as described previously.[7,22] Most of the following compounds were prepared on a 0.5-g. scale.

p-**Nitro-^{15}N-acetanilide** was prepared by the nitration of acetanilide with 38% nitric ^{15}N acid (Merck Sharp and Dohme 97% ^{15}N), according to the procedures given by Vogel.[23]

p-**Nitro-^{15}N-aniline** was obtained by the acid hydrolysis of *p*-nitro-^{15}N-acetanilide.[23]

Nitrobenzene-^{15}N was prepared by the nitration of benzene with 38% nitric-^{15}N acid according to the method given by Vogel.[24]

p-**Bromonitrobenzene-^{15}N** was prepared by the nitration of bromobenzene with 38% nitric-^{15}N acid according to the method given by Vogel.[25]

p-**Chloronitrobenzene-^{15}N** was prepared by the nitration of chlorobenzene with 38% nitric-^{15}N acid using similar conditions to those in the *p*-bromonitrobenzene-^{15}N preparation.

p-**Fluoronitrobenzene-^{15}N** was prepared from fluorobenzene and 38% nitric-^{15}N acid in the presence of sulfuric acid at 70°. The product was isolated in the same way as for the nitrobenzene-^{15}N preparation.

p-**Nitro-^{15}N-anisole** was prepared by the reaction of *p*-bromonitro-^{15}N-benzene with sodium methoxide in dimethyl sulfoxide solution at room temperature. The reaction mixture was poured onto crushed ice, and the pale yellow crystals of nitro-^{15}N-anisole were removed by filtration. The yield was almost quantitative.

p-**Dinitro-^{15}N-benzene** (labeled with ^{15}N in one nitrogen) was prepared by diazotized *p*-nitro-^{15}N-aniline according to the procedure given by Hodgson, *et al.*[26]

p-**Nitro-^{15}N-benzonitrile** was prepared from *p*-nitro-^{15}N-aniline by the Sandmeyer reaction in a manner analogous to that previously described by Bogert and Hand.[27]

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Neighboring Carboxyl Group Participation in the Hydrolysis of Monoesters of Phthalic Acid. The Dependence of Mechanisms on Leaving Group Tendencies

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Abstract: The hydrolyses of the following monohydrogen phthalate esters have been investigated in the region of the carboxyl group pK_a': I, methyl; II, 2′-monochloroethyl; III, propargyl; IV, 2′,2′,2′-trifluoroethyl; and V, phenyl. In addition, the hydrolysis of (VI) O-phthaloyl-N-acetylserinamide was also investigated. The pK_a' values of the alcohols employed in the preparation of the esters fall in the order I > II > III = VI > IV > V. Esters I and II hydrolyzed with neighboring COOH group participation while esters IV and V hydrolyze with COO^- participation. Thus, the mechanism changes with increase in leaving tendencies. The crossover between mechanisms occurs for esters of alcohols with pK_a' values of *ca.* 13.5 so that esters III and IV are equally prone to COOH and COO^- neighboring group catalyzed hydrolysis. These results are considered in the light of the possible participation of carboxyl groups in the mechanism of esterases. Extrapolation from this study suggests that such participation could be effective but defies detection through conventional examination of pH–rate profiles.

In order to understand the mechanism of enzyme action, it is important that the functional groups on the protein necessary for activity be identified and that their mode of action in the catalytic process be elucidated. The study of intramolecular catalysis in model systems has been a most useful tool in this direction.[3] This paper deals with neighboring carboxyl group participation in the hydrolysis of half-esters of phthalic acid.

It has been demonstrated that carboxyl anion participates in the hydrolyses of acetyl salicylic acid,[4] substituted phenyl acid succinates and glutarates,[5] and mono-*p*-bromphenyl esters of substituted glutaric and succinic acids.[6] On the other hand, ethyl hydrogen phthalate[7] and methyl hydrogen 3,6-dimethylphthalate[8] hydrolyze with participation by the neighboring protonated carboxyl group. It would appear, then, that those monoesters of dicarboxylic acids having good leaving groups hydrolyze with assistance by neighboring carboxyl anion (COO^-) and those having poor leaving groups hydrolyze with the aid of the neighboring carboxyl group (COOH).

Carboxyl groups have been implicated in the mechanism of action of several enzymes. Thus, Bernhard and Gutfreund[9] have found that in the ficin-catalyzed hydrolysis of benzoyl arginine ethyl ester, a carboxyl anion apparently participates in the hydrolysis of the acyl enzyme. This has been interpreted to occur by means of a rate-limiting attack of the carboxyl anion

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presumably on an acylthiol to yield a mixed anhydride which is hydrolyzed by water in a fast step.[10] Bernhard, *et al.*,[11] also have speculated on the possibility of participation by the γ-carboxyl group in the sequence Asp-Ser; these authors suggest a bicyclic system in which an oxyanion acts as a nucleophile towards substrates (but see ref 12). Finally, Stewart, *et al.*,[13] offer evidence for the participation of a carboxyl group in the mechanism of action of chymotrypsin.

The present paper will show that carboxyl groups may participate in the hydrolysis of certain esters by a mixed mechanism. The implications of this with respect to the mechanism of enzyme action are discussed.

Experimental Section

Materials. Phthalic anhydride was Eastman White Label and was recrystallized from chloroform prior to use. 2-Monochloroethanol was also Eastman White Label and was redistilled, $n^{20}D$ 1.4414 (lit[14] $n^{20}D$ 1.4419). 2,2,2-Trifluoroethanol was Aldrich technical grade. The redistilled material (bp 72° had a density of 1.376 (lit[15] bp 74°, *d* 1.374). *t*-Butyl alcohol was J. T. Baker analytical reagent and was redistilled from calcium hydride. Phenol was Baker and Adamson analytical reagent. *p*-Dioxane was purified by the method of Fieser[16] and stored frozen. Propargyl alcohol was Aldrich technical grade and was redistilled, bp 111–112° (lit[17] bp 114–115°), $n^{20}D$ 1.4302 (lit[17] $n^{20}D$ 1.4306). All other chemicals were of reagent grade quality and were used without further purification. Water used in these experiments was double distilled.

Methyl hydrogen phthalate was prepared by the method of Eliel and Burgstahler,[18] mp 83–84.5° (lit mp 82–82.5°).

Anal. Calcd for $C_9H_8O_4$: C, 60.00; H, 4.47. Found: C, 60.04; H, 4.53.

Phenyl Hydrogen Phthalate. A suspension of sodium phenoxide (13.9 g, 0.12 mole) in 400 ml of anhydrous ether was added with stirring to a solution of phthalic anhydride (14.8 g, 0.10 mole) in 100 ml of benzene in a 1-l flask fitted with a condenser, stirring paddle, and drying tube. The resulting white suspension was refluxed with stirring for 27 hr. HCl gas (dried by passage through sulfuric acid) was introduced with stirring for 2 hr, and the sodium chloride was removed by filtration with suction. (The filtration should be done rapidly in order to prevent excessive absorption of atmospheric moisture.) Benzene (200 ml) was added to the filtrate and the ether was boiled off. Petroleum ether (300 ml, bp 30–60°) was added to the benzene solution, and the resulting white precipitate was collected. The product was recrystallized twice from 150 ml of benzene–150 ml of petroleum ether. (A small amount of phthalic acid was removed each time from the benzene solutions by filtration.) This material was dissolved in 50 ml of chloroform and the cloudy solution was filtered with suction through Celite. The clear filtrate was diluted to 100 ml with chloroform and 400 ml of petroleum ether was added. The resulting fine, white needles were collected and dried *in vacuo*. The over-all yield was 5.7 g (24% of theory), mp 94–96° (lit[19] mp sintering at 92°, melting at 103°).

Anal. Calcd for $C_{14}H_{10}O_4$: C, 69.42; H, 4.16. Found: C, 69.16; H, 4.14.

2′,2′,2′-Trifluoroethyl Hydrogen Phthalate. Phthalic anhydride (8.9 g, 0.06 mole) and redistilled trifluoroethanol (9 ml, 0.12 mole) were dissolved in 200 ml of sodium-dried benzene in a three-neck flask fitted with a paddle-blade stirrer, drying tube, and dropping funnel. Fifty milliliters of 1.0 *M* potassium *t*-butoxide in *t*-butyl alcohol then was added dropwise with vigorous stirring. The resulting thick, gelatinous mixture was stirred for 45 min at room temperature after the final addition of the *t*-butoxide solution and then allowed to stand overnight at room temperature. The white potassium salt of the half-ester was collected by suction filtration, washed with warm benzene, and dried in a vacuum desiccator over P_2O_5. The salt was suspended in 250 ml of dry benzene and HCl gas was introduced for 2 hr with stirring. The KCl was removed by filtration through a Celite pad, and the filtrate was concentrated to a small volume on a rotary evaporator at 40°. The desired product was precipitated by addition of excess petroleum ether. A second crop was collected by concentration of the filtrate from the first crop and addition of petroleum ether. Both crops melted at 80–82.5°, yield 5.5 g (44%). The product was recrystallized once from chloroform–petroleum ether and twice from benzene–petroleum ether. For analysis, the compound was recrystallized from chloroform–*n*-hexane, mp 82–83.5°.

Anal. Calcd for $C_{10}H_7O_4F_3$: C, 48.40; H, 2.85; F, 22.97. Found: C, 48.45; H, 3.00; F, 22.70.

O-Phthaloyl-N-acetylserinamide. N-acetylserinamide[20] (1.46 g, 0.01 mole) and phthalic anhydride (2.96 g, 0.02 mole) were dissolved in 80 ml of *t*-butyl alcohol at 70° in a three-neck flask fitted with a condenser, drying tube, dropping funnel, and paddle-blade stirrer. Ten milliliters of 1.0 *M* potassium *t*-butoxide in *t*-butyl alcohol then was added dropwise with vigorous stirring; the resulting gelatinous suspension was allowed to cool to room temperature and stirring was continued overnight. The mixture was reheated to 70° and quickly filtered with suction, and the white hygroscopic product was washed with hot benzene. The precipitate was dried *in vacuo* over KOH and P_2O_5. The product then was suspended in dried (molecular sieves) tetrahydrofuran, and HCl gas was introduced for 1 hr with stirring. The mixture was filtered through a fine sintered glass funnel, and the pale yellow filtrate was concentrated to a viscous yellow oil which turned into a glass when dried *in vacuo* over P_2O_5 and KOH. The material was dissolved in 10 ml of methanol, and 10 ml of carbon tetrachloride and 20 ml of petroleum ether were added. After several days of storage in the freezer compartment of a refrigerator, a precipitate appeared which was collected by suction filtration and washed with petroleum ether. The product was recrystallized from absolute methanol, yield 0.50 g (17%), mp 165.5°.

Anal. Calcd for $C_{13}H_{14}O_6N_2$: C, 53.06; H, 4.80; N, 9.52. Found: C, 52.87; H, 4.98; N, 10.10.

The neutralization equivalent of this compound was within 5% of theory. The infrared spectrum had absorptions at 1730, 1710, 1645, 1560, 1285, 1220, 1145, and 1080 cm^{-1}.

2′-Chloroethyl Hydrogen Phthalate. Phthalic anhydride (7.4 g, 0.05 mole) was dissolved in 20 ml (0.30 mole) of redistilled 2-monochloroethanol, and the solution was refluxed for 2.5 hr. The solution was concentrated to a clear yellow oil in a rotary evaporator. After several days in the refrigerator, the oil set to a white solid. The ester was recrystallized twice from benzene–petroleum ether, yield 5.8 g (51%), mp 76–78°.

Anal. Calcd for $C_{10}H_9O_4Cl$: C, 52.53; H, 3.97; Cl, 15.51. Found: C, 52.49; H, 4.32; Cl, 15.36.

Propargyl Hydrogen Phthalate. Phthalic anhydride (44.4 g, 0.30 mole) was dissolved in 150 ml (2.40 moles) of redistilled propargyl alcohol, and the solution was refluxed for 3 hr. The golden yellow oil obtained after concentration in a rotary evaporator (60°) crystallized spontaneously with chilling. The compound was recrystallized twice from benzene–petroleum ether and three times from chloroform–*n*-hexane, mp 110–112°.

Anal. Calcd for $C_{11}H_8O_4$; C, 64.71; H, 3.95. Found: C, 65.05; H, 4.12.

Kinetics. With the exception of methyl hydrogen phthalate, all kinetic experiments reported in this paper were performed at a calculated ionic strength of 1.0 *M* (with KCl). Buffers employed were potassium phosphate (pH 1.6 to 2.9), potassium formate (pH 3.0 to 3.5), and potassium acetate (pH 3.7 to 4.5). In the case of the methyl ester, potassium citrate buffers were used in the pH range

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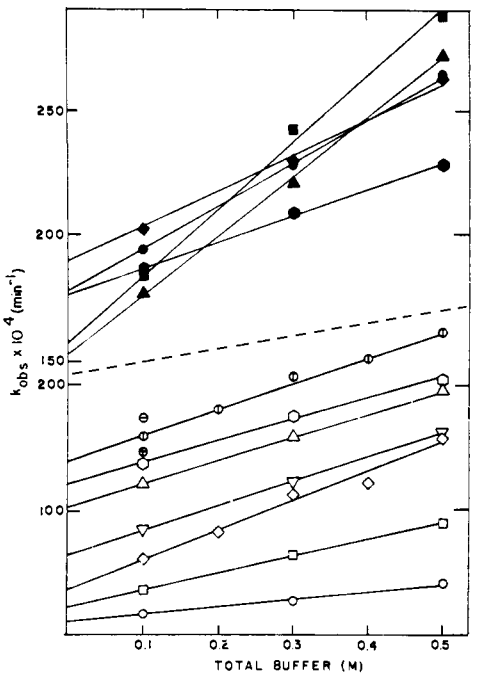

Figure 1. Plots of k_{obsd} *vs.* pH for methyl hydrogen phthalate, 100°: open symbols, potassium citrate buffers; filled symbols, potassium phosphate buffer; ○, pH 4.94; □, pH 4.36; ◇, pH 4.00; ▽, pH 3.79; △, pH 3.52; ⬡, pH 3.22; ⊕; pH 3.00; ⊖, pH 3.00 + 1.0 *M* KCl; ⊕, pH 3.00 + 0.1 *M* KCl; ■, pH 2.86; ●, pH 2.44; ▲, pH 2.30; ⬡, pH 2.08; ◆, pH 1.66.

3.0 to 4.9 and potassium phosphate buffers in the pH range 1.6 to 2.9. No attempt was made to control ionic strength in these experiments. However, the rates were only very slightly affected by the addition of KCl (pH 3.0, 0.1 *M* citrate buffer). The pH values of all solutions were measured before and after the kinetic runs at the temperatures corresponding to those employed in the kinetic experiments; pH control was usually good to ±0.05 pH units. At least three buffer concentrations (0.1 to 0.5 *M*) were employed at any single pH, and the observed rate constants (k_{obsd}) were extrapolated to 0 buffer concentration; extrapolations were linear.

In each case, phthalic acid was identified as a product of the reactions by comparison of the spectra of the reaction mixtures at infinite time (after diluting 1:5 with 1.0 *M* phosphate buffer at pH 7.4) with the spectrum of authentic phthalic acid under the same conditions.

Except for phenyl hydrogen phthalate and phthalic anhydride, kinetic measurements were made in the following manner. A solution of the ester (3 to 6 × 10^{-3} *M*) was made in the appropriate buffer and aliquots were dispensed into Pyrex ampoules which then were sealed and placed in a constant temperature bath. At appropriate time intervals the reactions were quenched by immersing the ampoules in ice–water. The reaction solutions then were diluted 1:5 with 1.0 *M* potassium phosphate buffer at pH 7.4. The optical densities of the resultant solutions were then measured at 279 mμ in a Zeiss PMQ II spectrophotometer against a reagent blank. The data were plotted in accordance with the first-order rate law $k_{obsd}t = 2.303 \log (OD_0 - OD_\infty)/(OD_t - OD_\infty)$. The plots were generally linear for at least two half-times.

The hydrolyses of phthalic anhydride and phenyl hydrogen phthalate were followed at 300 mμ in a thermostated (30 ± 0.1°) cell housing of a Zeiss PMQ II spectrophotometer. These compounds were added as 1 drop of a concentrated solution in purified dioxane to the prethermostated cells containing the desired buffers. In the case of phenyl hydrogen phthalate, it was observed that the optical density at 300 mμ initially rose and went through a maximum followed by a first-order decay. This indicated that an intermediate was being formed during the course of the reaction.

$$\text{monoester} \xrightarrow{k_1} \text{anhydride} \xrightarrow{k_2} \text{diacid} \qquad (1)$$

The scheme shown in eq 1 was verified by the fact that in the pH region where k_1 was sufficiently greater than k_2, the first-order

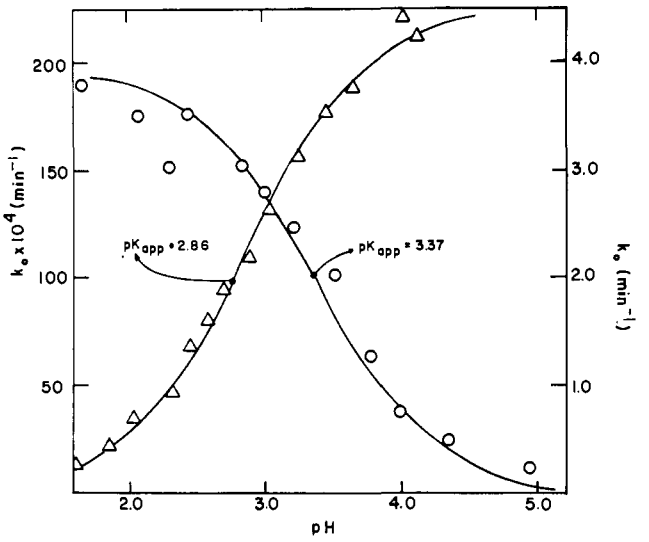

Figure 2. Plots of k_0 *vs.* pH: left ordinate and circles, methyl hydrogen phthalate (100°, μ = 0); right ordinate and triangles, phenyl hydrogen phthalate (30°, μ = 1.0 *M*).

portion of the data was identical with that for phthalic anhydride under the same conditions. The existence of the anhydride as an intermediate is therefore unequivocal. The latter compound has a flat pH–rate profile of hydrolysis (extrapolated to 0 buffer concentration) in the pH range 1.6 to 5.7 (k_2 = 0.739 min^{-1}; average of 15 values with a maximum deviation of 0.021 min^{-1}). Knowing the value of k_2 and the time of maximal absorption at 300 mμ, one can calculate the rate constant for the ring closure step, k_1, using the equation, $k_1 t_{max} = 2.3(K-1)^{-1} \log K$ where $K = k_2/k_1$.[21]

Apparatus. Optical densities were determined with a Zeiss PMQ II spectrophotometer fitted with a hollow brass cell holder through which was circulated water maintained at 30 ± 0.1° by a Haake constant-temperature bath. Spectra were recorded using a Perkin-Elmer Model 350 spectrophotometer. A Radiometer 22 pH meter equipped with a PHA 630 Pa scale expander and combined-type GK 2021C electrode was used to determine pH. High-temperature pH measurements were made with a Radiometer TTT 1a titrator equipped with a specially designed jacketed electrode housing around which solvents of the desired boiling point could be refluxed; a Metrohm Type EA 109 H high-temperature reference electrode was employed in these determinations. pK_a' determinations were carried out at 30° using a Radiometer TTT 1b titrator and PHA 630Ta scale expander according to the microtitration method described by Albert and Serjeant.[22] Two different types of baths were used to maintain constant temperature in the kinetic experiments. One was a specially designed insulated aluminum block drilled with holes of the desired depth and diameter which were stoppered with corks; temperature was kept constant by means of a heating element whose operation was controlled by a mercury contact regulator connected to an electronic relay box (Precision Instruments). The second was a Blue M Magniwhirl Utility water bath modified so that the bath temperature was controlled by means of a mercury contact thermometer and an electronic relay box. In both cases, temperature control was good to at least ±0.5°. Infrared spectra were measured on a Unicam SP 200 spectrophotometer. Elemental analyses were performed by Alfred Bernhardt, Max Planck Institut, Mulheim, Germany, and C. E. Geiger, Ontario, Calif.

Results

First-order rate constants for the hydrolysis of the monohydrogen phthalate esters were determined at constant pH and varying buffer concentration (see the Experimental Section). Plots of the experimentally determined first-order rate constants (k_{obsd}) *vs.* buffer

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Table I.[a] Hydrolytic Data for Monophthalate Esters

R	pK_a' of ROH (25°)	Temp, °C	Mechanism	$10^4 k_{rate}$, min^{-1}	pK_{app}[j]	pK_a'[k]
CH_3	15.54[b]	25.0	COOH	0.082[g,h]		
CH_3		30.0		0.152[g,h]		
CH_3		64.6		7.00[g,h]		
CH_3		78.5		25[h]		
CH_3		91.3		85[h]		
CH_3		100.0		200[i]	3.37 (μ = 0)	3.32 ± 0.05[m,n]
CH_3		100.0		160[h]		2.99 ± 0.05[l]
$(CH_2)_2Cl$	14.31[c]	91.3	COOH	65[i]	3.32	3.10 ± 0.03[l]
NASA[p]	13.6[d]	100.0	COOH + COO^-	(~100)		
$CH_2C{\equiv}CH$	13.55[b]	78.5	COOH + COO^-	41.6[o]		
CH_2CF_3	12.36[e]	64.6	COO^-	640[i]	2.88	
C_6H_5	9.98[f]	30.0	COO^-	45,000[i]	2.86	

[a] All data for μ = 1.0 except where noted. [b] P. Ballinger and F. A. Long, *J. Am. Chem., Soc.*, **82**, 795 (1960). [c] P. Ballinger and F. A. Long, *ibid.*, **81**, 2347 (1959). [d] T. C. Bruice, *et al.*, *Biochemistry*, **1**, 7 (1962). [e] P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **81**, 1050 (1959). [f] L. P. Fernandez and L. G. Hepler, *ibid.* **81**, 1783 (1959). [g] Obtained by calculation using the Arrhenius equation, $\log k = -E_a/2.3RT + C$. [h] Values for pH 2.00; μ = 1.0 with KCl. [i] Two times value at pK_{app}. [j] Kinetically apparent pK. [k] pK determined titrimetrically. [l] This study; μ = 1.0 with KCl; T = 30°. [m] This study; μ = 0; T = 30°. [n] The pK_a of this compound has been reported to be 3.22: F. H. Westheimer and O. T. Benfey, *J. Am. Chem. Soc.*, **78**, 5309 (1956). [o] Average of five values (extrapolated to 0 buffer concentration) in the pH region 2.0 to 4.3; maximum deviation = 5.6×10^{-4} min^{-1}. [p] N-Acetylserinamide.

concentration were found to be linear. The experimental data for the hydrolysis of methyl hydrogen phthalate are shown in Figure 1. Extrapolation of k_{obsd} to 0 buffer concentration supplies the pH dependent first-order rate constants k_0. These constants are shown plotted as a function of pH in Figure 2. The extrapolated rate constants fit a theoretical titration curve having a kinetically apparent pK_{app} of 3.37; the pK_a' of methyl hydrogen phthalate determined titrimetrically at essentially zero ionic strength[22] was found to be 3.32 ± 0.05 (Table I). The shape of the titration curve indicates that the hydrolysis of methyl hydrogen phthalate, in the pH range investigated, is dependent

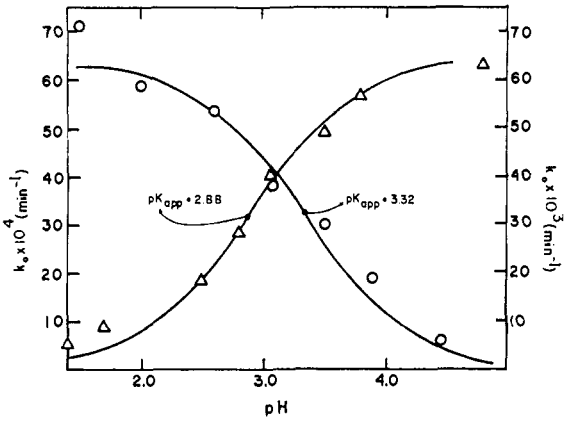


Figure 3. Plots of k_0 *vs.* pH: left ordinate and circles, 2′-chloroethyl hydrogen phthalate (91.3°, μ = 1.0); right ordinate and triangles, 2′,2′,2′-trifluoroethyl hydrogen phthalate (64.6°, μ = 1.0).

on the mole fraction of ester in the unionized form. Included in Figure 2 is a plot of the values of k_0, for phenyl hydrogen phthalate. The shape of the plot reveals that the hydrolysis of the phenyl ester is dependent on the mole fraction of ester in the ionized form. Therefore, the hydrolysis of the methyl ester occurs with neighboring COOH group participation while the phenyl ester hydrolyzes with COO^- group participation.

Table II. Values of k_0 and Activation Parameters[a] for the Hydrolysis of Methyl Hydrogen Phthalate, pH 2.00

k_0, sec^{-1}	Temp, °C	Kcal/mole $\Delta H^{\pm}$	$T\Delta S^{\pm}$	$\Delta F^{\pm}$
2.667×10^{-4}	100.0	22.44	−4.56	27.00
1.417×10^{-4}	91.3			
0.400×10^{-4}	78.5			

[a] Calculated from $\Delta H^{\pm} = E_a - RT$, $T\Delta S^{\pm} = \Delta H^{\pm} - \Delta F^{\pm}$, and $\Delta F^{\pm} = 2.303RT \log (kT/hk_r)$: A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, pp 95–97. Standard state is 1 *M*, T = 25°, time in seconds.

The activation parameters for the hydrolysis of methyl hydrogen phthalate have also been determined (μ = 1.0 with KCl; pH 2.00); see Table II.

In Figure 3 are plotted the values of k_0 *vs.* pH for 2′-chloroethyl hydrogen phthalate and for 2′,2′,2′-trifluoroethyl hydrogen phthalate. It can be seen that the trifluoroethyl ester hydrolyzes with COO^- participation whereas the hydrolysis of the chloroethyl ester proceeds with the assistance of the neighboring COOH group.

In Figure 4 there are plotted the values of k_0 *vs.* pH for propargyl hydrogen phthalate. The invariance of

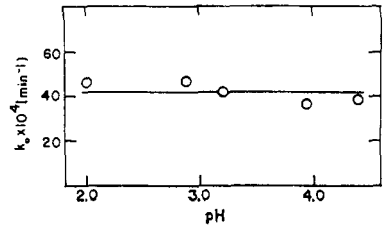


Figure 4. Plot of k_0 *vs.* pH for propargyl hydrogen phthalate (78.5°, μ = 1.0).

k_0 with pH in the range of the pK_a' of the ester establishes that the rate of hydrolysis is not dependent on the mole fraction of ester in the acidic or anionic form.

Discussion

In Table I are summarized the data obtained in these experiments. Obviously the mechanism of participa-

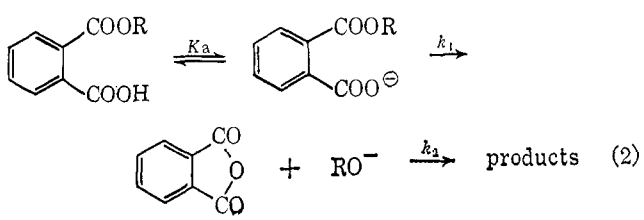
tion by the neighboring carboxyl group in the hydrolysis of phthalic acid monoesters is a function of the pK_a' of the leaving group, *i.e.*, those esters having good leaving groups hydrolyze with participation by neighboring carboxyl anion and those having poor leaving groups hydrolyze with participation by neighboring undissociated carboxyl group. A change in mechanism therefore occurs and it follows that there must be half-esters of phthalic acid which hydrolyze by both COOH and COO^- participation. If both COO^- and COOH participation were equally effective, a plot of k_0 *vs.* pH would be flat. As can be seen in Figure 4, the propargyl hydrogen phthalate approaches this situation within experimental error. The line drawn through the points in Figure 4 is an average of the points, the maximum deviation being 13.4%.

For the esters other than that of propargyl alcohol the true rate constant for hydrolysis with COOH or COO^- participation (k_{rate}) may be obtained by multiplying the value of k_0 at pK'_{app} by two. For the propargyl ester $k_{rate} = k_0$ at the plateau. A Brønsted plot of k_{rate} of hydrolysis of the half-esters employed in this study (relative to methyl hydrogen phthalate at the same temperatures) is shown in Figure 5. The change in mechanism clearly occurs at or around the pK_a' (13.55) of propargyl alcohol. It is of interest to note that the successful synthetic procedures for the preparation of the half-esters were the retrograde of the hydrolytic mechanism. Thus, the methyl, 2′-chloroethyl, and propargyl esters were prepared by refluxing the anhydride in the respective alcohols while the phenyl and 2′,2′,2′-trifluoroethyl esters were prepared by the reactions of the oxyanions with anhydride.

The change in mechanism at pK_a of ~13.5 for the alcohol portion of the ester is considered particularly significant in view of the fact that the pK_a' of the hydroxyl group of N-acetylserinamide is 13.6.[23] The implications of this with respect to the mechanism of enzyme action are significant. Such participation might not be experimentally detectable by conventional pH–rate profile studies since the net result of a group participating equally by two mechanisms would be a flat pH–rate profile.

We have attempted to determine the rates of hydrolysis of O-phthaloyl-N-acetylserinamide at 100° and μ = 1.0. The kinetic data, however, left something to be desired. Nevertheless, the rates of hydrolysis at pH 4.3 and 2.0 (extrapolated to 0 buffer concentration) appear to be approximately the same indicating that the pH–rate profile is flat throughout this pH region; the k_0 values are approximately 1.0×10^{-2} min^{-1}.

For those compounds which hydrolyze with carboxyl anion participation, the mechanism is presumably that shown in eq 2; the rate equation is $v = k_{obsd}(ester)_{total} = k_{rate}(K_a/K_a + a_H)(ester)_{total}$.



(2)

(23) T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, **1**, 7 (1962).

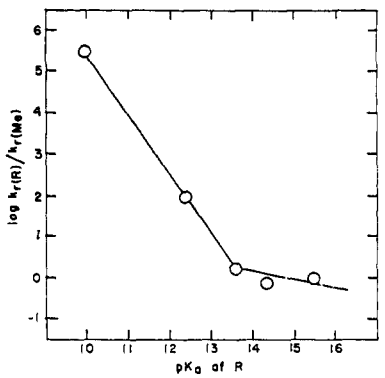
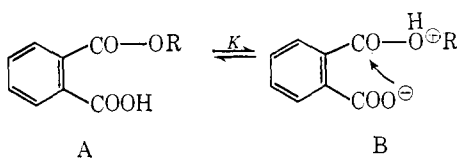
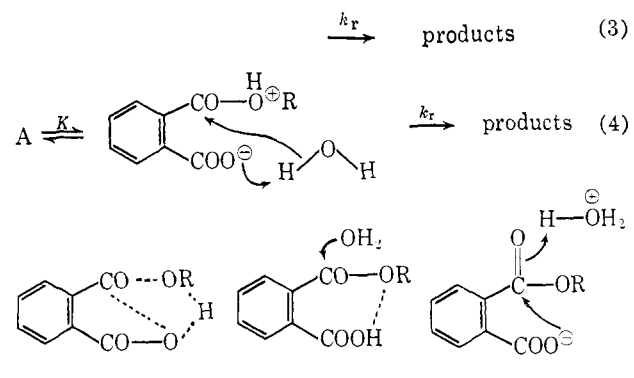


Figure 5. Plot of log $k_{r(R)}/k_{r(CH_3)}$ *vs.* pK_a of the conjugate acid of the leaving group.

The intermediacy of the anhydride has been established in the case of the phenyl ester since k_1 is greater than k_2 over most of the pH range studied, *i.e.*, above pH 2.2. Therefore the rate-limiting step is that associated with k_2 (*i.e.*, solvolysis of the anhydride). The latter rate constant has been determined in this study to be 0.739 min^{-1} (extrapolated to 0 buffer concentration, μ = 1.0; see the Experimental Section) in the pH range 1.6 to 5.7. This may be compared to a value of 0.276 min^{-1} at 25° reported by Rivett and Sidgwick.[24]

Compounds which hydrolyze with COOH participation may proceed by mechanisms 3 to 7 all of which obey the rate equation, $v = k_{obsd}(ester)_{total} = k_{rate}(a_H/(a_H + K_a)(ester)_{total}$. Mechanisms 3 and 4 both involve preequilibrium protonation; the latter also includes general base catalysis by the carboxyl anion. Mechanism 5 is strictly analogous to the four-center electro-





(5–7)

philic–nucleophilic process proposed by Bender, *et al.*,[25] for the hydrolysis of phthalamic acid and necessarily involves the formation of phthalic anhydride as an intermediate which would hydrolyze in a fast step. Mechanisms 4 and 6 do not require the anhydride as an

(24) A. C. D. Rivett and N. V. Sidgwick, *J. Chem. Soc.*, **97**, 1683 (1910).

(25) M. L. Bender, Y. L. Chow, and F. Chloupek, *J. Am. Chem. Soc.*, **80**, 5380 (1958).

intermediate. The necessity for the appearance of anhydride as an obligatory intermediate could be determined readily by isotope experiments of the type reported by Bender and co-workers[25] for the hydrolysis of phthalamic acid. Another likely mechanism is (7), specific acid catalysis with nucleophilic attack by carboxyl anion. We have excluded the possibility of a change in mechanism with temperature in the case of the methyl ester. Thus, the ratio of the observed rates at pH 4.0 and 3.0 at 100° was 0.39 (0.1 *M* citrate buffer) while the ratio under otherwise identical conditions at 70° was 0.41.

The activation parameters obtained for methyl hydrogen phthalate are those expected for an intramolecular reaction and are strikingly similar to those reported by Gaetjens and Morawetz for the hydrolyses of phenyl acid succinates and glutarates which proceed with participation by the neighboring carboxylate group.[5b] It has been demonstrated that the molecularity of a nucleophilic displacement reaction (for phenyl acetates) can be multiplied by approximately 5 kcal/mole in order to obtain the $T\Delta S^{\neq}$ value.[26] The present study of an intramolecular, first-order reaction has an experimentally determined $T\Delta S^{\neq}$ value of -4.6 kcal/mole, in good agreement with the conclusions of Bruice and Benkovic.[26] The large slope of the log k_{rel} *vs.* pK_a' plot for COO^- participation (Figure 5) is anticipated since electron withdrawal should favor the intramolecular nucleophilic attack. The large decrease in sensitivity to electronic effects observed for the cases of COOH participation also is anticipated on the basis that electron withdrawal should favor nucleophilic attack but disfavor protonation of the ester function (mechanisms 3 to 7).

We are unable to explain the total disagreement between our results for methyl hydrogen phthalate and those of Bender, *et al.*,[10] who report COO^- participation in the hydrolysis of this compound under similar conditions. In support of our data, we cite the results of Ågren and co-workers[7] and of Eberson[8] who present evidence for COOH participation in the hydrolysis of ethyl hydrogen phthalate and methyl hydrogen 3,6-dimethyl phthalate, respectively. A complete change in mechanism between the above-named compounds and methyl hydrogen phthalate is not readily conceivable on the basis of polar and/or steric factors.

It should be added that the k_{rate} for ethyl hydrogen phthalate (calculated from Ågren's data[7]) falls almost exactly on the line drawn in the Brønsted plot (Figure 5).

Acknowledgments. This work was supported by a grant from the National Institutes of Health. We should like to express our thanks to Mr. Clark Newcomb for his skilled technical assistance in collecting many of the rate data.

(26) T. C. Bruice and S. J. Benkovic, *J. Am. Chem. Soc.*, 86, 418 (1964).

Proximity Effects. XLIV. Stereospecific Synthesis and Solvolysis of *cis*- and *trans*-5-Phenylcyclooctyl and *cis*- and *trans*-5-Phenylcyclooctyl-1,2,2,8,8-d_5 Tosylates[1]

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Abstract: The isomeric *cis*- and *trans*-5-phenylcyclooctyl tosylates, whose structures have been verified by a stereospecific synthesis, were prepared and solvolyzed in anhydrous formic acid. The *cis* isomer gave mostly products from a transannular 1,5 hydride shift whereas the *trans* compound afforded mostly direct substitution or elimination products. Corresponding deuterated tosylates were prepared and solvolyzed. Degradation of the deuterated products established that no transannular phenyl migration had occurred.

The occurrence of hydride shifts during solvolytic reactions of medium-ring compounds is well documented.[3] The facility of this reaction led to speculation on the possibility of transannular alkyl or aryl migration. Earlier efforts to find phenyl or methyl migration in nine-membered rings[4] proved fruitless, but the recent observation of 1% phenyl migration during solvolysis of 5,5-diphenylcyclooctyl tosylate[5] spurred new interest in the problem. Two earlier cases of solvolysis of isomeric 5-substituted cyclooctyl tosylates have been reported.[6a,b]

The symmetrical phenonium ion (Figure 1) presented a very attractive hypothetical intermediate; this type of ion has been described for the case of 1,2 aryl participation.[7] Furthermore, a 5-phenylcyclooctyl tosylate is free from the possibly deleterious *gem*-dialkyl effect found in the 5,5-diphenyl derivative, which would force C-1 and C-5 apart.

The isomeric 5-phenylcyclooctyl tosylates also offer

(1) Supported in part by a research grant (NSF-GP-1587) from the National Science Foundation.

(2) National Institutes of Health Fellow, 1963–1964; Procter and Gamble Fellow, 1962–1963.

(3) For a general discussion see E. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 252 ff, and references contained therein; A. C. Cope, M. M. Martin, and M. A. McKervey, to be published.

(4) A. T. Blomquist and Y. C. Meinwald, *J. Am. Chem. Soc.*, **80**, 630 (1958); A. T. Blomquist and B. F. Hallam, *ibid.*, **81**, 676 (1959).

(5) A. C. Cope, P. E. Burton, and M. L. Caspar, *ibid.*, **84**, 4855 (1962).

(6) (a) A. C. Cope and D. M. Gale, *ibid.*, **85**, 3743 (1963); (b) N. L. Allinger and S. Greenberg, *ibid.*, **84**, 2394 (1962).

(7) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., Inc., New York, N. Y., 1959, p 457 ff.