Mechanisms of Monofunctional and Bifunctional Catalysis of an Ester Hydrolysis. The Kinetics of Methyl 2,6-Dicarboxybenzoate Hydrolysis[1a]

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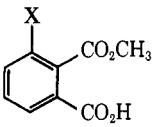
Abstract: The pH–rate profile for the hydrolysis of methyl 2,6-dicarboxybenzoate (**1**) reveals reactions of the protonated ester, neutral ester, monoanion, and dianion, and of hydroxide ion with dianion. At pH 4 the hydrolysis of **1** proceeds 10^7 times faster than hydrolysis of methyl mesitoate and 10^5 times faster than hydrolysis of methyl *p*-nitrobenzoate. Methyl 2-carboxy-6-nitrobenzoate (**2**) was found to have a pH-independent reaction at low pH with a rate constant similar to that of methyl hydrogen phthalate and one-half that of the neutral ester of **1**. Therefore, catalysis is assumed to be the same for these esters and to be monofunctional for the reaction of the neutral ester of **1**. Carboxyl group participation occurs in the reaction of the protonated ester since the solvent deuterium isotope effect of 1.1 is too large for other mechanisms. Only a nucleophilic mechanism for catalysis explains the failure to observe a pH-independent reaction in the hydrolysis of methyl 2-carboxy-3-nitrobenzoate (**4**) at low pH. The monoanion of **1** hydrolyzes 12 times faster than the neutral ester and 4000 times faster than the dianion. This is interpreted in terms of rate-limiting breakdown of the tetrahedral intermediate; the low reactivity of the dianion is also observed for the anion of **2** and results from unfavorable partitioning of the intermediate. Three possible mechanisms for bifunctional catalysis of the hydrolysis of the monoanion are given.

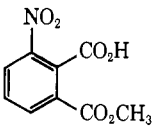
The necessity of enzymes to rely on catalytic groups which are combinations of the same groups that can act as catalysts in reactions of less complex organic molecules makes the study of functional and multifunctional group catalysis within simple organic structures essential for theories of enzyme mechanisms. Catalysis by carboxyl groups has been the subject of this investigation for a number of reasons. Among the numerous proteolytic enzymes that have a carboxyl group located in the active site, pepsin is prominently mentioned as one requiring participation of these groups in its catalysis,[2] and knowledge of the influence of carboxyl groups on the hydrolysis of acyl derivatives should assist the evaluation of these postulates.

Although monofunctional catalysis by a carboxyl group of the hydrolysis of alkyl esters has been studied,[3] the mechanism of hydrolysis of methyl hydrogen phthalate remains uncertain.[4] Bifunctional catalysis by carboxyl groups of the hydrolysis of alkyl esters has never been studied. This has been reported for an aryl ester,[5] an amide,[6] and a phosphate ester,[7] and might also be significant for alkyl esters.

The discovery of bifunctional catalysis of a benzoate ester hydrolysis by ortho-substituted hydroxyl groups[8] suggested that similar substitution by carboxyl groups would be successful. To evaluate the influence of a second ortho carboxyl group, methyl 2-carboxy-6-nitrobenzoate (**2**) and the previously studied methyl hydrogen phthalate (**3**) could be compared with methyl 2,6-dicarboxybenzoate (**1**). By comparison of these



1, X = CO_2H
2, X = NO_2
3, X = H



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esters with the methyl 2-carboxy-3-nitrobenzoate (**4**), information about the mechanism of the intramolecular catalysis of the former was sought.

Prediction of the mechanism for carboxyl group participation in these ester hydrolyses from known mechanisms of other esters is difficult. Examples of intermolecular nucleophilic or general catalysis of the hydrolysis of methyl esters by carboxylic acids or anions do not exist. For intramolecular participation of carboxyl groups in methyl ester hydrolyses, all examples with known mechanisms involve a nucleophilic mechanism,[3] but this mechanism would not be expected from the viewpoint of relative nucleophile and leaving groups basicities[9] if methoxide ion were the proposed leaving group or from the example of general participation of carboxylate ion in the hydrolysis of aspirin[10] which favors a general base mechanism. In the aspirin hydrolysis, however, nucleophilic catalysis suffers from the presence of the leaving group in the ortho position of the benzoate nucleophile.[10]

Experimental Section

Reagents. Acid solutions for kinetic experiments were prepared from reagent grade concentrated hydrochloric acid, sulfuric acid, deuterium chloride (Bio-Rad Laboratories or ICN Corp.), and sulfuric acid-d_2 (ICN Corp.) solutions which were standardized against potassium hydrogen phthalate. Deuterium oxide (Bio-

(1) (a) This research was supported by grants from the National Science Foundation and the National Institutes of Health. (b) National Institutes of Health Postdoctoral Fellow, 1967–1968, Special Fellow, 1969–1970.

(2) (a) M. L. Bender and F. J. Kézdy, *Annu. Rev. Biochem.*, **34**, 49 (1965); (b) T. R. Hollands and J. S. Fruton, *Proc. Nat. Acad. Sci. U. S.*, 62, 1116 (1969), and references therein.

(3) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y., 1966, p 175.

(4) J. W. Thanassi and T. C. Bruice, *J. Amer. Chem. Soc.*, 88, 747 (1966).

(5) H. Morawetz and I. Oreskes, *ibid.*, 80, 2591 (1958).

(6) H. Morawetz and J. Shafer, *ibid.*, **84**, 3783 (1962).

(7) Y. Murakami and A. E. Martell, *ibid.*, 86, 2119 (1964).

(8) F. L. Killian and M. L. Bender, *Tetrahedron Lett.*, 1255 (1969).

(9) J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, 86, 837 (1964).

(10) A. R. Fersht and A. J. Kirby, *ibid.*, **89**, 4857 (1967).

Rad Laboratories) was 99.88% pure. All salts, buffers, and bases used in rate measurements were reagent grade.

Methyl 2,6-Dicarboxybenzoate. 1,2,3-Benzenetricarboxylic acid (K & K Chemical Co.) melted at 189–191° after recrystallization from water (lit.[11] mp 190°). The anhydride (mp 195–196°) was prepared by sublimation at 210°, taking care to prevent hydrolysis from moisture collecting on the condenser during the transfer operation, or by simply heating 2 g for 45 min at 210° under vacuum.

To 3.6 g of hemimellitic anhydride was added 45 ml of freshly distilled methanol (Fisher Spectranalyzed Reagent) and the mixture was warmed to dissolve the crystals. Crystals were collected upon standing and, after recrystalization from acetone–benzene (1:2) and air drying, melted at 205–210°.

The structure proofs of Graebe and Leonhardt[12] and Wenkert, *et al.*,[13] for the product of the reaction of methanolic hydrogen chloride and hemimellitic anhydride and the agreement of the melting points of their products (203–205°[12] and 204–210°)[13] with that of the present product prepared by a similar method established its structure. In addition, the nmr spectrum supplied proof that the benzene ring was symmetrically substituted. The nmr spectrum (dimethyl sulfoxide-d_6) was interpreted as CH_3O δ 3.79 (s, 3), and an AB_2 system for the aromatic protons, $\nu_A = 7.71$, $\nu_B = 8.15$ ppm, $J_{AB} = 8$ Hz. The aromatic protons ortho to the carboxyl groups are equivalent whereas a difference near 0.17 ppm would be expected for the unsymmetrical ester based on differences between benzoic acid and methyl benzoate ortho protons. Unsymmetrical derivatives of hemimellitic acid such as the anhydride and some amides exhibited a distinctive narrow multiplet in their nmr spectra.

Evidence of the correct structure of the methyl ester was found by Wenkert, *et al.*,[13] by sublimation of hemimellitic anhydride from pure methyl ester and elimination of methanol rather than water which would have been lost from the isomeric methyl ester. This was attempted once, but sublimation was difficult and apparently yielded a mixture of sublimated anhydride and ester (mp 192–196°, ir more complex than ir of anhydride).

Anal. Calcd for $C_{10}H_8O_6$: C, 53.58; H, 3.60. Found: C, 53.47; H, 3.75.

Methyl 2-Carboxy-6-nitrobenzoate. The decomposition point (217–218°) of previously opened 3-nitrophthalic anhydride (Matheson Co., Inc.) corresponds to the diacid (lit.[14] mp 207°). It was converted to the anhydride, mp 161–162° (lit.[14] mp 161–162°), by the method of Bogert and Boroschek.[14] A solution of the anhydride in methanol was evaporated and the residue dissolved in benzene. Crystals from this solution were recrystallized from benzene and from ether yielding crystals of mp 152–153° (lit.[15] mp 152–153°). The ir spectrum (KBr) had carbonyl bands at 5.79 and 5.89 μ; nmr (dimethyl sulfoxide-d_6) CH_3O δ 3.97 (s, 3), aromatic protons, 7.83, 7.98, 8.10 (1), and 8.40, 8.48, 8.60 ppm (2).

Methyl 2-Carboxy-3-nitrobenzoate. Following the literature procedure,[16] 1 g of 3-nitrophthalic acid was dissolved in 20 ml of methanol and 0.5 ml of concentrated sulfuric acid and was then refluxed 16 hr. After cooling, water was added and the crystals were collected, washed, and recrystallized from ether, mp 157–159° (lit.[16] mp 157–158°). The ir spectrum (KBr) had carbonyl bands at 5.69 and 5.89 μ; nmr (dimethyl sulfoxide-d_6) CH_3O δ 3.97 (s, 3), aromatic protons, 7.71, 7.85, 7.98 (1), and 8.20, 8.34, 8.46 ppm (2).

The assignment of structures to the esters of 3-nitrophthalic acid was possible based on the assumptions of Wegescheider and Lipschitz,[16] and their experiments were repeated with the same results.

Buffer Solutions. Buffer solutions were made up to a total concentration of 0.2 *M* buffer and were adjusted to an ionic strength of 0.3 with potassium chloride. The pH regions covered by each buffer system at 99° were HCl–KCl, 0.648–2.92; chloroacetic acid, 2.23–3.67; acetic acid, 3.81–4.76; formic acid, 4.23; KH_2PO_4–Na_2HPO_4, 5.78–7.80; $NaHCO_3$–Na_2CO_3, 8.71; Na_2HPO_4–Na_3PO_4, 9.91.

Citric acid buffers were found to be unstable under these conditions. Chloroacetic acid buffer was tested by measuring the absorbance of hemimellitic acid in this buffer at pH 4 before and after 80 and 140 min at 99° in sealed tubes. A negligible absorbance decrease was found, but longer time periods were unacceptable. The pH remained constant throughout all rate experiments in this buffer except one where the pH recorded for initial and final points was 3.17 and 3.03.

pH Measurements. The pH of solutions used in rate determinations was measured at 99° (or 40.8° in earlier experiments) both before and after hydrolysis, and, with the exception mentioned previously, these agreed within 0.02 pH unit. A Radiometer pH meter, Model 26, was used with a Radiometer type G202BH glass electrode designed for 40–120° over the pH range 0–14 and a high-temperature calomel electrode, type K4016. The pH meter was calibrated at 95° with tartrate and phosphate buffers at pH 3.674 and 6.886.[17] All pH values are corrected for zero shift between 95 and 99°.[18] The H_0 values of solutions above an acid strength of 0.3 *M* and the pH of the 1 *M* NaOH solution were determined by calculation of the H_0 value from the equation of Johnson, *et al.*,[19] and the autoprotolysis constant[20] of 12.3 for water at 100°, respectively.

pK_a Measurements. The procedure of Albert and Serjeant[21] for the determination of the pK_a values of a dibasic acid was followed for **1** at 40° and for isophthalic acid at 40 and 99°. A Radiometer Titrator 11 assembly was used for the titration cell. Steam was circulated through the jacketed cell to maintain temperatures of 99° and thermostated water was circulated for 40° temperatures. The pH meter was calibrated at 95° (or 40°) before and after titration with tartrate and phosphate buffers. At the half-titration point solutions were 0.001 *M* in substrate and the ionic strength was 0.3. Boiled, distilled water was used for the preparation of solutions.

The pK_a values of **2** and **4** at 99° were found by a similar method.[22] At the half-titration point the ester concentration was 0.005 *M* and the ionic strength was 0.3. The accuracy of the pK_a determination for **4** was reduced because of the similarity of the logarithm of the ester concentration and the pK_a value. Errors from hydrolysis of these esters during the titration were negligible for this ester but could have caused an error of 5% perhaps in the pK_a of **2**.

Kinetic Method. All rates were measured by following the change in ultraviolet absorption upon hydrolysis. No absorbance change during hydrolysis of these esters was found in the low and high pH solutions, and a concentrated formate buffer was added to adjust the pH to 3 prior to measurement. The more concentrated acid solutions were neutralized slowly in an ice–water bath with a sodium hydroxide–sodium formate mixture. In the pH region of ionization of reactant and product carboxylic acid groups, changes in absorbance were observed directly, and a 0.002 *M* concentration of **1** was followed at a wavelength of 297.5 nm. Rates measured for ester concentrations differing tenfold were identical, and first-order plots of log $(A_{inf} - A_t)$ *vs.* time were linear over 2–4 half-lives. The slopes of these plots were determined by the least-squares method.

Solutions for kinetics were prepared by addition of 50 ml of stock buffer solution, previously adjusted to an ionic strength of 0.3, to a 100-ml volumetric flask heated in the boiling water bath. An 8-mm diameter glass tube equipped with a suction bulb extended through the rubber stopper into the solution to allow rapid transfer of samples to ice-cooled test tubes. After a 30-min equilibration period, a 50-μl pipet was used to add that quantity of 2 *M* methyl 2,6-dicarboxybenzoate (or, for the 3-nitrophthalate esters, 0.4 *M*) stock solution to the equilibrated solution. Samples were removed quickly, stoppered in the cold test tubes, then measured in a Cary recording spectrophotometer, Model 14, at 25°. Infinity points were taken by sealing samples in ampoules and leaving them in the bath at least 10 half-lives. In 1 *M* NaOH the reaction vessel was changed to a tube of alkali resistant glass. For reactions in pH regions of low reactivity, sealed tubes were employed, and solutions of pH 9.91 and 11.22 at 99° require alkali resistant glass ampoules. Kinetic determinations were reproducible within 10%.

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(12) C. Graebe and M. Leonhardt, *Justus Liebigs Ann. Chem.*, **290**, 217 (1896).

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(14) M. T. Bogert and L. Boroschek, *J. Amer. Chem. Soc.*, **23**, 744 (1901).

(15) (a) A. McKenzie, *J. Chem. Soc.*, **79**, 1141 (1901); (b) R. Kahn, *Ber.*, **35**, 3857 (1902).

(16) R. Wegscheider and A. Lipschitz, *Monatsh. Chem.*, **21**, 796 (1900).

(17) R. C. Weast, Ed., "Handbook of Chemistry and Physics," 48th ed, The Chemical Rubber Publishing Co., Cleveland, Ohio, 1967, p D-80.

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(22) Reference 21, p 34.

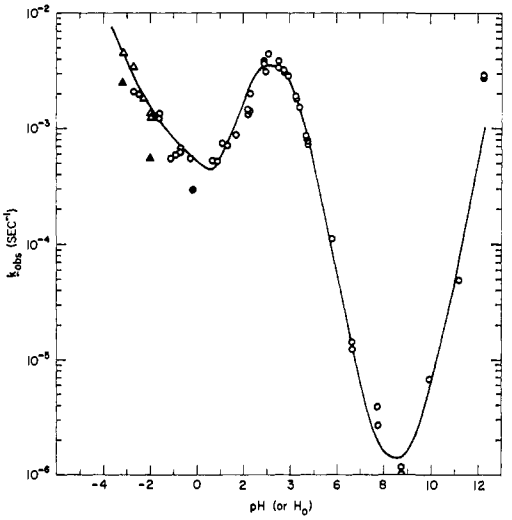


Figure 1. The pH–rate profile of the hydrolysis of methyl 2,6-dicarboxybenzoate at 99°. The points are experimental and the curve is calculated from eq 1. The various symbols represent the following solutions: Δ, H_2SO_4; ▲, D_2SO_4; O, HCl, buffer, or NaOH; ●, DCl.

Stock solutions of the esters were prepared by dissolving the ester in a slight excess of aqueous NaOH to give a final solution of pH 8 or 9. These solutions were stable indefinitely at room temperature except for that of **4** which apparently hydrolyzed slowly in basic solutions. Therefore this ester was dissolved directly in the buffer solution by brief heating, then the clear solution was filtered and distributed to the ampoules. The base solution for preparation of a stock solution in deuterium oxide was prepared by treating sodium with deuterium oxide.

Precipitation occurred upon addition of the stock solution of **1** to the hot, concentrated acid solutions, but this difficulty was completely avoided when the stock solution was first added to a portion of the water used in the preparation of the acid solution. Addition of this larger volume of aqueous solution to the preheated solution required more time before temperature equilibration occurred and limited the range of acid concentrations in which rates could be measured.

The wavelengths selected for following the hydrolysis of **2** and **4** were 252.5 and 320.0 nm for the former and 265.0 and 320.0 for the latter.

The temperature of the boiling water bath was constant during a kinetic run to within 0.3° and the maximum difference between runs was 0.9° but usually varied within 0.5°. The corrected temperature was 99.1°. Constant-temperature baths for activation parameter determinations were maintained at 62.4, 83.0, and 113.0° by a water constant-temperature bath, boiling 2-propanol, and boiling toluene, respectively.

Results

Methyl 2,6-Dicarboxybenzoate. The irregularities of the dependence of rates of hydrolysis of this ester on pH shown in Figure 1 provide evidence of numerous changes in the mechanism between pH regions. The theoretical curve drawn in Figure 1 was calculated from eq 1,[23] where N, M, and D refer to neutral ester, monoanion, and dianion, respectively. This equation gives the dependence of the observed rate constants on the rate constants for the reaction of the protonated ester, k_{H^+}, of the neutral ester, k_N, of the monoanion, k_M, of the dianion, k_D, and of hydroxide ion with the dianion, k_{OH^-}, where these constants are understood to refer to charge equivalent forms of the reacting molecules as well, and on factors giving the amount of the ester in each form determined by the acidity of the solutions and the ionization constants of the ester. The values of these constants are shown in Table I. The pH-independent reactions of neutral ester and dianion required by the kinetic data do not produce visible plateaus as a result of competing reactions. However, a bell shape is clearly visible and this was found at 40° also, but the data were less reproducible than at 99°.

$$k_{obsd} = k_{H^+}(h_0^m) + \left[k_N\left(\frac{[H^+]}{K_1}\right) + k_M + k_D\left(\frac{K_2}{[H^+]}\right)\right]\left(\frac{1}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}}\right) + k_{OH^-}[OH^-] \quad (1)$$

(23) M. Dixon and E. C. Webb, "Enzymes," 2nd ed, Academic Press, New York, N. Y., 1964, p 118.

Table I. Rate and Equilibrium Constants Calculated from Equation 1 for Hydrolysis of Methyl 2,6-Dicarboxybenzoate at 99°

Rate constant	Equilibrium constant
$k_{H^+} = 1.6 \times 10^{-4}\ M^{-1}\ sec^{-1}$	$K_1 = 5.0 \times 10^{-3}$
$k_N = 3.6 \times 10^{-4}\ sec^{-1}$	$K_2 = 8.0 \times 10^{-5}$
$k_M = 4.4 \times 10^{-3}\ sec^{-1}$	$k_M/k_N = 12$
$k_D = 1.0 \times 10^{-6}\ sec^{-1}$	$k_M/k_D = 4.4 \times 10^3$
$k_{OH^-} = 4.3 \times 10^{-4}\ M^{-1}\ sec^{-1}$	

The equilibrium constants calculated from eq 1 could not be directly compared with titrimetric values at 99° because rapid hydrolysis of the ester prevented their determination. However, titrimetric values were obtained at 40° and the constants derived from the kinetics at 99° can be evaluated by comparing the temperature effect on isophthalic acid. The pK_a values of isophthalic acid measured titrimetically at 40 and 99° are shown in Table II. The effect of temperature on the first ionization of both compounds is the same, although this is not true of the second ionization where structural differences should make the comparison difficult since the dielectric of the medium between two groups will influence charge–charge interactions more than charge–dipole interactions Therefore the kinetic pK_a values appear to be true ionization constants

Table II. pK_a Values

Ester	Temp, °C	pK_1	pK_2
Methyl 2,6-dicarboxybenzoate	40	2.75	3.85
	99	2.30[a]	4.10[a]
Isophthalic acid	40	3.70	4.45
	99	3.40	4.37
Methyl 2-carboxy-6-nitrobenzoate	99	2.7	
Methyl 2-carboxy-3-nitrobenzoate	99	2.3	

[a] Calculated from kinetic data and eq 1.

The absence of buffer catalysis was evident from the equality of rates in overlapping pH regions of different buffer systems and the agreement of three rates measured at constant pH but varying acetate buffer concentration. A constant ionic strength of 0.3 was maintained except in solutions of greater acid or base concentration. Salt effects on this ester were not determined due to the complexity of the rate processes in low pH solutions, and at high pH it is obvious that there is a positive salt effect

on the reaction of hydroxide ion with the dianion in 1 *M* base.

Rates measured for the most concentrated hydrochloric acid solutions were difficult to reproduce even with the sealed ampoule procedure, but sulfuric acid solutions were reliable Hydrolyses in 62.5, 74.1, and 93.7% H_2SO_4 were all too fast to measure ($k > 10^{-2}$ sec^{-1}).

The dependence of the rates of hydrolysis of methyl esters on water activity has been shown by Bunnett[24] and by Yates and McClelland[25] to change markedly when the mechanism changes from $A_{Ac}2$ to $A_{Ac}1$. Therefore, four values of log $k_{obsd} + H_0$ or log $k_{obsd} + mH_0$ were plotted against log a_{H_2O} (although values of log a_{H_2O} at 25° were used,[24] there is some justification for this[26]). These two plots become identical when unprotonated methyl benzoate is used as a model for the Hammett base behavior since m is close to 1.0.[27] For protonated methyl benzoate m = 0.86.[27] The slopes were found to be 2.1 and 1.8, respectively, and since the Hammett base behavior of the protonated ester is required, the latter is more appropriate.

The deuterium solvent isotope effects are shown in Table III to differ in the strong acid and low-pH regions. It is significant that the isotope effect in strong acid is larger than one.

Table III. Deuterium Solvent Isotope Effects

Ester	Ionization state	Medium	k_{H_2O}/k_{D_2O}
Methyl 2,6-dicarboxybenzoate	Neutral[a]	0.7 *M* DCl	1.6
	Protonated[b]	34% D_2SO_4	1.1
		48% D_2SO_4	1.2
Methyl 2-carboxy-6-nitrobenzoate	Neutral	1.0 *M* DCl	1.8
Methyl 2-carboxy-3-nitrobenzoate	Protonated	1.0 *M* DCl	1.0

[a] Calculated using the k_N obtained from eq 1 for the water reactions and the k_N in deuterium oxide obtained by subtraction of the rate constant for reaction of protonated ester at this acid concentration from the observed rate constant. [b] Contributions of neutral ester reaction to the observed rate constants were subtracted before calculation.

Hydrolysis of hemimellitic anhydride proceeds extremely fast relative to the rate of hydrolysis of the methyl ester, and attempts to form an unreactive, unsymmetrical amide in order to trap anhydride that might be an intermediate in the ester hydrolysis failed.

Activation parameters were determined for **1** at 1.0 *M* HCl and 0.3 *M* HCl, but the results were very different. Owing to the discovery of considerable competition of the protonated ester or of the monoanion reactions with the neutral ester reaction at these acidities, these results were concluded to be meaningless. Competition of the protonated ester reaction with the neutral ester reaction is also a possibility for the methyl hydrogen phthalate hydrolysis even at pH 2 since this ester is considerably more basic. This possibility and the interference of a small thermodynamic contribution from the ionization of methyl hydrogen phthalate at this pH suggest that the values of 22.4 kcal/mol and −12.2 eu reported by Bruice, *et al.*,[4] for the enthalpy and entropy of activation cannot be used for comparisons.

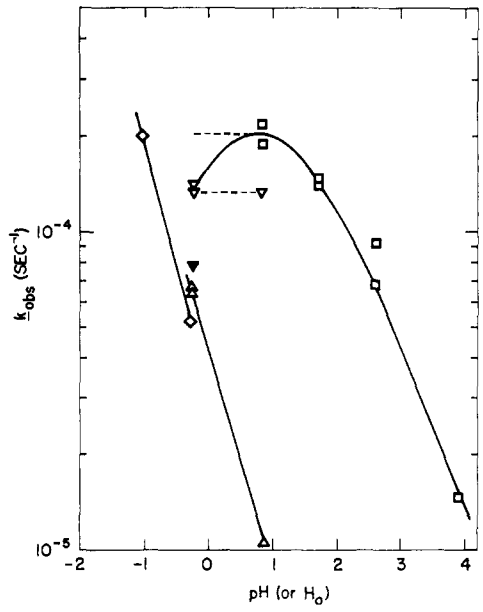


Figure 2. Partial pH–rate profiles of the monomethyl esters of 3-nitrophthalic acid at 99°: 1-methyl ester: (Δ) μ = 1.0, (◇) μ = 3.0, (▲) 1 *M* DCl; 2-methyl ester: (□) μ = 0.3, (▽) μ = 1.0, (▼) 1 *M* DCl.

Methyl 2-Carboxy-6-nitrobenzoate. A partial pH–rate profile for hydrolysis of this ester is shown in Figure 2. The bell shape is replaced by a plateau when salf effects are removed by addition of sodium bromide to provide constant ionic strength solutions. The decline in rates from pH 1 to 4 was caused by ionization of the carbonyl group, the pK_a of which was measured titrimetrically at 99° (Table II). Prediction of the observed rate constants at higher pH values was possible from the fraction of un-ionized ester and the rate constant at the maximum of the pH–rate profile. At pH 3.86 the major form of the ester is the anion so that it can be estimated that the rate of hydrolysis of the anion is at least 1000 times slower. It can also be concluded that buffer catalysis is nonexistent since the rate in acetic acid buffer is correctly predicted from rates in HCl–KCl buffers.

The deuterium solvent isotope effect was measured to provide a more reliable value of the isotope effect in the neutral ester reaction than could be determined from the hydrolysis of ester **1** where competing reactions introduce errors. As shown in Table III the agreement is reasonable.

The agreement of the activation parameters found for this ester in 0.3 and 1.0 *M* HCl solutions indicates that there are not competing pathways for hydrolysis at these acidities. These values are shown in Table IV, and it can be seen that the entropy of activation is surprisingly negative.

Methyl 2-Carboxy-3-nitrobenzoate. The pH–rate profile for this ester is similar to that of ester **1** in the

(24) J. F. Bunnett, *J. Amer. Chem. Soc.*, 83, 4956 (1961).
(25) K. Yates and R. A. McClelland, *ibid.*, 89, 2686 (1967).
(26) (a) K. Yates and J. C. Riordan, *Can. J. Chem.*, 43, 2328 (1965); (b) a referee has questioned whether $\Delta H^{\ddagger}$ remains medium independent over the acid range studied and stated that significant changes in log a_{H_2O} slopes have been found in that laboratory. Since the acid range covered here is not large, the assumption will be made that such effects do not alter the slopes to the extent that they would be assigned to different categories of mechanisms.
(27) C. C. Greig and C. D. Johnson, *J. Amer. Chem. Soc.*, 90, 6453 (1968).

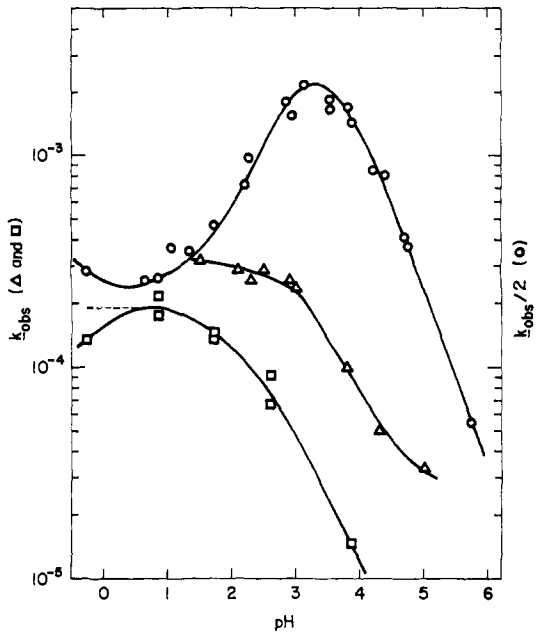


Figure 3. Comparison of pH–rate profiles of methyl 2,6-dicarboxybenzoate (99°, right ordinate and circles), methyl hydrogen phthalate (100°,[4] left ordinate and triangles), and methyl 2-carboxy-6-nitrobenzoate (99°, left ordinate and squares).

strong acid region but astonishingly different from those of esters **1** and **2** in the low-pH region (Figure 2). Instead of observing a plateau the rate increases with acid concentration. At pH 0.88 the rate should be near the maximal rate for the reaction of the un-ionized ester

Table IV. Rate Constants and Activation Parameters for Hydrolysis of Methyl 2-Carboxy-6-nitrobenzoate

[HCl], *M*	Temp, °C	Rate constants, sec^{-1}	$\Delta H^{\pm}$, kcal/mol	$-\Delta S^{\pm}$, eu (T = 371.6°K)
0.3	62.4	7.5×10^{-6}	19.4	21.5
	98.5	2.0×10^{-4}		
	113.6	3.6×10^{-4}		
1.0	62.4	6.6×10^{-6}	19.8	21.2
	83.0	4.1×10^{-5}		
	98.5	1.3×10^{-4}		
	113.6	5.4×10^{-4}		

since not much of the ester is ionized and the protonated ester reaction must be relatively slow at this pH. Nonetheless, it will be shown that the observed rate is slow enough to be accounted for by the reaction of the protonated ester, and to require the reaction of un-ionized ester **4** to be at least tenfold slower than that of un-ionized ester **2**. Although the rate increases only 6.5-fold per pH unit decrease at an ionic strength of 1.0, it seems likely that this is less than tenfold because medium effects on the reaction of protonated ester change the activity coefficients of reactants and transition states and reduce water activities and not because of a pH-independent reaction. The medium effects in solutions of ionic strength 3.0 reduce the slope a reasonable amount to 3.9, whereas, if these small slopes were a result of contributions from a pH-independent reaction, the relative slopes should have been reversed and less similar. Also, if there were a contribution from the pH-independent reaction, it should be significant near pH 0, but the low deuterium solvent isotope effect in 1 *M* HCl indicates that protonated ester is the primary reactant (Table III).

The reactivity of the anion of **4** was tested at pH 4.8 and a half-life of about 30 hr at 113° was estimated. Therefore this anion reacts at least ten times slower than the dianion of **1**.

Discussion

Evidence of Carboxyl Group Participation. The hydrolysis of methyl 2,6-dicarboxybenzoate in solutions of low hydroxide ion concentrations is catalyzed by one or both carboxyl groups depending on the pH. This is apparent from the agreement of the pK_a values calculated from the kinetics with the expected titrimetric values (Table II), and the observation of large rate enhancements relative to expected rates for hydronium or hydroxide ion catalyzed reactions. Participation of only one carboxyl group in the hydrolysis of the neutral ester, most evident between pH 0 and 1, can be concluded from the similarity of the rates of hydrolysis for the pH-independent reaction of ester **1** at low pH and for that of the un-ionized (2) shown in Figure 3. However, both carboxyl groups participate in the hydrolysis of the monoanion since the monoanion reacts faster than the neutral ester while the reverse is true when participation by a second group is impossible, as in the 2-methyl and phthalate esters. An argument against the alternative possibility of a hydronium ion reaction with the dianion will be presented below.

Unfortunately, distinguishing between general acid or base and nucleophilic participation is not simple since absolute proof of anhydride formation by isolation or spectral identification of the anhydride or an amide derivative of the anhydride is impossible due to their rapid hydrolysis.

Significance of Carboxyl Group Participation. That participation by carboxyl groups substantially lowers the hydrolytic stability of an ester in dilute acid solutions can be seen by comparison of **1** with methyl mesitoate and methyl *p*-nitrobenzoate. The methyl mesitoate comparison is appropriate since both steric and electronic differences are small and opposite in influence. Extrapolation of rates found for methyl mesitoate in dilute acid solutions from pH 0, where the mechanism is known to be $A_{Ac}1$,[28] to pH 4, where the $A_{Ac}1$ mechanism should still be favored over the $A_{Ac}2$ mechanism, gives a rate that will be an upper limit for comparison. The acceleration of ester hydrolysis by carboxyl participation in methyl 2,6-dicarboxybenzoate at 99° compared to methyl mesitoate in 60% aqueous dioxane at 90° is calculated to be $>4 \times 10^7$ at pH 4. Compared with methyl *p*-nitrobenzoate in 60% aqueous methanol at 100°,[29] an ester with similar basicity to the monoanion but with no steric hindrance, an acceleration of 2×10^5 at pH 4 accrues from carboxyl participation. Alternatively, the hydrolysis rate of methyl *p*-nitrobenzoate in 7 *M* hydrochloric acid would reach the rate of methyl 2,6-dicarboxybenzoate at pH 4.

Rate enhancements of such a magnitude are of special interest because of the possibilities for carboxyl

(28) (a) C. T. Chmiel and F. A. Long, *J. Amer. Chem. Soc.*, 78, 3326 (1956); (b) M. L. Bender, H. Ladenheim, and M. C. Chen, *ibid.*, 83, 123 (1961); (c) M. L. Bender and M. C. Chen, *ibid.*, 85, 37 (1963).

(29) E. W. Timm and C. N. Hinshelwood, *J. Chem. Soc.*, 862 (1938).

group participation in enzyme-catalyzed reactions. Since enzymes do not have such high temperatures available, direct application of these results to them is precluded. However, it may be that although enzymic hydrolysis of methyl esters requires a more basic functional group, hydrolysis of amides or aryl esters might follow the path of carboxyl participation demonstrated here except reaction would be much more rapid because of a change in the rate-limiting step and therefore in the influence of catalysis. This is especially likely for hydrolysis of anhydrides, and this mechanism of catalysis may operate in pepsin-catalyzed reactions since the acyl enzyme could have an anhydride structure.[2a,30] In the decomposition of the acyl enzyme intramolecular carboxyl group participation could be efficient and prevent reaction of the acyl enzyme with added nucleophiles. This would explain the failure to observe reaction of the acyl enzyme with [^{14}C] methanol reported by Knowles, *et al.*[31]

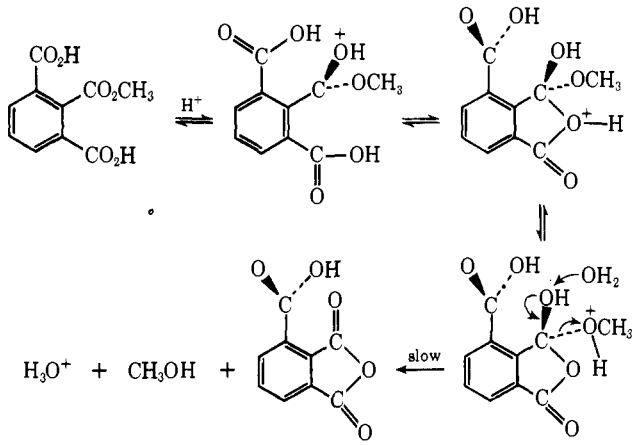
Mechanisms. The striking similarity in the rates of hydrolysis of the un-ionized esters **1**, **2**, and **3** shown in Figure 3 and the similarities of the structures of these esters suggest a common mechanism for hydrolysis of the neutral esters, and this will be assumed. In the following analysis identification of the active species will progress from the simplest to the most complex situation.

Protonated Ester. In strong acid solutions the log-rate increases proportionately to H_0 and therefore protonated ester is the major reactant. Hydrolysis by an $A_{Al}1$ mechanism cannot be considered since it has been demonstrated by Hopkinson[32] that methyl esters of dinitrobenzoates, which should be even more susceptible to alkyl oxygen cleavage at 65° in 100% sulfuric acid, react by an $A_{Ac}1$ rather than by an $A_{Al}1$ mechanism.

An $A_{Ac}1$ mechanism is unacceptable because the slopes of the Bunnett plot[24] and the Yates modification of it[25] are near +2 instead of near 0 and because methyl mesitoate hydrolyzes more slowly than **1**. An $A_{Ac}2$ mechanism would predict a large difference between the rates of the highly hindered ester **1** and methyl *p*-nitrobenzoate rather than the observed close similarity. Furthermore, it cannot explain the solvent isotope effect of 1.1 since all known examples have ratios less than one.[33] A mechanism of rate-limiting cyclization by the attack of the un-ionized carboxyl group on the protonated ester would not account for the presence of water in the transition state established by the acidity dependence.

The acidity dependence and solvent isotope effect are explained by the mechanism shown in Scheme I. Protonated ester first forms the tetrahedral intermediate by rapid and reversible attack of the carboxyl group on the ester group, the a proton shifts to the methoxyl oxygen atom, and methanol is lost slowly. One water molecule would act to remove the proton from the carbonyl oxygen and initiate breakdown of the intermediate. The observed isotope effect is the product of a factor <1 for the thermodynamic isotope effect on the protonation of the ester and a factor >1 for the kinetic isotope effect on proton abstraction from the intermediate. Only the latter influences the hydrolysis of the neutral ester and, as expected, its observed isotope effect is larger.

Scheme I



The immeasureably fast reactions in 94% and particularly in 74% sulfuric acid solutions suggest that the mechanism in these less aqueous solutions no longer includes water in the transition state. Other methyl esters hydrolyze relatively slowly in these media.[25] Further investigation is necessary to evaluate the possibility of a crossover to an $A_{Ac}1$ mechanism here.

Hydroxide Ion Reaction. The base hydrolysis of the doubly ionized ester observed in solutions above pH 10 shows a first-order dependence on hydroxide ion, and must represent the reaction of hydroxide ion with the dianion. General base or intramolecular nucleophilic mechanisms cannot reasonably involve hydroxide ion, so that the $B_{Al}2$ and $B_{Ac}2$ mechanisms are left as possibilities. The high-temperature base hydrolysis of methyl mesitoate has been proven to proceed by the $B_{Ac}2$ pathway[34] and the assumption can be made that methyl 4-bromo-2,6-dimethylbenzoate does also. The latter has very similar electronic and steric substituent effects to the dianion of **1**, leaving only electrostatic effects as causes of rate differences, and hydrolyzes 15-fold faster than **1** at the same temperture and base concentration.[35] Therefore, comparison with methyl mesitoate shows that hydroxide ion attack on the methyl group of **1** is unlikely, and comparison with methyl 4-bromo-2,6-dimethylbenzoate shows that the electrostatic repulsion of hydroxide ion attack at the acyl group is small.

Dianion. The pH-independent reaction observed near pH 9 can be explained as the reaction of the dianion with water, but not as the reaction of hydroxide ion with monoanion. Concentrations of hydroxide ion and monoanion are so low that a rate constant of 10^2 M^{-1} sec^{-1} would be required for this reaction to produce the observed rate constant whereas the rate constant should be less than 4×10^{-2} M^{-1} sec^{-1} based on the rate constant for dianion and the minor influence of electrostatic repulsion.

Because an approximate determination of the rate of ester 4 at pH 5 shows that this ester when ionized hydrolyzes slower than the dianion, we consider a mechanism of nucleophilic attack by carboxylate anion to form a tetrahedral intermediate that occasionally pre-

(30) M. Akhtar and J. M. Al-Janabi, *Chem. Commun.*, 859 (1969).
(31) A. J. Cornish-Bowden, P. Greenwell, and J. R. Knowles, *Biochem. J.*, 113, 369 (1969).
(32) A. C. Hopkinson, *J. Chem. Soc. B*, 203 (1969).
(33) (a) W. E. Nelson and J. A. V. Butler, *ibid.*, 957 (1938); (b) J. C. Hornel and J. A. V. Butler, *ibid.*, 1361 (1936); (c) D. S. Noyce and R. M. Pollack, *J. Amer. Chem. Soc.*, 91, 119, 7158 (1969).
(34) M. L. Bender and R. S. Dewey, *ibid.*, 78, 317 (1956).
(35) H. L. Goering, T. Rubin, and M. S. Newman, *ibid.*, 76, 787 (1954).

fers water-assisted elimination of methoxide to elimination of carboxylate ion to be more acceptable than a general base mechanism. The relative rates for these two esters would be expected for steric reasons to be reversed if a general base mechanism were operative. It would be difficult for a water molecule (hydroxide ion) to approach the center carbonyl group **1** compared to its approach to the carbonyl in the ester group in **4** which is not shielded by two ortho groups. On the other hand, a mechanism of nucleophilic attack by carboxylate ion would predict a slower rate for the anion **4** than for the dianion of **1** for stereochemical reasons to be discussed.

Neutral Ester. For the pH-independent reaction at low pH, the reactions of monoanion with hydronium ion, or of neutral ester or its zwitterion either internally or with water, or of protonated ester with hydroxide ion are consistent with the kinetics. The last reaction can be rejected since the estimated concentrations of reactants are so small that a rate constant much faster than diffusion rate constants is necessary. Comparison of the rates of hydrolysis of the neutral ester and the 2-methyl ester **2** (Figure 3) indicates that monofunctional catalysis occurs.

If a reaction of monoanion with hydronium ion occurred, it would probably require protonation of the departing methoxide ion by hydronium ion following carboxylate anion cyclization. One argument against such a mechanism is that it would predict a larger difference in the observed rates of hydrolysis of esters **2** and **3** because of additive substituent effects of the ortho nitro or hydrogen on the basicity of the methoxyl oxygen and on the ease of departure of methanol.

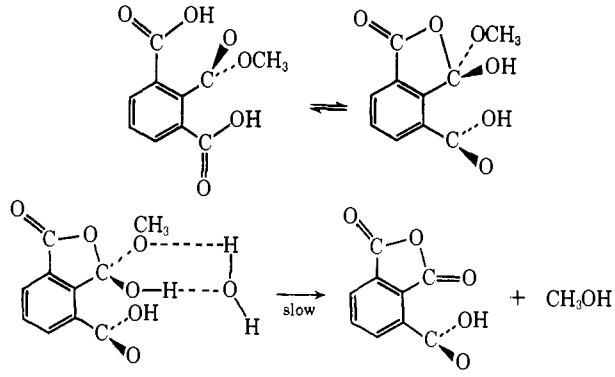
Comparison of the neutral ester with ester **2** (Figure 3) indicates that only monofunctional catalysis occurs. Mechanisms of either general acid catalysis of water attack on neutral ester or zwitterion formation followed by general base catalysis of water attack by carboxylate anion can be eliminated by comparison of the two nitrobenzoate esters **2** and **4**. The steric relationships of groups for intramolecular hydrogen bonding appear essentially equal in both esters but the carboxyl group in **4** is stronger, zwitterion formation is favored in **4**, and accessibility of the ester function to external nucleophiles is greater in **4**. Therefore, a faster rate is predicted for **4** by either mechanism. However, **4** hydrolyzes with the slowest rate, and there is no evidence of a reaction of un-ionized **4**. A pH-independent reaction would have been visible if it proceeded faster than one-tenth the rate of un-ionized **2**. Disappearance of this reaction pathway cannot be explained by either of these mechanisms.

If the carboxyl group participates in the hydrolysis of a methyl ester by nucleophilic mechanism, a rate-limiting breakdown of the tetrahedral intermediate is more tenable than rate-limiting cyclization to the tetrahedral intermediate for reasons clearly expressed previously.[36]

A mechanism of nucleophilic catalysis in which cyclization is not rate limiting is illustrated in Scheme II. Concerted formation of the carboxyl oxygen–ester carbonyl carbon bond and proton donation to the ester carbonyl oxygen occur in this mechanism.[37]

(36) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, 90, 2622 (1968).

Scheme II



Since the rate-limiting step is the loss of methanol, the mechanism in Scheme II correctly predicts a sizable isotope effect since water must abstract a proton from the intermediate in the transition state. The activation entropy of the methyl ester agrees with such a bimolecular interaction. Isotope effects and activation entropies found by Milstien and Cohen[38] in an example of rate-limiting breakdown of tetrahedral intermediate assisted by solvent are nearly the same as those found for esters **1** and **2**.

Furthermore, the absence of a pH-independent reaction for the 1-methyl ester is explained by this mechanism. Compared with esters **2** and **4**, the preequilibrium cyclization of **4** should shift toward ring-opened reactants since models indicate that three groups must rotate 90° for the carboxylate oxygen, which becomes coplanar with the ring, to approach the ester function, now forced perpendicular to the plan of the ring. This geometric relationship probably exists initially in the esters **1** and **2**.

Monoanion. The reaction of the monoanion can be considered to be the reaction of hydronium ion with the dianion or as a reaction of the monoanion by a general base or a nucleophilic mechanism.

The large rate difference between ionized ester **2** and the monoanion of **1** excludes general base catalysis alone as a possibility and makes the hydronium ion reaction unlikely. This difference is 1000 which is a large acceleration to arise from the increased electrostatic forces of the dianion for the hydronium ion relative to singly ionized **2**.

An intramolecular general acid catalysis of an intramolecular general base catalyzed hydrolysis of the monoanion cannot explain this large rate difference either. Although it is difficult to compare a different system, the fact that Kupchan, *et al.*,[39] observed a factor of only 40 for bifunctional general catalysis of the hydrolysis of an alkyl ester indicates smaller rate accelerations may result from this type of catalysis. More significant, perhaps, is the evidence that un-ionized **1**, **2**, and **4** do not hydrolyze by formation of a zwitterion and general base catalysis of water attack, as discussed previously, since this mechanism is similar to general acid–base catalysis of the monoanion.

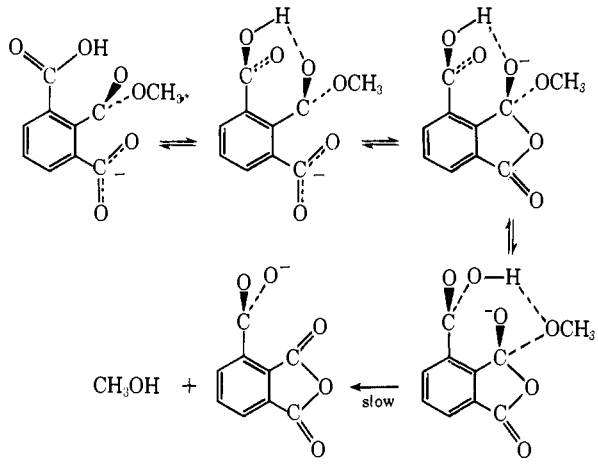
Nucleophilic mechanisms for participation of the carboxylate group in the hydrolysis of the monoanion in which cyclization is slow and loss of methanol is

(37) S. L. Johnson, *Advan. Phys. Org. Chem.*, 5, 312 (1967).
(38) S. Milstien and L. A. Cohen, *J. Amer. Chem. Soc.*, 91, 4585 (1969).
(39) S. M. Kupchan, S. P. Eriksen, and Y. Shen, *ibid.*, 85, 350 (1963).

rapid do not explain the greater reactivity of un-ionized 2-methyl ester relative to the ionized ester. To postulate the inability of carboxylate anion to attack the ester is inconsistent with the observation of efficient nucleophilic carboxylate anion catalysis of phenyl hydrogen phthalate.[4] Therefore, the absence of a proton hinders the loss of methanol, and a mechanism for the monoanion reaction must involve the protonation of the departing methoxyl function by a carboxyl group or incipient hydronium ion rather than by water as must occur in the dianion reaction. Proton donation is the critical factor for determining the partitioning of the cyclic intermediate since ring opening does not require protonation but methanol elimination does require it.

A mechanism of rapid, reversible carboxylate anion cyclization and slow loss of methanol, therefore, explains the relative rates of monoanion and dianion. The pH maximum can be explained by bifunctional catalysis of the following types. One possibility, shown in Scheme III, involves attack by the carboxylate

Scheme III



anion on the hydrogen-bonded ester function, a proton shift, and rate-limiting loss of methanol assisted by the acid group. A proton shift is unnecessary if the carboxyl group can rotate, but bonding of the carboxyl group to the methoxyl oxygen atom in the transition state is necessary. This mechanism is an example of bifunctional catalysis of tetrahedral intermediate formation and monofunctional catalysis of its breakdown.

Another mechanism that is less important perhaps is one of unassisted cyclization by the carboxylate anion followed by catalysis of the breakdown of the intermediate by the acid group. It differs from the previous mechanism in that cyclization occurs while the carboxyl group is hydrogen bonded to the methoxyl group or is free. This represents a type of bifunctional catalysis where each of two steps is catalyzed by single but different groups.

Alternatively, there may be concerted nucleophilic carboxylate anion cyclization and protonation of the ester carbonyl oxygen by the acid group, then breakdown of the intermediate by water in the manner shown in Scheme II. Only bifunctional catalysis of the first step occurs in this mechanism.

In conclusion, similar mechanisms were believed to be operating in all pH regions except high pH where the very reactive hydroxide ion is able to overcome the energy barrier to external attack. A mechanism of nucleophilic participation by the carboxyl group in the protonated ester and neutral ester reactions and by the carboxylate anion in the monoanion and dianion reactions is proposed. Bifunctional catalysis is not much more effective than the monofunctional catalysis by the carboxyl group observed for the neutral ester but is very effective compared to the monofunctional catalysis by the carboxylate anion observed for the dianion. This is explained by rate-limiting loss of methanol in the second step of the reaction and the necessity for protonation of the leaving group. Bifunctional catalysis is likely to be extremely effective in the hydrolysis of aryl esters or amides where the first step of the reaction is rate limiting.

The Silver Ion Assisted Solvolysis of α-Bromoisobutyrophenone[1]

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Abstract: The silver ion assisted solvolysis of α-bromoisobutyrophenone is kinetically complex. The rate equation which best fits all the data contains three terms, one first order in halide and silver ion, one first order in halide, silver ion, and hydrogen ion, and one first order in halide and second order in silver ion. Extensive amounts of rearranged products (dimethylphenylacetic acid and the ethyl ester) are observed. The kinetic and product distribution data are interpreted in terms of a mechanism involving an acid-catalyzed addition of solvent to the carbonyl group of α-bromoisobutyrophenone prior to the silver ion assisted solvolysis step.

Previous studies in our and other laboratories have been devoted to the study of the role of neighboring ketone functional groups in the solvolysis of halides and tosylates. Three different modes of neighboring group participation have been delineated. In a study of the silver ion assisted solvolysis of alkyl and aryl ω-chloro-

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