[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY]

Intramolecular Catalysis of Hydrolytic Reactions. IV. A Comparison of Intramolecular and Intermolecular Catalysis[1,2]

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The kinetics of the acetate ion-catalyzed hydrolyses of phenyl acetate and *p*-nitrophenyl acetate have been determined at three temperatures. The activation parameters of these processes have been calculated. The hydrolysis of 2,4-dinitrophenyl benzoate at *p*H 5.5 using acetate-O^{18} as catalyst led to benzoic acid containing about 75% of one oxygen-18 atom of the original acetate ion catalyst. The result of this tracer experiment suggests acetylbenzoyl anhydride as intermediate in this acetate ion catalysis and suggests the existence of anhydride intermediates in other acetate ion-catalyzed hydrolyses. The possibility of acylimidazole, acid anhydride, ester and lactone intermediates in nucleophilic catalysis is discussed, both in intramolecular and intermolecular cases. A kinetic comparison between a number of corresponding cases of intermolecular and intramolecular catalysis of hydrolytic reactions indicates the powerful nature of intramolecular catalysis and further suggests that in a rigidly held enzyme–substrate complex nucleophilic and/or electrophilic catalysis is responsible for the powerful action of enzymes in hydrolytic reactions.

Introduction

In previous papers of this series,[2,4,5] it has been demonstrated that catalysis of hydrolysis of carboxylic acid derivatives may be effected by nucleophilic or electrophilic groups within the molecule. It has been suggested[4,6] that the powerful catalytic activity of enzymes is in some measure due to the similarity of enzymatic action to intramolecular catalysis since 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. It was therefore of interest to carry out a quantitative kinetic comparison of corresponding intermolecularly- and intramolecularly-catalyzed reactions in order to determine the relative efficiencies of these two types of catalysis.

The hydrolyses of two phenyl esters, aspirin[7] and mono-*p*-nitrophenyl glutarate (as well as a related copolymer)[6] and one methyl ester, methyl hydrogen phthalate,[2] have been shown to involve intramolecular catalysis by carboxylate ion, involving anhydride intermediates. Data are presented here for the kinetics of the intermolecular analogs of the first two reactions cited above, namely, the acetate ion-catalyzed hydrolyses of phenyl, acetate and *p*-nitrophenyl acetate.

Experimental

Materials.—The preparation of *p*-nitrophenyl acetate has been described before.[8] Phenyl acetate was an Eastman Kodak Co. white label material, n^{20}_{D} 1.5025. 2,4-Dinitrophenyl benzoate was prepared from benzoyl chloride and 2,4-dinitrophenol in pyridine solution. Recrystallization from ethanol gave light yellow crystals, m.p. 134–135°. Acetic acid-O^{18} was prepared from acetyl chloride and H_2O^{18} (1.4 atom % oxygen-18; Stuart Oxygen Co., San Francisco, Calif.) in ethereal solution. After the exothermic reaction, the ether was removed and the acetic acid was distilled, b.p. 115–117°.

Kinetics of Hydrolysis.—The kinetics of hydrolysis of *p*-nitrophenyl acetate were determined by following the appearance of *p*-nitrophenol at 330 mμ (ϵ 7200), and for phenyl acetate by observing phenol at 272 mμ (ϵ 1560). All hydrolysis runs were carried out at *p*H 5.50 in acetic acid–sodium acetate buffers in which an adequate concentration of sodium chloride was added in order to maintain constant ionic strength (0.7455 *M*) throughout any set of kinetic determinations. The reactions were ordinarily carried out in sealed ampoules in a constant temperature bath and the spectrophotometric measurements were made with a Beckman DK2 spectrophotometer. The method of Guggenheim was used for the determination of the first-order rate constants.[9]

Hydrolysis of 2,4-Dinitrophenyl Benzoate by Acetate Ion-O^{18}.—2,4-Dinitrophenyl benzoate (230 mg.) was dissolved in 40 ml. of 50% dioxane–water solution which was one molar in a buffer consisting of acetic acid-O^{18} and sodium acetate-O^{18} at *p*H 5.5. The reaction mixture was refluxed for 20 hours and then acidified to *p*H 3 with concentrated hydrochloric acid. The solution was concentrated on a hot-plate to 10 ml., removing most of the dioxane solvent. The residue was allowed to cool, at which time light yellow needles, which were a mixture of benzoic acid and 2,4-dinitrophenol, crystallized. The product was fractionally crystallized from petroleum ether (b.p. 50–70°), yielding pure benzoic acid, m.p. 122–123°. The oxygen-18 content of the benzoic acid was determined by conversion of the oxygen in the organic compound to carbon dioxide according to the method of Doering and Dorfman[10] followed by mass spectrometric analysis of the carbon dioxide with a Consolidated–Nier isotope ratio mass spectrometer, model 21-201.[11]

Results and Discussion

Acetate Ion-catalyzed Hydrolysis of Phenyl Esters.—Acetate ion has been shown to catalyze the hydrolysis of 2,4-dinitrophenyl acetate and of *p*-nitrophenylacetate[12] by the standard method of determination of an increase in the observed first-order rate constant with an increase in the concentration of the catalytic species at constant ionic strength and constant *p*H. In the same manner it is demonstrated that acetate ion catalyzes the hydrolysis of *p*-nitrophenyl acetate and phenyl

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(2) Previous paper in this series: M. L. Bender, F. Chloupek and M. C. Neveu, THIS JOURNAL, **80**, 5384 (1958). A portion of this research was presented at a symposium on "Reaction Mechanisms and Solvent Effects" at Queen Mary College, London, July, 1957.

(3) From the Ph.D. thesis of M. C. Neveu.

(4) M. L. Bender, Y. L. Chow and F. Chloupek, THIS JOURNAL, **80**, 5380 (1958).

(5) M. L. Bender, *ibid.*, **79**, 1258 (1957).

(6) (a) H. Morawetz and P. E. Zimmering, *J. Phys. Chem.*, **58**, 753 (1954); H. Morawetz and E. W. Westhead, Jr., *J. Polymer Sci.*, **16**, 273 (1955); P. E. Zimmering, E. W. Westhead, Jr., and H. Morawetz, *Biochem. Biophys. Acta*, **25**, 376 (1957); (b) G. L. Schmir and T. C. Bruice, THIS JOURNAL, **80**, 1173 (1958).

(7) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950); E. R. Garrett, THIS JOURNAL, **79**, 3401 (1957).

(8) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1652 (1957).

(9) E. A. Guggenheim, *Phil. Mag.*, [7] **2**, 538 (1926).

(10) W. E. Doering and E. Dorfman, THIS JOURNAL, **75**, 5595 (1953).

(11) See M. L. Bender and K. C. Kemp, *ibid.*, **79**, 116 (1957), for a description of the details of this analytical procedure together with an analysis of the errors involved.

(12) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1656 (1957); T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958).

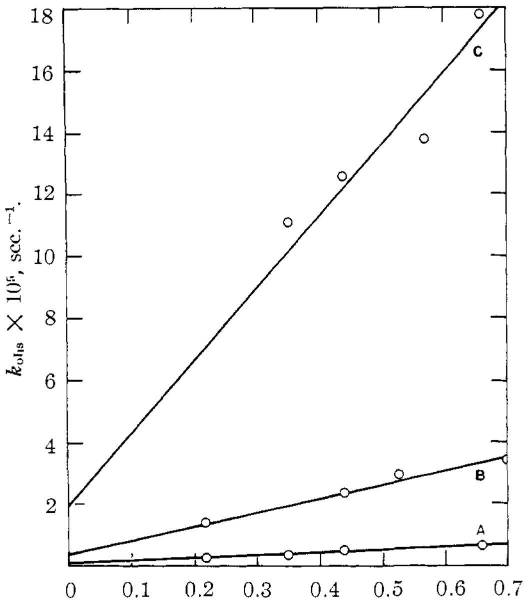


Fig. 1.—Acetate ion-catalyzed hydrolysis of phenyl acetate; A, 63°; B, 85°; C, 109°.

acetate as shown in Figs. 1 and 2. The catalytic rate constants for these hydrolyses are given in Table I together with the enthalpies and entropies of activation for these processes.

TABLE I

THE ACETATE ION-CATALYZED HYDROLYSIS OF PHENYL ACETATE AND *p*-NITROPHENYL ACETATE

Ester	Solvent	T, °C.	$k_c \times 10^5$, l./mole sec.	$\Delta H^{\ddagger}$, kcal./mole	$\Delta S^{\ddagger}$, e.u.
Phenyl acetate	Water	63	1.03		
	Water	85	5.02	16.6	−31.2
	Water	109	24.1		
p-Nitrophenyl acetate	5% dioxane Water	22.6	0.55		
	Water	47.8	4.85	15.7	−28.7
	Water	85	64.5		

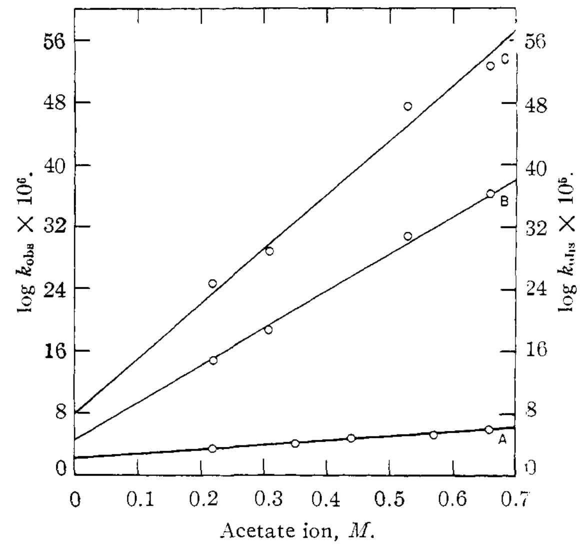
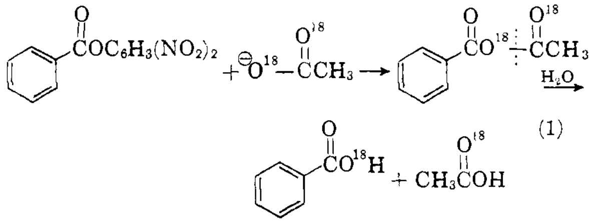


Fig. 2.—Acetate ion-catalyzed hydrolysis of *p*-nitrophenyl acetate: A, 22.6°; B, 47.8°; C, 85° (right-hand scale).

It is postulated that these intermolecular catalyses by acetate ion proceed through nucleophilic attack of the acetate ion on the carbonyl carbon atom of the ester, with the formation of an anhydride intermediate which is subsequently hydrolyzed by water in a fast step to result in the products of the reaction. This is analogous to the proposal for intramolecular catalysis by carboxylate ion.[2] Although it was possible to obtain direct spectrophotometric evidence for the acetylimidazole intermediate postulated in the imidazole-catalyzed hydrolysis of an ester,[12] direct evidence for the existence of anhydride intermediates is not obtainable since the subsequent hydrolysis of the anhydride is usually faster than its formation. However, the isotopic tracer experiment illustrated in eq. 1 has provided 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 acetylbenzoyl anhydride should occur at the bond denoted by the dotted line if the cleavage follows the relative reactivity of the two acyl groups.[13] 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 from one of the two labeled oxygen atoms of the acetate ion. It has been found that the benzoic acid isolated from this hydrolysis contains about 75% of the oxygen-18 derived from one of the oxygen atoms of the acetate-O^{18} ion. It should be pointed out that the maximum amount of oxygen-18 that could be incorporated in the benzoic acid by ordinary acid-catalyzed oxygen exchange with the solvent under the conditions of the reaction would be of the order of 1% of the excess oxygen-18 observed.[14]



(1)

Intermediates in Nucleophilic or Electrophilic Catalysis of Hydrolysis.—The isotopic tracer experiment reported above is interpreted as evidence for the existence of an anhydride intermediate in the intermolecular catalysis of hydrolysis by acetate ion. Previously tracer experiments have been reported which indicate that anhydride intermediates occur in the intramolecularly-catalyzed hydrolysis of phthalamic acid[4] and in the intramolecularly-catalyzed hydrolysis of aspirin.[2] Furthermore, spectrophotometric evidence for the existence of an acetylimidazole intermediate was

[13] *Cf.* D. B. Denney and M. A. Greenbaum, THIS JOURNAL, **79**, 979 (1957), for the reactions of mixed anhydrides with liquid ammonia which yield the amide derived from the acyl group of greater reactivity.

[14] See M. L. Bender, R. R. Stone and R. S. Dewey, *ibid.*, **78**, 319 (1956), for the kinetics of the acid-catalyzed oxygen exchange of substituted benzoic acids.

TABLE II

A KINETIC COMPARISON OF INTERMOLECULAR AND INTRAMOLECULAR CATALYSIS OF HYDROLYSIS

Substrate	Hydrogen ion, $M \times 10^6$	Temp., °C.	Catalysis Intramolecular k_1, sec.$^{-1}$	Catalysis Intermolecular k_2, l./mole sec.	$\Delta H^{\ddagger}$, kcal./mole	$\Delta S^{\ddagger}$, e.u.	Reference
Aspirin anion	1	60.3	83.8×10^{-6}		17.6	−24.7	7
Phenyl acetate + acetate ion	3	63.0		10.2×10^{-6}	16.6	−31.2	[a]
p-Acetoxybenzoic acid	1	60.3	1.9×10^{-6k}				[c]
Mono-*p*-nitrophenyl glutarate	3	0	3×10^{-4}		19.4	− 3.5	[b]
Copolymer of acrylic acid and *p*-nitrophenyl methacrylate	3	0	2×10^{-3}				[b]
p-Nitrophenyl acetate + acetate ion	3	22.6		5.7×10^{-6}	15.7	−28.7	[a]
Methyl hydrogen phthalate	1	109	5.9×10^{-5}		33.7	+ 7.5	2
Methyl benzoate + acetate ion	1	109		$0\text{–}1.2 \times 10^{-6}$			2
Phthalamic acid	1000	47.3	2.35×10^{-4}		20.7	−12.4	4, 5
Benzamide + acetic acid	1000	47.3					4, 5
Benzamide + hydrogen ion	1000	48.7	3.1×10^{-9k}	3.1×10^{-5}	22.8	−13.9	[f]
o-Nitrobenzamide + hydrogen ion	1000	48.7	1×10^{-10k}	1×10^{-7}			[g]
Glycyl-L-asparagine	1000	90	1.92×10^{-4}		24.0	− 9.4	[h]
L-Leucyl-L-asparagine	1000	90	2.48×10^{-4}		22.2	−14.1	[h]
L-Asparagine + hydrogen ion	1000	90		3.97×10^{-4}	19.8	−19.5	[h]
o-Carboxyphenyl phosphate	150	80	1.3×10^{-2}		23.5	− 1.2	[i,e]
Phenyl phosphate	15^k	80	3.2×10^{-3}		28.4	+ 0.9	[j,d]
m-Carboxyphenyl phosphate	15^k	80	3.3×10^{-5}		27.1	− 2.6	[j,e]
p-Carboxyphenyl phosphate	15^k	80	4.1×10^{-5}		26.8	− 3.1	[j,e]
4-(2'-Acetoxyphenyl)-imidazole	0.1	30	2×10^{-3}				[c]
p-Nitrophenyl acetate + substd. imidazole	0.1	30					[c]

[a] This research. [b] P. E. Zimmering, Thesis, Polytechnic Institute of Brooklyn, 1955, p. 89. [c] Extrapolated from the ref. 6b. [d] Rate constant of the monoanion. [e] Rate constant of the dianion. [f] Extrapolated from the data of B. S. Rabinovitch and C. A. Winkler, *Can. J. Research*, 20B, 76 (1942). [g] Extrapolated from the data of E. E. Reid, *Am. Chem. J.*, **21**, 327 (1899). [h] Calculated from ref. 23. [i] Extrapolated from ref. 21a. [j] Calculated from ref. 21c. [k] This rate constant is a pseudo first-order constant which pertains to an intermolecular catalysis.

found in the intermolecular catalysis of ester hydrolysis by imidazole.[8] These results in conjunction with one another strongly indicate that reactive carboxylic acid derivatives are quite feasible intermediates in the hydrolysis of carboxylic acid derivatives.

It is certainly possible that other intermediates are formed in nucleophilic and/or electrophilic catalysis. Some examples involving nucleophilic catalysis include the intermediate formation of an ester in the hydrolysis of an amide such as the acid hydrolysis of N-benzoyl-ψ-ephedrine which proceeds through O-benzoyl-ψ-ephedrine to produce ψ-ephedrine and benzoic acid.[15] The intermediate formation of a lactone has been postulated to account for the fact that 1-carbomethoxy-2-hydroxytriptycene is saponified much faster than 1-carbomethoxytriptycene itself.[16] Some examples involving electrophilic catalysis include the reaction in which a neighboring hydroxyl group facilitates the hydrolysis of an ester by hydrogen bonding to the alcoholic oxygen of the ester[17] and the catalysis of ester hydrolysis by complex formation with polyvalent metal ions.[18–21]

Kinetic Comparison of Intermolecular and Intramolecular Catalysis.—In Table II is shown a summary of the kinetic results of a number of intermolecular and intramolecular catalyses of the hydrolysis of carboxylic acid derivatives. In addition the important case of the intramolecular and intermolecular hydrolysis of phosphate esters is listed.[22] 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.[2,4–6,21]

A quantitative comparison of the two kinds of catalysis 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 because the corresponding intermolecular

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[15] L. H. Welsh, THIS JOURNAL, **69**, 128 (1947); **71**, 3500 (1949).

[16] P. D. Bartlett and F. D. Greene, *ibid.*, **76**, 1088 (1954); see also M. L. Wolfrom, R. B. Bennett and J. D. Crum, *ibid.*, **80**, 944 (1958).

[17] A. S. Hallsworth, H. B. Henbest and T. I. Wrigley, *J. Chem. Soc.*, 1969 (1957).

[18] H. Kroll, THIS JOURNAL, **74**, 2036 (1952).

[19] M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1889 (1957).

[20] R. B. Martin and C. Niemann, *ibid.*, **79**, 5828 (1957).

[21] J. I. Hoppe and J. E. Prue, *J. Chem. Soc.*, 1775 (1957).

[22] (a) J. D. Chanley, E. M. Gindler and H. Sobotka, THIS JOURNAL, **74**, 4347 (1952); (b) J. D. Chanley and E. M. Gindler, *ibid.*, **75**, 4035 (1953); (c) J. D. Chanley and E. Feageson, *ibid.*, **77**, 4002 (1955).

processes whereby a methyl ester or an amide is hydrolyzed by carboxylate ion or carboxylic acid, respectively, have not as yet been found. It should be pointed out that there is some equivocation connected with these statements since it has been reported that acetic acid catalyzes the hydrolysis of acetamide to a very small extent[4] and further that acetate ion catalyzes the hydrolysis of methyl benzoate,[2] although the experimental data in the latter case can be interpreted simply in terms of salt effects.

In the asparagine series,[23] N-acylated derivatives of asparagine undergo intramolecular hydrolysis while asparagine itself undergoes an intermolecular catalysis. This may be explained by the fact that in the dipeptides, the free amide group participates in an intramolecular hydrogen bonding such as has been suggested by Mizushima.[24] This internal hydrogen bonding may provide the necessary rigidity for the molecule to undergo catalysis by the intramolecular carboxylic acid group in the manner suggested for phthalamic acid.[4]

In the phosphate series[22] which includes a classical example of intramolecular catalysis, not of a carboxylic acid derivative but rather of a phosphate ester, the large differential in rate between the intramolecular process and intermolecular process is again evident.

From a general consideration of Table II, the kinetic differences between inter- and intramolecular catalysis cannot be ascribed either to the enthalpy or entropy of activation. It has been noted by Chanley, *et al.*,[22] and Leach and Lindley[23] that in certain instances the entropy of activation of intramolecular processes is less negative than that of intermolecular processes. Chanley,[22c] moreover, has pointed out that "the enormous increase in the speed of hydrolysis observed with *o*-carboxy-substituted aromatic phosphates over that of the corresponding non-substituted and *m*- and *p*-carboxy-substituted phosphates is associated primarily with a large decrease in the energy of activation accompanying the change in the mechanism of the hydrolysis." If one wishes, however, to compare intramolecular and intermolecular catalysis of identical mechanistic type, such as catalysis by carboxylate ion in the two cases, it is seen that there are only three comparisons possible from Table II. These are comparison 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, and comparison of intramolecular and intermolecular imidazole catalysis. 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 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) indicating the possibility of phenomenal differences in rate between an enzymatic process which occurs by primary complex formation and the equivalent process which does not involve complex formation.

Comparison of the enthalpies and entropies of intermolecular reactions with those of enzymatic processes is complicated by the meager data available at present on enzymatic reactions, which often are difficult to interpret.[25]

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(23) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 921 (1953).

(24) S. Mizushima, T. Shimanouchi, M. Tsuboi and T. Arakawa, THIS JOURNAL, **79**, 5357 (1957).

(25) See, however, I. B. Wilson and E. Cabib, *ibid.*, **78**, 202 (1956), for the acetylcholinesterase-catalyzed hydrolysis of acetylcholine which shows a positive $\Delta S^{\ddagger}$ for the step postulated for acetylenzyme formation.