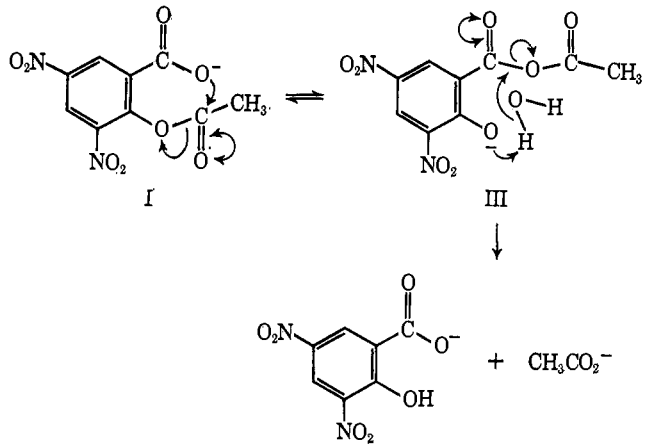
the hydrolysis of derivatives of salicylic acid, the nucleophilic catalysis becomes relatively less favorable, and the pK_a's of nucleophile and leaving group are approximately equal at the borderline. But if the leaving group is lost, as it is from derivatives of dicarboxylic acids, nucleophilic catalysis 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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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.

by about 50 times by intramolecular nucleophilic catalysis,[3] so that the hydrolysis of the free acid, which is some 30 times faster still, must be more strongly catalyzed, presumably by the free carboxyl group. Furthermore, this catalysis is presumably available also to those aspirin derivatives showing the classical pH–rate profile for hydrolysis. We have therefore investigated the scope, as well as the mechanism, of this catalysis.

Experimental Section

Materials. Inorganic salts were either purified reagent grade or analytical grade. The preparation of the substituted acetylsalicylic acids has been described previously.[2,3] Aspirin methyl ester was prepared from methyl salicylate and acetic anhydride containing a trace of concentrated H_2SO_4, and had mp 48.5–49° (lit.[6] mp 49°). Solvents were purified as before.[3]

Kinetic Methods and Results. The hydrolysis rates of the substituted aspirins were measured spectrophotometrically, as previously described,[3] at 39° and ionic strength 1.0, using the wavelengths listed in Table I. Final absorbances for runs followed by initial rates were measured by tenfold dilution with the correct buffer, maintained at ionic strength 1.0 with added KCl. The methyl esters were added from stock solutions in dioxane, so that the final reaction mixtures contained 3% of dioxane.

Table I. Rate Constants for the Hydrolysis of Substituted Aspirin Acids, at 39° (Ionic Strength 1.0)

Substituted salicylic acid[a]	Followed at (mμ)	$k_{hyd} \times 10^4$, min^{-1}	$k_{H^+} \times 10^3$, M^{-1} min^{-1}
Aspirin	298.5[b]	0.59 ± 0.02	4.72 ± 0.05
Aspirin, methyl ester	298.5[b]	0.038 ± 0.001	...
5-Chloroaspirin	311[c]	1.59 ± 0.18	4.28 ± 0.05
5-Bromoaspirin	315[c]	1.50 ± 0.16	4.32 ± 0.04
5-Methoxyaspirin	330[d]	0.52 ± 0.02	5.30 ± 0.07
4-Chloroaspirin	304[c]	2.1 ± 0.25	3.88 ± 0.07
4-Bromoaspirin	302[c]	1.95 ± 0.20	3.96 ± 0.05
4-Methoxyaspirin	297[d]	0.79 ± 0.01	4.35 ± 0.04
4-Nitroaspirin	347[d]	4.98 ± 0.32	3.88 ± 0.08
3-Nitroaspirin	347[d]	32.5 ± 0.6	1.02 ± 0.02
5-Nitroaspirin	317[d]	23.2 ± 0.7	3.71 ± 0.11
5-Nitroaspirin in D_2O	308[c]	16.3 ± 0.3	4.5 ± 0.1[e]
5-Nitroaspirin at 49.2°	308	80.1 ± 3.7	9.81 ± 0.48
5-Nitroaspirin at 59.4°	308	240 ± 10	24.5 ± 1.2

For 5-nitroaspirin, $\Delta H_{av}^{\ddagger} = 23 \pm 0.6$ kcal/mol; $\Delta S_{39}^{\ddagger} = -5 \pm 3$ eu[f]

[a] The iodo compounds were too insoluble to use. [b] Isosbestic point for aspirin anion, acid, and methyl ester. [c] Absorption maximum for the substituted acid. [d] Wavelength of maximum difference in absorption between aspirin and salicylic acid produced. [e] k_2 for catalysis by D^+. [f] Calculated from the rate constants given for 39, 49.2, and 59.4°.

The spontaneous rate constants for the hydrolysis of the aspirin acids were measured by extrapolation to zero acid concentration of the good straight lines obtained by plotting the observed hydrolysis rates against the concentration of HCl. In each case six runs at HCl concentrations between 0.1 and 0.6 *M* gave the spontaneous and acid-catalyzed rate constants, listed in Table I. The values are corrected for residual contributions from hydrolysis of the anions. The accuracy of the low rate constants for aspirin and its 4- and 5-methoxy derivatives was improved by three extra runs in HCl solutions near pH 2. The large contributions to the observed rate from the hydrolysis of the anions at this pH were calculated from the known rate constants for anion hydrolysis,[3] using the Henderson–Hasselbach equation. The pK_a's of these acids were measured under the experimental conditions by the spectrophotometric method.[7] pH's for these measurements, routinely at the end of each kinetic run, were measured using a Vibron electrometer, fitted with a C-33B pH-measuring attachment. The results of the pK_a measurements, at 39° and ionic strength 1.0, were for aspirin 3.36 ± 0.05, for 5-methoxyaspirin, 3.37 ± 0.04, and for 4-methoxyaspirin, 3.89 ± 0.04. Detailed hydrolysis data for 3,5-dinitroaspirin are given in Table II.

Table II. Hydrolysis Data for 3,5-Dinitroaspirin Acid at 39° (Ionic Strength 1.0[a])

Conditions	pH	k_{hyd}, min^{-1}
1.0 *M* HCl		0.746
1.0 *M* HCl, ionic strength 3.0 (KCl)		1.15
2.9 *M* HCl, ionic strength 2.9		1.04
1.0 *M* DCl in D_2O		0.562
0.5 *M* HCl		0.741
0.5 *M* HCl at 32.1°		0.338
0.5 *M* HCl at 24.9°		0.140
0.2 *M* HCl		0.730
0.1 *M* HCl		0.682
0.05 *M* HCl	1.39	0.582
0.033 *M* HCl	1.57	0.534
0.02 *M* HCl	1.75	0.468
0.01 *M* HCl	2.06	0.344
0.005 *M* HCl	2.35	0.232
Formate buffer[b]	2.59	0.157
	2.87	0.099
	3.57	0.047
Acetate buffer[b]	4.89	0.028
	5.60	0.027
Phosphate buffer[b]	6.44	0.027
Carbonate buffer[b]	8.75	0.048
	9.68	0.176
	10.10	0.386
Corrected rate constant for free acid		0.746 ± 0.001
Corrected rate constant for anion		2.68 ± 0.02 × 10^{-2}
Rate constant for hydrolysis of methyl ester[c]		3.6 ± 0.1 × 10^{-3}
For free acid, $\Delta H_{av}^{\ddagger} = 21.4 \pm 0.7$ kcal/mol; $\Delta S_{39}^{\ddagger} = 1.4 \pm 3$ eu[d]		

[a] Followed at 307 mμ for the acid form, and at 338 mμ for the anion, as described previously.[3] [b] Extrapolated to zero buffer concentration. [c] Followed in phosphate buffers, pH 6.4, and extrapolated to zero buffer concentration. [d] Based on first-order rate constants in 0.5 *M* HCl.

Hydrolysis in 50% Aqueous Methanol. A 4.2-mg sample of 3,5-dinitroaspirin was hydrolyzed for ten half-lives at 39° in 250 ml of a solvent composed of equal volumes of methanol and 2 *M* HCl. The hydrolysis mixture was then compared spectrophotometrically with solutions of authentic samples of 3,5-dinitrosalicylic acid and its methyl ester, in the same solvent (Table III).

Table III

Wavelength λ, mμ	OD_λ/OD_{350}: Reaction product	3,5-Dinitrosalicylic acid	Methyl 3,5-dinitrosalicylate
285	1.8206	1.6842	3.8192
325	1.6542	1.6083	2.4385
340	1.3034	1.2763	1.5962
350	1.0000	1.0000	1.0000

The composition of the reaction mixture was calculated from these ratios, using the ratio of the absorbtivities of the two components at 350 mμ ($\epsilon_{acid}/\epsilon_{ester} = 2.055$). This gave an estimate for the composition of the reaction product of 12.6 ± 0.6% of methyl ester. Two further determinations at room temperature gave the similar figure of 12.2 ± 0.7% methyl ester. When the experiment

(6) H. Erdmann, *Chem. Ber.*, 32, 3572 (1899).

(7) A. Albert and E. J. Sergeant, "Ionization Constants of Acids and Bases," Methuen and Co., Ltd., London, 1962.

Table IV. Apparent Second-Order Rate Constants for the Reactions of Oxy Anions with Substituted Aspirins at 39° (Ionic Strength 1.0)

Oxy anion	pH	% of free base in buffer	Concn range of free base, M	No. of runs	k_2,[a] M^{-1} min^{-1}
		3,5-Dinitroaspirin			
Methoxyacetate	6.4[b]	100	0–0.8	4	1.04×10^{-2}
	3.45	50	0.1–0.8	6	2.12×10^{-2}
	2.94	25	0.05–0.4	6	4.05×10^{-2}
Formate	6.4[b]	100	0–0.8	4	9.9×10^{-2}
	3.57	50	0.1–0.7	5	0.192
	2.83	16.7	0.02–0.16	6	0.63
	2.49	9.1	0.02–0.16	6	0.72
Acetate[c]	6.45[b]	100	0–0.8	6	3.32×10^{-2}
	5.65	91	0.1–0.8	6	3.08×10^{-2}
	4.95	67	0.1–0.8	6	2.87×10^{-2}
	3.57	9.1	0.02–0.16	6	3.66×10^{-2}
		3-Nitroaspirin			
Formate	6.3[b]	100	0–0.9	4	3.0×10^{-4}
	3.56	50	0.1–1.0	5	9.1×10^{-3}
		4-Methoxyaspirin			
Acetate	5.7	91	0.1–0.8	6	6.0×10^{-5}
	4.4	33	0.1–0.8	6	1.7×10^{-4}
		Aspirin			
Formate	6.3[b]	100	0–0.9	6	5.4×10^{-5}
	5.6[d]	99	0–0.9	9	9.5×10^{-5}
	3.54	50	0.2–0.8	3	9.1×10^{-4}
	3.24	33	0.2–0.8	6	11.6×10^{-4}

[a] Data for the sum of the reactions of the anion and the acid form. [b] In 0.05 M phosphate buffer, 50% free base. [c] See below, and Table VI. [d] In 0.1 M acetate buffer, 90% free base.

Table V. Second-Order Rate Constants for Attack of Oxy Anions on Substituted Aspirins at 39° (Ionic Strength 1.0)

Ester	Oxy anion	k_2 for free acid, M^{-1} min^{-1}	k_2 for anion, M^{-1} min^{-1}
3,5-Dinitroaspirin	Methoxyacetate	0.34 ± 0.03	1.0×10^{-2}
	Formate	4 ± 1	9.8×10^{-2}
3-Nitroaspirin	Formate	0.1[a]	9.1×10^{-3}
4-Methoxyaspirin	Acetate	5×10^{-4} [a]	6×10^{-5} [a]
Aspirin	Formate	$2.04 \pm 0.07 \times 10^{-3}$	5.4×10^{-5}

[a] Approximate value only.

was repeated using 400 mg of 3,5-dinitroaspirin in 16 ml of the mixed solvent, the ester crystallized on cooling to 0°, and 36 mg (10%) was isolated, and identified by melting point, mixture melting point, and ir spectrum as methyl 3,5-dinitrosalicylate. 3,5-Dinitrosalicylic acid gave no ester under these conditions.

Catalysis by Oxy Anions. The second-order rate constants for catalysis of the hydrolysis of 3,5-dinitroaspirin by formate ion were found to increase with decreasing pH of the medium, above the values measured for the reaction with the aspirin anion.[3] Subsequent experiments showed that the same phenomenon is observed for the reactions of other weakly basic oxy anions, and with substituted aspirins covering the whole range of reactivity. The methods used to measure the rate constants have been described previously;[2,3] the data are given in Table IV. It appears that the oxy anions react considerably faster with the aspirin acids than they do with the anions. Second-order rate constants for attack on the aspirin acids, calculated from the data of Table IV, are listed in Table V.

Inhibition of Acetate Catalysis by Acetic Acid. Although formate and methoxyacetate catalyze the hydrolysis of 3,5-dinitroaspirin acid more effectively than that of the anion, the reverse appears to be the case, marginally, for acetate, which is slightly less effective at lower pH. We suspected that this might be an effect of the increasing concentration of acetic acid in the solvent at low pH, and carried out the series of experiments summarized in Table VI, on the methyl ester of 3,5-dinitroaspirin, and on 2,4-dinitrophenyl acetate. In each case the apparent second-order rate constant for attack by acetate ion is decreased by increasing concentrations of acetic acid. This accounts for the difficulty in demonstrating acetate catalysis of the hydrolysis of 3,5-dinitroaspirin acid, which could only be observed at low pH. The same effect is also responsible for the curvature we have often observed in second-order plots of data for acetate catalysis of the hydrolysis of various esters, which has led us to prefer to measure catalytic constants for carboxylate anions in a phosphate carrier buffer where possible.

Table VI. Inhibition of Acetate Catalysis by Acetic Acid, at 39° (Ionic Strength 1.0)

Ester	Concn of acetate, M	Concn of acetic acid, M	k_{obsd}, min^{-1} [a]
3,5-Dinitroaspirin, methyl ester	0.9	0.09	0.194
	0.9	0.45	0.150
2,4-Dinitrophenyl acetate	1.0	0.1	0.118
	1.0	0.5	0.104
	1.0	1.0	0.090

[a] There is no significant change in the spontaneous hydrolysis rates of the esters over pH range involved in these measurements.

Discussion

The pH–rate profile for the hydrolysis of 3,5-dinitroaspirin, I (Figure 1), shows two pH-independent regions, but otherwise differs strikingly from that for aspirin itself because hydrolysis in this case is faster in the lower pH-independent region. It seems clear that these two regions represent the separate reactions of 3,5-dinitroaspirin acid and its anion. The curve of Figure 1 is that calculated from the Henderson–Hassel-

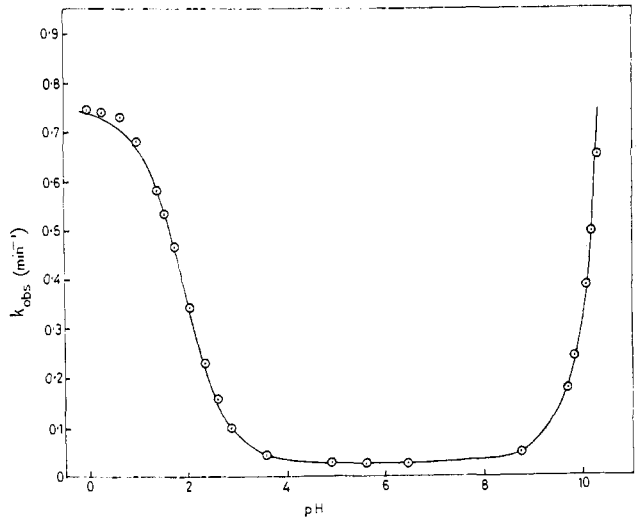
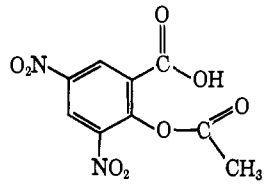


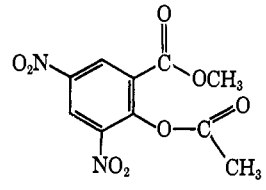
Figure 1. pH–rate profile for the hydrolysis of 3,5-dinitroaspirin at 39° and ionic strength 1.0; data are from Table II.

bach equation, using an estimated value[8] of 1.94 for the pK_a of the carboxyl group, and the rate constants for hydrolysis in the pH-independent regions.

Since the rate of hydrolysis of the 3,5-dinitroaspirin anion is itself considerably enhanced by intramolecular catalysis, it is clear from the pH–rate profile that the hydrolysis of the free acid must be accelerated also. We have obtained an estimate of the factor involved by comparing the rates of hydrolysis of 3,5-dinitroaspirin (I) and its methyl ester (II). At 39° the acid is hydrolyzed 207 times faster.



I



II

If, as seems likely, this acceleration is due to catalysis of hydrolysis by the free carboxyl group of 3,5-dinitroaspirin, this catalysis is presumably also possible for the hydrolysis of other, singly substituted aspirins. These compounds generally show the classical pH–rate profile for aspirin hydrolysis,[4] with the anions more reactive than the acid forms. So since the hydrolysis of the anions of aspirin and its 3,5-dinitro derivative is accelerated by roughly the same factor (about 50 times in each case), it would appear that catalysis is relatively less effective for aspirin acid and its singly substituted derivatives. We have confirmed this by a comparison of the rates of hydrolysis of aspirin and its methyl ester. In this case the acid form is hydrolyzed only some 14 times faster than the aspirin ester (Table I).

Thus this form of catalysis is more effective either for a more strongly acidic carboxyl group, or for a better group, or, of course, for both. We can separate the effects of single substituents on the two groups by a modified Hammett plot,[9] as used previously[2] for the data for the corresponding anion reactions. In the case of the substituted aspirin acids we find that the plot of $1/\sigma_1 \log (k/k_0)$ against σ_2/σ_1[10] does not correlate the hydrolysis data significantly better than does the simple Hammett plot, shown in Figure 2. This is because the rate of hydrolysis is significantly sensitive only to the effects of substituents on the phenol oxygen, and is insensitive, within the limits of experimental error, to the pK_a of the carboxylic acid group. From the modified Hammett plot (not shown) we can estimate a value of 0.03 ± 0.17 for ρ_{acid}. ρ_{phenol}, describing the effects of substituents on the leaving group, is 1.47 ± 0.15. The same value is obtained from the Hammett plot of Figure 2.

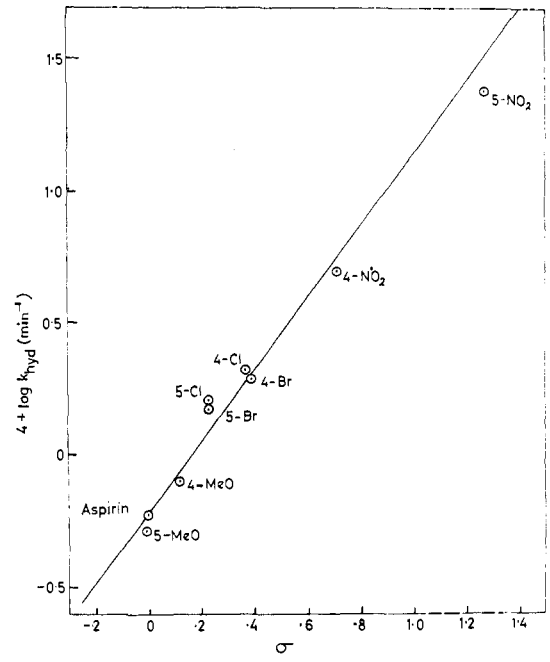


Figure 2. Hammett plot of the data of Table I for the hydrolysis of substituted aspirin acids, at 39° and ionic strength 1.0.

Since the rates of hydrolysis of the monosubstituted aspirin anions are notably insensitive to the effects of substituents, with a ρ_{phenol} of 0.96 largely offset by a ρ_{acid} of −0.52,[2] the ρ value of 1.47 describing the sensitivity to substitution of the hydrolysis rates of the substituted acids is sufficient to bring about a gradual change in the relative rates of hydrolysis of aspirin anions and free acids. It is this relative change which accounts for the shape of the pH–rate profile for the hydrolysis of 3,5-dinitroaspirin, shown in Figure 1. Only the 5-nitro group is sufficiently strongly electron withdrawing to make the free acid more reactive to hydrolysis than the anion in the case of a singly substituted aspirin. In that case the acid form is less than twice as reactive, so that intramolecular catalysis is almost equally effective for the anion and the free acid; thus the pH–rate profile has (Figure 3) almost precisely the form expected in the absence of catalysis. (The rate constants involved are, of course, a good deal larger.) These changes with substitution are illustrated by Figure 3, in which the pH–rate profiles for the hydrolysis of 5-nitro- and 3,5-dinitroaspirin are compared with that of the parent compound.

(8) This is the value obtained by subtracting $2\sigma_m$ for the NO_2 group from the pK_a of aspirin (3.36[1]). The pK_a could not be measured because the free acid has a half-life of less than 1 min in aqueous solution at 39°.

(9) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953); *Science*, **118**, 246 (1953).

(10) The symbols are defined in ref 2.

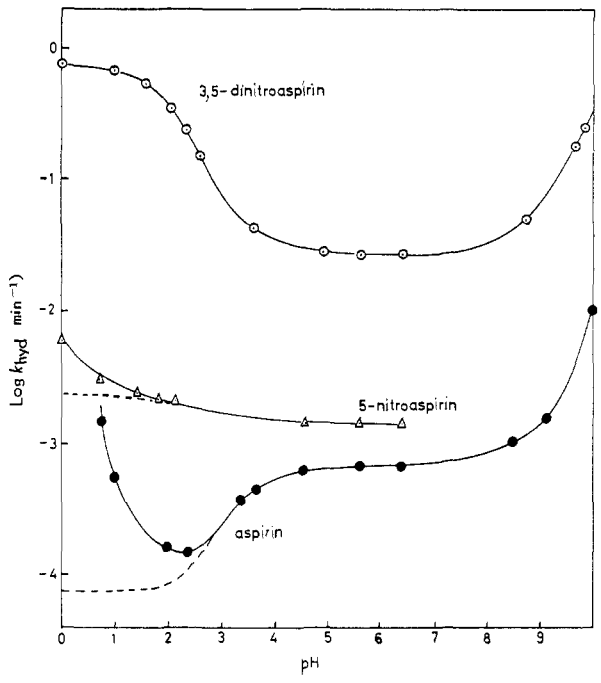
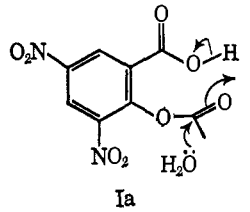


Figure 3. A comparison of the pH–rate profiles (logarithmic) for the hydrolysis of aspirin and its 5-nitro and 3,5-dinitro derivatives at 39° and ionic strength 1.0. Data are from this study and ref 2. The broken lines represent the spontaneous hydrolysis of the acid forms and have been corrected for external acid catalysis of hydrolysis using the data of Tables I and II.

The Hydrolysis Mechanism. Since the most reactive, and also one of the least reactive, aspirin acids measured both appear to undergo intramolecular catalysis of hydrolysis, and the Hammett plot of Figure 2 correlates the data for all the monosubstituted aspirin acids tested, it is reasonable to assume a common mechanism for all these reactions. We have investigated the mechanism of hydrolysis in detail for 3,5-dinitroaspirin, since catalysis is most significant for this ester, but we would expect our conclusions to apply to the other cases also.

The fact that the hydrolysis rates for the aspirin acids are fast compared with those of the corresponding methyl esters seems to rule out catalysis by dipole–dipole interactions between the adjacent ester carbonyl groups, and implicates specifically the proton of the acetylsalicylic acid, or its kinetic equivalent. Two different mechanisms are consistent with the observed kinetics, involving either intramolecular general acid catalysis by the carboxyl group, or intramolecular nucleophilic catalysis, closely similar to the mechanism established for participation by the ionized carboxyl group in the hydrolysis of 3,5-dinitroaspirin anion.[3]

We have suggested previously that the hydrolysis of 3,5-dinitroaspirin acid involves intramolecular general acid catalysis by the carboxyl group of the attack of a molecule of water on the neighboring ester group (Ia).



Ia

This mechanism is in accord with the observation that the attack of other nucleophiles than water is also subject to catalysis. We have observed rate increases of up to 40 times for the attack of oxy anions on aspirin acids, as compared with attack on the corresponding anions. And St. Pierre and Jencks[11] have found even larger increases for neutral amine nucleophiles (which are not subject to electrostatic repulsion): these they too attribute to general acid catalysis by the neighboring carboxyl group.

The kinetic behavior of the hydrolysis reaction, however, is difficult to reconcile with the rate-determining step represented by Ia. This requires two molecules to come together in the transition state, yet the observed entropy of activation is near zero. It involves a proton transfer in the slow step of the reaction, and yet the observed deuterium isotope effect is very small (k_H/k_D = 1.3), and it implicates the carboxylic acid group directly, although the observed ρ_{acid} (corresponding to α for general acid catalysis[2]) is close to zero. Finally, it directly contradicts the principle that catalysis occurs where it is most needed,[12] in that catalysis is most effective for the most reactive ester.

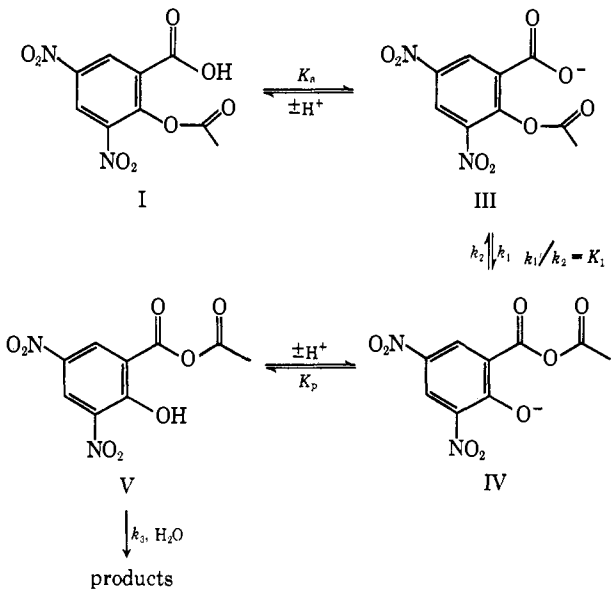
Concerned at this catalog of contraindications, we felt compelled to reexamine the single piece of evidence that seemed to rule out the most reasonable alternative mechanism, that of intramolecular nucleophilic catalysis. This was our observation that the solvolysis of 3,5-dinitroaspirin acid in 50% aqueous methanol gave a quantitative yield of 3,5-dinitrosalicylic acid,[5] and no methyl ester. We have repeated this experiment, *and are satisfied that our previous observation was wrong.* The yield of 3,5-dinitrosalicylic acid is in fact only about 88%, and significant quantities of methyl 3,5-dinitrosalicylate are formed. The ester was isolated directly from the reaction mixture in 10% yield, and its final concentration was estimated spectrophotometrically as about 12% of the total solvolysis products (see Experimental Section). This observation is evidence for the formation of an anhydride intermediate in the hydrolysis reaction, and thus specifically supports a mechanism involving intramolecular nucleophilic attack of the carboxyl group on the acetyl group. We must therefore conclude that there is no good evidence that the mechanism of hydrolysis of 3,5-dinitroaspirin acid involves intramolecular general acid catalysis.

The alternative mechanism, in which a mixed salicylic–acetic anhydride is an intermediate, presumably includes several of the fast steps involved in the hydrolysis of the anion.[3] If we assume that in a reaction involving several steps the tetrahedral intermediates are unlikely to be kinetically significant, the mechanism can be represented by Scheme I.

The proton transfers will be fast, as must the stage III → IV, because of the observed kinetic dependence on the neutral species. So the rate-determining step must be the hydrolysis of the anhydride V. This is reasonable, since the hydrolysis of the anhydride anion IV is the slow step in the hydrolysis of 3,5-dinitroaspirin anion, even though it is subject to intramolecular general base catalysis and should therefore be more reactive than the protonated form V. In which case, since the

(11) T. St. Pierre and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 3817 (1968).
(12) J. E. Reimann and W. P. Jencks, *ibid.*, **88**, 3973 (1966).

Scheme I

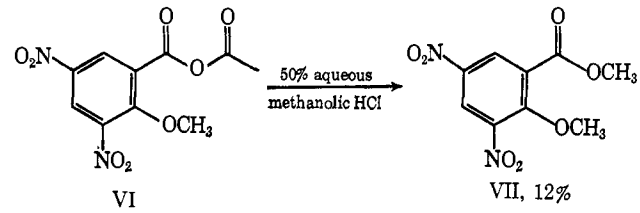


over-all rate of hydrolysis of the acid form is 28 times faster than that of 3,5-dinitroaspirin anion, the anhydride intermediate (V) must be present in much higher concentration than is its anion (IV) during the hydrolysis of the ester anion. In this latter reaction we have estimated that the maximum possible concentration of anhydride anion (IV) is about 0.03% of that of unreacted ester, and that this would imply a 17-fold acceleration of hydrolysis by intramolecular catalysis.[3] If the hydrolysis of V is not accelerated, its concentration must therefore be in the region of 0.03 × 17 × 28, some 10–15% of that of unreacted 3,5-dinitroaspirin acid.

We consider that this more favorable equilibrium for the formation of the mixed anhydride V is the source of the relatively more efficient catalysis of the hydrolysis of the protonated form of the aspirin acid. Because the phenolate oxygen atom of the anion IV is almost completely protonated in the acid region, the back-reaction (k_2) to regenerate starting materials is suppressed. The forward reaction, III → IV, is affected less at any pH below the pK_a of the carboxyl group because this is lower than the pK_a of the phenol group. So in acid the back-reaction is relatively less favorable, and a higher concentration of anhydride is formed at equilibrium.[13]

The anhydride V could not be detected spectrophotometrically, perhaps not surprisingly, since the chromophore is very similar to that of the product, 3,5-dinitrosalicylic acid. An indication that the pathway through V can account quantitatively for the reaction is given by a comparison with the methyl ether, VI.[3] Under the conditions used for the solvolysis of the 3,5-dinitroaspirin acid, in 50% aqueous methanol, this ether anhydride also gave 12% of the corresponding methyl salicylate (VII).

(13) Recently we have observed an even more striking instance of this phenomenon (A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, **90**, 5833 (1968). The hydrolysis of the dianion of 3-acetoxyphthalate involves intramolecular nucleophilic catalysis by the neighboring carboxyl group, and is some 20 times faster than that of aspirin anion. But the hydrolysis of the monoanion is over 6000 times faster still, simply because of the more favorable equilibrium constant for the formation of the protonated form of the mixed anhydride intermediate.



Although the phenomenological description of mechanism seems clear, the interpretation of some kinetic parameters is less straightforward. In the mechanistic scheme above, if the slow step of the reaction is that represented by k_3, the hydrolysis of the anhydride V, then the observed rate of the reaction is given by

$$k_{obsd}[I] = k_3[V] = \frac{k_3[V][I]}{[I]}$$

The equilibrium constant [V]/[I] is a composite constant, given by $(K_a/K_p)\,K_1$, where K_a and K_p are the dissociation constants of the carboxylic group of I and the phenolic group of V, respectively. Therefore

$$k_{obsd} = k_3[V]/[I] = k_3K_1K_a/K_p$$

Given the complex nature of the observed rate constant it is evident that simple interpretations of the kinetic parameters are unlikely to be successful. The small deuterium isotope effect (k_H/k_D = 1.3) and near-zero entropy of activation, neither characteristic of the neutral hydrolysis of an anhydride,[14] are presumably accounted for in this way. Analysis of the Hammett ρ values is a little more straightforward, and it is possible to show that the observed values are compatible with crude predictions based on Scheme I, as in eq 1. If

$$k_{obsd} = k_3K_1K_a/K_p = (k_3k_1/k_2)(K_a/K_p)$$

$$\log k_{obsd} = \log k_3 + \log k_1 - \log k_2 + \log K_a - \log K_p \quad (1)$$

Separating, as we have done previously,[2] the effects of substituents on the carboxylic acid (a) and phenolic (p) groups, we can write

$$\log k_1 = \rho_1\sigma_a + \rho_2\sigma_p$$

$$\log k_2 = \rho_3\sigma_a + \rho_4\sigma_p$$

$$\log k_3 = \rho_5\sigma_a$$

(the phenol group plays no part in the hydrolysis of the anhydride V, so this reaction should be independent of σ_p).

Rough estimates of the ρ values involved can be made as follows. k_1 describes the nucleophilic attack of a carboxylate anion on a substituted phenyl acetate. In such reactions the sensitivity to changes in the nucleophile is given by the Brønsted β value, which is generally taken as about 0.8.[15] Since ρ for the ionization of substituted benzoic acids is about 1.[2]

$$\rho_1 \simeq -0.8$$

For reactions of this sort the sensitivity to the leaving group, $\rho_{phenol} = \rho_2 \simeq 1.6$.[2] For the reverse reaction,

(14) A. R. Butler and V. Gold, *J. Chem. Soc.*, 2212 (1962); C. A. Bunton, N. A. Fuller, S. G. Perry, and V. J. Shiner, *ibid.*, 2918 (1963).

(15) W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, **82**, 1778 (1960).

k_2 describes the nucleophilic attack of a phenolate anion on an anhydride. Taking β again as about 0.8, and since ρ for the ionization of substituted phenols is about 2.1,[16] $\rho_{phenol} = \rho_4 \simeq -1.7$. ρ_3 and ρ_5 both describe the sensitivity to changes in substitution in the aromatic group for attack on a mixed anhydride $ArCO \cdot O \cdot OCH_3$, and will be similar, so that

$$\rho_3 \simeq \rho_5$$

Thus from eq 1 the expected ρ values are

$$\begin{aligned}\rho_{acid} &= \rho_{ionization} + \rho_1 - \rho_3 + \rho_5 \\ &= 1 - 0.8 \\ &= 0.2\end{aligned}$$

$$\begin{aligned}\rho_{phenol} &= -\rho_{ionization} + \rho_2 - \rho_4 \\ &= -2.1 + 1.6 + 1.7 \\ &= 1.2\end{aligned}$$

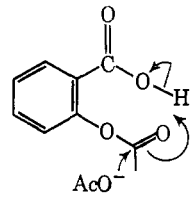
Thus a very small value for ρ_{acid} and a moderate, positive value for ρ_{phenol} are expected, and the observed figures (0.03 ± 0.17 and 1.47 ± 0.15) are compatible with the proposed mechanism.

Reactions with Other Nucleophiles. The data in Tables IV and V show that there is catalysis of the attack of oxy anions on aspirin acids, since the rate constants are up to 40 times larger than for the attack on the aspirin anions, and about an order of magnitude greater than those for attack on the methyl ester.[3] Accurate data are difficult to obtain, since the more reactive oxy anions are largely protonated at the low pH values necessary to ensure a substantial concentration of aspirin acid (pK_a's between about 2 and 3.4), and the weakly basic oxy anions used effect relatively small rate increases against the background of the strongly catalyzed hydrolysis reactions.

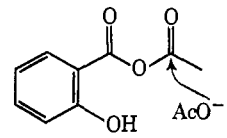
In every case the reaction with the acid form of the aspirin is fastest for formate anion. Reaction with acetate is complicated by inhibition by acetic acid, and could only be measured for 4-methoxyaspirin, which has a particularly high pK_a, but formate reacts about ten times faster than does methoxyacetate with 3,5-dinitroaspirin, and probably with aspirin itself, where catalysis by methoxyacetate could not be detected. This exceptional reactivity of formate anion is characteristic of reactions in which it acts as a nucleophile, rather than as a general base,[3] and suggests that the mechanism of catalysis of hydrolysis by oxy anions involves either intramolecular general acid catalysis of nucleophilic attack, VIII, or rate-determining nucleophilic attack on the anhydride intermediate, IX.

Since we have concluded above that the hydrolysis reaction involves the anhydride as an intermediate, and that this is probably present in considerable concentration, it seems certain that the reactions with oxy

(16) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).



VIII



IX

anions must involve this intermediate also. The full extent of catalysis of the attack of oxy anions is not easily measured. Formate, for example, attacks the acid form of aspirin itself more than 40 times faster than it attacks the anion (Table V) but the true extent of catalysis is greater than this factor would suggest, since the figure for attack on the anion represents general base catalysis.[1] It is relevant that the larger factors observed by St. Pierre and Jencks[11]—up to 150-fold for the acceleration of the attack of semicarbazide on aspirin acid—are for nitrogen nucleophiles, where attack on the anion also is likely to be nucleophilic.

St. Pierre and Jencks[11] have also considered the question of the anhydride intermediate in the hydrolysis of aspirin. Like us, they rule out the possibility for the anion reactions, and agree further that if an anhydride intermediate is involved in the hydrolysis of aspirin acid, its hydrolysis must be rate determining. Their experimental evidence is relevant mainly to the anion reaction; but in one experiment they searched for a leveling off in the second-order rate constant for the reaction with hydroxylamine at pH 3.74 where some 30% of aspirin is in the acid form, expecting that anhydride formation would become rate determining at high hydroxylamine concentrations. However, we now know, from our very recent results with 3-acetoxypthalate,[13] that the rate constant for the formation of the anhydride is probably greater than 1 min^{-1}, 20 times faster than the fastest reaction with NH_2OH measured by St. Pierre and Jencks,[11] so that no leveling off is to be expected under the conditions of their experiments. We cannot rule out a contribution from intramolecular general acid catalysis in cases we have not studied in detail, although the extrapolation from 3,5-dinitroaspirin seems reasonably sound: the kinetic parameters are almost identical for 3,5-dinitroaspirin and the 5-nitro compound, and the latter falls on the Hammett plot for aspirin and its monosubstituted derivatives. But in the absence of any positive evidence for intramolecular general acid catalysis it seems likely that the nucleophilic mechanism is generally valid.

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