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Catalysis of Ester Hydrolysis by *N*-(2-Dimethylaminoethyl)Acetohydroxamic Acid (*I*)

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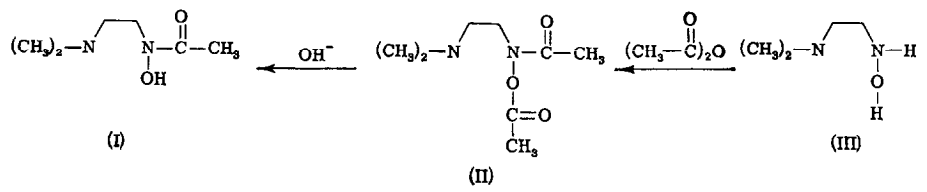
N-(2-dimethylaminoethyl)acetohydroxamic acid was synthesized. This compound, which incorporates a dimethylamino group as a second functionality into the hydroxamic acid molecule, catalyzes the hydrolysis of *p*-nitrophenyl acetate faster than acetohydroxamic acid itself does. The function of the dimethylamino group is to labilize the intermediate formed in the reaction, thus assisting deacylation intramolecularly. The dimethylamino group carries out this function by intramolecular general base catalysis. Nucleophilic catalysis is ruled out by the sizable deuterium oxide solvent isotope effect ($k_{H_2O}/k_{D_2O} = 2.05$) found. General acid–hydroxide ion catalysis is ruled out by determination of the lack of reaction with azide ion, which does not possess a dissociable proton, with the intermediate in this reaction. The deuterium oxide solvent isotope effect on the azide ion reaction of the intermediate also rules out a general acid–hydroxide ion reaction.

INTRODUCTION

The *N*-alkylhydroxamate ion was shown to be an efficient nucleophilic catalyst in the neutral pH region in the hydrolysis of ester substrates forming an intermediate which had reasonable lability leading to the formation of the final product and the regeneration of the catalyst (*2*). However, the rate-determining step of the reaction was the decomposition of the intermediate. Therefore we looked for ways to speed this reaction and found that the introduction of a second functionality, specifically the dimethylamino group in a stereochemically correct manner, assists this reaction which corresponds to enzymatic deacylation. This corresponds to intramolecular assistance (of the deacylation reaction), a process which is known in some cases to give large rate accelerations (3–5). The internal dimethylamino group could theoretically function as a nucleophilic catalyst, a general basic catalyst, or a general acid–hydroxide ion catalyst, which are all kinetically equivalent to one another. Experiments were performed to show that it is in fact acting as a general basic catalyst, by the use of deuterium oxide solvent isotope effects and the use of azide ion in water and deuterium oxide.

EXPERIMENTAL

N-(2-dimethylaminoethyl)acetohydroxamic acid (**I**) was prepared by the basic hydrolysis of *N,O*-diacetyl-2-(dimethylamino)ethylhydroxylamine (**II**), which was in turn synthesized by the acetylation of *N*-(2-dimethylaminoethyl)hydroxylamine (**III**).



SCHEME 1

MATERIALS

N-(2-dimethylaminoethyl)hydroxylamine (**III**) was synthesized by diborane reduction of dimethylaminoacetaldoxime, prepared by the method of Somin and Kuznetsov (*6*), according to Feuer and Vincent (*7*), followed by acidic hydrolysis of the boronic acid ester. Dimethylaminoacetaldoxime, 9.0 g (0.088 mol), was dissolved in 50 ml of anhydrous tetrahydrofuran (THF); 200 ml (0.1 mol) of diborane in THF was added dropwise below 5°C. The solution was stirred for 1 hr at 5°C and then HCl and NaOH were added.

Hydroxylamine formation was tested for with Fehling's solution. The THF was separated and the aqueous phase was extracted with diethyl ether. The combined THF and combined ether extracts, after drying over sodium sulfate, were evaporated to give 8.9 g of a clear liquid (95% yield).

Thin-layer chromatography of the crude hydroxylamine product on silica gel (Eastman Chromatoplates) with methanol as a solvent, showed one spot ($R_f = 0.89$) upon development with Fehling's solution.

The nmr spectrum (CD_3CN, TMS external) exhibited peaks at δ 4.25 (2H, singlet, hydroxylamine protons), 2.90 (4H, broad multiplet, ethylene protons), and 2.45 (6H, singlet, dimethylamine protons).

N,*O*-diacetyl-2-(dimethylamino)ethylhydroxylamine (**II**) was synthesized by acetic anhydride acetylation of **III**. The hydroxylamine, 8.9 g (0.85 mol of the ether extract product), was dissolved in 100 ml of acetonitrile and cooled to 2°C. Then 27 g (0.27 mol) of acetic anhydride dissolved in 25 ml acetonitrile was added dropwise below 15°C. The solution was stirred for 2 hr at 25°C and then the solvent and excess acetic anhydride were removed *in vacuo*. The acetic acid was separated by fractional distillation *in vacuo* on a Vigreaux column. On further distillation at 0.4 mm, 5.4 g of **II** was collected; bp, 75–80°C; yield, 30%. The sample of **II** which was used for elemental analysis and kinetic studies was distilled two additional times on a concentric tube column: 1) bp, 50–54°C (0.05 mm); 2) bp, 56–57°C (0.1 mm). The final sample was clear, odorless, and initially showed no color upon admixture with 5% $FeCl_3$ in 0.1 *N* HCl.

Anal. Calcd for $C_8H_{16}N_2O_3$: C, 51.05; H, 8.57; N, 14.88. Found: C, 51.55; H, 8.72; N, 14.64.

The nmr spectrum showed singlets at δ 1.90 (3H, *N*-acetyl protons) and 2.20 (9H, *O*-acetyl and dimethylamino protons) and triplets at 2.45 (2H, $J = 6.7$ Hz, ethylene protons) and 3.75 (2H, $J = 6.7$ Hz, ethylene protons). The infrared spectrum had λ_{max} (liquid film, sodium chloride plates) at 3.39 and 3.60 (weak, C—H), 5.59 (strong, $-OCOCH_3$), 5.95 (strong, $\rangle NCOCH_3$), 6.89, 7.13, and 7.28 (weak), and 8.49 (strong).

N-(2-dimethylaminoethyl)acetohydroxamic acid (**I**) was prepared by both the basic hydrolysis of **II** and by the chromatography of **II** on a silica gel column with methanol elution. For aqueous hydrolysis, 1.0 g (5.2 mmol) of **II** was dissolved in 10 ml of water, and, at 5°C, 5.2 ml of 1 *N* sodium hydroxide (5.2 mmol) was added dropwise. The solution was allowed to stand at room temperature and then extracted continuously with diethyl ether.

For the synthesis of **I** by chromatography, 3.0 g (15.6 mmol) of the diacetylhydroxylamine was applied to a 3 × 35-cm silica-gel column and eluted with methanol. By removing the methanol on a rotary evaporator, 2.1 g of viscous oil was recovered. Both products were fractionally distilled *in vacuo* on a short path distillation head; bp, 80–85°C (0.5 mm). This material was rechromatographed on silica gel and distilled bulb-to-bulb in a Kügelrohr apparatus; bp, 85–90°C (0.1 mm).

Anal. Calcd for $C_6H_{14}N_2O_2$; C, 49.30; H, 9.65; N, 19.16. Found: C, 49.21; H, 9.72; N, 19.18.

The nmr spectrum had peaks at δ 2.10 (3H, singlet, *N*-acetyl protons), 2.30 (6H, singlet, dimethylamino protons), 2.60 (2H, triplet, $J = 6.0$ Hz, ethylene protons), 3.80 (2H, triplet, $J = 6.0$ Hz, ethylene protons), and 9.65 (1H, singlet, hydroxyl proton). The infrared spectrum had λ_{max} (liquid film, salt plates) at 3.16 (medium, OH), 3.40 and 3.60 (strong, C-H), 6.12 (strong, $NCOCH_3$), and 6.86 and 8.30 (strong).

Kinetics of the N-*(2-Dimethylaminoethyl)Acetohydroxamic Acid-Catalyzed Hydrolysis of* p-*Nitrophenyl Acetate*

a. Pseudo-first-order kinetics. Borate buffer solutions containing **I** were used within a week after preparation and kept cold between experiments. For studies with *p*-nitrophenyl acetate in excess, the appearance of *p*-nitrophenolate ion product adhered to pseudo-first-order kinetics. The hydrolysis of *p*-nitrophenyl acetate was followed at 464 nm. For runs at high pH, rate constants were calculated from the usual first-order plots. For slow reactions at lower pH, the reactions were not followed to completion and pseudo-first-order rate constants were determined by Eq. [1] (*8*).

$$A_t = (A_{t+\Delta})\, e^{\Delta \cdot k_{obs}} + A_\infty(1 - e^{\Delta \cdot k_{obs}}). \qquad [1]$$

b. Burst kinetics with ester in excess. For the reaction of **I** with a 20-fold excess of *p*-nitrophenyl acetate, the biphasic release of *p*-nitrophenolate ion was followed at 464 or 453 nm.

For the zero-order portion of the curve, the experimental slopes, $[P_1]/t$, were calculated from Eq. [2] using two absorbance values in the zero-order portion of the curve, A_{t_1} and A_{t_2}, and 2670 for the extinction coefficient of *p*-nitrophenolate ion, ε_{PNPO^-}, at 464 nm in 32% acetonitrile at pH = 9.09, $I = 0.2$ *M*.

$$[P_1]/t(M\,\text{sec}^{-1}) = \frac{A_{t_1} - A_{t_2}}{t_1 - t_2} \cdot \frac{1}{\varepsilon_{PNPO^-}}. \qquad [2]$$

Steady-state rate constants were calculated from the 464-nm data only since these data proved to be the most reliable. The pH of each reaction solution after the burst (ca. 5% completion) was found to be the same as the initial pH.

c. The hydrolysis of N,O-*diacetyl-2-(dimethylamino)ethylhydroxylamine* (**II**). The solvolysis of **II** in 30% acetonitrile–water and in deuterium oxide was followed by

monitoring the release of hydroxamate anion product at 263–270 nm. All reactions gave linear pseudo-first-order plots to 90% completion. Deuterium oxide buffer solutions were prepared from 99.88% D_2O, solid buffer reagents, and potassium chloride.

d. The reaction of N,O-*diacetyl-2-(dimethylamino)ethylhydroxylamine with azide ion.* This reaction was followed by observing the release of hydroxamate anion at 263 nm. Upon completion of the reaction of the acetyl hydroxamates with azide ion, the absorbance was observed to be about 20% higher than that for the hydrolysis of the acyl hydroxamate in the absence of azide ion at the same pH. Also, in the azide ion experiments, the absorbance decreased very slowly after completion of the reaction. These two observations are in accord with the formation followed by the slow hydrolysis of an acetyl azide intermediate (*9*).

RESULTS AND DISCUSSION

A number of studies were made of the *N*-(2-dimethylaminoethyl)acetohydroxamic acid-catalyzed hydrolysis of *p*-nitrophenyl acetate.

1. Pseudo-First-Order Kinetics with Ester in Excess

When 6.34×10^{-4} *M* **I** was reacted with threefold excess *p*-nitrophenyl acetate (PNPA) at pH 8.26, 30.5% acetonitrile, the release of *p*-nitrophenolate ion followed pseudo-first-order kinetics to at least 95% completion of the reaction with $k_{obs} = 4.30 \times 10^{-4}$ sec^{-1}, spontaneous hydrolysis, $k_{obs} = 1.8 \times 10^{-5}$ sec^{-1}. Therefore **I** catalyzes PNPA hydrolysis by 25-fold (with threefold excess ester).

TABLE 1

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF *p*-NITROPHENYL ACETATE IN THE PRESENCE OF *N*-(2-DIMETHYLAMINOETHYL)ACETOHYDROXAMIC ACID[a]

p*H*[b]	$k_{obs}^{HA} \times 10^4$ (sec^{-1})	$k_{obs}^{0} \times 10^5$ (sec^{-1})
8.24	3.12	1.81
8.30	3.91	2.02
8.99	8.20	5.88
9.51	13.6	15.2
9.76	18.7	21.4
9.99	24.4	36.3
10.46	30.7	73.9
10.94[c]	50.9	210

[a] [I] = 5.96×10^{-4} *M*, [Ester] = 1.61×10^{-3} *M*, 0.2 *M* borate buffer, *I* = 0.2 *M*, 30.2% acetonitrile.

[b] Measured pH.

[c] 0.2 *M* carbonate buffer.

The catalysis of *p*-nitrophenyl acetate hydrolysis by **I** was studied as a function of pH under catalytic conditions slightly different from those above (5.96×10^{-4} *M* hydroxamic acid). The pseudo-first-order rate constants for hydrolysis in buffer alone, k^0_{obs}, and in the presence of hydroxamic acid, k^{HA}_{obs}, are recorded in Table 1. A plot of $-\log(k^{HA}_{obs} - k^0_{obs})$ as a function of pH is shown in Fig. 1. The upper curve in Fig. 1 is a theoretical line, calculated on the basis of the ionization of the hydroxamic acid. Assuming that the catalyst concentration remains constant throughout the reaction, the rate constant of the hydroxamic acid, $k_{HA} = 5.0\ M^{-1}\ sec^{-1}$.

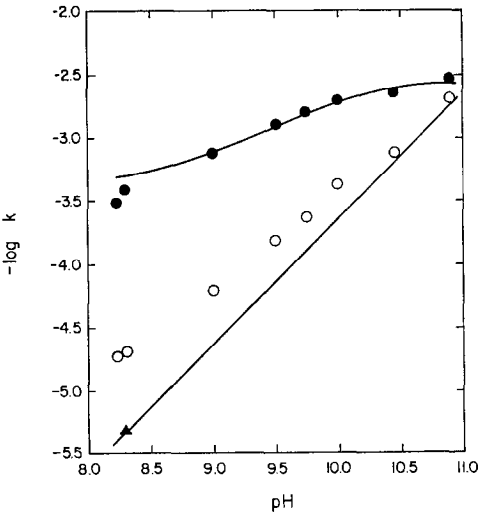


FIG. 1. pH rate profile for the *N*-(2-dimethylaminoethyl)acetohydroxamic acid-catalyzed hydrolysis of *p*-nitrophenyl acetate. $k = (k^{HA}_{obs} - k^0_{obs})$, (-●-●-); $k = k^0_{obs}$, (-○-○-); $k = k_{hydr}$ at zero buffer concentration (-▲-).

For comparison, Fig. 1 also includes the data for k^0_{obs} (lower line has a slope of 1) from Table 1 and one rate constant which is corrected for buffer catalysis, k_{hydr}. By extrapolation to zero buffer concentration, the buffer-independent rate constant was found to be $6.3 \times 10^{-6}\ sec^{-1}$. From this value, k_{OH^-} for the hydrolysis of *p*-nitrophenyl acetate is $7.1\ M^{-1}\ sec^{-1}$ in 30% acetonitrile; k_{H_2O} could not be detected. For *p*-nitrophenyl acetate hydrolysis with less than 1% acetonitrile, $I = 1.0\ M$, $k_{OH^-} = 9.50\ M^{-1}\ sec^{-1}$ (*35*).

2. *Biphasic Kinetics with Ester in Large Excess*

When 2.5×10^{-4} *M* **I** is reacted with $5\text{–}20 \times 10^{-3}$ *M p*-nitrophenyl acetate at pH 9.09 (increased ester and decreased catalyst over the previous experiment), the release of *p*-nitrophenolate ion with time is a biphasic curve as shown in Fig. 2. There was an initial rapid liberation, "burst," of *p*-nitrophenolate ion, followed by a slower, zero-order, release. The observation of biphasic kinetics can be interpreted in terms of

Eq. [3]. For a reaction which follows Eq. [3], if experimental conditions are such that $k_1[S]_0 > k_2$ and $[S]_0 \gg [C]_0$, the release of P_1 will follow Eq. [4] (*41*).

$$\begin{array}{c} C + S \rightarrow CS' \rightarrow C + P_2 \\ + \\ P_1 \end{array} \qquad [3]$$

$$[P_1] = \frac{k_1[S]_0[C]_0(k_2 - b)}{-b^2} \cdot (1 - e^{-bt}) + \frac{k_1 \cdot k_2[S]_0[C]_0}{b} \cdot t + k_3[S]_0 t. \qquad [4]$$

In Eq. [4] $[S]_0$ and $[C]_0$ are the initial substrate and catalyst concentrations, respectively, k_1 is equal to $k_{HA} \cdot \alpha_{HA}$, k_2 is the rate constant for the hydrolysis of intermediate

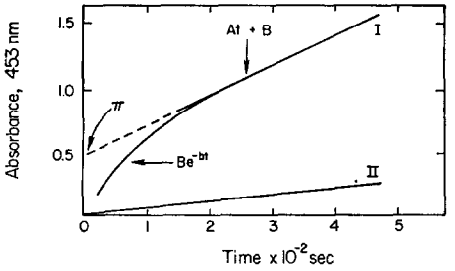


FIG. 2. Absorbance versus time curves for the hydrolysis of 7.0×10^{-3} *M p*-nitrophenyl acetate at pH 9.09, 32% acetonitrile. Curve I, hydrolysis in the presence of 2.5×10^{-4} *M* I. Curve II, spontaneous hydrolysis at pH 9.09.

CS, b is defined as $k_1[S]_0 + k_2$, and α_{HA} is the degree of ionization of the hydroxamic acid. By collecting constants in Eq. [4], it may be reduced to

$$[P_1] = At + B(1 - e^{-bt}), \qquad [5]$$

where A and B are constants.

The first-order rate constants, b, measured at several substrate concentrations, are presented in Table 2. Since $b = k_1[S]_0 + k_2$, a plot of b versus $[S]_0$ should give a straight

TABLE 2

DEPENDENCE OF THE FIRST-ORDER RATE CONSTANT, b, ON ESTER CONCENTRATION FOR THE REACTION OF *p*-NITROPHENYL ACETATE WITH *N*-(2-DIMETHYLAMINO)ACETOHYDROXAMIC ACID[a]

$[S]_0 \times 10^3$ (*M*)	$b \times 10^2$ (sec^{-1})	No. of runs
5.30	1.13 ± 0.07	4
7.04	1.48 ± 0.12	9
8.44	1.53 ± 0.05	3
9.36	1.85 ± 0.08	5
1.13	2.02 ± 0.08	4

[a] $[I] = 2.50 \times 10^{-4}$ *M*, 32.0–33.5% (v/v) acetonitrile, $I = 0.2$ *M*, pH 9.09, 0.2 *M* borate buffer.

line of slope k_1 and intercept k_2. Such a plot for the data in Table 2 was made and from the least squares line of this plot, $k_1 = 1.46 \pm 0.15\ M^{-1}\ \text{sec}^{-1}$ and $k_2 = 3.89 \pm 1.3 \times 10^{-3}$ sec^{-1}.

3. Kinetics with Catalyst in Excess

To check the validity of the kinetics under catalytic conditions, *p*-nitrophenyl acetate was hydrolyzed in the presence of a 10- to 50-fold excess of **I**. The results at pH 9.09 are summarized in Table 3. From Fig. 3, $k_{HA}\alpha_{HA} = 1.67 \pm 0.04\ M^{-1}\ \text{sec}^{-1}$. Within experimental error, this is the same value obtained for $k_{HA} \cdot \alpha_{HA}(k_1)$ from analysis of the biphasic kinetics under turnover conditions.

TABLE 3

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE REACTION OF *p*-NITROPHENYL ACETATE WITH EXCESS *N*-(2-DIMETHYLAMINOETHYL)ACETOHYDROXAMIC ACID[a]

Hydroxamic acid ($\times 10^3\ M$)	k_{obs}^{HA} ($\times 10^3\ \text{sec}^{-1}$)	No. of runs
1.93	3.08	3
3.91	6.16	3
4.82	8.00	3
7.49	12.3	1

[a] [Ester] $= 1.53 \times 10^{-4}\ M$, pH 9.09, $I = 0.2\ M$, 0.2 *M* borate buffer, 32.2% (v/v) acetonitrile.

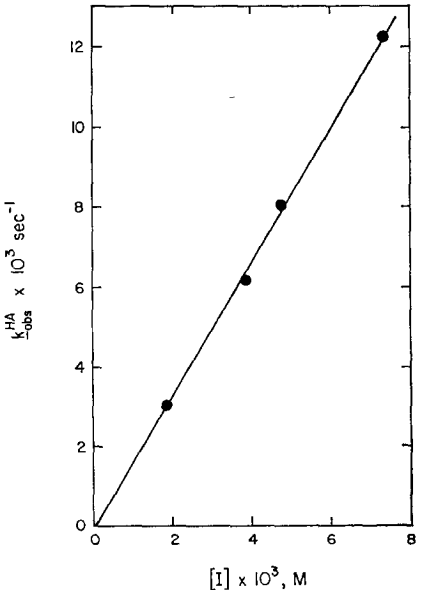


FIG. 3. Pseudo-first-order rate constant versus [I] plot for the reaction of *p*-nitrophenyl acetate with excess *N*-(2-dimethylaminoethyl)acetohydroxamic acid, pH 9.09, 32.2% acetonitrile.

Since at pH 9.09 the hydroxamic acid functionality of *N*-(2-dimethylaminoethyl)-acetohydroxamic acid is 27% ionized, $k_{HA} = 6.2 \pm 0.5\ M^{-1}\ sec^{-1}$ when determined with excess catalyst. This is in good agreement with the pseudo-first-order kinetics under catalytic conditions where k_{HA} was found to be $5.0\ M^{-1}\ sec^{-1}$. This result shows conclusively that *N*-(2-dimethylaminoethyl)acetohydroxamic acid is acting as a true catalyst for the hydrolysis of *p*-nitrophenyl acetate.

4. Hydrolysis of N,O-*Diacetyl*-N-(*2-Dimethylaminoethyl*)*hydroxylamine in 30.6% Acetonitrile*

The observation of a burst in the *N*-(2-dimethylaminoethyl)acetohydroxamic acid-catalyzed hydrolysis of *p*-nitrophenyl acetate is strong evidence that there is an intermediate on the catalytic pathway. Since the nucleophilicity of hydroxamate ions toward labile esters is far greater than that of the dimethylamino group (*35*), it is reasonable to

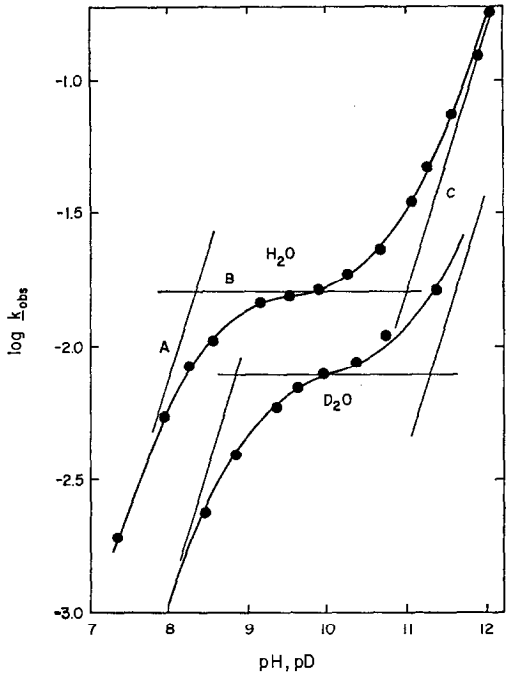


FIG. 4. pH log(k_{obs}) profiles for the solvolysis of *N,O*-diacetyl-2-(dimethylamino)ethylhydroxylamine in H_2O and D_2O. [II] = 2.68×10^{-4} *M*, ionic strength = 0.2 *M*, 0.8% acetonitrile; 99.68–99.75% deuterium oxide, ionic strength = 0.8 *M* at pH 8.23. The curve for H_2O is a theoretical line (see text).

suggest that this intermediate is *N,O*-diacetyl-*N*-(2-dimethylaminoethyl)hydroxylamine (**II**). Support for this hypothesis was obtained by hydrolysis of **II** under the same conditions as in the catalysis experiments. At pH 9.09, $I = 0.2\ M$, 0.2 *M* borate buffer, 30.6% acetonitrile, the hydrolysis rate of **II** is $4.53 \times 10^{-3}\ sec^{-1}$. Within experimental error, this was the same rate constant that was obtained for the hydrolysis of the intermediate observed kinetically under biphasic conditions. The pH-dependence of

the pseudo-first-order rate constant for the hydrolysis of **II** was determined in 30.6% acetonitrile. The logarithms of the k_{obs} values were plotted against pH as solid circles (Fig. 4).

The rate equation for the hydrolysis reaction is derived assuming there are two reactive forms of **II**, one with a protonated amino group, A, and one with an unprotonated amino group, B. The total hydroxylamine, $A + B$, will be represented by T. Terms are included for hydroxide ion (k_{AOH}, k_{BOH}), hydronium ion (k_{AH}, k_{BH}), and water (k_{AW}, k_{BW}) catalysis (*32*). Buffer catalysis may be neglected. Since it is sterically possible for the dimethylamino group of **II** to act as an intramolecular nucleophile, a nucleophilic term is included, k_N. Since the reactions were carried out above neutrality, the terms involving $[H^+]$ may be neglected. Equation [6] may then be derived by use of the relationships $T = A + B$ and $K_a = ([H][B]/[A])$. When plotting $\log(k_{obs})$ versus pH, at low pH where $[H^+] \gg K_a$, terms involving k_{AOH}, k_{BW}, and k_N describe a straight-

$$-\frac{dT}{dt} = k_{obs} = \frac{T}{H^+ + K_a} k_{AW}[H_2O]\cdot[H^+] + k_{AOH}[OH^-]\cdot[H^+] + K_a k_{BW}[H_2O] + K_a k_{BOH}[OH^-] + K_a k_N \quad [6]$$

line of slope one. Where $K_a \gg [H^+]$, these three terms describe a straight-line of slope zero. With $K_a \gg [H^+]$ the term involving k_{BOH} describes a line of slope 1. These three regions in the pH-$\log(k_{obs})$ profile were observed and are delineated by the solid straight lines in Figs. 4A, B, and C, respectively. The k_{AW} term in Eq. [6] is not observed. k_I is defined as given in Eq. [7].

$$k_I = \frac{k_{AOH}\cdot K_w}{K_a} + [H_2O]\cdot k_{BW} + k_N. \quad [7]$$

k_{obs} can be expressed as

$$k_{obs} = \frac{K_a\cdot k_I}{K_a + [H^+]} + (k_{BOH}\cdot[OH^-]). \quad [8]$$

(The symbol I is introduced to signify "intramolecular.")

The solid curved line (in H_2O) in Fig. 4 is the theoretical pH-$\log(k_{obs})$ profile calculated from Eq. [8] using the constants $k_I = 4.80 \times 10^{-3}$ sec^{-1}, $pK_a = 7.90$, and $k_{BOH} = 15.97\ M^{-1}$ sec^{-1}.

For comparison, the pH dependence of the hydrolysis of *N,O*-diacetyl-*N*-methylacetohydroxylamine was studied in 30.5% acetonitrile. Hydrolysis rates at a series of pH values were determined. From a plot of k_{obs} versus hydroxide ion concentration, $k_{OH^-} = 12.7 \pm 1\ M^{-1}$ sec^{-1} and $k_{H_2O} < 10^{-5}\ M^{-1}$ sec^{-1}. [These values agree well with those found by Kirsch and Jencks (*10*).]

Two comparisons can be made between the pH-log (rate) profile for the hydrolysis of *N,O*-diacetyl-*N*-methylacetohydroxylamine and the pH-log (rate) profile for the hydrolysis of **II**: 1) At high pH where the k_{BOH} term predominates in the hydrolysis of **II**, the hydrolysis rates of the two esters are almost identical. Thus, the steric and polar effects of the dimethylamino group are in this case very small. 2) At low pH the hydrolysis rate of **II** is far greater than that of *N,O*-diacetyl-*N*-methylhydroxylamine. By a comparison of the two, the dimethylamino derivative hydrolyzes 1800 times faster at low pH than does *N,O*-diacetyl-*N*-methylhydroxylamine. This rate acceleration is attributed to a specific catalytic effect of the dimethylamino group.

The experiments which follow are designed to establish the mechanism of this catalysis.

5. Hydrolysis of N,O-*Diacetyl*-N-(*2-Dimethylaminoethyl*)*hydroxylamine in Water and Deuterium Oxide*

In order to evaluate the effects of deuterium oxide on the hydrolysis of **II**, this compound was solvolyzed in H_2O and D_2O without added acetonitrile. A $\log(k_{obs})$ versus pH plot of the data is shown in Fig. 4. The upper line in Fig. 4 is the theoretical curve calculated from Eq. [8] using the following constants for the variable parameters: $k_I = 1.60 \times 10^{-2}$ sec^{-1}, p$K_a = 8.23$, and $k_{BOH} = 12.3\ M^{-1}$ sec^{-1}.

The pK_{app} value of 8.23 obtained from the pH-log(k_{obs}) profile (pK_{app} is the kinetically determined pK_a) agrees very well with the pK_a of 8.25 for **II** determined by potentiometric titration.

As in 30.6% acetonitrile, k_{BOH} for hydrolysis of **II** is very close to the second-order rate constant for the hydrolysis of *N,O*-diacetyl-*N*-methylhydroxylamine.

Figure 4 includes $\log k_{obs}$ data for the deuterolysis of **II** in 99.68–99.75 mol % deuterium oxide at different p*D* values. The p*D* values were calculated from measured pH readings and the known relationship between pH and p*D*, p*D* = meter pH + 0.4 (*11*). The solid line through the data points was calculated from Eq. [8] using the values: $k_I = 0.0078$ sec^{-1}, p$K_{app} = 8.82$, and $k_{BOD} = 17.5\ M^{-1}$ sec^{-1}.

From the k_I in water, $k_I^{H_2O}$, and the k_I in deuterium oxide, $k_I^{D_2O}$, the solvent deuterium isotope effect on k_I, $k_I^{H_2O}/k_I^{D_2O} = 2.05$.

The specific base-catalyzed hydrolysis of **II** shows an inverse kinetic solvent deuterium isotope effect, $k_{BOH}/k_{BOD} = 0.70$. This result is consistent with other studies where it is generally observed that the nucleophilicity of OD^- in D_2O is 20–40% higher than OH^- in H_2O (*13–16*).

6. The Reaction of N,O-*Diacetyl*-N-(*2-Dimethylaminoethyl*)*Hydroxylamine with Azide Ion at Low pH in Water and Deuterium Oxide*

Table 4 lists the pseudo-first-order rate constants for the reaction of **II** with different concentrations of azide ion in water and deuterium oxide. In the pH (p*D*) range of these studies, hydrozen azide is completely dissociated (p$K_a = 4.72$ for HN_3 (*17*) and the dimethylamino group of **II** is 98% protonated (p$K_a = 8.23$). In addition, in this pH range the azide reactions are pH independent as shown by the results at pH 6.58, 6.93, and 7.56 at constant azide concentration equal to 0.102 *M*. The small increase in k_{obs} at pH 7.56 is accounted for by the higher azide independent hydrolysis rate (see Fig. 5). Therefore, the dependence of the reaction rate on azide concentration should be described by Eq. [9]; where *A* is protonated **II**, k_{Az} is the second-order rate constant for the reaction of azide ion with *A*, and k_{obs}^0 is the hydrolysis rate of **II** in the absence of azide ion.

$$-(d[A]/dt) = k_{obs}[A] = k_{obs}^0[A] + k_{Az}[N_3^-][A]. \qquad [9]$$

With a great excess of azide ion, pseudo-first-order conditions, $k_{obs} = k_0 + k_{Az}[N_3^-]$. Thus a plot of k_{obs} versus azide concentration, $[N_3^-]$, should be a straight line of slope $= k_{Az}$ and intercept $= k_0$. Such a plot is shown in Fig. 5 for the data in Table 4 at pH 6.53–6.57 and p*D* 6.93–7.07. From the least squares line (solid line, Fig. 5) for

TABLE 4

RATE CONSTANTS FOR THE REACTION OF AZIDE ION WITH *N,O*-DIACETYL-2-(DIMETHYLAMINO)ETHYLHYDROXYLAMINE IN WATER AND DEUTERIUM OXIDE[a]

pH (p*D*)	Solvent	[Azide] (*M*)	k_{obs}[c] ($\times 10^2$ sec^{-1})
6.57	H_2O	0.0514	0.67 ± 0.07
6.58	H_2O	0.1025	1.14 ± 0.06
6.53	H_2O	0.1480	1.56 ± 0.25
6.93	H_2O	0.1017	1.28
7.56	H_2O	0.1024	1.61
6.99	D_2O[b]	0.0493	0.61 ± 0.08
6.93	D_2O[b]	0.1004	0.97
7.07	D_2O[b]	0.1497	1.48 ± 0.21
6.59	H_2O[d]	0	0.07
6.58	H_2O[e]	0	0.06
7.0	D_2O[f]	0	0.025

[a] [**II**] = 6.73×10^{-4} *M*, *I* = 0.2 *M*, 0.11–0.03 *M* phosphate buffer, 0.8% acetonitrile, 25°C.

[b] 99.85% D_2O, 0.03 *M* phosphate buffer, *I* = 0.2 *M* with KCl.

[c] Where standard deviations are given, values are averages of three runs.

[d] In 0.11 *M* phosphate buffer, *I* = 0.2 *M*.

[e] In 0.06 *M* phosphate buffer, *I* = 0.2 *M*.

[f] Estimated from Fig. 4 and k_{obs} in H_2O without added azide.

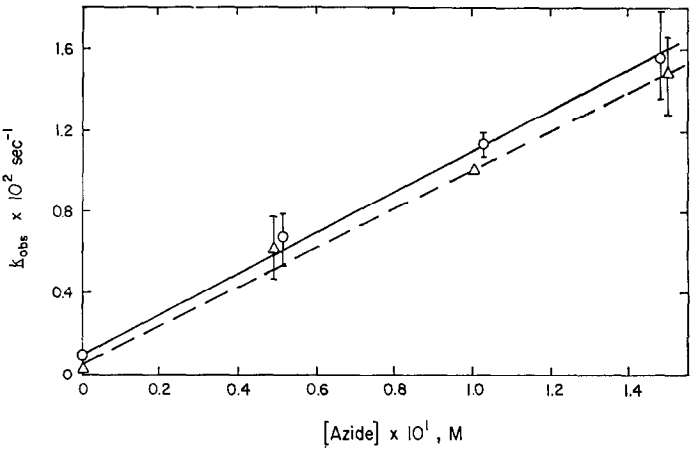


FIG. 5. Plot of pseudo-first-order rate constant versus azide concentration for the reaction of azide ion with *N,O*-diacetyl-2-(dimethylamino)ethylhydroxylamine in H_2O and D_2O. Reaction in H_2O (○). Reaction in D_2O (△).

the reaction in H_2O, $k_{Az} = 0.100 \pm 0.004\ M^{-1}$ sec^{-1} and the intercept = $1.0 \pm 0.4 \times 10^{-3}$ sec^{-1}, the same as k^0_{obs} within experimental error. For the reaction in D_2O (dashed line, Fig. 5), $k_{Az} = 0.095 \pm 0.006\ M^{-1}$ sec^{-1} and intercept = $6.4 \pm 5.5 \times 10^{-4}$ sec^{-1}, within experimental error the same as k^0_{obs}.

The mechanistically significant parameter from these azide ion experiments is the value of the kinetic solvent deuterium isotope effect, $k_{Az}^{H_2O}/k_{Az}^{D_2O}$. From the values of k_{Az} above, $k_{Az}^{H_2O}/k_{zAz}^{D_2O} = 1.06 \pm 0.08$. The estimation of error for this value is based on Eq. [37] of Beers (*18*).

7. The Reaction of N,O-*Diacetyl*-N-*Methylhydroxylamine with Azide Ion in Water*

N,O-diacetyl-*N*-methylhydroxylamine was reacted with azide ion under the same conditions used in the **II**-azide ion experiments. The pseudo-first-order rate constants for this reaction at different azide ion concentrations are given in Table 5.

TABLE 5

RATE CONSTANTS FOR THE REACTION OF AZIDE ION WITH *N,O*-DIACETYL-*N*-METHYLHYDROXYLAMINE IN WATER[a]

[Azide] (M)	$k_{obs} \times 10^3$ (sec^{-1})
0.0	0.02
0.0593	1.9 ± 0.5
0.1043	3.0 ± 0.2
0.1520	3.9

[a] pH = 6.0, $I = 0.2$ M, 0.30–0.11 M phosphate buffer, [*N,O*-diacetyl-*N*-methylhydroxylamine] = 7.1×10^{-4} M, 0.8% acetonitrile, reaction followed at 263 nm.

From the slope of a plot of k_{obs} versus azide ion concentration, $k_{Az} = 2.7 \pm 0.5 \times 10^{-2}$ M^{-1} sec^{-1} for *N,O*-diacetyl-*N*-methylhydroxylamine.

The introduction of a dimethylamino group into the hydroxamic acid profoundly affects the magnitude of the catalysis by this molecule. The dimethylamino group acts as a classical intramolecular catalyst. In the following sections we will try to probe the mechanism of its action.

8. The Mechanism of N,O-*Diacetyl*-N-(*2-Dimethylaminoethyl*)*Hydroxylamine* (**II**) *Hydrolysis*

To account for the plateau regions in Fig. 4 for the hydrolysis of **II** it was necessary to assume that the hydrolysis rate is dependent on the ionization state of the dimethylamino group and to postulate that this group affects a marked catalysis on the hydrolysis reaction. However, on the basis of the pH-rate profile it is not possible to speculate on the mechanism of this catalysis or even to assign the acidic or basic form of the dimethylamino group as the reactive species. In theory, any one or each of the terms given in Eq. [10] may contribute to the observed catalytic rate constant, k_I.

$$k_I = \frac{k_{AOH} \cdot K_w}{K_a} + k_{BW}[H_2O] + k_N. \qquad [10]$$

Associated with each term in Eq. [10] there is at least one reasonable mechanistic interpretation which can account for the observed catalysis by the dimethylamino group.

The k_{BW} term in Eq. [10] for the reaction of water with the unprotonated form of **II** could explain the observed catalysis on the basis of a general base mechanism. In this mechanism, the dimethylamino group assists water attack at the carbonyl function by acting as an intramolecular base for proton abstraction from water. This type of general base mechanism has been shown to account for the rapid hydrolysis rate of aspirin (*19*) and *p*-nitrophenyl 5-nitrosalicylate (*9*); these esters show intramolecular participation by the carboxylate and phenoxide ions, respectively. In addition, it has been suggested that the imidazole group acts as an internal general base in the hydrolysis of 4-(2′-acetoxyphenyl)-imidazole (*20*).

As previously discussed, the k_N term was included in Eq. [10] to accommodate the possibility of a direct nucleophilic attack by the dissociated dimethylamino group at the carbonyl functionality. Intramolecular nucleophilic catalysis by the dimethylamino group has been postulated to be operative in the hydrolysis of the phenyl esters of γ-(*N*,*N*-dimethylamino)butyric acid and δ-(*N*,*N*-dimethylamino)valeric acid (*21*). However, for these esters general base catalysis by the dimethylamino group has not been ruled out experimentally.

The k_{AOH} term for attack of hydroxide ion on the protonated form of **II** may be mechanistically described as a general acid-specific hydroxide ion catalysis. In this mechanism the dimethylammonium ion may assist the attack of hydroxide ion by polarization of the carbonyl group through hydrogen bonding. The general acid mechanism has been most favored by previous authors for the hydrolysis of carboxylic acid derivatives containing a neighboring hydroxyl (*14*, *22*) or amino group (*23*, *24*). This premise has been supported by the observation of intramolecular hydrogen bonding of the ammonium hydrogen atom ($-NH^+$) to the ester carbonyl group in chloroform solution (*24*). Recently, St. Pierre and Jencks (*19*) have presented compelling evidence that the acidic species of aspirin catalyzes the attack of weakly basic amines by an intramolecular general acid-assisted mechanism.

The acidic form of the dimethylamino group may also facilitate hydroxide ion attack by an electrostatic effect of the formal positive charge. Electrostatic catalysis by the dimethylammonium ion could result either from an increased local concentration of hydroxide ion in the vicinity of the carbonyl group (*25*, *26*), or from direct polarization of the carbonyl group (*27*). Studies of the hydrolysis of acetate esters containing the trimethylammonium functionality in the β and γ positions of the alcohol leaving group indicate that this mechanism increases the hydroxide ion rate 17- and 8-fold, respectively (*27*). Since the hydroxamate leaving group of **II** most resembles a γ-substituted alcohol, the electrostatic mechanism probably can be rejected on the basis of the observed 340-fold catalysis by the dimethylamino group (over the unsubstituted hydroxamic acid). However, the trimethylammonium ion might be a poor model for evaluation of the facilitation expected from the electrostatic mechanism since the methyl group could shield the formal charge at nitrogen more effectively than the hydrogen atom (*28*).

Although the pH-dependence of the hydrolysis of **II** cannot distinguish between the various mechanisms discussed above, analysis of the effect of deuterium oxide on k_I as detailed below can serve to rule out the nucleophilic and electrostatic mechanisms.

Since for the general base mechanism, $k_I = k_{BW}(H_2O)$, the correct comparison of the rate constant k_{BW} in H_2O and D_2O is given in Eq. [11]. Since $(D_2O)/(H_2O)$ is

approximately unity, $k_{BW}/k_{BD_2O} = k_I^{H_2O}/k_I^{D_2O} = 2.05$. This rate ratio is within the range of $k^{H_2O}/k^{D_2O} = 2$–3 generally observed for intermolecular general base catalysis (*30*).

$$(k_{BW}/k_{BD_2O}) = (k_I^{H_2O}/k_I^{D_2O}) \cdot (D_2O/H_2O). \quad [11]$$

In addition, it is congruent with the solvent deuterium isotope effects of 1.68 and 1.80, respectively, observed for the intramolecular general base-catalyzed hydrolysis of *p*-nitrophenyl 5-nitrosalicylate (*9*) and aspirin (*29*). Thus the kinetic deuterium oxide solvent isotope effect is consistent with the general base mechanism for the hydrolysis of **II**.

For the nucleophilic mechanism, $k_N^{H_2O}/k_N^{D_2O} = k_I^{H_2O}/k_I^{D_2O} = 2.05$. As discussed previously, for intermolecular nucleophilic reactions at the ester carbonyl, k^{H_2O}/k^{D_2O} ranges generally from 0.9 to 1.2 (*30*). On the basis of the observed deuterium isotope effect of 2.05, the nucleophilic mechanism can be eliminated.

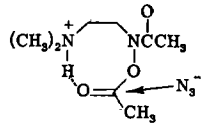
For the general acid-specific base mechanism either involving hydrogen bonding or electrostatics, $k_I = k_{AOH} \cdot (K_w/K_a)$ and the expression relating k_{AOH} in H_2O and D_2O is given in Eq. [12].

$$(k_{AOH}/k_{AOD}) = (k_I^{H_2O}/k_I^{D_2O}) \cdot (K_a^{H_2O}/K_a^{D_2O}) \cdot (K_{D_2O}/K_W). \quad [12]$$

From $K_a^{H_2O}/K_a^{D_2O} = 3.88$ and $K_{D_2O}/K_W = 0.156$, $k_{AOH}/k_{AOD} = 1.24$. In addition, it may be argued that since $k_{BOH}/k_{BOD} = 0.7$ for the reaction of hydroxide ion with neutral **II**, $k_{AOH}/k_{AOD} = (1.24/0.7) = 1.77$ is the appropriate value to compare with k^{H_2O}/k^{D_2O} for the general acid-catalyzed reactions of nucleophiles other than hydroxide ion (*32*). Thus the deuterium oxide solvent isotope effect is also reasonably consistent with the general acid mechanism for the hydrolysis of **II**.

The inability to distinguish between the general acid-specific base and general base mechanisms on the basis of deuterium oxide effects is a dilemma that has confronted other investigators (*9*, *19*, *31*). Definitive mechanistic assignments have been achieved in the cases of aspirin (*19*) and *p*-nitrophenyl salicylate (*9*) hydrolysis by studying the reactivity of nucleophiles such as imidazole and azide ion which are susceptible to general acid but not general base catalysis. The demonstration that the reaction rate of these nucleophiles with aspirin and *p*-nitrophenyl 5-nitrosalicylate is close to the rate of reaction with related esters which do not contain the catalytic group has served to rule out the general acid mechanism.

This argument may also be applied to results obtained for the reaction of azide ion with **II** and *N*,*O*-diacetyl-*N*-methylhydroxylamine. If hydrolysis occurs by a general base mechanism, it cannot be operative for reaction with azide ion since the nucleophilic atom of azide ion does not possess a dissociable proton. If catalysis occurs by general acid-assisted hydroxide attack, the azide ion reaction must be general acid-catalyzed as depicted below.



SCHEME 2

Therefore, it is of interest to compare the second-order rate constant $k_{Az} = 0.1\ M^{-1}$ sec^{-1} for the azide ion reaction of the acidic form of **II** with $k_{Az} = 0.03\ M^{-1}$ sec^{-1} for reaction with *N,O*-diacetyl-*N*-methylhydroxylamine. Since the ratio of the rate constants is about 3, there does appear to be some catalysis by the **II** dimethylamino group. However, the size of this catalysis is much smaller than the hypothetical 340-fold rate enhancement for the hydroxide ion which was assessed by comparing lines A and C in Fig. 4. On this basis the general acid mechanism appears to be unlikely.

However, this type of experiment has been criticized on the basis that the steric and electronic effects associated with deletion of the catalytic group makes these comparisons inconclusive (*32*).

In view of the above criticism, it was considered desirable to find a new method of unequivocal mechanistic assignment which does not involve alteration of the ester molecule. The study of the kinetic deuterium oxide solvent isotope effect on the reaction of azide ion with **II** is such a method. For a general acid-catalyzed azide reaction $k_{Az}^{H_2O}/k_{Az}^{D_2O}$ should be in the range of 2–3. For an uncatalyzed nucleophilic reaction $k_{Az}^{H_2O}/k_{Az}^{D_2O}$ should be close to unity as observed for simple nucleophilic reactions. From the experimentally determined value of $k_{Az}^{H_2O}/k_{Az}^{D_2O} = 1.06 \pm 0.08$, the general acid mechanism may be ruled out. Therefore, the dimethylamino group most probably catalyzes the hydrolysis of **II** by a general base mechanism.

The slightly faster reaction of azide with **II** when compared to *N,O*-diacetyl-*N*-methylhydroxylamine is probably due to a relatively small electrostatic catalysis.

9. The Comparison of the General Base-Catalyzed and Uncatalyzed Reactions of N,O-*Diacetyl*-N-(*2-Dimethylaminoethyl*)*Hydroxylamine with Water*

The efficiency of catalysis by the dimethylamino group in the hydrolysis of **II** was previously assessed by taking the difference between lines A and C in Fig. 4. However, this comparison is useful only in an operational sense since lines A and C correspond to two different mechanisms; the azide ion experiments have shown that line A represents a general base-catalyzed reaction with water while line C represents an uncatalyzed reaction with hydroxide ion. To correctly assess the magnitude of catalysis, the rate constant for general base catalysis, $k_{BW} = k_1/(H_2O) = 1.60 \times 10^{-2}$ $sec^{-1}/55$ $M = 2.91 \times 10^{-4}\ M^{-1}$ sec^{-1}, must be compared with the rate constant for the uncatalyzed water (spontaneous) hydrolysis of **II**. Although the spontaneous hydrolysis rate of **II** was not measured experimentally, a close approximation to this value may be obtained by the calculations below.

Holmquist and Bruice (*34*) have studied the hydrolysis reactions of a series of *o*-nitrophenyl esters and found that Eq. [13] gives a good correlation between the hydroxide ion rate constants, k_{OH}, and spontaneous rate constants, k_{H_2O}.

$$\log k_{OH^-} = 0.84 \log k_{H_2O} + 8.00. \qquad [13]$$

This equation accurately correlates k_{H_2O} with k_{OH^-} for esters other than *o*-nitrophenyl acetates (*20*) and is apparently applicable to *O*-acylhydroxamates since k_{H_2O} for chloroacetyl *N*-methylacetohydroxamate hydrolysis, when calculated from Eq. [13] and $k_{OH^-} = 7.29 \times 10^3\ M^{-1}$ sec^{-1}, is within 20% agreement of the experimentally determined value. For **II**, $k_{BOH} = 16.8\ M^{-1}$ sec^{-1} (based on a_{OH}) and by Eq. [13] the uncatalyzed water rate constant, k_{BW}^{un}, is $8.6 \times 10^{-9}\ M^{-1}$ sec^{-1}; this is a reasonable

value when compared to $k_{OH^-} = 9.5\ M^{-1}\ sec^{-1}$ and $k_{H_2O} = 7.8 \times 10^{-9}\ M^{-1}\ sec^{-1}$ or *p*-nitrophenyl acetate hydrolysis (*35*).

From the ratio k_{BW}/k_{BW}^{un}, the reaction of **II** with water is catalyzed approximately 34,000-fold by the dimethylamino group. When compared to the rate accelerations of other intramolecular general base catalysis reactions, the catalysis in **II** hydrolysis is the largest observed to date for a hydrolysis. For example, rate accelerations of 30-, 195-, 500-, and 3000-fold have been found for the hydrolyses of *p*-nitrophenyl 5-nitrosalicylate (*9*), aspirin (*19*), and 8-acetoxyquinoline (*20*), respectively.

In view of the apparent importance of general base catalysis in the deacylation reactions of serine enzymes (*36*), it is tempting to turn to some unusual effect such as "orbital steering" (*37*) or "strain mechanism" (*38*) to account for the very large 34,000-fold catalysis attendant in the hydrolysis of **II**. However, the greater efficiency of the intramolecular general base mechanism in **II** hydrolysis in comparison to related esters is probably due to improved stereochemistry of the dimethylamino group in relation to the ester carbonyl or simply the greater basicity of the dimethylamino group.

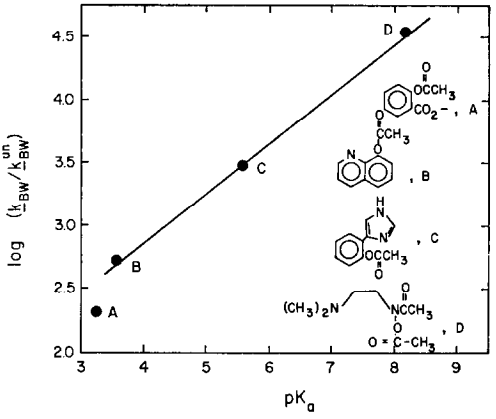


FIG. 6. Plot of pK_a versus logarithm of rate enhancements due to general base catalysis for aspirin, A (*19*); 8-acetoxyquinoline, B (*20*); 4-(2′-acetoxyphenyl)imidazole, C (*20*); and *N,O*-diacetyl-2-(dimethylamino)ethylhydroxylamine, D.

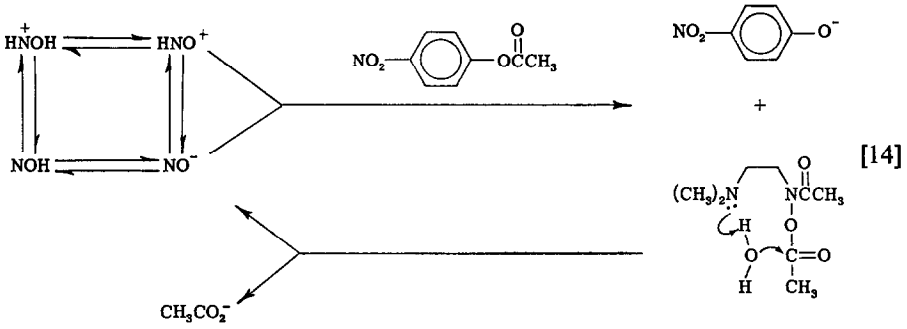
Evidence that the latter explanation is probably correct is seen from examination of the Brønsted-type plot in Fig. 6. The line in Fig. 6 is drawn through the compounds involving nitrogen bases and the slope of this line, 0.40, can be related to the α value in the Brønsted relationship, $\log k = \alpha \cdot pK_a + C$. It is noteworthy that the Brønsted slope is equal to 0.47 for the intermolecular general base catalysis of phenyl acetate hydrolysis by a series of oxygen and amine nucleophiles (*39*). Although the Brønsted relationship has found wide utility in the correlation of rate data for many types of general base reactions (*32*), the reasonable correlation in Fig. 6 is surprising since the rate data in this figure were obtained for esters of widely differing molecular structure.

10. The Overall Catalytic Mechanism

Since it has been shown that **II** is an intermediate in the catalytic reaction, the first step in this reaction must be the nucleophilic attack of **I** on the carbonyl carbon of

p-nitrophenyl acetate. However, the occurrence of general base catalysis by the amino group in the deacylation of **II** suggests that acylation may also be catalyzed in this fashion. Nevertheless, the pH-dependency of the acylation rate constant appears to be identical to the pH-dependency of the total concentration of hydroxamate ion as determined spectroscopically. Also, **I** does not exhibit any high reactivity when compared to the second-order rate constants for the reaction of different *N*-methylhydroxamic acids with *p*-nitrophenyl acetate.

Therefore, the overall catalytic process is the one presented in Eq. [14] where the –NO– structures represent the different ionization states of **I**. The size of the transition state (7 not counting H) is larger than the most stable ring. However, it should be noted that models of this transition state are compatible with this mechanism, as are the experimental data.



[14]

SCHEME 3

As pointed out earlier, good nucleophiles are usually not good catalysts for ester hydrolysis because of the intrinsic inverse relationship between nucleophilicity and leaving group ability. The selective general base catalysis of the deacylation step in the case of **I** overcomes this difficulty and thereby enhances the overall catalytic efficiency. Thus, when the catalytic efficiency is expressed in terms of the theoretical catalytic limit, **I** is characterized by a value of 450 in water and 2300 in 30% acetonitrile. In this case the comparison is made by taking the difference between line A in Fig. 4 and a line which represents $k_{OH^-} = 9.5\ M^{-1}\ \text{sec}^{-1}$ for *p*-nitrophenyl acetate hydrolysis.

11. Relationship to Enzyme Models

This bifunctional catalytic system was designed in an effort to increase the overall catalytic efficiency of hydroxamic acids. The detailed mechanism was established in order to examine closely the origin of improved catalytic efficiency in a simple model. In addition, the desirability of studying this system was based on the practical synthetic potential of introducing *N*-(2-dimethylaminoethyl)-acetohydroxamic acid into cyclohexaamylose which reversibly binds aromatic esters. This accomplishment is described in the following paper.

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