# Hydroxamic Acids IV: Hydroxide Effect in Hydroxylaminolysis of Ethyl Acetate

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**Abstract** ☐ The kinetics and yields of acetohydroxamic acid from ethyl acetate were studied in order to assess the ability of excess hydroxide to decrease the sensitivity of the hydroxamic acid assay of a simple ester. Apparent first-order rate constants for hydroxylaminolysis of ethyl acetate were calculated from pH-stat data in the pH range 9.5–11.0 in the presence of 0.2–0.75 *M* hydroxylamine at 40°. First-order rate constants for ethyl acetate hydrolysis in the absence of hydroxylamine were determined under similar experimental conditions. The percent yields of acetohydroxamic acid were calculated as a function of pH and hydroxylamine concentration using the colorimetric assay of the hydroxamic acid–iron complex at 515 nm. Excess hydroxide was shown to increase the rate of reaction without decreasing hydroxamic acid yields. Based on these data, a rapid assay for ethyl acetate, acetylcholine, methacholine, and pilocarpine was developed, with high yields of the corresponding hydroxamic acids. Proposed mechanisms for participation by hydroxide ion in hydroxamic acid formation from simple esters are discussed.

**Keyphrases** ☐ Ethyl acetate—hydroxylaminolysis rate constants, hydroxide-ion effect, colorimetric assay ☐ Acetohydroxamic acid—from ethyl acetate, rate constants, hydroxide-ion effect, colorimetric assay ☐ Acetylcholine—colorimetric assay ☐ Methacholine—colorimetric assay ☐ Pilocarpine—colorimetric assay ☐ Hydroxamic acids—formation by hydroxylaminolysis, hydroxide-ion effect, colorimetric assay ☐ Colorimetry—monitoring, assay, hydroxamic acids

The formation of a hydroxamic acid and the subsequent colorimetric determination of its iron complex [Fe (III)–hydroxamic acid complexes absorb at about 515 nm.] provide a potentially useful method of analysis for: (*a*) drugs for which no direct UV or colorimetric assay exists, and (*b*) cases where the assays do not adequately differentiate between the drug, its degradation product, metabolic product, or other components in a formulation or a biological sample, provided that the drug will form a hydroxamic acid.

Hydroxamic acids can be formed from a variety of functional groups including esters, amides, acid chlorides, anhydrides, lactones, and imides (1). However, compounds possessing one or more of these functional groups do not all appear amenable to commonly used hydroxamic acid assay conditions typically employing 2 *M* $NH_2OH$ and an excess of 1 *M* NaOH (2). Negative results have been reported for several compounds of pharmaceutical interest including phenobarbital, pentobarbital, succinimide, glutamine, nicotinamide, nikethamide, prostigmine, and meperidine (3, 4). There are at least three possible reasons for obtaining little or no color formation in the usual method of assay: (*a*) the hydroxamic acid did not form, (*b*) the hydroxamic acid formed but degraded, or (*c*) the hydroxamic acid formed but did not complex.

This situation prompted a systematic investigation into the factors involved in the formation of the colored complex (1) and the kinetics and mechanisms controlling the formation of hydroxamic acids (2, 5). Succinimide, previously reported as poorly reactive, was shown to give good color yield at pH 11–12 and 40°; but in the presence of excess NaOH, the hydroxamic acid was found to undergo hydrolysis (5). Furthermore, a more sensitive assay (sixfold) was developed by kinetically controlling the yield of the intermediate *N*-hydroxysuccinimide and measuring its UV absorption (5).

Excess NaOH was also assumed to be deleterious to yields of acetohydroxamic acid from ethyl acetate due to the simultaneous hydrolysis of the ester to acetic acid (2, 6). The present study was undertaken to investigate the role of NaOH in the hydroxylaminolysis of ethyl acetate and to apply these results to the analysis of more complex structures. Contrary to the *a priori* assumption that excess NaOH decreases hydroxamic acid yields due to ester hydrolysis, the yields were found to be invariant over the range of from $1 \times 10^{-4}$ to 2.4 *M* NaOH at 40°. The use of 0.75 *M* $NH_2OH$ and 2.4 *M* NaOH at 40° was shown to give high yields in just 5 min., and ethyl acetate, acetylcholine, methacholine, and pilocarpine assays were satisfactorily carried out under these conditions.

## EXPERIMENTAL AND RESULTS

**Kinetics and Yields of Ethyl Acetate–Hydroxylamine Reactions**—It was previously demonstrated (2) that the apparent first-order rate constants for reactions between hydroxylamine and ethyl acetate in alkali may be determined from data obtained by the pH-stat method or by colorimetric analysis of the iron complex of *N*-hydroxyacetamide (acetohydroxamic acid). In the present study, the time course of each reaction was followed by pH-stat because of its simplicity, and the experimental methods and equipment were identical to those previously described (2). At the completion of each reaction, the yield of *N*-hydroxyacetamide was determined by complexing it with iron and spectrophotometrically determining the resultant absorption at 515 nm. according to the procedures previously developed (1).

Rate constants for the hydrolysis of ethyl acetate in the absence of hydroxylamine were also determined using the pH-stat method.

Experimental conditions used in these studies are listed in Table I.

**Effect of [$NH_2OH$] on Apparent First-Order Rate Constants**—Data obtained from the pH-stat were used to calculate the apparent first-order rate constants, $k_1$, from the equation:

$$\ln (V_\infty - V_t) = \ln V_\infty - k_1 t \qquad \text{(Eq. 1)}$$

where $V_\infty$ is the final volume of NaOH added, and $V_t$ is the volume at time $t$. Thus, a plot of $\ln (V_\infty - V_t)$ *versus* $t$ is linear with slope $-k_1$, where $k_1$ is the rate constant for formation of both acetic acid and acetohydroxamic acid under the specific reaction conditions studied. Table I lists the reaction conditions and the corresponding values obtained for $k_1$. Figure 1 illustrates the linear dependency of the apparent first-order rate constants on the total hydroxylamine concentration. The intercept value at each pH corresponds to the apparent first-order rate constant for ethyl acetate hydrolysis in the absence of hydroxylamine.

**Table I**—Experimental Conditions, Apparent First-Order Rate Constants, and Acetohydroxamic Acid Yields from Ethyl Acetate, 0.016 *M*, at 40°

| pH | $NH_2OH$, *M* | $10^2k_1$, $min.^{-1}$ | Acetohydroxamic Acid, % Yield[a] |
|---|---|---|---|
| 9.50 | 0.00[b] | 0.255[d] | 00 |
| | 0.12 | 0.389 | 28 |
| | 0.15[c] | 0.547 | 28 |
| | 0.20 | 0.535 | 43 |
| | 0.25[c] | 0.822 | 47 |
| | 0.35[c] | 1.05 | 64 |
| | 0.50[b] | 1.37 | 75 |
| | 0.65[c] | 1.73 | 75 |
| | 0.75[b] | 2.24 | 87 |
| | 0.83[c] | 2.28 | 86 |
| | 1.12[c] | 2.98 | 84 |
| 9.75 | 0.75 | 3.17 | 87 |
| 10.00 | 0.00 | 0.634[d] | 00 |
| | 0.75 | 4.72 | 89 |
| 10.25 | 0.00 | 1.09 | 00 |
| | 0.20 | 2.81 | 38 |
| | 0.50 | 5.77 | 74 |
| | 0.75 | 8.23 | 88 |
| 10.30 | 0.75 | 8.40 | 90 |
| 10.35 | 0.20 | 3.28 | 42 |
| | 0.50 | 5.52 | 75 |
| | 0.69 | 8.34 | 85 |
| | 0.75 | 8.42 | 87 |
| 10.40 | 0.75 | 10.1 | 86 |
| 10.45 | 0.20 | 4.82 | 42 |
| | 0.50 | 8.95 | 79 |
| | 0.75 | 13.2 | 88 |
| 10.50 | 0.75 | 13.2 | 91 |
| 10.60 | 0.00 | 2.25[d] | 00 |
| | 0.12 | 4.02 | 29 |
| | 0.20 | 6.17 | 44 |
| | 0.50 | 11.7 | 75 |
| | 0.72 | 16.3 | 88 |
| 10.75 | 0.00 | 2.62[d] | 00 |
| | 0.15 | 8.84 | 40 |
| | 0.20 | 9.72 | 47 |
| | 0.30 | 15.9 | 66 |
| | 0.75 | 28.9 | 88 |
| 11.00 | 0.00 | 5.92[d] | 00 |
| | 0.12 | 12.6 | 35 |
| | 0.15 | 18.0 | 42 |
| | 0.20 | 20.0 | 50 |
| | 0.75 | 67.1 | 92 |
| 11.20 | 0.00 | 8.35[d] | 00 |
| 11.25 | 0.20 | 30.4 | 56 |
| NaOH = 2.4 *N* | 0.75 | 5-min. reaction | 80 |

[a] Determined colorimetrically as the iron complex (1). [b] Average values of current study and those reported in *Reference 2*. [c] Taken from *Reference 2*. [d] Rate constants for hydrolysis at $\mu$ = 0.75 (NaCl) are (pH, $10^2k$): 9.5, 0.346; 10.00, 1.10; 10.25, 2.25; 10.35, 2.42; 10.45, 2.97; 10.60, 3.97; 10.75, 5.60; and 11.00, 8.35. At $\mu$ = 0.20: 10.00, 8.03; 10.75, 4.64; and 11.00, 7.07. At $\mu$ = 0.50: 10.00, 1.02; 10.75, 5.51; and 11.00, 9.71. All other studies have $\mu$ = $NH_2OH$ concentration due to NaCl formed in neutralization of $NH_2OH \cdot HCl$.

**Effect of pH on Apparent First-Order Rate Constants**—Plots of $k_1$ *versus* [$NH_2OH$] were found to increase in slope with increasing pH values (Fig. 1). Thus, it is apparent that for a given concentration of hydroxylamine, the rate constant, $k_1$, increases as the activity of hydroxide ion increases. The hydroxide-ion activity was calculated for each pH in Table I by using $K_w = 2.917 \times 10^{-14}$, which is the value at 40° (7).

A typical example of the effect of hydroxide-ion activity on $k_1$ is shown in Fig. 2 for the case where [$NH_2OH$] was kept constant at 0.75 *M*. A plot of the rate constants for the hydrolysis of ethyl acetate *versus* hydroxide-ion activity under identical experimental conditions (40°, $\mu$ = 0.75 with NaCl) is also included in Fig. 2 for comparison. It is readily apparent that the hydrolysis of ethyl acetate in the absence of hydroxylamine cannot account for the observed increase in $k_1$ as a function of hydroxyl-ion activity.

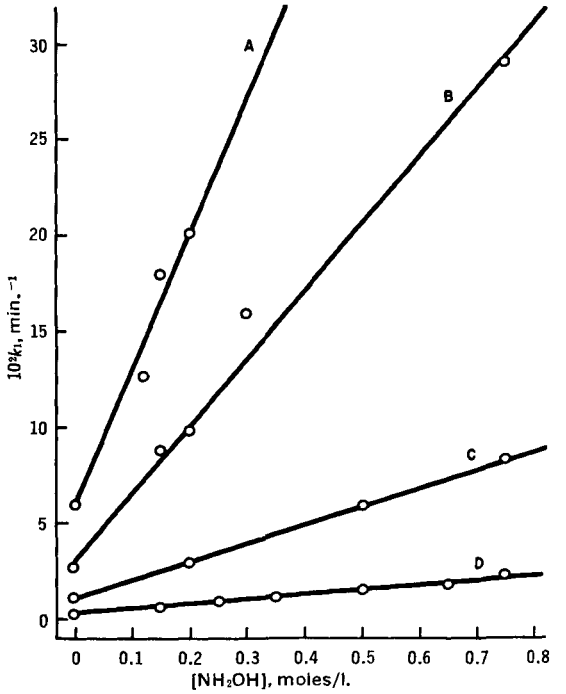



**Figure 1**—*Apparent first-order rate constants for the reaction of 0.016* M *ethyl acetate at 40° as a function of hydroxylamine concentration at pH: (A) 11.00, (B) 10.75, (C) 10.25, and (D) 9.50.*

**Effect of pH on Yields**—The percent yield of *N*-hydroxyacetamide, calculated on the basis of initial ethyl acetate concentration,

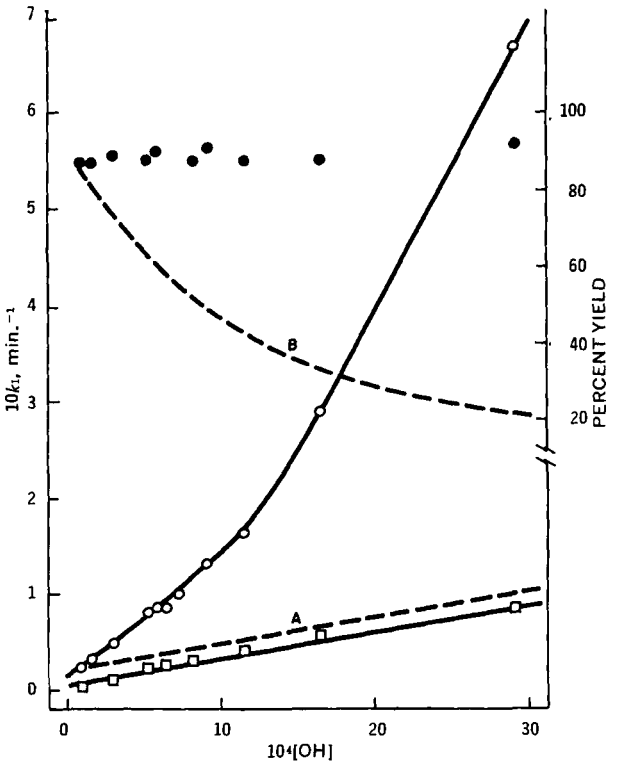



**Figure 2**—*Apparent first-order rate constants (left ordinate) for hydrolysis (□) and hydroxylaminolysis with 0.75* M *$NH_2OH$ (○) of 0.016* M *ethyl acetate at 40°, $\mu$ = 0.75, as a function of hydroxyl-ion activity and corresponding percent yields (right ordinate) of N-hydroxyacetamide obtained during the hydroxylaminolysis reactions (●). The dashed lines are calculated values of the rate constants (A) and yields (B) based on Eq. 7.*

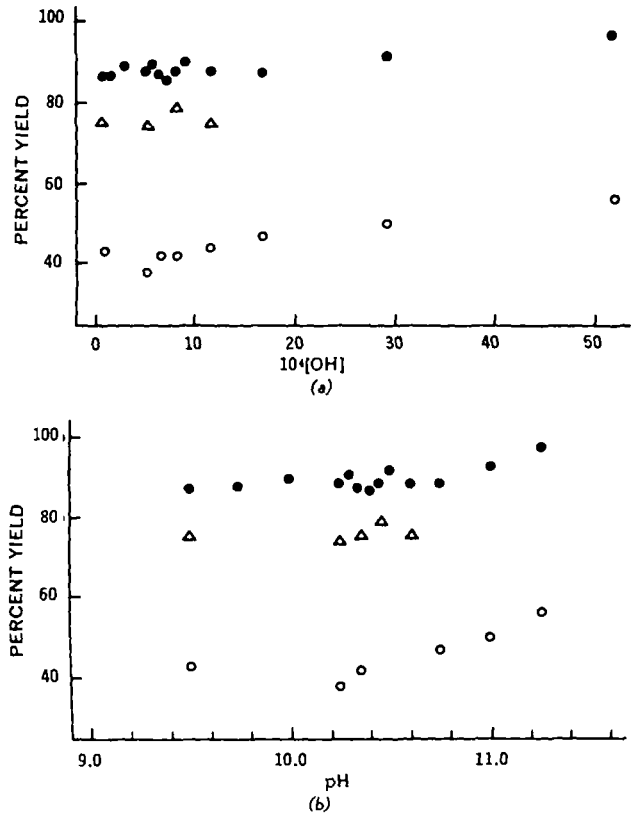



**Figure 3**—*The percent yields of* N-*hydroxyacetamide obtained by reacting 0.016* M *ethyl acetate with* $NH_2OH$ (●, *0.75* M; Δ, *0.50* M; *and* ○, *0.20* M) *at 40° as a function of* (a) *hydroxyl-ion activity and* (b) *pH.*

was determined for each kinetic run and the results are given in Table I. The effect of pH on the yield of *N*-hydroxyacetamide is illustrated in Fig. 3*b* for three concentrations of hydroxylamine (0.20, 0.50, and 0.75) in the pH range 9.5–11.25. For a given hydroxylamine concentration, the percent yield of *N*-hydroxyacetamide appears to be independent of pH in this region. These results cover a wide range of hydroxyl-ion activity. Figure 3*a* shows the same yields as a function of the hydroxyl-ion activity which was varied more than 50-fold in the case of 0.20 and 0.75 *M* hydroxylamine without significant change in the yield of hydroxamic acid. To increase the possibility of hydrolysis by excess hydroxide, ethyl acetate was reacted with 0.75 *M* hydroxylamine in the presence of 2.4 *M* NaOH and the resulting yield of *N*-hydroxyacetamide was determined. As shown in Table I, the yield after 5 min. of reaction at 40° was found to be 80%, which is in reasonable agreement with the values obtained for 0.75 *M* hydroxylamine in the pH region 9.5–11.0.

**Hydroxamic Acid Assay of Selected Compounds**—Since the hydroxyl-ion concentration was shown to increase the reaction rate without decreasing the yields of hydroxamic acid, it should be possible to analyze appropriate esters quickly in strong alkali without loss of sensitivity. Three compounds were chosen to test this hypothesis by comparison with ethyl acetate (Table II). Mixtures containing varying initial concentrations of substrate in the presence of 0.75 *M* $NH_2OH$ and 2.4 *M* NaOH were allowed to react for 5 min. at 40°. Samples were then withdrawn and added to ferric perchlorate reagent, and the resulting absorbance was determined at 515 nm. (1). The absorbance values were plotted *versus* initial

$$CH_3COOC_2H_5 \xrightarrow[NH_2OH]{k_N} CH_3CONHOH + CH_3CH_2OH$$

$$CH_3COOC_2H_5 \xrightarrow[OH^-]{k_H} CH_3COOH + CH_3CH_2OH$$

*Scheme I*

**Table II**—Hydroxamic Acid–Iron Complex Analyses in 0.75 *M* $NH_2OH$ and 2.4 *M* NaOH at 40° for 5 min.

| Compound | Apparent Absorptivity[a] |
|---|---|
| Ethyl acetate | 950 |
| Acetylcholine chloride | 915 |
| Methacholine chloride | 1032 |
| Pilocarpine hydrochloride | 926 |

[a] Determined from the slope of a Beer's law plot based on initial concentration of substrate.

concentration, and the slopes were calculated as representing the "apparent absorptivity" for each compound.

These values are lower than the absorptivity of the corresponding hydroxamic acids since the yields would not be expected to be 100%. In the case of ethyl acetate, the absorptivity of the color complex of *N*-hydroxyacetamide under these conditions is 1180 (1). The apparent absorptivity of 950, as given in Table II, corresponds to an 80% yield of the hydroxamic acid in 5 min.

## DISCUSSION

**Kinetics and Yields of Ethyl Acetate–Hydroxylamine Reactions**—Under conditions of constant alkaline pH and sufficient excess of hydroxylamine, ethyl acetate undergoes pseudo-first-order simultaneous reactions to yield acetic acid and acetohydroxamic acid (2). In its simplest form, this reaction may be represented by Scheme I, where $k_N$ is the rate constant for formation of the hydroxamic acid by all routes and $k_H$ is for the formation of acetic acid. Regardless of the mechanism involved, it may be assumed *a priori* that hydroxylamine and hydroxyl ion are in competition for the substrate. It would seem likely that a large excess of hydroxide would decrease the yield of hydroxamic acid formed from esters, and this supposition was previously suggested (2, 6). Since the pKa of $NH_2OH^+$ is 6.0 (8), its concentration is negligible under the present conditions with pH values of 9.5 and higher. Nucleophilic displacements by $NH_2OH$ attack on esters in alkali are commonly assumed to pass through a tetrahedral intermediate with $NH_2OH$ acting as a nitrogen nucleophile:

$$RCOOR' + NH_2OH \rightleftharpoons RC(O^-)(^+NH_2OH)OR' \rightarrow RC(=O)NHOH + R'OH \quad \text{(Eq. 2)}$$

or an oxygen nucleophile:

$$RCOOR' + NH_2OH \rightleftharpoons RC(O^-)(^+HONH_2)OR' \rightarrow RC(=O)NH_2 + R'OH \quad \text{(Eq. 3)}$$

In the presence of sufficient excess of hydroxylamine, the *O*-acylhydroxylamine would be converted to the hydroxamic acid. In either case, the rate of loss of ester would be dependent on the concentration of ester and hydroxylamine. Under pseudo-first-order conditions of excess $NH_2OH$, the rate constants at a given pH show a linear dependence on $NH_2OH$ concentration (Fig. 1). The rate of conversion of ethyl acetate to acetohydroxamic acid at a given pH might, therefore, be expressed:

$$-dE/dt = k_N[E] = k_N'[NH_2OH][E] \quad \text{(Eq. 4)}$$

The rate of hydrolysis of ethyl acetate is also linear with hydroxyl-ion activity and may be written:

$$-dE/dt = k_H[E] = k_H'[OH][E] \quad \text{(Eq. 5)}$$

Therefore, if the hydroxylamine concentration were held constant and the hydroxyl-ion activity were increased, the overall rate constant, $k_1$, would increase due to increased hydrolysis, provided that:

$$-dE/dt = k_1[E] = (k_N'[NH_2OH] + k_H'[OH])[E] \quad \text{(Eq. 6)}$$

and:

$$k_1 = k_N'[NH_2OH] + k_H'[OH] \quad \text{(Eq. 7)}$$

accurately describe the reaction. This is not the case. Equation 7 has been used to predict the values for $k_1$ as a function of pH beginning with the observed values at pH 9.5 and 0.75 *M* $NH_2OH$. The results, shown in Fig. 2 (dashed line, A), illustrate that the increase in $k_1$ with increasing hydroxyl-ion activity cannot be attributed to the hydrolysis of ethyl acetate. Furthermore, the percent yield of hydroxamic acid would decrease, as illustrated in curve B of Fig. 2, if hydrolysis increased and the rate constant for hydroxylamine attack, $k_N$, remained constant. However, the hydroxamic acid yields in the presence of 0.75 *M* $NH_2OH$ and other concentrations studied (Fig. 3) are independent of the hydroxyl-ion activity. This implies that the hydroxyl-ion activity is involved in both the hydrolysis and the hydroxylaminolysis reactions and that it is the same order in both. If this is the case, then the rate expression should be written:

$$-dE/dt = k_1[E] = (k_N''[NH_2OH][OH] + k_H'[OH])[E] \quad \text{(Eq. 8)}$$

and:

$$k_1 = (k_N''[NH_2OH] + k_H')[OH] \quad \text{(Eq. 9)}$$

At constant hydroxylamine concentration, this may be written:

$$k_1 = k[OH] \quad \text{(Eq. 10)}$$

and:

$$\log k_1 = \log k + \log [OH] \quad \text{(Eq. 11)}$$

so that:

$$\log k_1 = \log k - pKw + pH \quad \text{(Eq. 12)}$$

Thus, a plot of log $k_1$ *versus* pH should be linear with a slope of 1 and an intercept value of (log $k$ − pKw). Figure 4 shows the experimental points and the least-squares regression lines for the hydrolysis of ethyl acetate and for its reaction with 0.75 and 0.20 *M* hydroxylamine as a function of pH. The slope of the least-squares regression line for hydrolysis is 0.928 and that for hydroxylaminolysis is 1.028 in the case of 0.20 *M* hydroxylamine and 0.971 for 0.75 *M*, all in good agreement with the expected slope of 1.

Two kinetically equivalent mechanisms may be considered for participation of hydroxyl ion in the formation of the hydroxamic acid. One possibility is the conversion of $NH_2OH$ to its more reactive conjugate base:

$$NH_2OH + OH^- \rightleftharpoons {}^-NHOH + H_2O \quad \text{(Eq. 13)}$$

or:

$$NH_2OH + OH^- \rightleftharpoons NH_2O^- + H_2O \quad \text{(Eq. 14)}$$

which would then react to form the hydroxamic acid according to Scheme II.

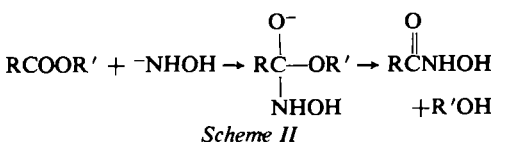
There is some evidence that the proton attached to the oxygen is more acidic than the proton on the nitrogen (9). A dissociation constant could be defined for either case. For example:

$$K_a = \frac{[NH_2O^-]}{[NH_2OH][OH^-]} \quad \text{(Eq. 15)}$$

so that:

$$[NH_2O^-] = K_a[NH_2OH][OH^-] \quad \text{(Eq. 16)}$$

Thus, as the hydroxyl-ion activity increases, more $NH_2O^-$ (or ${}^-NHOH$) is formed and the nucleophilic attack on the carbonyl carbon would be enhanced (Figs. 1, 2, and 4).




*Scheme II*

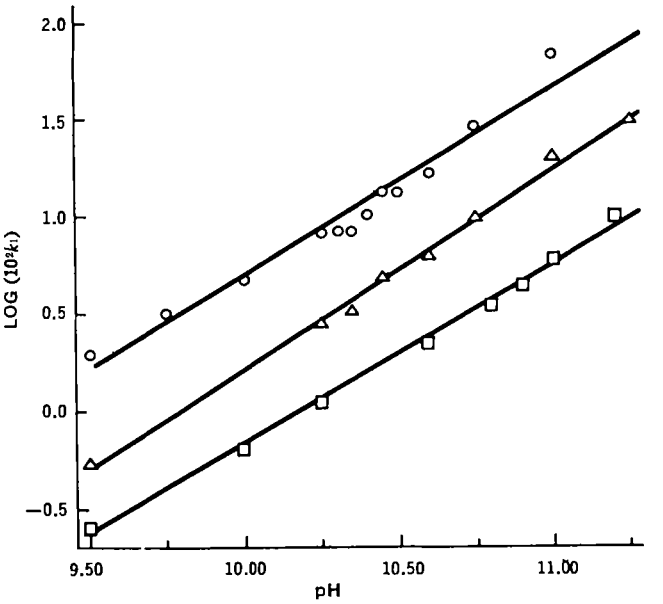



**Figure 4**—*Experimental values of log* k versus *pH for: ethyl acetate hydrolysis* (□) *and hydroxylaminolysis with* $NH_2OH$ *0.20* M (Δ) *and 0.75* M (○) *at 40° and the least-squares regression lines for the data.*

There is little, if any, evidence in the literature for nucleophilic attack by anionic hydroxylamine (${}^-NHOH \rightleftharpoons NH_2O^-$). It has been stated that hydroxylamine does not generally react with esters *via* the species $NH_2O^-$ (10). Bruice and Bruno (11) postulated three mechanisms for the reaction of hydroxylamine with valerolactones in the pH region 6.5–9. All three involve the species $NH_2O^-$, which behaves either as the nucleophile itself or as a general-base catalyst in agreement with the observed rate equation:

$$V = k[NH_2OH]^2[OH^-][L] \quad \text{(Eq. 17)}$$

where $V$ is the reaction velocity and [*L*] is the concentration of lactone. Attempts to determine experimentally the value for $K_a$ (Eq. 15) by the half-neutralization technique were unsuccessful, presumably due to the weak acidity of $NH_2OH$. It was concluded that the value for the pKa of $NH_2OH$ was far beyond the pH range of the study (6.5–9) and, therefore, $H_2NO^-$ could only be present in trace amounts.

Later, Pearson and Keaton (12) were able quantitatively to convert hindered ketones to the corresponding oximes by use of potassium *tert*-amylate in *tert*-amyl alcohol at room temperature with prolonged standing. They suggested that a sufficiently strong base could be employed to convert hydroxylamine to the dianion:

$$NH_2OH \overset{OH^-}{\rightleftharpoons} ({}^-NH_2OH \rightleftharpoons NH_2O^-) \overset{OH^-}{\rightleftharpoons} {}^-NHO^- \quad \text{(Eq. 18)}$$

However, they surmised that the attacking species in their system was the monanion, although no direct evidence was presented in support of this conclusion.

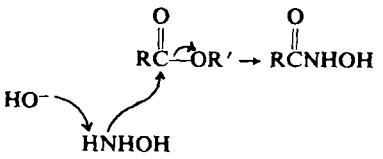
The experimental conditions employed here were higher in pH (9.5–11.25; also in 2.4 *M* NaOH) than the lactone studies (6.5–9) but not as strongly basic as the hindered ketone work with potassium *tert*-amylate. Nucleophilic attack by anionic hydroxylamine (${}^-NHOH \rightleftharpoons NH_2O^-$) is consistent with the observed rate expression (Eq. 8). Substitution into Eq. 8 with Eq. 16 yields:

$$-dE/dt = \{(k_N''/K_a)[NH_2O^-] + k_H'[OH]\}[E] \quad \text{(Eq. 19)}$$

Thus, formation of both the hydroxamic acid and acetic acid would increase with increasing hydroxyl-ion activity (and, thus $NH_2O^-$) and the observed rate constant would be defined:

$$k_1 = (k_N''/K_a)[NH_2O^-] + k_H'[OH] \quad \text{(Eq. 20)}$$

It is not possible to solve for the individual rate constants and further test this mechanism since the value for $K_a$ is not known.



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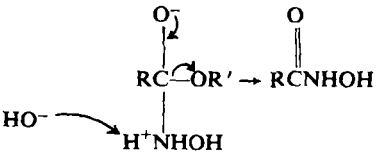

*Scheme III*

An equivalent mechanism involves specific hydroxyl-ion catalysis of the nucleophilic attack by neutral hydroxylamine as shown in Scheme III. Similar schemes can be written for *O*-attack by hydroxylamine, but both mechanisms satisfy the rate expression given in Eq. 8 being first-order in $NH_2OH$, $OH^-$, and ester.

**Significance in Analysis of Esters**—To analyze completely the discussed kinetics, a method for calculating the fraction of each hydroxylamine species present at a given hydroxyl-ion activity is required. This necessitates a knowledge of the value of $K_a$ defined in Eq. 15. This value has not previously been reported. At least one attempt was made to determine its value, but it was unsuccessful due to the weakness of $NH_2OH$ to behave as an acid (11). The lack of this information does not represent an obstacle to the use of the present kinetic studies in the development of optimum reaction conditions for the hydroxamic acid assay of esters. Since hydroxyl ion is involved to the first power in both hydrolysis and hydroxylaminolysis, an excess of NaOH serves primarily to increase the rate of reaction without decreasing the yield. The general considerations for developing optimum conditions for analysis by hydroxamic acid formation were outlined previously (5). One obvious goal would be that of sufficiently fast reaction conditions to allow a rapid and convenient method of analysis. Thus, for simple esters, one can employ sufficient NaOH to achieve this goal without loss of sensitivity.

The authors recommend using only the amount of NaOH found necessary to give a convenient reaction time. This practice will avoid the possibility of loss of yield due to hydrolysis of the hydroxamic acid to the corresponding carboxylic acid. It will also make easier the adjustment of the ferric perchlorate reagent solution, which should contain (after addition of the reaction aliquot) about 0.14 *M* $HClO_4$ and at least a fourfold excess of $Fe(ClO_4)_3$ over the $NH_2OH$ concentration for maximum color stability (1). In the present case, high yields (probably 80% or greater) of hydroxamic acids were produced within 5 min. at 40° in the presence of 2.4 *M* NaOH and 0.75 *M* $NH_2OH$ for a number of compounds (Table II). In the case of ethyl acetate, this yield may be considered as being maximum for 0.75 *M* $NH_2OH$ as it does not vary significantly over the pH range studied. Thus, the notion that ester hydrolysis would be increased with a resultant decrease in assay sensitivity (2, 6) has been unequivocally proven to be unfounded for the simple ester, ethyl acetate. Other compounds are currently under study in this laboratory. The assay of cyclic imides related to succinimide is also under study and, as in the case of succinimide, the most sensitive assay results are obtained by maintaining the pH below 10 and examining the UV absorption of the intermediate which is rapidly destroyed in the presence of excess NaOH (5).

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