by P. D. Boyer, H. Lardy, and K. Myrbäck, 2nd edition, Vol. 1, p. 123. Academic Press, Inc., New York (1959).
(166) FROST, A. A., AND PEARSON, R. G.: *Kinetics and Mechanism,* p. 205. John Wiley and Sons, Inc., New York (1953).
(167) FRUTON, J. S., JOHNSTON, R. B., AND FRIED, M.: J. Biol. Chem. **190,** 39 (1951).
(168) FRUTON, J. S., AND SIMMONDS, S.: *General Biochemistry,* 2nd edition, Chap. 29. John Wiley and Sons, Inc., New York (1958).
(169) FUSON, N., JOSIEN, M.-L., AND SHELTON, E. M.: J. Am. Chem. Soc. **76,** 2526 (1954).
(169a) GARRETT, E. R.: J. Am. Chem. Soc. **79,** 3401 (1957).
(170) GARRETT, E. R.: J. Am. Chem. Soc. **79,** 5206 (1957).
(171) GARRETT, E. R.: J. Am. Chem. Soc. **80,** 4049 (1958).
(172) GARRETT, E. R.: J. Am. Chem. Soc. **82,** in press (1960).
(173) GAWRON, O., AND DRAUS, F.: J. Am. Chem. Soc. **80,** 5392 (1958).
(174) GLADNER, J. A., AND LAKI, K.: J. Am. Chem. Soc. **80,** 1263 (1958).
(175) GOLD, V.: Trans. Faraday Soc. **44,** 506 (1948).
(176) GOLD, V., AND JEFFERSON, E. G.: J. Chem. Soc. **1953,** 1406, 1409.
(177) GOLD, V., AND JEFFERSON, E. G.: J. Chem. Soc. **1953,** 1416.
(178) GOLDSCHMIDT, H.: Ber. **28,** 3218 (1895).
(179) GOODHUE, E. A., AND DUNLAP, H. L.: J. Am. Chem. Soc. **50,** 1920 (1928).
(180) GORDON, M., MILLER, J. G., AND DAY, A. R.: J. Am. Chem. Soc. **70,** 1946 (1948).
(181) GREEN, A. L., AND NICHOLLS, J. D.: Biochem. J. **72,** 70 (1959).
(182) Green, M., AND HUDSON, R. F.: Proc. Chem. Soc. **1959,** 149.
(183) GROGGINS, P. H.: *Unit Processes in Organic Synthesis,* 5th edition, p. 696. McGraw-Hill Book Company, Inc., New York (1958).
(184) GROOCOCK, C. M., INGOLD, C. K., AND JACKSON, A.: J. Chem. Soc. **1930,** 1039.
(185) GUTFREUND, H., AND HAMMOND, B. R.: Biochem. J. **73,** 526 (1959).
(186) GUTFREUND, H.: Trans. Faraday Soc. **51,** 441 (1955).
(186a) GUTFREUND, H.: Discussions Faraday Soc. **20,** 167 (1955).
(187) GUTFREUND, H., AND STURTEVANT, J. M.: Biochem. J. **63,** 656 (1956).
(188) GUTFREUND, H., AND STURTEVANT, J. M.: Proc. Natl. Acad. Sci. U.S. **42,** 719 (1956).
(189) HALL, H. K., JR., BRANDT, M. K., AND MASON, R. M.: J. Am. Chem. Soc. **80,** 6420 (1958).
(190) HAMMETT, L. P.: *Physical Organic Chemistry,* Chap. VII. McGraw-Hill Book Company, Inc., New York (1940).
(191) HAMMOND, B. R., AND GUTFREUND, H.: Biochem. J. **61,** 187 (1955).
(192) HAMMOND, B. R., AND GUTFREUND, H.: Biochem. J. **72,** 349 (1959).
(193) HAMMOND, G. S.: J. Am. Chem. Soc. **77,** 334 (1955).
(193a) HANSEN, B.: Acta Chem. Scand. **12,** 324 (1958).
(194) HARTLEY, B. S., AND KILBY, B. A.: Biochem. J. **50,** 672 (1952).
(195) HARTLEY, B. S., AND KILBY, B. A.: Biochem. J. **56,** 288 (1954).

(196) HARTLEY, B. S., AND MASSEY, V.: Ann. Repts. on Progr. Chem. (Chem. Soc. London) **51**, 311 (1954).
(197) HARTLEY, B. S., AND MASSEY, V.: Biochim. et Biophys. Acta **21**, 58 (1956).
(198) HARTMAN, L.: J. Am. Oil Chemists' Soc. **33**, 129 (1956).
(199) HASKELL, V. C., AND HAMMETT, L. P.: J. Am. Chem. Soc. **71**, 1284 (1949).
(200) HAUROWITZ, F., AND HOROWITZ, J.: J. Am. Chem. Soc. **77**, 3138 (1955).
(201) HAWKINS, P. J., AND PISCALNIKOW, I.: J. Am. Chem. Soc. **77**, 2771 (1955).
(202) HAWKINS, P. J., AND TARBELL, D. S.: J. Am. Chem. Soc. **75**, 2982 (1953).
(203) HEILBRONN, E.: Acta Chem. Scand. **12**, 1492 (1958).
(204) HEINICKE, R. M., AND MORI, R.: Science **129**, 1678 (1959).
(205) HELE, P.: J. Biol. Chem. **206**, 671 (1954).
(206) HENBEST, H. B., AND LOVELL, B. J.: J. Chem. Soc. **1957**, 1965.
(207) HESTRIN, S.: Biochim. et Biophys. Acta **4**, 310 (1950).
(208) HIGUCHI, T., AND LACHMAN, L.: J. Am. Pharm. Assoc. **44**, 521 (1955).
(209) HIGUCHI, T., AND MARCUS, A. D.: J. Am. Pharm. Assoc. **43**, 530 (1954).
(210) HIGUCHI, T., MARCUS, A. D., AND BIAS, C. D.: J. Am. Pharm. Assoc. **43**, 129 (1954).
(211) HILL, R. L., AND SMITH, E. L.: Biochim. et Biophys. Acta **19**, 376 (1956).
(212) HINE, J.: *Physical Organic Chemistry*. McGraw-Hill Book Company, Inc., New York (1956).
(212a) HIRSHBERG, Y., LAVIE, D., AND BERGMANN, E. D.: J. Chem. Soc. **1951**, 1030.
(213) HOGNESS, D. S., AND NIEMANN, C.: J. Am. Chem. Soc. **75**, 884 (1953).
(214) HOLLAND, G. F., DURANT, R. C., FRIESS, S. L., AND WITKOP, B.: J. Am. Chem. Soc. **80**, 6031 (1958).
(215) HOLMBERG, B.: Ber. **45**, 2997 (1912).
(216) HOPPÉ, J. I., AND PRUE, J. E.: J. Chem. Soc. **1957**, 1775.
(217) HUANG, H. T., AND NIEMANN, C.: J. Am. Chem. Soc. **73**, 3223 (1951).
(218) HUBERT, A., BUIJLE, R., AND HARGITAY, B.: Nature **182**, 259 (1958).
(219) INGOLD, C. K.: *Structure and Mechanism in Organic Chemistry*. Cornell University Press, Ithaca, New York, (1953).
(220) INGOLD, C. K., AND INGOLD, E. H.: J. Chem. Soc. **1932**, 756.
(221) JANNSEN, F., WINITZ, M., AND FOX, S. W.: J. Am. Chem. Soc. **75**, 704 (1953).
(222) JANSEN, E. F., NUTTING, M.-D. F., AND BALLS, A. K.: J. Biol. Chem. **179**, 201 (1949).
(223) JANSEN, E. F., NUTTING, M.-D. F., JANG, R., AND BALLS, A. K.: J. Biol. Chem. **179**, 189 (1949).
(224) JAFFÉ, H. H.: Chem. Revs. **53**, 191 (1953).
(224a) JANSZ, H. S., POSTHUMUS, C. H., AND COHEN, J. A.: Biochim. et Biophys. Acta **33**, 387, 396 (1959).
(225) JENCKS, W. P.: Biochim. et Biophys. Acta **24**, 227 (1957).
(226) JENCKS, W. P.: J. Am. Chem. Soc. **80**, 4581 (1958).
(227) JENCKS, W. P.: J. Am. Chem. Soc. **80**, 4585 (1958).
(228) JENCKS, W. P.: J. Am. Chem. Soc. **81**, 475 (1959).
(229) JENCKS, W. P., AND CARRIUOLO, J.: J. Am. Chem. Soc. **82**, in press (1960).
(229a) JENCKS, W. P., AND CARRIUOLO, J.: J. Am. Chem. Soc. **82**, in press (1960).
(230) JENCKS, W. P., AND CARRIUOLO, J.: J. Biol. Chem. **234**, 1272 (1959).
(231) JENCKS, W. P., AND CARRIUOLO, J.: J. Biol. Chem. **234**, 1280 (1959).
(232) JOHNSTON, R. B., MYCEK, M. J., AND FRUTON, J. S.: J. Biol. Chem. **187**, 205 (1950).
(233) JONES, D. E., AND VERNON, C. A.: Nature **176**, 791 (1955).
(234) JONES, M. E.: Federation Proc. **12**, 708 (1953).
(235) JONES, R. N., FORBES, W. F., AND MUELLER, W. A.: Can. J. Chem. **35**, 504 (1957).
(236) KAGANOVA, I. L., AND OREKHOVICH, V. N.: Doklady Akad. Nauk. S.S.S.R. **95**, 1259 (1954).
(237) KENNEDY, E. P., AND KOSHLAND, D. E., JR.: J. Biol. Chem. **228**, 419 (1957).
(238) KENYON, J., AND THAKER, K.: J. Chem. Soc. **1957**, 2531.
(239) KERN, W., HEROLD, W., AND SCHERHAG, B.: Makromol. Chem. **17**, 231 (1956).
(240) KILPATRICK, M., JR.: J. Am. Chem. Soc. **50**, 2891 (1928).
(241) KILPATRICK, M., JR.: J. Am. Chem. Soc. **52**, 1410 (1930).
(242) KILPATRICK, M., JR., AND KILPATRICK, M. L.: J. Am. Chem. Soc. **52**, 1418 (1930).
(242a) KIMMEL, J. R., AND SMITH, E. L.: J. Biol. Chem. **207**, 515 (1954).
(243) KINDLER, K.: Ann. **452**, 90 (1927); Ber. **69B**, 2792 (1936).
(244) KLAGES, F.: Chem. Ber. **82**, 358 (1949).
(245) KOPPLE, K. D., AND KATZ, J. J.: J. Am. Chem. Soc. **78**, 6199 (1956).
(245a) KOPPLE, K. D., AND SOSNOVSKY, G.: Personal communication.
(246) KOSHLAND, D. E., JR.: Biol. Revs. Cambridge Phil. Soc. **28**, 416 (1953).
(247) KOSHLAND, D. E., JR.: J. Am. Chem. Soc. **74**, 2286 (1952).
(248) KOSHLAND, D. E., JR.: In *The Enzymes*, edited by P. D. Boyer, H. Lardy, and K. Myrbäck, 2nd edition, Vol. 1, p. 305. Academic Press, Inc., New York (1959).
(249) KOSHLAND, D. E., JR.: In *The Mechanism of Enzyme Action*, edited by W. D. McElroy and B. Glass, p. 608. Johns Hopkins Press, Baltimore (1954).
(250) KOSHLAND, D. E., JR., AND ERWIN, M. J.: J. Am. Chem. Soc. **79**, 2657 (1957).
(251) KOSHLAND, D. E., JR., AND HERR, E. B., JR.: J. Biol. Chem. **228**, 1021 (1957).
(252) KRIEBLE, V. K., AND HOLST, K. A.: J. Am. Chem. Soc. **60**, 2976 (1938).
(253) KROLL, H.: J. Am. Chem. Soc. **74**, 2036 (1952).
(254) KROLL, H.: J. Am. Chem. Soc. **74**, 2034 (1952).
(254a) KUPCHAN, S. M., AND JOHNSON, W. S.: J. Am. Chem. Soc. **78**, 3864 (1956).
(255) KURSANOV, D. N., AND KUDRYAVTSEV, R. V.: Zhur. Obschef Khim. **26**, 1040 (1956).
(256) KWART, H.: Personal communication.
(257) LACHMAN, L., GUTTMAN, D., AND HIGUCHI, T.: J. Am. Pharm. Assoc. **46**, 36 (1957).
(258) LACHMAN, L., RAVIN, L. J., AND HIGUCHI, T.: J. Am. Pharm. Assoc. **45**, 290 (1956).
(259) LAIDLER, K. J.: Discussions Faraday Soc. **20**, 83 (1955).
(260) LAIDLER, K. J., AND LANDSKROENER, P. A.: Trans. Faraday Soc. **52**, 200 (1956).
(261) LANGENBECK, W., AND MAHRWALD, R.: Chem. Ber. **90**, 2423 (1957).
(262) LANGENBECK, W., AND MAHRWALD, R.: Ann. **611**, 1 (1958).
(263) LEACH, S. J., AND LINDLEY, H.: Trans. Faraday Soc. **49**, 921 (1953).
(264) LEE, W., AND TURNBULL, J. H.: Biochim. et Biophys. Acta **30**, 655 (1958).
(264a) LEES, E. B., AND SAVILLE, B.: J. Chem. Soc. **1958**, 2262.
(265) LEISTEN, J. A.: Chem. & Ind. (London) **1959**, 397.
(266) LEISTEN, J. A.: J. Chem. Soc. **1959**, 765.
(267) LEVIN, Y., BERGER, A., AND KATCHALSKI, E.: Biochem. J. **63**, 308 (1956).

(268) Li, N. C., Doody, Br. E., and White, J. M.: J. Am. Chem. Soc. **79,** 5859 (1957).
(269) Linderstrøm-Lang, K. U., and Schellman, J. A.: In *The Enzymes,* edited by P. D. Boyer, H. Lardy, and K. Myrbäck, 2nd edition, Vol. 1, p. 443. Academic Press, Inc., New York (1959).
(270) Lipmann, F.: J. Biol. Chem. **155,** 55 (1944).
(271) Long, F. A., and Friedman, L.: J. Am. Chem. Soc. **72,** 3692 (1950).
(272) Lowry, T. M.: J. Chem. Soc. **130,** 2554 (1927).
(273) Lumry, R.: In *The Enzymes,* edited by P. D. Boyer, H. Lardy, and K. Myrbäck, 2nd edition, Vol. 1, p. 199. Academic Press, Inc., New York (1958).
(273a) Lukton, A., and Olcott, H. S.: Biochim. et Biophys. Acta **32,** 267 (1959).
(274) Lutwack, R., Mower, H. F., and Niemann, C.: J. Am. Chem. Soc. **79,** 5690 (1957).
(274a) Lynen, F., Reichert, E., and Rueff, L.: Ann. **574,** 14 (1951).
(275) Marini, M. A., and Hess, G. P.: J. Am. Chem. Soc. **81,** 2594 (1959).
(276) Marsden, R. J. B., and Sutton, L. E.: J. Chem. Soc. **1936,** 1383.
(277) Martin, C. J., Golubow, J., and Axelrold, A. E.: Biochim. et Biophys. Acta **27,** 430 (1958).
(278) Martin, R. B., and Niemann, C.: J. Am. Chem. Soc. **79,** 5828 (1957).
(279) Massey, V., Harrington, W. F., and Hartley, B. S.: Discussions Faraday Soc. **20,** 24 (1955).
(280) Massey, V., and Hartley, B. S.: Biochim. et Biophys. Acta **21,** 361 (1956).
(281) McCasland, G. E.: J. Am. Chem. Soc. **73,** 2295 (1951).
(282) McConnan, J., and Titherley, W.: J. Chem. Soc. **89,** 1318 (1906).
(283) McDonald, C. E., and Balls, A. K.: J. Biol. Chem. **221,** 993 (1956).
(284) McDonald, C. E., and Balls, A. K.: J. Biol. Chem. **227,** 727 (1957).
(285) McElroy, W. D., and Glass, B., Editors: *The Mechanism of Enzyme Action,* Part III. Johns Hopkins Press, Baltimore (1954).
(286) Meerwein, H., and Sönke, H.: Ber. **64B,** 2375 (1931).
(287) Mehrotra, R. C.: J. Am. Chem. Soc. **76,** 2266 (1954).
(288) Meriwether, L., and Westheimer, F. H.: J. Am. Chem. Soc. **78,** 5119 (1956).
(288a) Merrifield, R. B., and Woolley, D. W.: Federation Proc. **17,** 275 (1958).
(289) Michaelis, A., and Kerkhof, W.: Ber. **31,** 2172 (1898).
(290) Mill, P. J., and Crimmin, W. R. C.: Biochim. et Biophys. Acta **23,** 432 (1957).
(291) Miller, S. I.: J. Am. Chem. Soc. **81,** 101 (1959).
(292) Miller, S. I., and Yonan, P. K.: J. Am. Chem. Soc. **79,** 5931 (1957).
(293) Mizushima, S., Shimanouchi, T., Tsuboi, M., and Arakawa, T.: J. Am. Chem. Soc. **79,** 5357 (1957).
(294) Moelwyn-Hughes, E. A.: J. Chem. Soc. **1938,** 779.
(295) Moelwyn-Hughes, E. A.: Proc. Roy. Soc. (London) **A164,** 295 (1938).
(296) Montanari, F.: Gazz. chim. ital. **87,** 149 (1957).
(297) Morawetz, H., and Gaetjens, E.: J. Polymer Sci. **32,** 526 (1958).
(297a) Morawetz, H., and Gaetjens, E.: Personal communication.
(298) Morawetz, H., and Oreskes, I.: J. Am. Chem. Soc. **80,** 2591 (1958).
(299) Morawetz, H., and Westhead, E. W., Jr.: J. Polymer Sci. **16,** 273 (1955).
(300) Morawetz, H., and Zimmering, P. E.: J. Phys. Chem. **58,** 753 (1954).
(301) Morse, B. K., and Tarbell, D. S.: J. Am. Chem. Soc. **74,** 416 (1952).
(302) Mounter, L. A.: Biochim. et Biophys. Acta **27,** 219 (1958).
(303) Nachmansohn, D., and Wilson, I. B.: Advances in Enzymol. **12,** 259 (1951).
(303a) Neurath, H., and Hartley, B. S.: J. Cellular Comp. Physiol., in press (1960).
(304) Newman, M. S., Editor: *Steric Effects in Organic Chemistry,* Chap. 4 and 13. John Wiley and Sons, Inc., New York (1956).
(305) Neveu, M. C.: Ph.D. Thesis, Illinois Institute of Technology (1959).
(306) Norton, H. M., and Quayle, O. R.: J. Am. Chem. Soc. **62,** 1170 (1940).
(307) Oosterbaan, R. A., Jansz, H. S., and Cohen, J. A.: Biochim. et Biophys. Acta **20,** 402 (1956).
(308) Oosterbaan, R. A., and Adrichem, M. E. van: Biochim. et Biophys. Acta **27,** 423 (1958).
(309) Ostwald, W.: Quoted in R. P. Bell, *Acid-Base Catalysis,* p. 2. Oxford University Press, London (1941).
(310) Ostwald, W.: J. prakt. Chem. **30,** 39 (1884).
(311) Overbeek, J. T. G., and Koningsberger, V. V.: Proc. Koninkl. Ned. Akad. Wetenschappen **B57,** 464 (1954).
(312) Pascual, J., Sistaré, J., and Regas, A.: J. Chem. Soc. **1949,** 1943.
(313) Pauling, L., and Corey, R. B.: J. Am. Chem. Soc. **74,** 3964 (1952).
(314) Penland, R. B., Mizushima, S., Curran, C., and Quagliano, J. V.: J. Am. Chem. Soc. **79,** 1575 (1957).
(315) Perlmann, G. E.: Nature **173,** 406 (1954).
(316) Petersen, Q. R.: J. Am. Chem. Soc. **77,** 1743 (1955).
(317) Phillips, A. P.: J. Am. Chem. Soc. **75,** 4725 (1953).
(318) Phillips, A. P., and Baltzly, R.: J. Am. Chem. Soc. **69,** 200 (1947).
(319) Polanyi, M., and Szabo, A. L.: Trans. Faraday Soc. **30,** 508 (1934).
(320) Potts, J. E., Jr., and Amis, E. S.: J. Am. Chem. Soc. **71,** 2112 (1949).
(321) Rabinovitch, B. S., and Winkler, C. A.: Can. J. Research **20B,** 76 (1942).
(322) Reid, E. E.: Am. Chem. J. **21,** 284 (1899).
(323) Rhodes, W. C., and McElroy, W. D.: J. Biol. Chem. **233,** 1528 (1958).
(324) Robinson, R. A.: Trans. Faraday Soc. **26,** 217 (1930).
(325) Rolfe, R. A., and Hinshelwood, C. N.: Trans. Faraday Soc. **30,** 935 (1934).
(326) Ronwin, E.: J. Am. Chem. Soc. **75,** 4026 (1953).
(327) Ronwin, E.: Enzymologia **16,** 81, 179 (1953).
(328) Rose, I. A., Grunberg-Manago, M., Korey, S. R., and Ochoa, S.: J. Biol. Chem. **211,** 737 (1954).
(329) Rydon, H. N.: Nature **182,** 928 (1958).
(330) Rylander, P. N., and Tarbell, D. S.: J. Am. Chem. Soc. **72,** 3021 (1950).
(331) Sachs, G.: Ber. **54,** 1849 (1921).
(332) Samelson, H., and Hammett, L. P.: J. Am. Chem. Soc. **78,** 524 (1956).
(333) Schaffer, N. K., Engle, R. E., Simet, L., Drisko, R. W., and Harshman, S.: Federation Proc. **15,** 347 (1956).
(334) Schaffer, N. K., May, S. C., Jr., and Summerson, W. H.: J. Biol. Chem. **202,** 67 (1953).
(335) Schaffer, N. K., May, S. C., Jr., and Summerson, W. H.: J. Biol. Chem. **206,** 201 (1954).
(336) Schaffer, N. K., Simet, L., Harshman, S., Engle, R. R., and Drisko, R. W.: J. Biol. Chem. **225,** 197 (1957).

(337) SCHMIR, G. L., AND BRUICE, T. C.: J. Am. Chem. Soc. **80,** 1173 (1958).
(338) SCHMIR, G. L., AND BRUICE, T. C.: Unpublished observations.
(339) SCHNEIDER, W. G.: J. Chem. Phys. **23,** 26 (1955).
(340) SCHONBAUM, G. R., AND BENDER, M. L.: Abstracts of Papers Presented at the 136th Meeting of the American Chemical Society, Atlantic City, New Jersey, September, 1959, p. 51-P.
(341) SCHONBAUM, G. R., AND BENDER, M. L.: Unpublished results.
(342) SCHONBAUM, G. R., NAKAMURA, K., AND BENDER, M. L., J. Am. Chem. Soc. **81,** 4746 (1959).
(343) SCHULLER, W. H., AND NIEMANN, C.: J. Am. Chem. Soc. **74,** 4630 (1952).
(344) SCHULTZ, J., AND DELAVAN, L.: Abstracts of Papers Presented at the 134th meeting of the American Chemical Society, Chicago, Illinois, September, 1958, p. 59-C.
(345) SCOTT, J. E.: Nature **172,** 777 (1953).
(346) SIMON, E. J., AND SHEMIN, D.: J. Am. Chem. Soc. **75,** 2520 (1953).
(347) SMITH, A., AND OLIN, B.: Z. physik. Chem. **177,** 131 (1936).
(348) SMITH, E. L.: J. Biol. Chem. **233,** 1392 (1958).
(349) SMITH, E. L., CHAVRÉ, V. J., AND PARKER, M. J.: J. Biol. Chem. **230,** 283 (1958).
(350) SMITH, E. L., FINKLE, B. J., AND STOCKELL, A.: Discussions Faraday Soc. **20,** 96 (1955).
(351) SMITH, E. L., AND PARKER, M. J.: J. Biol. Chem. **233,** 1387 (1958).
(352) SONDHEIMER, E., AND HOLLEY, R. W.: J. Am. Chem. Soc. **76,** 2467 (1954).
(353) SPENCER, T., AND STURTEVANT, J. M.: J. Am. Chem. Soc. **81,** 1874 (1959).
(354) SPRINSON, D. B., AND RITTENBERG, D.: Nature **167,** 484 (1951).
(355) STADTMAN, E. R.: In *The Mechanism of Enzyme Action,* edited by W. D. McElroy and B. Glass, p. 581. Johns Hopkins Press, Baltimore (1954).
(356) STADTMAN, E. R., AND WHITE, F. H., JR.: J. Am. Chem. Soc. **75,** 2022 (1953).
(357) STEARN, A. E.: J. Gen. Physiol. **18,** 301 (1935).
(358) STEIN, S. S., AND KOSHLAND, D. E., JR.: Arch. Biochem. Biophys. **39,** 229 (1952).
(359) STEIN, S. S., AND KOSHLAND, D. E., JR.: Arch. Biochem. Biophys. **45,** 467 (1953).
(360) STEINBERGER, R., AND WESTHEIMER, F. H.: J. Am. Chem. Soc. **73,** 429 (1951).
(361) STERN, J. R.: J. Biol. Chem. **221,** 33 (1956).
(362) STERN, J. R., COON, M. J., CAMPILLO, A. DEL, AND SCHNEIDER, M. C.: J. Biol. Chem. **221,** 15 (1956).
(363) STEWART, R., AND YATES, K.: Personal communication.
(364) STEWART, R., AND YATES, K.: Abstracts of Papers Presented at the 133rd meeting of the American Chemical Society, San Francisco, California, April, 1958, p. N-30.
(365) STIEGLITZ, J.: Am. Chem. J. **39,** 29 (1908).
(366) STILES, M.: J. Am. Chem. Soc. **81,** 2598 (1959).
(367) STILES, M., AND FINKBEINER, H. L.: J. Am. Chem. Soc. **81,** 505 (1959).
(368) STOCKELL, A., AND SMITH, E. L.: J. Biol. Chem. **227,** 1 (1957).
(369) STURTEVANT, J. M.: J. Am. Chem. Soc. **75,** 2016 (1953).
(370) STURTEVANT, J. M.: J. Am. Chem. Soc. **77,** 1495 (1955).
(371) SULLIVAN, D. G., AND SADLER, P. W.: J. Chem. Soc. **1957,** 4144.
(372) SWAIN, C. G.: J. Am. Chem. Soc. **72,** 4578 (1950).
(373) SWAIN, C. G., AND BROWN, J. F., JR.: J. Am. Chem. Soc. **74,** 2534 (1952).
(374) SWAIN, C. G., AND BROWN, J. F., JR.: J. Am. Chem. Soc. **74,** 2538 (1952).
(375) SWAIN, C. G., DIMILO, A. J., AND CORDNER, J. P.: J. Am. Chem. Soc. **80,** 5983 (1958).
(375a) SWAIN, C. G., AND SCOTT, C. B.: J. Am. Chem. Soc. **75,** 141 (1953).
(376) SWARTS, F.: Bull. soc. chim. Belges **35,** 414 (1926).
(377) SYRKIN, YA. K., AND MOISEEV, I. I.: Uspekhi Khim. **27,** 717 (1958).
(378) TAFT, R. W., JR.: In *Steric Effects in Organic Chemistry,* edited by M. S. Newman, p. 556. John Wiley and Sons, Inc., New York (1956).
(379) TAUBER, H.: J. Am. Chem. Soc. **74,** 847 (1952).
(380) TAYLOR, T. W. J.: J. Chem. Soc. **1930,** 2741.
(381) TAYLOR, D. B.: Enzymologia **2,** 310 (1938).
(382) TIROUFLET, J., DABARD, R., AND LAVIRON, E.: Bull. soc. chim. France **24,** 570 (1957).
(383) TOMMILA, E., KOIVISTO, A., LYYRA, J. P., ANTELL, K., AND HEIMO, S.: Ann. Acad. Sci. Fennicae Ser. A, II, No. **47,** 3 (1952).
(384) TROLLE-LASSEN, C.: Arch. Pharm. Chemi. **60,** 689 (1953).
(385) TURBA, F., AND GUNDLACH, G.: Biol. Z. **327,** 186 (1955).
(385a) VAJDA, T.: Chem. & Ind. (London) **1959,** 197.
(386) VASLOW, F.: Compt. rend. Lab. Carlsberg **30,** 4 (1956).
(387) VERKADE, P. E., AND WEPSTER, B. M.: Industrie chim. belge **20,** 1281 (1955).
(387a) VISWANATHA, T.: J. Cellular Comp. Physiol., in press (1960).
(388) WADSÖ, I.: Acta Chem. Scand. **11,** 1745 (1957).
(389) WALEY, S. G., AND WATSON, J.: Biochem. J. **57,** 529, 538 (1954).
(390) WALEY, S. G., AND WATSON, J.: Proc. Roy. Soc. (London) **199A,** 499 (1949).
(391) WATANABE, W. H., AND DE FONSO, L. R.: J. Am. Chem. Soc. **78,** 4542 (1956).
(392) WEINGARTEN, H.: J. Am. Chem. Soc. **80,** 352 (1958).
(392a) WEIL, L., JAMES, S., AND BUCHERT, A. R.: Arch. Biochem. Biophys. **46,** 266 (1953).
(393) WELLMAN, J. W.: U.S. patent 2,720,503 (October 11, 1955); Chem. Abstracts **50,** 2204 (1956).
(394) WELSH, L. H.: J. Am. Chem. Soc. **69,** 128 (1947).
(395) WELSH, L. H.: J. Am. Chem. Soc. **71,** 3500 (1949).
(396) WEPSTER, B. M., AND VERKADE, P. E.: Rec. trav. chim. **67,** 411, 425 (1948).
(397) WESTHEAD, E. W., JR., AND MORAWETZ, H.: J. Am. Chem. Soc. **80,** 237 (1958).
(398) WESTHEIMER, F. H.: Trans. N.Y. Acad. Sci. **18,** 15 (1955).
(398a) WESTHEIMER, F. H.: Proc. Natl. Acad. Sci. U.S. **43,** 969 (1957).
(399) WESTHEIMER, F. H., AND SHOOKHOFF, M. W.: J. Am. Chem. Soc. **62,** 269 (1940).
(400) WHEELER, O. H.: J. Am. Chem. Soc. **79,** 4191 (1957).
(401) WHELAND, G. W.: *Resonance in Organic Chemistry.* John Wiley and Sons, Inc., New York (1955).
(401a) WHITAKER, J. R., AND DEATHERAGE, F. E.: J. Am. Chem. Soc. **77,** 3360 (1955).
(402) WHITAKER, J. R., AND JANDORF, B. J.: J. Biol. Chem. **223,** 751 (1956).
(403) WHITE, J. M., MANNING, R. A., AND LI, N. C.: J. Am. Chem. Soc. **78,** 2367 (1956).
(403a) WHITMORE, F. C.: *Organic Chemistry,* p. 339. D. Van Nostrand Company, Inc., New York (1937).
(404) WIBERG, K. B.: J. Am. Chem. Soc. **75,** 3961 (1953).
(405) WIBERG, K. B.: J. Am. Chem. Soc. **77,** 2519 (1955).
(406) WIBERG, K. B.: Chem. Revs. **55,** 719 (1955).
(406a) WIELAND, T., AND SCHNEIDER, G.: Ann. **580,** 159 (1953).

(406b) WIELAND, T., AND STIMMING, D.: Ann. **579,** 97 (1953).
(407) WILSON, I. B.: In *The Mechanism of Enzyme Action,* edited by W. D. McElroy and B. Glass, p. 642. Johns Hopkins Press, Baltimore (1954).
(408) WILSON, I. B.: Discussions Faraday Soc. **20,** 119 (1955).
(409) WILSON, I. B., BERGMANN, F., AND NACHMANSOHN, D.: J. Biol. Chem. **186,** 781 (1950).
(410) WILSON, I. B., AND CABIB, E.: J. Am. Chem. Soc. **78,** 202 (1956).
(411) WILSON, I. B., GINSBERG, S., AND MEISLICH, E. K.: J. Am. Chem. Soc. **77,** 4286 (1955).
(412) WOLFROM, M. L., BENNETT, R. B., AND CRUM, J. D.: J. Am. Chem. Soc. **80,** 944 (1958).
(413) WYNESS, K. G.: J. Chem. Soc. **1958,** 2934.
(414) ZIMMERING, P. E.: Ph.D. Thesis, Polytechnic Institute of Brooklyn, 1955, p. 89.
(415) ZIMMERING, P. E., WESTHEAD, E. W., JR., AND MORAWETZ, H.: Biochim. et Biophys. Acta **25,** 376 (1957).