# Neighboring-Group Participation of Aldehydes and Ketones in Ester Hydrolysis. Mechanism of Hydrolysis of *O*-Acetylsalicylaldehyde

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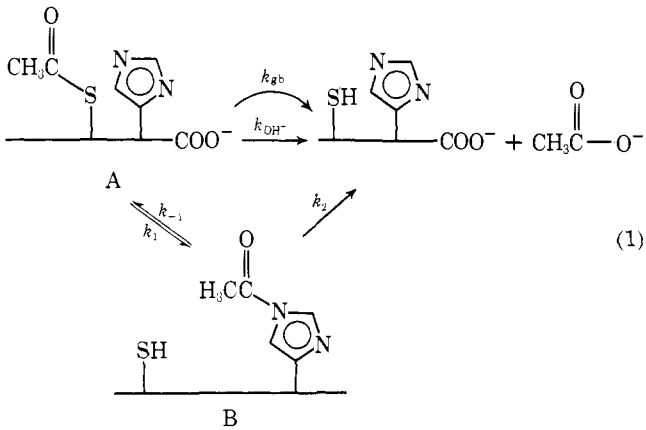
**Abstract:** The mechanism of hydrolysis of *O*-acetylsalicylaldehyde has been established by isotopic tracer studies of the reaction carried out in $H_2{}^{18}O$. The hydrate of the aldehyde operates as a nucleophilic catalyst. It attacks the neighboring phenyl ester to yield the hemiacylal of acetic acid and salicylaldehyde, which then rapidly undergoes elimination of acetate. The rate-limiting step of the hydrolysis is the hydration of the aldehyde. Since in the usual scheme for multifunctional catalysis of ester hydrolysis a so-called endocyclic system such as *O*-acetylsalicylaldehyde is formed as an intermediate, the intramolecular aldehyde group may prove of general use in the development of such multifunctional catalytic systems. In the following paper we present an initial application of this mechanism to the catalytic hydrolysis of activated phenyl esters.

In efforts to develop a synthetic catalyst for ester hydrolysis similar to the proteolytic enzymes, considerable attention has been devoted to the construction of multifunctional systems.[1-6] Recently we investigated a series of synthetic cysteine-histidine peptides as models for the cysteine proteases, to determine the catalytic potential of bifunctional thiol-imidazole intramolecular systems for hydrolytic reactions.[7] With substituted phenyl acetates as substrates, catalysis was limited by the slow rate of hydrolysis of the S-acetylated peptide A, formed as an intermediate in the reaction pathway.

Three mechanisms for the hydrolysis of A are shown in eq 1: general base catalysis by the imidazole moiety of histidine



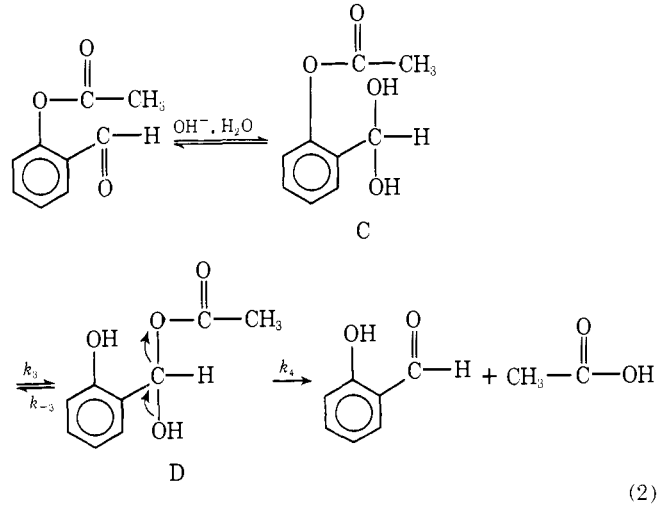
(1)

($k_{gb}$); nucleophilic catalysis by the imidazole group proceeding through the acyl imidazole intermediate B; and direct $OH^-$ attack on the thiol ester ($k_{OH^-}$). Hydroxide ion attack proved to be the predominant pathway for the reaction; the imidazole group was without effect as either a general base or nucleophilic catalyst. With the use of thiol-specific trapping reagents, however, the existence of a rapid reversible intramolecular transfer of the acetyl group between the cysteine and histidine residues, greatly favoring cysteine, was revealed. Thus, in the absence of trapping reagent, the potentially effective nucleophilic pathway was blocked by the back-attack of cysteine.

Similar relationships for the nucleophilic and general base mechanisms of intramolecular catalysis have been reported by Fersht and Kirby for the hydrolysis of aspirin derivatives.[8] These relationships can also be recognized in several other intramolecular catalytic systems by comparisons of the so-called exocyclic and endocyclic arrangements.[7,9,10] In general, the nucleophilic mechanism, which forms a high free energy acyl intermediate, is undermined by the back-attack of the primary nucleophile, the thiol group of cysteine in eq 1, while general base catalysis, which encounters no such difficulty, offers only a modest, or in some instances negligible, enhancement of the rate.

From this we conclude that the most promising approach to an efficient synthetic catalyst for ester hydrolysis should utilize for the deacylation step a nucleophilic mechanism in which the back-attack by the primary nucleophile is circumvented. Trapping of the intermediate can be effected in two ways: (1) by retarding the rate for the reversed transacetylation of the intermediate (decreasing $k_{-1}$), or (2) by enhancing the rate of hydrolysis of the intermediate (increasing $k_2$).

The rate of hydrolysis of *O*-acetylsalicylaldehyde (*o*-acetoxybenzaldehyde) has been reported to be $10^4$ times greater than that of *p*-acetoxybenzaldehyde.[11] This large increase in rate indicates that the neighboring aldehyde group participates directly in the hydrolytic process. Indirect evidence obtained for related systems suggests mechanism 2.[12-14] In this scheme, the hydrated aldehyde functions as a nucleophilic catalyst.



(2)

If this mechanism is indeed correct, the question immediately arises as to why back-attack by the primary nucleophile (the phenoxide group of D) does not undermine the nucleophilic pathway, as in the hydrolyses of the *S*-acetylcysteine-histidine peptides and aspirin.[7,8] A provocative explanation is that cleavage of the intermediate ester D occurs not by the direct attack of water (or hydroxide ion) on the ester carbonyl group but rather by elimination as shown in eq 2. From studies of related systems we would predict this reaction to proceed

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by specific base catalysis at or near the diffusion-limited rate.[15] Overall this scheme would then represent a pathway of nucleophilic catalysis in which back-attack by the primary nucleophile is circumvented by rapid breakdown of the acyl intermediate. It becomes germane, therefore, to establish the mechanism of hydrolysis of *O*-acetylsalicylaldehyde so as to determine the potential scope of this mechanism for the development of synthetic catalysts for hydrolytic reactions.

## Experimental Section

**Materials.** Acetic acid and salicylaldehyde were obtained from commercial sources. Salicylaldehyde was twice purified by fractional distillation in vacuo. *O*-Acetylsalicylaldehyde was synthesized by the procedure of Neuberger[16] and purified by vacuum fractional distillation: mp 36.5–37.5 °C (lit.[16] 37 °C). $H_2{}^{18}O$, 96% isotopic purity, was obtained from Matheson Research Corp.

**Kinetics.** The hydrolysis of *O*-acetylsalicylaldehyde was followed with a Cary 14 spectrophotometer at 320 nm. The reaction was studied at 25 °C over the pH range 6.0–8.5 in 0.025 and 0.05 M phosphate buffers and 0.1 M veronal buffer, ionic strength maintained at 1.0 M with KCl. Pseudo-first-order rate constants were determined by least-squares analysis of the appropriate semilogarithmic plots.

**Hydrolysis of *O*-Acetylsalicylaldehyde in Presence of $H_2{}^{18}O$.** One milliliter of either $H_2{}^{16}O$ or $H_2{}^{18}O$, 96% isotopic purity, was added to a small vial containing ~60 mg of *O*-acetylsalicylaldehyde. The mixture was stirred with a small magnetic stirring bar. The pH, monitored with a Beckman Model 3550 pH meter, was maintained between 7 and 8 by the addition of 10 M NaOH, 20–30 μL being required during the course of the hydrolysis reaction. The reactions were allowed to continue until the production of acid ceased, typically for a period of 90 min. Such long reaction times were necessary because of the slow rate of solution of *O*-acetylsalicylaldehyde.

Much of the salicylaldehyde produced settled out as an oil. When the reaction was complete, the supernatant was removed by pipet, acidified to pH 2.0 by the addition of ~15 μL of concentrated HCl, and extracted with 10 mL of ethyl ether. The ether layer was separated from the aqueous layer by pipet and concentrated to 1.0 mL by evaporation. Such ether samples were then analyzed directly by combined gas chromatography–mass spectrometry.

Gas chromatograms and mass spectra were obtained with an interfaced system (Hewlett-Packard Model 5930A). The gas chromatography was run on a ⅛ in. (o.d.) by 6 ft stainless steel column packed with 2% Carbowax 20M on a 80–100 mesh WHP support. Oven temperature was 150 °C and the flow rate 30 mL/min. The gas chromatogram of the ether extract of the *O*-acetylsalicylaldehyde hydrolysate revealed only salicylaldehyde and acetic acid. These species were separated sufficiently by gas chromatography to reveal the individual mass spectrum of each compound.

## Results

The pseudo-first-order rate constants for the hydrolysis of *O*-acetylsalicylaldehyde, $k_{hyd}$, from pH 6.0 to 8.5, fit the equation

$$k_{hyd} = k_0 + k_5[OH^-] \tag{3}$$

In this equation, $k_0$, the rate constant for the spontaneous or water-catalyzed reaction path, has the value $1.7 \times 10^{-3}$ s$^{-1}$, and $k_5$, the rate constant for the hydroxide ion catalyzed reaction, is $1.2 \times 10^4$ M$^{-1}$ s$^{-1}$. The water rate, like the hydroxide ion catalyzed rate, is greatly enhanced (~$10^4$-fold) over that for reference phenyl esters with leaving groups comparable with salicylaldehyde.[17,18] Hence the neighboring aldehyde group must participate directly in both the hydroxide ion and water-catalyzed reactions.

The interpretation of the $^{18}O$ tracer experiments is based on the following rationale. If one assumes a steady state for the concentrations of species C and D, then $k_{hyd}$, according to the mechanism in eq 2, is given by

$$k_{hyd} = (k_6 + k_7[OH^-])\alpha \tag{4}$$

$$\alpha = \frac{k_3k_4}{k_8(k_{-3} + k_4) + k_3k_4}$$

In these equations, $k_6$ and $k_7$ represent the rate constants for the water- and hydroxide-ion catalyzed hydration of *O*-acetylsalicylaldehyde, respectively, $k_8$ is the rate constant for the dehydration of C, and the other constants are those defined in eq 2. The factor $\alpha$ represents the probability that the hydrated aldehyde C will proceed to products rather than dehydrate to regenerate *O*-acetylsalicylaldehyde. Since each of the rate constants that comprise $\alpha$ would likely contain a pH-dependent term, $\alpha$ itself might also be pH dependent. However, if the nucleophilic mechanism (eq 2) is correct, $\alpha$ must be pH independent over the range studied so that eq 4 conforms to eq 3.

Let us define $f_{CH_3C^{16}O_2H}$ as the fraction of acetic acid in the isotopic form $CH_3(^{16}O{=})C{-}^{16}OH$ produced from the hydrolysis of *O*-acetylsalicylaldehyde in $H_2{}^{18}O$. For the mechanism of hydrolysis in eq 2, $f_{CH_3C^{16}O_2H}$ depends on the relative rate of the dehydration step ($k_8$). If the dehydration step is very slow relative to the subsequent reactions, then once C is formed it will go on to produce $CH_3CO_2H$ and salicylaldehyde. After one hydration step in $H_2{}^{18}O$, the two O atoms of the hydrated aldehyde will have an isotopic distribution of 1:1 for $^{18}O$ and $^{16}O$. Therefore, in the relatively fast succeeding step leading to D, there is a 50% chance that $^{16}O$ will be the nucleophilic oxygen covalently linked to the acetyl group. Consequently $f_{CH_3C^{16}O_2H}$ would be 0.5. On the other hand, if the dehydration step were sufficiently fast so as to occur to some extent before hydrolysis proceeds, then additional hydration steps would be completed before the conversion of C to products. These repeated hydrations would place more $^{18}O$ in the hydrated aldehyde before the final hydrolysis reaction. Hence in the nucleophilic attack ($k_3$) there would be a greater probability that $^{18}O$ will be linked to the acetyl group, and consequently that $CH_3(^{16}O{=})C{-}^{18}OH$ will be produced; thus $f_{CH_3C^{16}O_2H} < 0.5$.

A quantitative expression for $f_{CH_3C^{16}O_2H}$ for the nucleophilic mechanism, can be derived as follows.[19] First we recognize that

$$f_{CH_3C^{16}O_2H} = \sum_{i=1}^{\infty} P_iX_i \tag{5}$$

where $P_i$ is the probability that hydrolysis will occur on the $i$th hydration of the aldehyde, and $X_i$ is the probability that this hydrolysis will lead to the formation of $CH_3C^{16}O_2H$. It can be easily shown that

$$P_i = (1 - \alpha)^{i-1}\alpha \tag{6}$$

and

$$X_i = (1/2)^i \tag{7}$$

where $\alpha$ is defined as in eq 4. Substituting eq 6 and 7 into 5, we obtain

$$f_{CH_3C^{16}O_2H} = \sum_{i=1}^{\infty} (1 - \alpha)^{i-1}\alpha(1/2)^i \tag{8}$$

which readily yields

$$f_{CH_3C^{16}O_2H} = \alpha/(1 + \alpha) \tag{9}$$

As shown above, the upper limit of $f_{CH_3C^{16}O_2H}$ is 0.5. A lower limit may be estimated as follows. The rate constant for the addition of hydroxide ion to acetaldehyde is $8 \times 10^4$ M$^{-1}$ s$^{-1}$.[20] The rate constant, $k_7$, for the addition of hydroxide ion to *O*-acetylsalicylaldehyde should be less than this value since the aldehyde is conjugated with a benzene ring. If the nucleophilic mechanism of eq 2 is correct, then the measured rate constant for the hydroxide ion catalyzed hydrolysis of *O*-acetylsalicylaldehyde, $1.2 \times 10^4$ M$^{-1}$ s$^{-1}$, is equal to $k_7\alpha$ (eq 4). If $k_7$ in the aromatic *O*-acetylsalicylaldehyde is as high as that for acetaldehyde, then $\alpha = 0.15$. In this circumstance

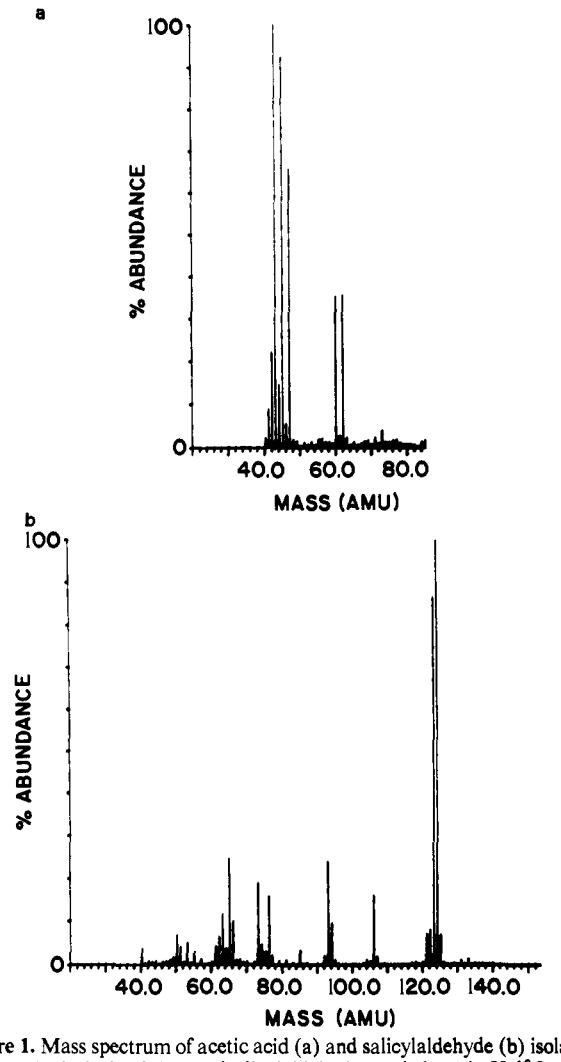



**Figure 1.** Mass spectrum of acetic acid (a) and salicylaldehyde (b) isolated from an hydrolysis of *O*-acetylsalicylaldehyde carried out in $H_2{}^{18}O$, 96% isotopic purity.

$f_{CH_3C^{16}O_2H} = 0.13$ (eq 9). At the other extreme, the measured rate constant could be actually $k_7$, in which case the probability of hydrolysis of C, as opposed to dehydration, becomes unity, i.e., $\alpha = 1$, and once again we find that $f_{CH_3C^{16}O_2H} = 0.5$. Therefore $0.15 \leq \alpha \leq 1$ and hence $0.13 \leq f_{CH_3C^{16}O_2H} \leq 0.5$. That is, if the nucleophilic mechanism is correct, we must detect some acetic acid that has incorporated $^{16}O$ from the aldehyde when the hydrolysis is carried out in $H_2{}^{18}O$. Only nucleophilic catalysis by the hydrated aldehyde could lead to this result. Other possible mechanisms would lead exclusively to the production of $CH_3(^{16}O{=})C{-}{}^{18}OH$; i.e., to $f_{CH_3C^{16}O_2H} = 0.0$.[21]

Acetic acid and salicylaldehyde produced from the hydrolysis of *O*-acetylsalicylaldehyde in $H_2{}^{16}O$ gave mass spectra identical with those of the known compounds with natural isotopic abundance. Mass spectra of acetic acid and salicylaldehyde isolated from an hydrolysis in $H_2{}^{18}O$, 96% isotopic purity, are shown in Figure 1a and 1b, respectively. The carbonyl oxygen of salicylaldehyde exchanges with water rapidly on the time scale of the experiment. As a result we observe in Figure 1b molecular ion peaks for salicylaldehyde at 122 and 124 amu (the latter due to the incorporation of $^{18}O$ into the aldehyde). From the ratio of these peaks we can determine the percentage of $H_2{}^{18}O$ in the sample, in this case 91%. This value is slightly less than the starting 96% owing to some dilution with $H_2{}^{16}O$ upon addition of the NaOH and HCl solutions (see Experimental Section).

Acetic acid, unlike salicylaldehyde, would not be expected to exchange oxygen with water under the conditions of our experiment.[22] This was verified by a separate experiment in which acetic acid was incubated in $H_2{}^{18}O$ and the sample treated in the same fashion as the hydrolyzates.

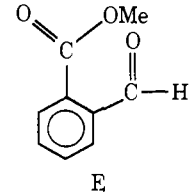
The mass spectrum of acetic acid isolated from the hydrolysis run in $H_2{}^{18}O$ (Figure 1a) reveals molecular ion peaks at 60 and 62 amu of very nearly equal intensity. That these peaks do in fact represent $CH_3(^{16}O{=})C{-}{}^{16}OH$ and $CH_3(^{16}O{=})C{-}{}^{18}OH$, respectively, was verified by analysis of the peak intensities at 43, 45, and 47 amu. For a 1:1 mixture of these two isotopic species, we would predict a ratio of peak intensities of 100:96.5:63.1, respectively.[23] A ratio of 100:92.5:65.7 was found.

## Discussion

The fraction of $CH_3(^{16}O{=})C{-}{}^{16}OH$ produced in the hydrolysis of *O*-acetylsalicylaldehyde in $H_2{}^{18}O$, $f_{CH_3{}^{16}O_2H}$, was, within experimental error, 0.5. This result establishes unequivocally that nucleophilic catalysis by the hydrated aldehyde is the mechanism of hydrolysis both for the hydroxide ion and water-catalyzed reactions. The latter accounted for ~25% of the hydrolysis in the $^{18}O$ experiment. Since $f_{CH_3C^{16}O_2H}$ is 0.5, $\alpha$ (eq 9) must equal 1. The hydration of the aldehyde is, therefore, the rate-limiting step in the reaction. Hence $k_6$ and $k_7$, the rate constants for the addition of water and of hydroxide ion to *O*-acetylsalicylaldehyde, are equal to the corresponding hydrolysis constants $k_0$ ($1.7 \times 10^{-3}$ s$^{-1}$) and $k_5$ ($1.2 \times 10^4$ M$^{-1}$ s$^{-1}$).[24] These values are less than those for addition to acetaldehyde ($7.8 \times 10^{-3}$ s$^{-1}$ and $8 \times 10^4$ M$^{-1}$ s$^{-1}$)[20] as expected. Furthermore, cleavage of the acyl intermediate D must occur by elimination as shown in eq 2 rather than by direct attack of solvent (water or hydroxide ion) on the ester carbonyl group. This follows since the latter would lead exclusively to the production of $CH_3(^{16}O{=})C{-}{}^{18}OH$ and thus to $f_{CH_3C^{16}O_2H} = 0.0$.

The efficiency of nucleophilic catalysis of ester hydrolysis is dependent on circumventing the back-attack of the acyl intermediate by the primary nucleophile.[7-10] This is achieved in the hydrolysis of *O*-acetylsalicylaldehyde by the rapid elimination reaction ($k_4$) of the acyl intermediate D.[15]

Our principal objective in studying the mechanism of *O*-acetylsalicylaldehyde hydrolysis was to determine the potential scope of this reaction for the development of synthetic catalysts for ester hydrolysis. The finding of importance in this respect is that the rate-limiting step of the hydrolysis is the hydration of the aldehyde. We are led to conclude, therefore, that this mechanism should be applicable to the deacylation of a variety of nucleophiles and, in particular, to those stronger than the phenoxide group of salicylaldehyde needed for the rapid cleavage of ester substrates. Indeed Bender and co-workers[13] have observed catalysis of the hydrolysis of a methyl ester by the intramoleclar aldehyde group in system E. The hydroxide




E

ion rate for the hydrolysis of E is 2000 M$^{-1}$ s$^{-1}$, almost equal to that for *O*-acetylsalicylaldehyde. Even in this case, hydration of the aldehyde may well be the rate-limiting step. Observations of similar catalytic effects with ketones, albeit at slower rates than with the corresponding aldehydes, extend even further the potential utility of this reaction.[12,14] In the following paper, we present an initial application of this mechanism to the catalytic hydrolysis of activated phenyl esters.[25]

**Acknowledgment.** We are grateful to Dr. Hoying Hung of the Analytical Services Laboratory of the Chemistry Department for assistance in obtaining GC–mass spectra. This investigation was supported in part by a grant from the National Science Foundation.

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$$f_{CH_3C^{18}O_2H} = (1 - f_{H_2{}^{16}O})[\alpha/(1 + \alpha)] + f_{H_2{}^{16}O}$$

where $f_{H_2{}^{16}O}$ is equal to the fraction of $H_2{}^{16}O$ in the sample. For our present purposes, eq. 9 suffices.
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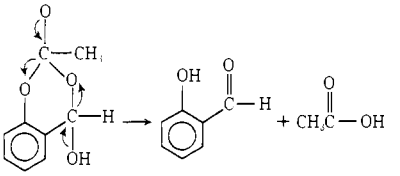

(22) D. R. Llewellyn and C. O'Connor, *J. Chem. Soc.*, 545 (1964).
(23) This follows from the fact that in the natural abundance mass spectrum of acetic acid there occur peaks at 43 and 45 amu, owing to $CH_3C^+{=}O$ and $O{=}COH^+$, respectively, in a ratio of 100:94.7.
(24) The rate constant reported in ref 11 for the hydroxide ion catalyzed hydrolysis of *O*-acetylsalicylaldehyde at 25 °C is $11 \times 10^4$ $M^{-1}$ $s^{-1}$, nearly tenfold greater than the value that we obtained. This discrepancy led us to repeat the pH–rate profile for the hydrolysis of *O*-acetylsalicylaldehyde several times, but the same value for this constant, namely $1.2 \times 10^4$ $M^{-1}$ $s^{-1}$, was always found, within experimental error. Interestingly Shalitin and Bernhard[14] have reported this same value for the hydrolysis of *O*-cinnamoylsalicylaldehyde. Such agreement is precisely what one would expect since the rate-limiting step of the reaction is the hydration of the aldehyde which should occur at very nearly the same rate for *O*-acetylsalicylaldehyde and *O*-cinnamoylsalicylaldehyde.
(25) R. S. Johnson, J. A. Walder, and I. M. Klotz, following paper in this issue.

# Catalysis of the Hydrolysis of a Nitrophenyl Ester by *o*-Hydroxybenzaldehyde in the Presence of a Poly(ethylenimine) Derivative

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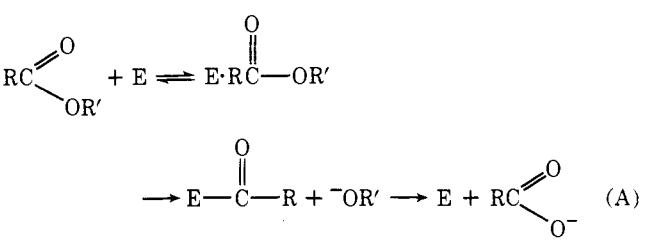
**Abstract:** *o*-Hydroxybenzaldehyde, a bifunctional reagent, has been shown to be effective in catalyzing the hydrolysis of nitrophenyl esters. The phenoxide ion acts as the primary nucleophile, attacking the ester and forming an acylated intermediate; the proximal hydrated formyl group acts as the secondary nucleophile to deacylate the acylated intermediate. For the reaction in the presence of a binding polymer, lauryl quaternized poly(ethylenimine), a kinetic equation has been derived under the conditions of concentration of polymer > *o*-hydroxybenzaldehyde > 3-nitro-4-acetoxybenzoic acid. The second-order rate constant calculated using this equation is 160 times greater than the value observed in the absence of polymer. This acceleration reflects the binding of the anionic reactants to the positively charged polymer and also a decrease in the $pK_a$ of the phenolic group (by 0.9 units) of *o*-hydroxybenzaldehyde. That true catalysis occurs was demonstrated in the regeneration of 96% of the original *o*-hydroxybenzaldehyde in an experiment in which a 2.5-fold excess of ester was hydrolyzed through the acylsalicylaldehyde pathway.

There have been numerous attempts to reproduce enzyme-like catalytic behavior with synthetic polymers.[1-4] Most of the investigators in this area have tried to reproduce the esterolytic properties of the serine proteases, since this class of enzymes has been well characterized.[5]

It is generally agreed that, in the enzymatic hydrolysis of labile ester substrates, such as *p*-nitrophenyl acetate, the first step is the formation of a noncovalent complex between the enzyme and ester. This is followed by the formation of an acyl enzyme intermediate, which is subsequently hydrolyzed to regenerate the original enzyme (reaction A).



(A)

Since the first step in the overall process is the binding of the ester substrate to the macromolecule, the polymer selected for

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