Intramolecular Nucleophilic Catalysis of Ester Hydrolysis by the Ionized Carboxyl Group. The Hydrolysis of 3,5-Dinitroaspirin Anion

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Abstract: Oxygen-18 is incorporated into the 3,5-dinitrosalicylic acid produced on hydrolysis of acetyl 3,5-dinitrosalicylate in the enriched solvent; methyl 3,5-dinitrosalicylate is the major product of solvolysis in 50% aqueous methanol. This evidence, together with the kinetic data, is consistent with a mechanism for hydrolysis involving intramolecular nucleophilic attack by the neighboring carboxylate group, to form a mixed salicylic acetic anhydride intermediate. This is kinetically significant only in the hydrolysis reaction; intermolecular reactions with oxy anion nucleophiles involve uncatalyzed nucleophilic attack on the ester. Comparison with model compounds shows that intramolecular catalysis causes an increase of at least 50 times in the rate of hydrolysis of 3,5-dinitroaspirin. The slow step is the hydrolysis of the anhydride intermediate, even though this is itself subject to intramolecular general base catalysis. A general discussion of the point of changeover from nucleophilic to general base catalysis leads to the conclusion that this point is displaced in a predictable way in intramolecular catalysis relative to its position in intermolecular reactions. It appears that the nucleophilic mechanism is important in the hydrolysis of 3,5-dinitroaspirin only because the hydrolysis of the anhydride intermediate is catalyzed. This mechanism, involving two separate intramolecular catalytic processes, approaches the order of complexity associated with some enzymic reactions.

We showed recently[1] that the carboxylate group of aspirin is involved in the hydrolysis of the neighboring ester function as a general base, rather than as a nucleophile. Oxy anions, such as acetate and phosphate, also catalyze the hydrolysis of aspirin, and of monosubstituted acetylsalicylic acids,[2] and these intermolecular reactions also involve general base catalysis.

In symmetrical reactions of this sort, in which one oxy anion catalyzes the displacement of another from the carbonyl group, the choice between general base catalysis and the direct displacement of the nucleophilic mechanism depends largely on the relative basicities of the nucleophile and the leaving group. Gold[3] has shown that acetate does not catalyze the hydrolysis of substituted phenyl acetates by the nucleophilic mechanism if the leaving group is more than 3–4 pK units more basic than the catalyst. In the case of aspirin the leaving group is at least 6–7 pK units more basic than the carboxylate group.[1]

By an appropriate choice of substituents it is possible to lower the basicity of the leaving group of aspirin relative to that of the carboxylate group. Two nitro groups *ortho* and *para* to the phenolic oxygen would be expected to reduce its basicity by up to 6 pK units, while reducing the pK_a of the carboxyl group by only some $2 \times \sigma_m = 1.4$ units. This change in relative basicity could be sufficient to change the mechanism of hydrolysis of acetyl-3,5-dinitrosalicylic acid to predominantly intramolecular nucleophilic catalysis. Intermolecular catalysis of the hydrolysis of this ester by oxy anions would certainly be expected to involve nucleophilic attack, since this has been shown to be the mechanism of the reaction of acetate with 2,4-dinitrophenyl acetate. In this paper we describe the evidence that both inter- and intramolecular catalysis of the hydrolysis of acetyl-3,5-dinitrosalicylic acid do involve nucleophilic mechanisms, and discuss the changeover from nucleophilic to general base catalysis, with special reference to intramolecular reactions.[4]

Experimental Section

Materials. Inorganic salts were either of analytical grade or were purified before use. Distilled water was glass distilled twice more and dioxane dried over CaH_2 before distillation from $LiAlH_4$. 3-Nitro-, 5-nitro-, and 3,5-dinitrosalicylic acids were obtained commercially, and were acetylated by the method of Ciampa.[5] The 5-nitroaspirin has been described previously.[2] Acetyl-3-nitrosalicylic acid had mp 109–110° (from chloroform). *Anal.* Calcd for $C_9H_7NO_6$: C, 48.0; H, 3.11; N, 6.23. Found: C, 48.15; H, 3.30; N, 6.12.

Acetyl-3,5-dinitrosalicylic acid had mp 93–94° dec from aqueous ethanolic HCl. *Anal.* Calcd for $C_9H_6N_2O_8$: C, 40.00; H, 2.22; N, 10.38. Found: C, 40.06; H, 2.44; N, 10.34.

Methyl 3,5-dinitrosalicylate was prepared by methanolysis of the chloride, generated from the salicylic acid using thionyl chloride, mp 128–129° from ethanol (lit.[6] mp 128°).

Methyl acetyl-3,5-dinitrosalicylate was prepared by acetylating methyl 3,5-dinitrosalicylate by heating at 70° for 1.5 hr in acetic anhydride containing a trace of concentrated H_2SO_4. On recrystallization from ethanol it had mp 82–82.5°. *Anal.* Calcd for $C_{10}H_8N_2O_8$: C, 42.25; H, 2.82; N, 9.86. Found: C, 42.36; H, 3.12; N, 9.83. 2,4-Dinitrophenyl acetate was prepared by Chattaway's method,[7] using an excess of acetic anhydride, and had mp 70.5–71.5° (lit.[8] mp 72°).

Acetic 2-methoxy-3,5-dinitrobenzoic anhydride was prepared by passing ketene into an ice-cooled solution of 2-methoxy-3,5-dinitrobenzoic acid[9] (400 mg) in the minimum volume of ether (15 ml). After 30 min, monitoring by ir indicated that reaction was complete, and the anhydride crystallized when the apparatus was dismantled. The near-quantitative yield of anhydride obtained in this way had mp 80–80.5°, unchanged on recrystallization from ether. *Anal.* Calcd for $C_{10}H_8N_2O_8$: C, 42.25; H, 2.82; N, 9.86. Found: C, 42.25; H, 2.88; N, 10.1.

(1) A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **89**, 4857 (1967).
(2) A. R. Fersht and A. J. Kirby, *ibid.*, **89**, 4853 (1967).
(3) D. G. Oakenfull, T. Riley, and V. Gold, *Chem. Commun.*, 1966, 385.
(4) Preliminary communication: A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **89**, 5960 (1957).
(5) G. Ciampa, *Ann. Chim.* (Rome), **54**, 975 (1964).
(6) Th. Zncke, *J. Prakt. Chem.*, **82**, 23 (1910).
(7) F. D. Chattaway, *J. Chem. Soc.*, 2495 (1931).
(8) J. Blanksma, *Chem. Weekbl.*, **6**, 725 (1909).
(9) F. Ullmann, *Ann.*, **366**, 32 (1909).

Kinetic Methods and Results

The hydrolysis reactions of the acetates were measured by following the release of the phenolate anion at the wavelengths listed in Table I, at 39 ± 0.05° and ionic strength 1.0 (KCl). The hydrolysis rates of the mononitro compounds were measured by following the initial rates of release of phenolate anion, as described previously.[2] The reactions of the dinitro compounds were followed for three to four half-lives, and end points taken after at least ten, and gave excellent pseudo-first-order semilogarithmic plots. Reactions were started by adding 0.10 ml of a stock solution of the ester to 3.0 ml of the reaction mixture, in cuvettes in the thermostated cell compartment of the Zeiss PMQ II spectrophotometer. The stock solutions were in water, except for those of the methyl esters, which were dioxane. Thus the reactions of the methyl esters were measured in solutions containing a little more than 3% of dioxane.

The pH of the reaction mixture was measured at the end of each run at 39°, using a Vibron electrometer fitted with an E.I.L. C-33B pH-measuring attachment and a Pye-Ingold combined glass-reference electrode.

Buffer catalysis was measured by varying the concentration of the sodium salt of the oxy anion, usually in a 0.05 *M* phosphate carrier buffer, at pH 6.4. This procedure avoids competition from the faster reactions with the aspirin free-acid, described in the following paper.[10] The second-order rate constants were obtained as the slopes of excellent linear plots of observed first-order rate constants against oxy anion concentration. The results are summarized in Tables II, III, and IV. Rate constants for the hydrolysis reaction of 3,5-dinitroaspirin were obtained by extrapolation of the appropriate second-order plots to zero buffer concentration, and are given in Table V.

Table I. Wavelengths Used to Follow the Release of Phenolate Anions

Phenolate anion[a]	λ,[c] mμ
3,5-Dinitrosalicylate	338
	348[b]
Methyl 3,5-dinitrosalicylate	366
3-Nitrosalicylate	347
5-Nitrosalicylate	317
2,4-Dinitrophenolate	360

[a] These are all monoanions, except for 3,5-dinitrosalicylate at high pH (second $pK_a \sim 7$); see note *b*. [b] Isosbestic point for mono- and dianion. [c] Wavelength corresponds to an absorption maximum for the monoanion, unless otherwise stated.

Table II. Second-Order Rate Constants for Catalysis by Oxy Anions of the Hydrolysis of 3,5-Dinitroaspirin at 39° (Ionic Strength 1.0)

Oxy anion[a]	pK_a[b]	pH range	Concn range	No. of runs	$k_2 \times 10^2$, M^{-1} min^{-1}
Chloroacetate	2.87	6.4[c]	0–0.8	4	0.25 ± 0.06
Phenoxyacetate	3.12	6.4[c]	0–0.8	4	0.89 ± 0.18
Methoxyacetate	3.53	6.4[c]	0–0.8	4	1.04 ± 0.04
m-Bromobenzoate	3.81	6.4[c]	0–0.8	4	1.73 ± 0.11
Formate	3.75	6.4[c]	0–0.8	4	9.80 ± 0.13
2-Chloropropionate	4.08	6.4[c]			
Acetate	4.76	5.6–5.7[d]	0.1–0.8	6	3.08 ± 0.12
Acetate at 46.9°		5.6–5.7[d]	0.1–0.8	6	6.24 ± 0.10
Acetate at 55.2°		5.6–5.7[d]	0.1–0.8	6	12.93 ± 0.14
Acetate in D_2O			0.1–0.8	6	2.83 ± 0.03
Succinate	5.64	6.4[c]	0–0.4	4	7.60 ± 0.34
Phosphate	7.21	6.4–6.6[e]	0.025–0.75	7	11.11 ± 0.22
Carbonate	10.4	8.75[f]	0.01–0.05	5	215 ± 8
Hydroxide	15.7	8.7–10.3[g]	...	5	(919 ± 20) × 10^2

$\Delta H_{av}^{\ddagger}$ for acetate catalysis = 17.4 kcal/mol; $\Delta S_{39}^{\ddagger}$ for acetate catalysis = −17.8 eu

[a] Na salts used throughout. [b] pK_a of conjugate acid (thermodynamic). [c] In 0.05 *M* phosphate buffer, 50% monoanion, 50% dianion. [d] 91% free base buffer. [e] 50% free base buffer. [f] 9% free base buffer. [g] In carbonate buffers.

Table III. Second-Order Rate Constants for the Reactions of Oxy Anions with Methyl-3,5-dinitroaspirin, at 39° (Ionic Strength 1.0)

Oxy anion	pH	Concn range	No. of runs	k_2, M^{-1} min^{-1}
(H_2O)	6.4	(55)	3	6.65 × 10^{-6}
Chloroacetate	5.6[a]	0.1–0.9	3	0.017
Methoxyacetate	6.4	0.1–0.9	3	0.055
Formate	4.6	0.1–0.5	5	0.675
Acetate	5.6–5.7	0.1–0.5	5	0.219
Phosphate	6.4–6.6	0.025–0.225	3	0.112
Carbonate	8.9	0.005–0.05	10	4.10
Hydroxide	9.4–10.0	...	5	1940

[a] In acetate carrier buffer, 91% sodium acetate–9% acetic acid.

Table IV. Second-Order Rate Constants for the Reactions of Oxy Anions with 2,4-Dinitrophenyl Acetate at 39° (Ionic Strength 1.0)

Oxy anion	Concn range	No. of runs	k_2, M^{-1} min^{-1}
Cyanoacetate[a]	0–0.8	4	1.6 × 10^{-3}
Chloroacetate[b]	0–0.8	4	3.7 × 10^{-3}
Methoxyacetate	0–0.8	4	2.4 × 10^{-2}
Formate	0–0.8	4	0.20
β-Chloropropionate	0–0.8	4	5.2 × 10^{-2}
Acetate	0–0.8	4	0.12
Phosphate[c]	0.025–0.225	4	0.70

[a] In acetate carrier buffer, pH 5.6. [b] In phosphate carrier buffer at pH 6.4–6.6. [c] Phosphate buffer, 50% free base, pH 6.4–6.6.

Hydrolysis in $H_2{}^{18}O$. The extent of incorporation of ^{18}O into the salicylic acid produced on hydrolysis in the enriched solvent was measured for 3,5-dinitroaspirin, and for the 3- and 5-mononitro compounds, by calculating the relative increase in the M + 2 peaks in the mass spectrum, as described previously for

(10) A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **90**, 5826 (1968).

Table V. Hydrolysis Data for 3,5-Dinitroaspirin at 39° (Ionic Strength 1.0)

Buffer[a]	pH	$k_0 \times 10^2$,[b] min^{-1}
Acetate	5.6–5.7	2.71 ± 0.06
Acetate in D_2O	c	1.32 ± 0.02
Acetate at 46.9°	5.6–5.7	5.39 ± 0.05
Acetate at 55.2°	5.6–5.7	10.67 ± 0.07
Phosphate	6.4–6.6	2.68 ± 0.02
Hydrolysis rate, corrected for ionization and hydroxide attack		2.68 ± 0.02
$\Delta H_{av}^{\ddagger}$ = 16.7 kcal/mol, $\Delta S_{39}^{\ddagger}$ = −20.6 eu		

[a] For conditions see Table I. [b] By extrapolation to zero buffer concentration. [c] pH 5.6–5.7 in water.

aspirin.[1] Incorporation was negligible in the case of the mononitro compounds, which were hydrolyzed in 22.5% enriched $H_2{}^{18}O$; but considerable for 3,5-dinitroaspirin, which was hydrolyzed in the 11% enriched solvent. 3,5-Dinitrosalicylic acid gives a strong molecular ion peak, and the results are more accurate than for the mononitro compounds, which gave rather weak molecular ion peaks. The results of these experiments appear in Table VI.

Table VI. Incorporation of ^{18}O into the Salicylic Acids Produced on Hydrolysis of Substituted Aspirins in $H_2{}^{18}O$[a]

Ester	Incorporation, %
3,5-Dinitroaspirin	39 ± 1[b]
3,5-Dinitrosalicylic acid[c]	0 ± 0.2
3-Nitroaspirin	0.4 ± 0.3
5-Nitroaspirin	0.18 ± 0.12
Aspirin	0.05 ± 0.04[d]

[a] Incubated for 15 half-lives; at 39°, ionic strength 1.0, acetate buffer pH 5.6. [b] Actual figure (18.9 ± 0.2%) corrected for acetate-catalyzed reaction, which is assumed not to lead to incorporation. [c] Control experiment to estimate possible incorporation after hydrolysis is complete. [d] Datum from ref 1.

Hydrolysis in Aqueous Methanol. 3,5-Dinitroaspirin was hydrolyzed in 50% v/v aqueous methanol, 0.05 *M* in phosphate buffer (50% free base, pH 6.4 in water), and the ratio of salicylic acid to methyl ester produced measured spectrophotometrically, in the following way. 3,5-Dinitrosalicylate and its methyl ester have an isosbestic point at 342 mμ (ε 11,465). The absorbance of the solvolysis mixture was therefore measured at this wavelength to obtain the initial concentration of aspirin. Measurements of the absorbance at two further wavelengths, 300 and 368 mμ, which are points of maximum difference in absorbtivity between the salicylate ester and anion, then gave two values for the methyl ester concentration, with a mean amounting to 60 ± 2% of solvolysis products.

This experiment was repeated on a preparative scale. A 1.4-g sample of acetyl-3,5-dinitrosalicylic acid was dissolved with warming in 100 ml of 50% v/v aqueous methanol, containing 0.5 *M* phosphate buffer, 50% free base. After standing for 10 min (at least ten half-lives) at 39°, acidification with concentrated HCl precipitated a 56% yield of methyl 3,5-dinitrosalicylate, identical by melting point, mixture melting point, and ir spectrum with an authentic sample (see above).

A few kinetic measurements in the mixed solvent established that the solvolysis rate increases considerably with increasing methanol concentration.

Hydrolysis of Acetic 2-Methoxy-3,5-dinitrobenzoic Anhydride. The hydrolysis of acetic 2-methoxy-3,5-dinitrobenzoic anhydride was conveniently followed at 25°, at which temperature the fastest reaction measured had a half-time of about 20 sec. The procedure was as described above for methyl-3,5-dinitroaspirin, using the wavelength of maximum change in absorbance at 325 mμ. Excellent pseudo-first-order plots at 0.01, 0.02, and 0.03 *M* acetate (concentrations of free base in 50% free base buffer, pH 4.3) gave a second-order rate constant for acetate catalysis of 25.4 ± 0.2 M^{-1} min^{-1}. Extrapolation to zero buffer concentration gave the hydrolysis rate constant, 0.142 min^{-1}.

The aromatic product from the solvolysis of the anhydride in 50% aqueous methanol were measured spectrophotometrically. For the neutral solvolysis 6.8 mg of anhydride in 1 ml of dioxane was added to 50% ml of 50% v/v aqueous methanol. After 15 min the volume was made up to 250 ml. A similar experiment was carried out using aqueous methanol 1 *N* in HCl. Subsequent operations were identical in the two cases.

Five milliliters of this 250 ml of solution was added to 5 ml of aqueous 2 *M* NaOAc. The absorbances of these solutions were then measured at 280 (isosbestic point of methyl ester and carboxylate anion), 320, and 325 mμ (points of maximum difference in absorbtivity between the two species). Comparison with separate solutions of the same concentrations of 2-methoxy-3,5-dinitrobenzoate and methyl ester allowed the calculation of the composition of the reaction products.

The percentages of methyl ester obtained in this way were (for hydrolysis in 1 *N* acid) 11.9 ± 0.5% and (for neutral hydrolysis) 15.3 ± 0.1%.

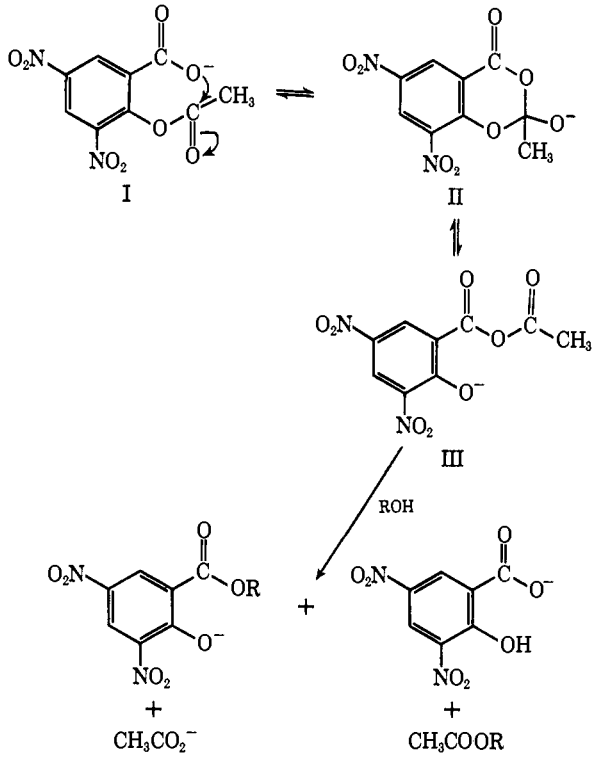
Discussion

The rate of hydrolysis of 3,5-dinitroaspirin, like that of aspirin itself,[11] is independent of pH between pH 4 and 8.[10] In this case it is not self-evident that hydrolysis is catalyzed by the ionized carboxyl group, because the anion is hydrolyzed less rapidly than the free acid.[10] There are in fact several pieces of evidence which show that the hydrolysis of the anion is some 50 times faster than expected, and these are referred to below. But the results of the experiments in $H_2{}^{18}O$ and aqueous methanol, described above, show conclusively that the carboxyl carbonyl group is involved in the hydrolysis mechanism, because it acylates the solvent during the course of the reaction. No mechanism that does not involve the aryl carboxylic acid group could lead to the incorporation of ^{18}O into the 3,5-dinitrosalicylic acid produced on hydrolysis.

Furthermore, the acylation of the solvent requires covalent bond formation to one of the carboxylate oxygen atoms during the course of the reaction, and of the three kinetically equivalent mechanisms possible in the pH-independent region[1] this observation is uniquely consistent with intramolecular nucleophilic catalysis. Thus at least 39% of hydrolysis in water and more than 60% of solvolysis in 50% aqueous methanol goes by the mechanism given in Scheme I. This is the classical mechanism for intramolecular nucleophilic catalysis, as proposed originally for the hydrolysis of aspirin (for a list of references see ref 1), but does not

(11) L. J. Edwards, *Trans. Faraday Soc.*, 46, 723 (1950).

Scheme I



specify which step is rate determining. It seems unlikely that the breakdown of the tetrahedral intermediate (II) will be slow, because it bears two good leaving groups; the choice appears to be between the initial nucleophilic attack, a unimolecular reaction, and the bimolecular solvolysis of the anhydride III, which would presumably be present in very low concentration.

The kinetic data are consistent with rate-determining attack on the anhydride. Thus the solvent isotope effect, $k_H/k_D = 2.05$ (Table II), is significantly greater than that expected for nucleophilic attack by an oxy anion ($k_H/k_D = 1.3$ for intermolecular attack by acetate ion, as discussed below); the entropy of activation for the hydrolysis reaction is −20.6 eu (Table II), a value consistent with a bimolecular rate-determining step;[12] and the rate of solvolysis increases rapidly at the concentration of methanol in the mixed aqueous solvent is increased. All these figures are closely similar to those observed for the hydrolysis of aspirin,[1] for which k_H/k_D is 2.2, $\Delta S^{\ddagger}$ is −22.6 eu, and the solvolysis rate is increased by factors of 3.39 and 10.12, relative to water, on going to 20 and 50% methanol. The corresponding increases for 3,5-dinitroaspirin (Table VII) are almost identical, the factors being 3.39 and 10.04.

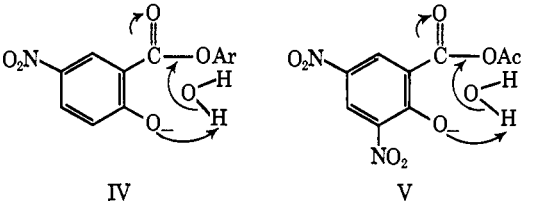
Table VII. Rate Constants for Solvolysis of 3,5-Dinitroaspirin in Water–Methanol Mixtures at 39° (Ionic Strength 0.1)[a]

Vol % MeOH	k_{obsd}, min^{-1}	k/k_{H_2O}
0	2.68×10^{-2}	1.0
20	9.08×10^{-2}	3.39
50	26.92×10^{-2}	10.04

[a] Ionic strength and pH control maintained with 0.05 *M* phosphate buffer, 50% free base.

(12) The entropy of activation for any mechanism involving a rate-determining bimolecular reaction of the anhydride will be a composite figure, containing both $\Delta S^{\ddagger}$ for the slow step and ΔS for the equilibrium I ⇌ III. This argument assumes that the entropy change for the equilibrium is small.

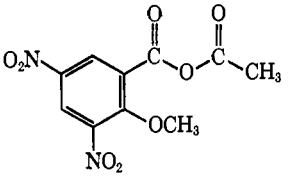
Quantitatively, therefore, the reaction closely resembles a hydrolysis known to involve intramolecular general base catalysis. This is not inconsistent with rate-determining hydrolysis of the anhydride anion III, since it is well established that the phenolate oxygens of salicylate ester anions act as general bases to catalyze the hydrolysis of the neighboring ester group.[13] Thus the hydrolysis of *p*-nitrophenyl 5-nitrosalicylate involves the mechanism represented by IV[13a] so that



the hydrolysis of the anhydride III can reasonably be expected to involve the similar process V.

There is direct evidence that the attack of water on the anhydride must be catalyzed, if this is the rate-determining step. A comparison of the relative rates of attack of water and other nucleophiles on 3,5-dinitroaspirin and its methyl ester (see below) shows that only the attack of water is catalyzed. All the other nucleophiles involved are oxy anions which are more strongly nucleophilic than water, and would attack the anhydride intermediate III more rapidly than would water in the absence of catalysis. Since the anhydride is not kinetically significant for the attack of other nucleophiles, its hydrolysis must be selectively catalyzed, as expected for intramolecular general base catalysis (mechanism IV).[13]

This argument is based on the assumption that the rate-determining step in the hydrolysis of 3,5-dinitroaspirin is the attack of water on the anhydride. There is other evidence to suggest that the attack of water on the anhydride III is catalyzed, which does not require this assumption. We have made the anhydride VI, and have studied its solvolysis in water and in 50% aqueous methanol. In the mixed solvent the yield of methyl 2-methoxy-3,5-dinitrobenzoate was 15%. Neglecting the water reaction, which is considerably slower than methanolysis under these conditions, this figure is similar to the 25% of hydrolysis of acetic benzoic anhydride going by attack on the benzoyl carbonyl group, under similar conditions.[14] The aromatic carbonyl carbon of VI is more reactive than that of a benzoyl group, but probably sterically hindered.



VI

Solvolysis of 3,5-dinitroaspirin, by way of the anhydride III, gives 60% of methyl 3,5-dinitrosalicylate under these conditions. The electronic effect of the *o*-phenolate oxygen will be to *reduce* the reactivity of

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

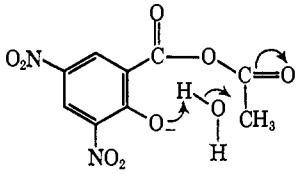
(14) M. L. Bender and M. C. Neveu, *J. Amer. Chem. Soc.*, **80**, 5388 (1958).

the salicoyl carbonyl group of III to nucleophilic attack, relative to that of the model compound VI. So the fact that methyl 3,5-dinitrosalicylate is the major product of solvolysis in 50% aqueous methanol is a strong indication that there is catalysis of the attack of solvent on the salicoyl carbonyl group of the anhydride intermediate, III.

Using the kinetic results obtained with the anhydride VI, it is possible to estimate the efficiency of this catalysis. The second-order rate constant for the attack of acetate ion is 25.4 M^{-1} min^{-1}. The figure for attack on 3,5-dinitroaspirin is 7.9×10^{-3} M^{-1} min^{-1}. Assume now that the two anhydrides, III and VI, show similar reactivity toward acetate (this is reasonable, since nucleophilic attack can only lead to catalysis of hydrolysis if it occurs at the acetyl carbonyl group); then the concentration of III in solution during the hydrolysis of 3,5-dinitroaspirin cannot be greater than $7.9 \times 10^{-3}/25.4$, or some 0.03% of that of the ester. Now even if it were present at this maximum concentration, III would have to be hydrolyzed 17 times more rapidly than the model compound VI to account for the observed rate of hydrolysis of 3,5-dinitroaspirin anion. Since there is no evidence that the anhydride III is an intermediate in the reaction of acetate with 3,5-dinitroaspirin, the actual concentration of anhydride in solution is probably significantly less than the maximum value, obtained by assuming that the acetate reaction involves the anhydride route exclusively. So the hydrolysis of the anhydride intermediate III appears to be accelerated by a factor greater, and probably considerably greater, than 17.

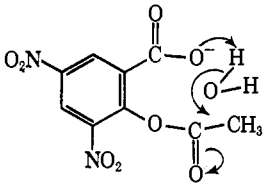
One implication of this result is particularly noteworthy. We estimate below that intramolecular catalysis causes an increase of only some 50 times in the rate of hydrolysis of 3,5-dinitroaspirin. It is apparent, therefore, that this factor could be entirely accounted for by the acceleration of the hydrolysis of the anhydride intermediate III. In other words, the nucleophilic pathway could be significant only because the hydrolysis of the anhydride is catalyzed.

To summarize thus far, we consider that a major reaction pathway in the hydrolysis of 3,5-dinitroaspirin involves the rapid, preequilibrium formation of a low concentration of the anhydride III (Scheme I), followed by its hydrolysis in a rate-determining step which involves intramolecular general base catalysis by the displaced phenolate group, as in V. It is not clear, however, that this is the only important reaction path. The experiment in $H_2{}^{18}O$ shows that 39% of the reaction goes by hydrolysis of the anhydride III at the salicoyl carbonyl group, but attack at the acetyl carbonyl group of III does not necessarily account for the remaining 61% of reaction. If, for example, intramolecular general base catalysis of the hydrolysis of the anhydride III were specific for attack at the salicoyl carbonyl group, as shown in V, then hydrolysis could involve virtually *exclusive* attack at this position. This does not in fact seem likely, since the phenolate oxygen can also catalyze the attack of water on the acetyl carbonyl group of the anhydride, as in VII.[15] But we cannot rule out the possibility that the hydrolysis of 3,5-



VII

dinitroaspirin anion involves a significant contribution from intramolecular base catalysis by the carboxylate group, VIII, as proposed for aspirin itself.[1] It is rele-



VIII

vant that there is no significant incorporation of ^{18}O into the salicylic acids produced from 3-nitro- or 5-nitroaspirin on hydrolysis in $H_2{}^{18}O$ (Table VI), so that the changeover from general base to nucleophilic catalysis does occur after the introduction of the first nitro group. Since the two mechanisms likely to be involved are expected to have very similar characteristics, no clear distinction can be drawn on kinetic grounds. We can, however, make a rough estimate of the expected rate of the general base-catalyzed reaction, by assuming that the effects of the 3-nitro and 5-nitro groups on log k_{hyd} for aspirin are additive. The hydrolysis rate constants for 3-nitro- and 5-nitroaspirin are 4.65×10^{-3} and 1.40×10^{-3} min^{-1}, respectively (data from this study and ref 2), and lead to an estimate of 9.8×10^{-3} min^{-1} for the general base catalyzed hydrolysis of 3,5-dinitroaspirin. This is 37% of the observed hydrolysis rate, so that this calculation strengthens the possibility that the hydrolysis of 3,5-dinitroaspirin involves both nucleophilic and general base catalysis.

Reactions with Oxy Anions. The hydrolysis of 3,5-dinitroaspirin is catalyzed by the anions of carboxylic and other oxy acids, but the data are not satisfactorily correlated by the Brønsted equation (Figures 1 and 2). The Brønsted plot for the reactions with anions of oxy acids with pK_a's between 2 and 7 (Figure 1) shows marked curvature, and the point for formate anion shows a large positive deviation (see below). This behavior is difficult to explain if the oxy anions are acting as general bases, but is not unexpected for reactions involving nucleophilic catalysis. Thus Jencks has shown[16] that the Brønsted plots correlating data for nucleophilic catalysis by oxy anions of the hydrolysis of acetate esters with a wide range of leaving group are generally nonlinear. The slopes of the lines for nucleophiles with pK values less than the pK of the leaving group are steeper than those for nucleophiles with pK values larger than the pK of the leaving group, as observed here.

(15) This process would be intramolecular general base catalysis through an eight-membered cyclic transition state, with very similar steric and electronic requirements to that for the general base catalyzed hydrolysis of aspirin.[1]

(16) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968). Our own data are not extensive enough to establish conclusively that Brønsted plots of the data for nucleophilic catalysis of ester hydrolysis by oxy anions are naturally curved: a fortuitous combination of steric effects could conceivably be the cause of the observed nonlinearity. We are directly concerned to establish only the similarities in behavior of 3,5-dinitroaspirin and model compounds, where the mechanism of catalysis is not in doubt.

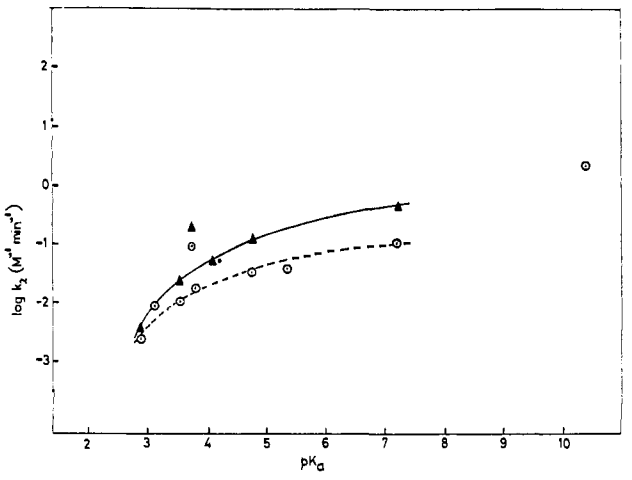


Figure 1. Brønsted plots comparing catalysis by oxy anions of the hydrolysis of 2,4-dinitrophenyl acetate (▲) and 3,5-dinitroaspirin anion (○); data from Tables II and IV are for (in increasing order of basicity) chloroacetate, phenoxyacetate, methoxyacetate, formate (positive deviation), *m*-bromobenzoate, acetate, succinate, phosphate, and carbonate.

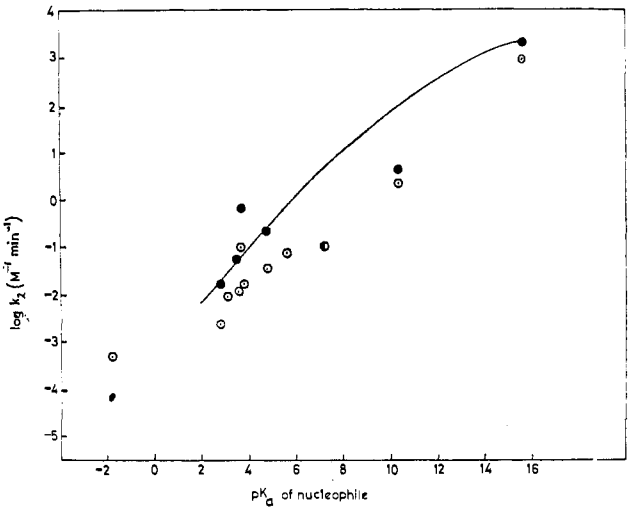
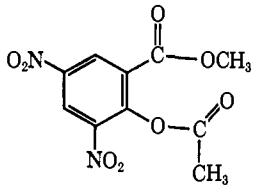


Figure 2. Brønsted plots comparing second-order rate constants for catalysis by oxy anions of the hydrolysis of 3,5-dinitroaspirin anion (○) and its methyl ester VI (●); data from Tables II and V are for (in increasing order of basicity) water, chloroacetate, phenoxyacetate, methoxyacetate, formate, *m*-bromobenzoate, acetate, succinate, phosphate, carbonate, and hydroxide. The curve drawn through the points for the methyl ester has the form expected for a Brønsted plot for an ester of this type.[16]

To illustrate and confirm this point we have compared the data for the reactions of oxy anions with 3,5-dinitroaspirin with those for attack on two representative model compounds. These are the methyl ester, IX, and 2,4-dinitrophenyl acetate. Catalysis of the hydrolysis



IX

of 2,4-dinitrophenyl acetate by acetate ion is known to involve the nucleophilic mechanism,[3] and its reactions with oxy anions of similar and higher basicity will be expected to do so also.

In Figure 1 the data for the reactions of oxy anions with 3,5-dinitroaspirin anion are compared with those for attack on 2,4-dinitrophenyl acetate. It is evident that the reactivity of the two esters is closely similar, both qualitatively and quantitatively. The Brønsted plots show similar curvature, and the same positive deviation for the point for formate. We conclude that intermolecular nucleophilic catalysis is involved in each case.

Other kinetic evidence is consistent with this interpretation. The solvent deuterium isotope effect for acetate catalysis of the hydrolysis of 3,5-dinitroaspirin is 1.3 (Table II), similar to that (1.4) found for the nucleophilic component of the acetate-catalyzed hydrolysis of *p*-nitrophenyl acetate.[3] The same authors[3] measured the entropies of activation for the two components of this reaction and obtained values of −16 and −39 eu, respectively, for the nucleophilic and general base catalyzed pathways. The entropy of activation for the acetate-catalyzed hydrolysis of 3,5-dinitroaspirin is −17.8 eu (Table II).

Figure 2 is a Brønsted plot using data for a wider range of nucleophiles, comparing reactivity toward 3,5-dinitroaspirin anion with that toward its methyl ester, IX. This ester reacts at about the same rate as 2,4-dinitrophenyl acetate with most oxy anions, but the Brønsted plot shows several irregularities. The positive deviation of the point for formate occurs as before, and indeed appears to be diagnostic for nucleophilic, as opposed to general base catalysis.[17,18] Phosphate dianion, on the other hand, shows a negative deviation, and attacks the methyl ester at the same rate as it attacks 3,5-dinitroaspirin itself, although other anions are up to seven times more reactive toward the methyl ester. It is likely that this deviation marks the appearance of steric hindrance; the oxygen atoms of the tetrahedral phosphate anion are more hindered than those of carboxylate ions, and the acetyl carbonyl group of the ester IX is adjacent to a seriously hindered position.

Apart from phosphate, oxy anions react with the methyl ester of 3,5-dinitroaspirin several times faster than with 3,5-dinitroaspirin itself. Acetate ion, for example, attacks the methyl ester IX twice as fast as it attacks 2,4-dinitrophenyl acetate, which is itself attacked about three times more rapidly than 3,5-dinitroaspirin. The effects of steric hindrance and electrostatic repulsion are clearly not large, and these relative rates are similar to those expected for reactions involving the same mechanism, which, it seems certain, is intermolecular nucleophilic catalysis. In particular, it is clear that the attack of acetate on 3,5-dinitroaspirin is not catalyzed significantly by the neighboring carboxylate group. So we can rule out any very large contribution from a pathway involving the anhydride III (Scheme I) as an intermediate.

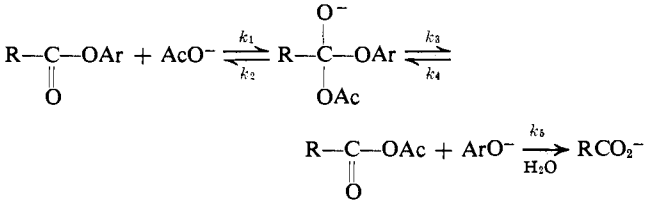
(17) In all three reactions described here formate shows a degree of reactivity expected for the anion of an oxy acid of pK_a near 6. It also shows positive[8] deviations from Brønsted plots for the data for SN2 reactions; for example, for the attack of oxy anions on chloroacetate.[18] So a simple test of mechanism is to compare the second-order rate constants for attack by formate and acetate; formate is generally more reactive as a nucleophile, but less reactive when acting as a general base.

(18) G. F. Smith, *J. Chem. Soc.*, 513 (1943).

The other important difference between the Brønsted plots for 3,5-dinitroaspirin and its methyl ester is that although oxy anions are several times more reactive toward the methyl ester, the reverse is the case for water. Thus the ratio k_{OAc}/k_0 for the methyl ester is 65 at 39°, a typical value for an ester of this type;[3] but k_{OAc}/k_0 for 3,5-dinitroaspirin is only 1.2. Thus the hydrolysis of the anion is 50 times faster than expected (strictly, the attack of water is catalyzed some 50 times more effectively than that of acetate ion), and this factor is a measure of the effectiveness of catalysis by the ionized carboxyl group.

Nucleophilic *vs.* General Base Catalysis. We have concluded above that the nucleophilic mechanism could well only be important in the hydrolysis of 3,5-dinitroaspirin because the hydrolysis of the anhydride intermediate is itself catalyzed. This would mean that the borderline between nucleophilic and general base catalysis was displaced to favor the former, and would otherwise lie so that 3,5-dinitroaspirin was hydrolyzed by a mechanism involving intramolecular general base catalysis. Now the pK_a of the carboxyl group of 3,5-dinitroaspirin is about 2, and the effective pK_a of the phenol group of the anhydride III will be between 3 and 4; so that a simple analogy with intermolecular catalysis, where acetate ion, for example, can displace a phenolate group which is 2.5 pK units more basic from its acetate ester,[3] would suggest that nucleophilic catalysis ought to be an important mechanism for the hydrolysis of this ester even if the hydrolysis of the anhydride were not catalyzed. In the light of our conclusions this analogy is evidently suspect, and it is of some interest to examine the problem of the change-over from nucleophilic to general base catalysis in rather general terms, with particular reference to the transition in intramolecular catalysis.

Several authors have distinguished the two types of catalysis of ester hydrolysis by oxy anions,[19] and have identified borderline cases which involve concomitant nucleophilic and general base catalysis. A well-authenticated example is the catalysis by acetate ion of the hydrolysis of *p*-nitrophenyl acetate.[3,20] Oakenfull, Riley, and Gold[3] have pointed out that a decisive factor is the partitioning of the tetrahedral intermediate X.



Nucleophilic catalysis will only be significant if the ratio k_3/k_2 is sufficiently large. If the tetrahedral intermediate breaks down preferentially to regenerate starting materials ($k_2 > k_3$), the addition step (k_1) cannot be rate determining. The slow step of the reaction will then be either the breakdown of the tetrahedral intermediate, or the attack of water on the anhydride. In intermolecular reactions it is probable that the breakdown of the tetrahedral intermediate is rate determining, but only because the concentration of water is so much greater than that of the ester (about 10^5 times greater under the pseudo-first-order conditions normally used in measurements of these reactions). This is apparent if we compare the attack of water on the anhydride with the *reverse* of the breakdown of the tetrahedral intermediate, which involves the attack of aryl oxide on the anhydride. The aryl oxide ion could be up to 10^{12} times more basic than water, but it would also be much more basic than that the group it displaces from the anhydride. Since such reactions are expected to be rather insensitive to the basicity of the nucleophile with a Brønsted slope of about 0.3,[16] this corresponds to an advantage of only 10^3 to 10^4 in rate constant. This is too small to overcome the concentration difference, so that the rate-determining step in intermolecular nucleophilic catalysis by an oxy anion which is a weaker base than the leaving group is expected to be the breakdown of the tetrahedral intermediate.

On the other hand, in intramolecular reactions of the sort discussed so far in this paper, where the leaving group remains attached to the molecule bearing the nucleophilic center (see Scheme I), the attack of aryl oxide on the anhydride is itself an intramolecular reaction, and would be expected[21] to be faster than the attack of water, which will then become rate determining. This should be a general point of difference in the mechanisms of inter- and intramolecular nucleophilic catalysis, at least by oxy anions, where the nucleophile is a weaker base than the group displaced.

This difference in the rate-determining step can account for the different positions of the borderline between nucleophilic and general base catalyzed mechanisms in inter- and intramolecular reactions, because it means that part of the advantage in rate of the intramolecular process is lost in the nucleophilic mechanism. The enhanced rates of intramolecular reactions, compared with the corresponding intermolecular processes, are due principally to their more favorable entropies of reaction, and these in turn reflect the higher probability of forming transition states in which fewer molecules are required to come together. In a given system the entropy advantage of the intramolecular reaction should be similar for both nucleophilic and general base catalysis, since the difference in each case is that the catalyst is already part of the molecule. In general base catalysis the rate-determining step is unlikely to be the breakdown of the tetrahedral intermediate, especially one formed from an aryl ester, and should be the initial attack on the carbonyl group for both inter- and intramolecular reactions. Thus the rate of the intramolecular reaction will be increased by a factor corresponding to the entropy advantage over the intermolecular process.

In the nucleophilic mechanism, on the other hand, although the breakdown of the tetrahedral intermediate (the slow step in the intermolecular reaction) will be accelerated by the same factor in the intramolecular process as is general base catalysis, the observed rate enhancement will be smaller, because another step, the attack of water on the anhydride, becomes rate determining. The hydrolysis of the anhydride is probably not significantly faster in the intramolecular reaction;

(19) S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 237 (1967).
(20) A. R. Butler and V. Gold, *J. Chem. Soc.*, 1334 (1962).

(21) In the case of 3,5-dinitroaspirin, for example, the initial intramolecular attack by the carboxylate anion on the ester group, which will certainly be slower than that of aryl oxide on the anhydride, is very much faster than the uncatalyzed attack by water.

its rate of formation is increased because it involves an intramolecular displacement, but so is the rate of the reverse reaction, for the same reason, so that the equilibrium concentration is unaffected.

The significant conclusion to be drawn from these arguments is that nucleophilic catalysis becomes less favorable, relative to general base catalysis, on going from an intermolecular to the corresponding intramolecular process. That is, the borderline between nucleophilic and general base catalysis is expected to be shifted in favor of the latter in intramolecular catalysis of this type.

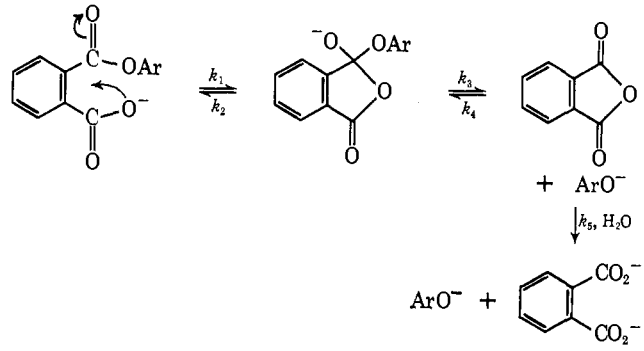
We have no way of estimating the relative advantage of intramolecular general base catalysis, but it is possible to set an upper limit. The entropy advantage of an intramolecular process often lies between 10 and 20 eu, giving a rate enhancement of some 10^3 to 10^4 times; this then is a maximum figure for the relative advantage of intramolecular general base catalysis, and corresponds to a change of up to 3–4 in the pK_a of the nucleophile or leaving group; since the slopes of the logarithmic plots of the rate constants for intermolecular nucleophilic catalysis against the pK_a of either nucleophile or leaving group both approach 1.0 as the leaving group becomes more basic than the nucleophile.[16]

The borderline, where nucleophilic and general base catalyzed reactions proceed side by side at equal rates, has been identified in the case of intermolecular catalysis by acetate ion of the hydrolysis of substituted phenyl acetates as falling where the pK_a of the leaving group is about 7.2,[3] some 2.5 pK units more basic than the nucleophile. If intramolecular nucleophilic catalysis is relatively less favorable by a factor corresponding to a difference of up to 3–4 in pK_a, the borderline in intramolecular catalysis should lie in the region of equal pK_a of nucleophile and leaving group. This is consistent with our conclusions in the case of 3,5-dinitroaspirin.

It should be stressed that this relative disadvantage of the nucleophilic mechanism occurs only in that class of intramolecular reactions in which the leaving group remains attached. If the leaving group is bound to the molecule only by the ester linkage, and is displaced completely by nucleophilic attack, the situation is different. Typical of this class of compound are derivatives of dicarboxylic acids.[22] Here, as before, attack on the ester should be rate determining in both inter- and intramolecular general base catalyzed reactions, and the intramolecular reaction will be accelerated by the amount corresponding to its more favorable entropy of activation. In the nucleophilic mechanism (Scheme II) the reactions represented by k_3, k_4, and k_5 are similar in the inter- and intramolecular reactions, but k_1 and k_2 are both changed. The initial attack, represented by k_1, is now an intramolecular reaction, and is therefore accelerated by the same factor as is intramolecular general base catalysis. But the reverse of the formation of the tetrahedral intermediate, k_2, is relatively slower in the intramolecular reaction (Scheme II) because of a less favorable entropy term; in the intermolecular reaction two molecules are produced from one in this step, whereas there is no change in the number of molecules in the intramolecular case.

(22) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966, p 173 ff.

Scheme II



Thus *both* the rate and the equilibrium constant for the formation of the tetrahedral intermediate, and thus of the anhydride also, are increased in intramolecular nucleophilic catalysis, relative to the intermolecular reaction, and the second factor constitutes a relative advantage of the nucleophilic mechanism over general base catalysis on going from an intermolecular to a intramolecular process. Thus the borderline in this type of intramolecular catalysis is also displaced, relative to that in intermolecular reactions, but this time in favor of the nucleophilic mechanism.

The entropy factor responsible for this advantage is again that saved by requiring one less molecule to join an aggregate, and will probably be of a similar magnitude, 10–20 eu, to that discussed previously, leading as before to a rate enhancement of 10^3 to 10^4 times. Consequently intramolecular nucleophilic catalysis would be expected to be more favorable in intramolecular reactions where the leaving group is lost, by a factor corresponding to a difference of 3–4 pK units in the basicity of the nucleophile or leaving group, compared with the corresponding intermolecular reactions. The borderline between nucleophilic and general base catalysis should therefore lie in the region where the leaving group is some 5.5–6.5 pK units more basic than the nucleophile. This limit just accommodates a group of cases of intramolecular catalysis, by the free carboxylate groups of the aryl half esters of dicarboxylic acids (such as succinic,[23] glutaric,[23] and phthalic[24] acids), which are known to involve the nucleophilic mechanism.

These arguments have been couched in general terms, and the figures quoted are not intended to indicate more than orders of magnitude. To simplify the discussion of mechanism we have often assumed that a single step is rate determining, even though in many cases there is little doubt that the kinetics will be complex, and that the rate of reactions will depend on several transition states. With these animadversions noted, the conclusions reached in this discussion can be simply summarized: the borderline between nucleophilic and general base catalysis by oxy anions of the hydrolysis of esters is known to lie in the region where the leaving group is some 2.5 pK units more basic than the nucleophile for intermolecular reactions. In intramolecular catalysis the borderline is displaced either way, depending on the class of compound concerned. If the leaving group remains attached, as in the case of

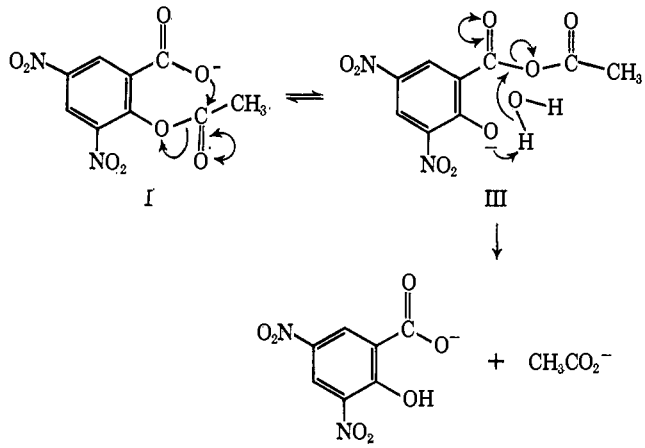
(23) E. Gaetjens and H. Morawetz, *J. Amer. Chem. Soc.*, **82**, 5328 (1960).

(24) T. C. Bruice and U. K. Pandit, *ibid.*, **82**, 5858 (1960); T. C. Bruice and J. Thanassi, *ibid.*, **88**, 747 (1966).

the hydrolysis of derivatives of salicylic acid, the nucleophilic catalysis becomes relatively less favorable, and the pK_a's of nucleophile and leaving group are approximately equal at the borderline. But if the leaving group is lost, as it is from derivatives of dicarboxylic acids, nucleophilic catalysis is relatively *more* favorable than in intermolecular reactions, and leaving groups up to about 6.5 pK units more basic than the nucleophile can be displaced.

Implications for Enzymic Catalysis. On the basis of the evidence and arguments presented in this paper we consider that the mechanism outlined in Scheme III is an important pathway for the hydrolysis of the anion of 3,5-dinitroaspirin. This mechanism achieves a potentiation of intramolecular nucleophilic catalysis of the hydrolysis of this ester by a second, independent, intramolecular process in which the leaving group of the first step catalyzes the further reaction of the intermediate formed. Such tight control and integration of consecutive intramolecular processes is a characteristic generally associated with enzymic catalysis. For example, the formation of an acyl enzyme with a second potentially nucleophilic group involved in the active site is probably a not uncommon situation. 3,5-Dinitroaspirin is a model for such a system; the acyl group migrates rapidly and reversibly between the two nucleophilic centers, and is hydrolyzed slowly relative to this process by one of several possible routes involving general species catalysis of the attack of water on the acyl group by the free nucleophilic center. In this paper we have shown that in the hydrolysis of 3,5-dinitroaspirin both nucleophilic centers can act as general base catalysts in this way. In the following paper we present evidence that the addition of a proton to the system, which might be expected to inhibit the nucleophilic mechanism, actually enhances catalysis.

Scheme III



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Intramolecular Nucleophilic Catalysis in the Hydrolysis of Substituted Aspirin Acids

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Contribution from the University Chemical Laboratory, Cambridge, England.
Received January 16, 1968

Abstract: The hydrolysis of acetyl 3,5-dinitrosalicylic acid is faster than that of the anion, even though the hydrolysis of the anion is already accelerated by intramolecular nucleophilic catalysis. The evidence does not support our earlier suggestion that this further acceleration is due to intramolecular general acid catalysis by the neighboring carboxyl group. Solvolysis of the acid in 50% aqueous methanol produces significant amounts of methyl 3,5-dinitrosalicylate, indicating that a mixed salicylic acetic anhydride is an intermediate, and therefore that intramolecular nucleophilic catalysis is involved in this case also. Catalysis is observed for the hydrolysis of aspirin itself, and for its monosubstituted derivatives, and is shown to assist the attack of other nucleophiles than water. Nucleophilic catalysis appears to be favored for the hydrolysis of the aspirin acids because of a more favorable equilibrium constant for the formation of the protonated form, rather than the anion, of the mixed anhydride intermediate.

We have shown that intramolecular catalysis of the hydrolysis of aspirin anion[1] and its singly substituted derivatives[2] involves the carboxylate group as a general base, and that the mechanism changes to nucleophilic catalysis for 3,5-dinitroaspirin.[3] This catalysis is apparent in the case of aspirin and the singly substituted compounds from their characteristic pH–rate profiles,[4] which show that the aspirin anions are hydrolyzed more rapidly than the protonated forms. The pH–rate profile for the hydrolysis of 3,5-dinitroaspirin (Figure 1) also shows a pH-independent region between pH 4 and 8, but in this case the free acid is considerably more reactive than the anion.[5] We have established that the hydrolysis of the anion is accelerated

(1) A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **89**, 4857 (1967).
(2) A. R. Fersht and A. J. Kirby, *ibid.*, **89**, 4853 (1967).
(3) A. R. Fersht and A. J. Kirby, *ibid.*, **90**, 5818 (1968).
(4) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).
(5) A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **89**, 5961 (1967); preliminary communication.