Series Nucleophilic Catalysis in the Hydrolysis of 3-Acetoxyphthalate. Intramolecular Catalysis of Ester Hydrolysis by the Carboxyl Group Once Removed

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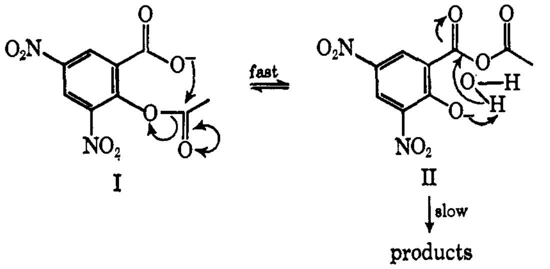
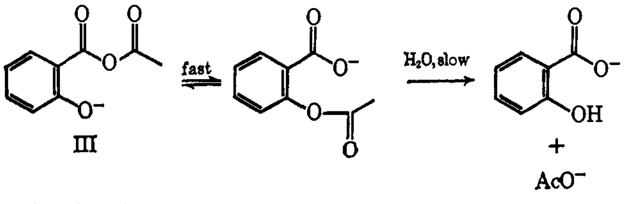
Abstract: The introduction of a second carboxyl group into the 6 position of aspirin results in a remarkable increase in the efficiency of intramolecular catalysis. Compared with the methyl ester of aspirin, the hydrolysis of the monoanion of 3-acetoxyphthalate is accelerated by a factor of about 10^6. The second carboxyl group, which cannot interact directly with the ester group, is responsible for an increase in the rate of hydrolysis of over 6000 times. The mechanism of this catalysis involves a series of equilibria in which the acetyl group is first transferred to the adjacent carboxyl group, then displaced from it, as acetate ion, by the second. The slow step of the reaction is the hydrolysis of 3-hydroxyphthalic anhydride, which can be isolated from the reaction mixture in the case of the monoanion, and is an intermediate also in the hydrolysis of the dianion. The scope and requirements of series nucleophilic catalysis are briefly discussed.

We have shown[1,2] that the hydrolysis of the anion of 3,5-dinitroaspirin (I) involves intramolecular nucleophilic catalysis by the ionized carboxyl group. The slow step of this reaction is the hydrolysis of the mixed anhydride II, and we consider[2] that the nucleophilic mechanism is important only because the hydrolysis of II is itself catalyzed by the phenolate group acting as a general base.



The hydrolysis of aspirin involves intramolecular general base catalysis by the carboxylate group, and no contribution from the nucleophilic mechanism can be detected.[3] All the steps of the nucleophilic pathway remain possible, and presumably rapid, but the equilibrium for the formation of the mixed anhydride is much less favorable.[1,2] In other words, the phenolate oxygen of the unsubstituted anhydride III attacks the neighboring acetyl group, to regenerate starting material, much more effectively than it acts as a general base to catalyze the hydrolysis to products (Scheme I).

Scheme I



If this picture is accurate it should be possible to extend the scope of the nucleophilic mechanism by making available more effective catalysis of the hydrolysis of the anhydride intermediate. It is known that the neighboring carboxylate group, in half-esters of phthalic acid,[4] is a much more effective catalyst for the hydrolysis of aromatic esters than is the phenolate oxygen of aryl salicylates.[5] Therefore we have examined the hydrolysis of 3-acetoxyphthalic acid.

Experimental Section

Materials. Inorganic salts were of analytical grade, or were purified before use. Distilled water was further twice glass distilled. 3-Hydroxyphthalic anhydride was prepared by a published procedure,[6] and had mp 200–201° (lit.[6] mp 200–201°). 3-Acetoxyphthalic anhydride[7] had mp 115–116° (lit.[8] mp 115°). **3-Acetoxyphthalic acid** was prepared by acid hydrolysis of the anhydride. 3-Acetoxyphthalic anhydride (900 mg) was dissolved in acetone (30 ml) and 1 *N* HCl (30 ml) added over 30 min. After standing a further 10 min, extraction with ether gave 700 mg of free acid. After recrystallization from acetone–chloroform this had mp 203–204° (rapid heating). *Anal.* Calcd for $C_{10}H_8O_6$: C, 53.57; H, 3.57. Found: C, 53.61; H, 3.82.

Kinetic Methods and Results

Measurements were made at 25° and ionic strength 1.0 (added KCl), in the thermostated cell compartment of a Zeiss PMQ II spectrophotometer. Reactions were generally begun by adding 0.10 ml of a stock solution of substrate in dioxane, to 3 ml of reaction mixture incubated in the cuvette. But for most measurements of the hydrolysis of 3-acetoxyphthalic acid the compound was not isolated, but prepared in solution in 1 *N* HCl from 3-acetoxyphthalic anhydride. This anhydride has a half-life of some 40 sec at 25° and pH 0, and the subsequent hydrolysis of 3-acetoxyphthalic acid is very much slower under these conditions; so that the anhydride is converted almost quantitatively to the free acid on standing for 6–7 min in 1 *N* HCl. 3-Acetoxyphthalic acid was isolated when this acid hydrolysis was carried out on a preparative scale (see above). A number of runs were then repeated using the purified compound,

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(1) A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **89**, 5960 (1967).

(2) A. R. Fersht and A. J. Kirby, *ibid.*, **90**, 5818 (1968).

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

(4) J. Thanassi and T. C. Bruice, *ibid.*, **88**, 747 (1966).

(5) M. L. Bender, F. J. Kezdy, and B. Zerner, *ibid.*, **85**, 3017 (1963); B. Capon and B. C. Ghosh, *J. Chem. Soc., B*, 472 (1966).

(6) E. L. Eliel, A. W. Burgstahler, D. E. Rivard, and L. Haefele, *J. Amer. Chem. Soc.*, **77**, 5092 (1955).

(7) D. S. Pratt and G. A. Perkins, *ibid.*, **40**, 228 (1918).

(8) H. Muhlemann, *Pharm. Acta Helv.*, **23**, 257 (1948); *Chem. Abstr.*, **43**, 2984*g* (1949).

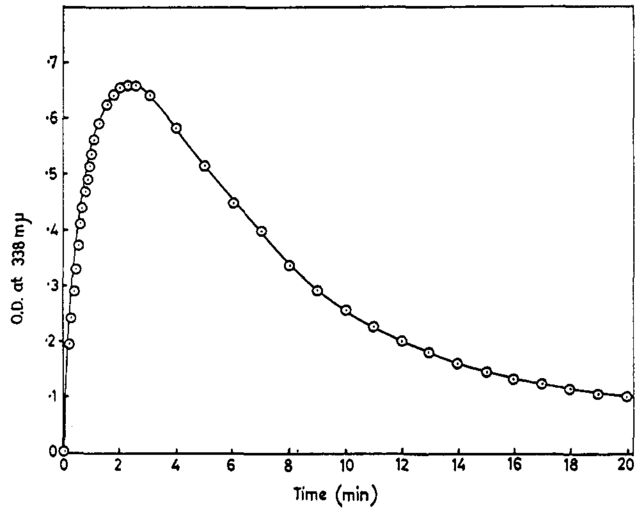

Figure 1. Spectroscopic evidence for the formation of 3-hydroxyphthalic anhydride as an intermediate in the hydrolysis of 3-acetoxyphthalate monoanion. Optical density at 338 mμ as a function of time during hydrolysis at pH 3.9.

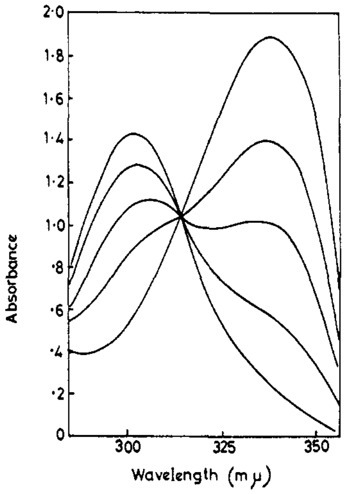

Figure 2. Determination of the isosbestic point for 3-hydroxyphthalic anhydride and its hydrolysis products. These are spectra of a solution of the anhydride in water at pH 4.0, at 25° and ionic strength 1.0, taken after 1, 3, 5, 10, and 30 min.

and the results were identical with those originally obtained with the acid prepared in solution.

In an initial series of experiments the rate of hydrolysis of 3-acetoxyphthalic acid was followed at 304 mμ, which is an isosbestic point for the three forms—dianion, monoanion, and neutral species—of 3-hydroxyphthalic acid. But in the region of fast hydrolysis near pH 4 the reaction did not follow simple first-order kinetics: a rapid initial change in optical density was succeeded by a second, slower process. This second reaction was identified as the hydrolysis of 3-hydroxyphthalic anhydride, as follows.

Repeated scanning of the reaction mixture showed that the rapid increase in optical density was associated with an absorption maximum at 338 mμ, which is a λ_{max} for 3-hydroxyphthalic anhydride under these conditions. When the reaction was followed at 338 mμ at pH 3.9 the optical density (Figure 1) rose to a maximum after about 140 sec. After 8 min, when the fast reaction was complete, the rate of disappearance of the 338-mμ absorption followed excellent pseudo-first-order kinetics, and was measured as 0.185 min^{-1}. The rate of hydrolysis of 3-hydroxyphthalic anhydride under these conditions (0.05 *M* acetate buffer, pH 3.9, ionic strength 1.0) was measured at 304 mμ and found to be identical, at 0.185 min^{-1}.[9] Finally, 3-hydroxyphthalic anhydride was isolated from the hydrolysis mixture when the reaction was carried out on a preparative scale. 3-Acetoxyphthalic acid (200 mg) was dissolved in 5 ml of acetate buffer (pH 4.3). After 30 sec a precipitate appeared. This was collected after 10 min and shown by melting point, mixture melting point, and ir spectrum, to be identical with an authentic sample of 3-hydroxyphthalic anhydride. The yield was 125 mg (85%).

It is evident from these results that 3-hydroxyphthalic anhydride is an intermediate, and is hydrolyzed at a rate comparable to that of 3-acetoxyphthalic acid in the region near pH 4. To isolate the step in which the acetyl group is lost we therefore carried out a series of experiments in which the reaction was followed at the isosbestic point for 3-hydroxyphthalic anhydride and its hydrolysis products. This was obtained by repeated scanning of a solution in which 3-hydroxyphthalic anhydride was being hydrolyzed (Figure 2). Since the hydrolysis products are a mixture of three ionic species

Table I. Rate Constants for the Hydrolysis of 3-Acetoxyphthalic Acid, at 25° and Ionic Strength 1.0

pH	Followed at, mμ	k_{hyd}, min^{-1}
0[a]	312.7	5.75×10^{-3}
1[a]	312.7	0.031
2.06[a]	313.0	0.128
2.61[b]	313.4	0.302
3.03[b]	313.5	0.442
3.21[b]	313.6	0.529
3.62[c]	313.7	0.637
3.66[c]	313.6	0.739
3.84[c]	313.4	0.789
3.89[c]	313.3	0.776
3.99[c]	313.1	0.740
4.11[c]	313.1	0.744
4.13[c]	313.2	0.726
4.28[c]	312.7	0.635
4.57[c]	312.7	0.511
4.82[c]	312.7	0.356
4.88[c]	312.7	0.274
8.99[d]	304	2.2×10^{-3}
9.8[d]	304	2.2×10^{-3}
12[e]	304	0.237
12.5[e]	304	0.746
Rate constant for hydrolysis of the monoanion[f]		0.97 ± 0.05
Rate constant for hydrolysis of the dianion		$1.8 \pm 0.1 \times 10^{-3}$
Second-order rate constant for carbonate		5.4×10^{-3} M^{-1} min^{-1}
Second-order rate constant for hydroxide		25 M^{-1} min^{-1}
Apparent pK_a's		3.11 ± 0.03, 4.6 ± 0.1[f]

[a] HCl solution. [b] Formate buffer (0.05 *M*). Buffer catalysis is insignificant compared with changes in rate due to small pH changes caused by varying buffer concentration. [c] Acetate buffer (0.05 *M*). See footnote *b*. [d] Carbonate buffers, extrapolated to zero buffer concentration. [e] NaOH solutions. [f] Calculated from the data in the pH 3–5 region.

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(9) This rate constant includes a small contribution from catalysis by the acetate buffer.

the isosbestic point is pH sensitive, and had to be measured separately for each buffer mixture. The wavelengths used are listed in Table I, together with the rate constants for hydrolysis obtained. Reactions were followed for at least three half-lives, and end points taken after at least ten. This procedure gave excellent pseudo-first-order semilogarithmic plots as long as the wavelength used was correct to within one ångström unit.

Above pH 8.9 the hydrolysis of 3-hydroxyphthalic anhydride becomes much faster than its formation, and the reaction could be followed directly from 3-acetoxyphthalate dianion to 3-hydroxyphthalate dianion, at 304 mμ. At pH 9 the contributions from the hydrolysis of the monoanion, and from hydroxide ion attack, are small, and the observed rate is almost entirely due to hydrolysis of the dianion. Since the rate of hydrolysis between pH 9 and 10 is some 13 times greater than that of aspirin anion, it seemed likely that nucleophilic catalysis was operative in the reaction of the dianion also. Two experiments were carried out to test this possibility.

First we attempted to detect 3-hydroxyphthalic anhydride directly. The pK_a of the phenol group of this anhydride lies in the region between 6 and 7 (it could not be measured accurately because hydrolysis is rapid) so that the compound will exist as the anion at pH 9. The anion is yellow, with an absorption maximum at 397 mμ (Table II), and is readily observed visually. A 0.10 *M* solution of 3-acetoxyphthalate was made up by dissolving the solid acid in a buffer initially 0.2 *M* in sodium carbonate and 0.6 *M* in sodium bicarbonate. The pH fell from 9.4 to 9.1 as the reaction proceeded, and a yellow coloration appeared immediately. Several scans of the reaction mixture showed an absorption maximum in the region of 397 mμ (λ_{max} for the anion of 3-hydroxyphthalic anhydride) with initial optical density 0.24. This was monitored at 25° in the spectrophotometer, and decreased steadily in intensity. The rate constant associated with the disappearance of this absorption was approximately 1.3×10^{-3} min^{-1}, similar to that (2.2×10^{-3} min^{-1}) for the over-all reaction. We conclude that this is evidence for the formation of a low, close-to-steady-state concentration of 3-hydroxyphthalic anhydride anion. Using our estimate of the extinction coefficient for the absorption involved (Table II) we calculate that the concentration of the intermediate is about one ten thousandth that of the starting material, 3-acetoxyphthalate dianion.

Table II. Ultraviolet Spectral Characteristics of 3-Acetoxyphthalate and Its Hydrolysis Products

	λ_{max}, mμ (ϵ)	Isosbestic point, mμ (ϵ)
3-Acetoxyphthalic acid	278.5 (1700)	
3-Acetoxyphthalate monoanion	*a*	
3-Acetoxyphthalate dianion	273.5 (930)	
3-Hydroxyphthalic anhydride	338 ($\sim$6000)[b]	$\sim$313[d]
3-Hydroxyphthalic anhydride anion	397 (>6000)[b]	
3-Hydroxyphthalic acid	301.5 (3260)	304 (3180)
3-Hydroxyphthalate monoanion	301 ($\sim$3200)[c]	
3-Hydroxyphthalate dianion	299 (3360)	

[a] Too rapidly hydrolyzed to measure. [b] Hydrolyzed too rapidly to measure accurately. [c] Estimated value. Never 100% of species present. [d] Varies with pH. See Table I.

To confirm that an anhydride intermediate is involved, and lies on the reaction pathway, we carried out the hydrolysis of the dianion in ^{18}O-enriched water. 3-Acetoxyphthalic acid (22.4 mg) was dissolved in 1 ml of 20% enriched $H_2{}^{18}O$, made up to 0.4 *M* in sodium carbonate and 0.6 *M* in sodium bicarbonate, using the solid salts. This gave a 0.10 *M* solution of the dianion, and a pH (measured in water in a trial run) of 9.2, falling to 8.9. The 3-hydroxyphthalic acid produced was isolated, after ten half-lives, by acidification and ether extraction, and examined for incorporation of ^{18}O as described previously for aspirin hydrolysis.[3] In this case the molecular ion of 3-hydroxyphthalic acid could not be observed, and measurements were made on the strong peak at *m/e* 164, which is due to 3-hydroxyphthalic anhydride. (Dehydration very likely precedes ionization, since the acid readily gives the anhydride on sublimation.[6]) Since much of the ^{18}O incorporated is likely to be lost in this reaction we used two controls: the acid obtained by hydrolysis of 3-acetoxyphthalic acid in natural abundance H_2O, and the acid obtained by hydrolysis of 3-hydroxyphthalic anhydride in 20% enriched $H_2{}^{18}O$. The ^{18}O content of the anhydride from the deliberately enriched acid was unexpectedly low, at 8 atom %; that from the product of hydrolysis of 3-acetoxyphthalate dianion in $H_2{}^{18}O$, surprisingly, was higher, at 16 atom %. It is likely that these low figures for incorporation are accounted for by a high proportion of nucleophilic catalysis by carbonate of hydrolysis of the anhydride. If the resulting mixed phthalic–carbonic anhydride, $ArCO{-}O{-}CO_2{}^-$, breaks down by decarboxylation, no incorporation of ^{18}O from the enriched solvent is to be expected. The lower incorporation into the acid produced on hydrolysis of the preformed 3-hydroxyphthalic anhydride could then be explained, since the final concentration of carbonate was higher in this experiment.

In any case it is clear that there is substantial incorporation of ^{18}O from the enriched solvent into the product of hydrolysis of the dianion, and thus that the anhydride observed spectrophotometrically is an intermediate in the hydrolysis reaction.

Absorption maxima and extinction coefficients for all the species involved are summarized in Table II.

One final complication was observed in the region pH 5–8. Once again simple first-order kinetics could not be observed. In this case the complication was traced to the hydrolysis of 3-hydroxyphthalic anhydride, which in this region shows a fast initial increase in optical density at 304 mμ, followed by a second, slower, process.[10] For this reason isosbestic points could not be obtained for the hydrolysis of the hydroxyanhydride in this region, and the rate of hydrolysis of 3-acetoxyphthalic acid could not be measured accurately. Since the shape of the pH–rate profile is well established by measurements at other pH's this did not create a serious difficulty.

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(10) We have not investigated this phenomenon. The phenolic group of the 3-hydroxyanhydride has a pK_a near 6–7 (see above) and it is possible that the phenolate oxygen of one molecule attacks the anhydride group of another, to form eventually an eight-membered bislactone, which is being hydrolyzed in the second, slower, step to 3-hydroxyphthalate. We are satisfied that the known reactivities of phenolate anions make this quantitatively possible, but have no relevant evidence.

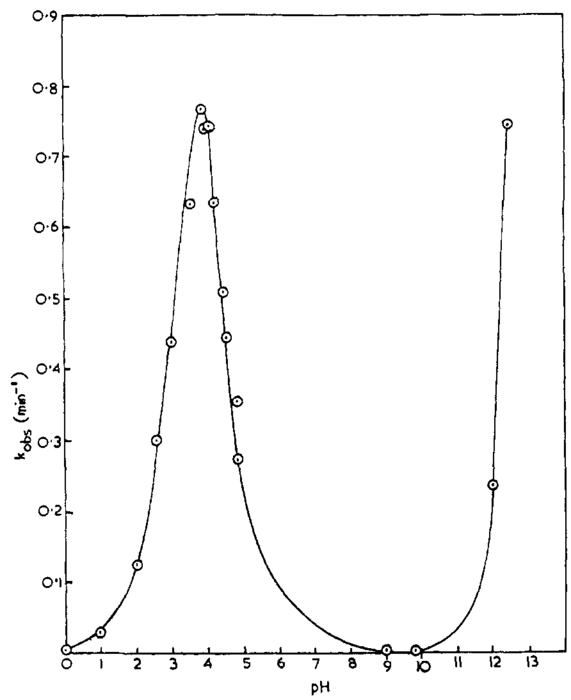


Figure 3. pH-rate profile for the hydrolysis of 3-acetoxyphthalic acid to 3-hydroxyphthalic acid and anhydride, at 25° and ionic strength 1.0. The maximum rate at pH 3.84 is 0.79 min^{-1}, and the minimum at pH 9.8 is 2.2 × 10^{-3} min^{-1}.

The pH of each reaction mixture was measured at the end of the run, using an E.I.L. Vibron electrometer fitted with a C-33B pH-measuring attachment and a Pye-Ingold combined glass-reference electrode.

Discussion

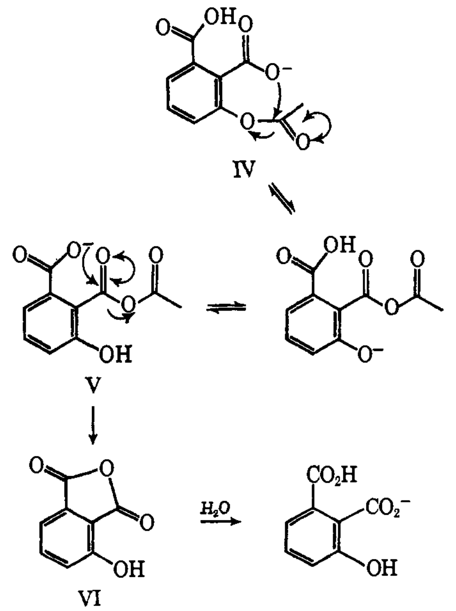
The pH-rate profile for the hydrolysis of 3-acetoxyphthalic acid is shown in Figure 3. The rate maximum at pH 3.86 is in the region where the concentration of the monoanion is expected to be a maximum. We interpret the pH-rate profile in terms of a rapid hydrolysis of the monoanion, and much slower hydrolyses of neutral 3-acetoxyphthalic acid, at low pH, and of the dianion between pH 9 and 10. The apparent pK_a's, calculated from the points defining the bell-shaped curve, are 3.1 and 4.6, much as expected for the ionization of 3-acetoxyphthalic acid. Above pH 10 the alkaline hydrolysis of the dianion becomes important.

Hydrolysis of the Monoanion. The dominating feature of the pH-rate profile is the region of fast hydrolysis near pH 4. In this region hydrolysis is a two-stage reaction, involving the 3-hydroxyphthalic anhydride as an intermediate. The evidence is given in the Experimental Section, and is overwhelming. The formation of the anhydride was demonstrated spectrophotometrically: the rate of hydrolysis of the intermediate is identical with that of the anhydride, and the anhydride was isolated, in 85% yield, from the reaction mixture. This demonstration of the presence of the intermediate in the reaction mixture was simplified by the fact that 3-hydroxyphthalic anhydride is formed up to five times faster than it is hydrolyzed. For this reason the reaction was followed in the region of fast hydrolysis at wavelengths known to be isosbestic points for 3-hydroxyphthalic anhydride and its hydrolysis products, so that the pH-rate profile of Figure 3 represents specifically the hydrolysis of the acetyl group, and the formation of 3-hydroxyphthalic anhydride plus 3-hydroxyphthalate.

Analysis of the data in the region of fast hydrolysis gives a first-order rate constant of 0.97 ± 0.05 min^{-1} for the hydrolysis of 3-acetoxyphthalate monoanion. This represents remarkably effective catalysis of the hydrolysis of this ester. The carboxylate group of aspirin is a relatively ineffective intramolecular catalyst; the aspirin anion is hydrolyzed only some 150 times faster than the methyl ester at 25°.[11] Yet the hydrolysis of the monoanion of 3-acetoxyphthalate, *in which the second carboxyl group cannot interact directly with the ester group*, is some 10^6 times faster (than aspirin methyl ester). The introduction of the second carboxyl group increases the rate of hydrolysis (compared with aspirin anion[11]) by a factor of nearly 6300.

The reaction pathway largely defines itself. Bifunctional catalysis is not a possibility, since only one of the carboxyl groups can interact with the ester group. The second carboxyl group cannot be acting as a general acid, since this could not assist the attack of the adjacent carboxylate anion on the ester, nor as a general base (removing a proton from the adjacent carboxylic acid group in the transition state), since general base catalysis cannot make a carboxyl group more reactive than its fully ionized form. The only reasonable way in which the second carboxyl group can take part in the reaction involves the intermediate formation of the mixed anhydride V, as a result of nucleophilic attack on the ester group by the carboxylate anion in the 2 position. Since the initial product of hydrolysis of the monoanion is 3-hydroxyphthalic anhydride, there can be little doubt that the reaction follows the course outlined in Scheme II.

Scheme II



The only important detail of mechanism outstanding concerns the step or steps which are rate determining for the formation of 3-hydroxyphthalic anhydride. Consider first the simplified scheme

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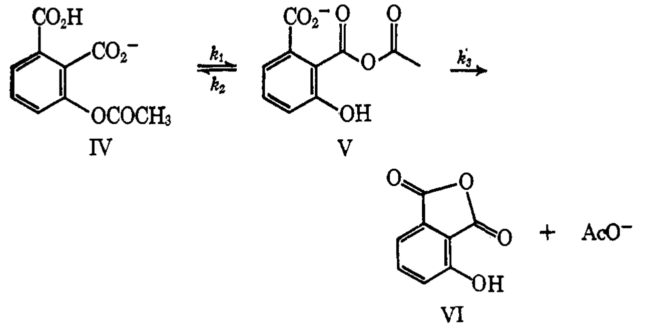
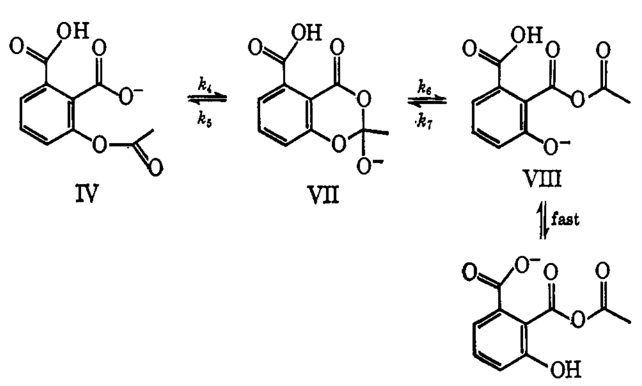
[11] T. St. Pierre and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 3817 (1968).



(the reverse of k_3, the reaction of acetate ion with the anhydride VI, can safely be disregarded since acetate actually catalyzes its hydrolysis). The carboxylate group of V is clearly a much more reactive nucleophile than is the phenolic hydroxyl group, so that $k_3 \gg k_2$. The rate-determining step must therefore be the *formation* of the mixed anhydride V and the observed rate constant approximately equal to k_1.

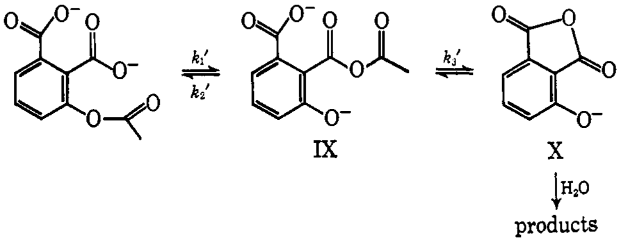
The formation of the mixed anhydride is itself a three-stage reaction.



We assume that the final proton transfer is not rate limiting, since the over-all rate of the reaction is less than 1 min^{-1}. Of the other rate constants involved, k_7, although undoubtedly a large rate constant, represents a reaction which is probably relatively slow in practice, since the equilibrium constant for the prototropic equilibrium VIII $\rightleftharpoons$ V will be of the order of 10^6, and the concentration of VIII will be very small. We can, therefore, consider the reaction simply in terms of the partitioning of the tetrahedral intermediate VII, and here it is clear that k_5, representing the elimination of carboxylate, is likely to be much greater than k_6, the elimination of the much poorer phenoxide leaving group. Thus, we conclude that the slow step in the formation of V, and therefore in the formation of 3-hydroxyphthalic anhydride also, is the breakdown of the first tetrahedral intermediate VII. The observed rate constant is therefore represented approximately by $k_{\text{obsd}} = k_6(k_4/k_5)$, and contains the unfavorable equilibrium constant for the formation of the tetrahedral intermediate VII. This result makes it clear that the limits of the effectiveness of catalysis in this system have not yet been reached. The bond-making and -breaking processes involved could be several orders of magnitude greater than even the very fast rate observed for the hydrolysis of 3-acetoxyphthalate monoanion, and it should be possible to increase the effectiveness of catalysis still further by changes designed to increase either k_6 or the equilibrium constant k_4/k_5.

Hydrolysis of the Dianion. The dianion is hydrolyzed about 13 times as fast as is the anion of aspirin. The rate of the intramolecular general base catalyzed attack of water, corresponding to the mechanism of aspirin hydrolysis,[3] is expected to be about four times faster in the case of 3-acetoxyphthalate, since the general base concerned is more strongly basic ($pK_2 = 4.6$ compared with 3.2 for aspirin at 25° and ionic strength 1.0, and Brønsted $\beta = 0.52$[12]). This difference suggests that the second carboxylate group may be involved in the hydrolysis of the dianion also, and the evidence makes it clear that this is so.

When 3-acetoxyphthalate is hydrolyzed in ^{18}O-enriched water there is substantial incorporation of ^{18}O into the 3-hydroxyphthalate produced. This is evidence for the formation of either the mixed anhydride IX or of the anion of 3-hydroxyphthalic anhydride (X), or, of course, both. In fact the formation of X can be demonstrated spectrophotometrically. When a concentrated (0.1 *M*) solution of 3-acetoxyphthalate was hydrolyzed at pH 9, an absorption maximum was observed at 397 mμ, which is λ_{max} for the anion of 3-hydroxyphthalic anhydride. From the optical density at 397 mμ the concentration of X was estimated as about 10^{-5} *M*. This absorption gradually disappeared as the hydrolysis proceeded with a first-order rate constant of about 1.3×10^{-3} min^{-1}, similar to the rate of the over-all reaction (2.2×10^{-3} min^{-1}). These two pieces of evidence indicate that the nucleophilic mechanism probably accounts for the major part of the hydrolysis of the dianion.



Compared with the similar reaction of the monoanion, the important difference of mechanism is that the reverse of the first step, k_2', is much faster, since it now involves the attack of a phenoxide anion rather than a neutral hydroxyl group on the acetyl group of the mixed anhydride, IX. The phenolate oxygen of IX is also a much better nucleophile than the carboxylate group, so that it is likely that $k_2' \gg k_3'$. In this case a rapid preequilibrium is set up, with the second stage, k_3', the formation of the 3-hydroxyphthalic anhydride anion, rate determining. The observed rate constant will therefore be approximately equal to $k_3'(k_1'/k_2')$. Since neither k_1' nor k_3' should be greatly affected by the change from monoanion to dianion, this effect on k_2' is decisive. Thus the much faster hydrolysis of the monoanion is a result of the more favorable equilibrium for the formation of the monoanion, rather than the dianion IX, of the mixed anhydride intermediate.

Series Nucleophilic Catalysis. The hydrolysis of the 3-acetoxyphthalate monoanion is subject to a highly efficient form of intramolecular catalysis, involving successive nucleophilic displacements at carbonyl centers. We have concluded that this is possible

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(12) A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **89**, 4853 (1967).

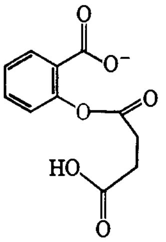
because the ester is in rapid equilibrium with the mixed anhydride V, which itself undergoes rapid, effectively irreversible, intramolecular nucleophilic displacement of acetate by the free carboxylate group.

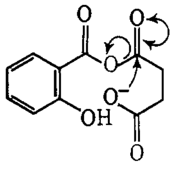
We consider that this type of catalysis, in which the acyl group is transferred, by successive nucleophilic displacements, from an unreactive to a highly reactive center, could be of general importance. This work has shown that the bond-making and -breaking processes can be fast enough for the reactive intermediate to be formed in a series of equilibria, disturbed only by the final, irreversible step. As long as all the steps involved are fast, therefore, their number is not limited to one, and more complex examples of series nucleophilic catalysis are to be expected, in which intermediate nucleophilic groups act only as carriers to relay the acyl group from its starting position to the reactive site.

In practice the requirement that the acyl transfers involved must be fast means that they must be either intramolecular, or between groups brought into very close proximity, as in an enzyme–substrate complex. Series nucleophilic catalysis is thus necessarily intramolecular catalysis under most conditions.

There seems no reason to doubt that similar reactions are possible involving nucleophilic and leaving groups other than phenolate and carboxylate, and we are investigating the possibility of such reactions. But it is clear that the carboxylate group is uniquely suited to series catalysis, since it becomes itself susceptible to nucleophilic attack when it acts as a nucleophile, converting the acyl group at the same time into a good leaving group. Our demonstration, in the case of 3-acetoxyphthalate, that the equilibria involved in the transfer of an acyl group to carboxylate from a relatively poor leaving group can be rapidly established, is therefore an important one.

The hydrolysis of 3-acetoxyphthalate provides an unambiguous example of series nucleophilic catalysis, since the second carboxyl group cannot interact with the acyl group until the first nucleophilic reaction has taken place. In cases where it is sterically possible for two catalytic centers to interact directly with the ester group alternative mechanisms are possible. The ester XI, for example, shows a pH–rate profile identical in form with that of 3-acetoxyphthalate, and the reactive monoanion is hydrolyzed at a very similar rate (about 3 min^{-1} at 25°). Morawetz and Oreskes[13] have interpreted this reaction in terms of bifunctional catalysis, with one carboxyl group assisting the attack of the second carboxylate group on the ester. In view of the efficiency of series intramolecular catalysis in the case of 3-acetoxyphthalate, the similar mechanism, involving the mixed anhydride intermediate XII is clearly a possibility in this case also. We believe that it is a strong possibility, since the rate-determining step should be the formation of the mixed anhydride in each case, and the similarity in rates is therefore predicted.


XI


XII

Implications for Enzymic Catalysis. We have considered[2] the anion of 3,5-dinitroaspirin as a model for an acyl enzyme with a second potentially nucleophilic center involved in the active site. The acyl group migrates rapidly between the two nucleophilic centers, and is hydrolyzed slowly relative to this process by routes involving general species catalysis of the attack of water on the acyl center by the free nucleophilic group or its conjugate acid. A weakness of this model is the relatively slow rate of the hydrolysis reaction; our results with 3-acetoxyphthalate show that problem can be largely overcome if both nucleophilic centers are carboxylate groups, or if the active site contains three nucleophilic centers, two of which are carboxyl groups. This ester can be regarded, therefore, as a model for an enzyme–substrate complex or an active site, since the processes concerned require only a degree of pH control and the proximity of the two carboxyl groups and the ester group.

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[13] H. Morawetz and I. Oreskes, *J. Amer. Chem. Soc.*, **80**, 2591 (1960).