A Kinetic Analysis of Nitrostyrene Hydrolysis and the Knoevenagel Condensation

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Abstract: The kinetics of addition of water to 3,4-methylenedioxy-β-nitrostyrene and both the dehydration and the cleavage of the resulting nitroalcohol have been studied from pH 1 to 13. The results are interpreted in terms of several ionic intermediates. Two inflection points in the pH-rate profile are caused by nucleophilic addition of hydroxide ion at high pH and by rate-controlling protonation of an anion formed by addition of water at pH 5–7. The small amount of methanol (1%) used to introduce the substrate into the aqueous solution had a large effect on the kinetics in alkaline solutions, due to the high nucleophilicity of methoxide ion relative to its basicity. Thiosulfate, but not thiocyanate, adds rapidly to the nitrostyrene. The rate and equilibrium constants of the base-catalyzed condensation of piperonal and nitromethane, the reverse of the hydrolysis, were measured and related to the forward reaction.

Our first studies of the Knoevenagel condensation of aromatic aldehydes with nitromethane to form β-nitrostyrenes were concerned with the function of primary amine catalysts. When the reactions were run in butylammonium acetate buffers in methanol, the observed induction period[1] and kinetics were consistent with nucleophilic catalysis and a Schiff-base intermediate.[2] This intermediate was again indicated when

$$\mathrm{ArCHO + BuNH_2 \rightleftharpoons ArCH{=}NBu + H_2O} \qquad (1)$$

$$\mathrm{ArCH{=}NBu + CH_3NO_2 \rightleftharpoons ArCH{=}CHNO_2 + BuNH_2} \qquad (2)$$

the rate of the same condensation in glacial acetic acid was inhibited by traces of water which measurably shift equilibrium 1 to the left.[3]

Since a better understanding of amine catalysis was not possible until the behavior of the system without primary amines but with general acids and bases was established, a kinetic study of the hydrolysis of 3,4-methylenedioxy-β-nitrostyrene (**1**) was made in aqueous solutions up to pH 6.[4] We now report results in more alkaline solutions, the conditions used in synthesis, under which hydroxide ion and organic anions become mechanistically important.

Experimental Section

Materials. The nitrostyrene **1** was prepared as previously described.[2] The β-deuterionitrostyrene was prepared by substituting CD_3NO_2, C_2H_5OD, and D_2O for the corresponding protium compounds in the preparation. β-Deuteration results in an M^+ peak of 194 in the mass spectrum and reduction of the nmr vinyl singlet at δ 8.2 from 2 H to 1 H. The nitroalcohol, 1-(3,4-methylenedioxyphenyl)-2-nitroethanol (**2**), was prepared by the method of Boileau.[5] A solution of 0.2 mol of nitromethane and 0.2 mol of piperonal in 50 ml of anhydrous ether was added to a mixture of 40 ml of 5 *M* sodium methoxide in methanol with 150 ml of ether, at 0–15°. The mixture was neutralized by slowly adding 0.195 mol of acetic acid in 50 ml of anhydrous ether and was stirred for 1 hr. After filtration of the precipitated sodium acetate, the filtrate was evaporated under vacuum at room temperature and the nitroalcohol recrystallized from carbon tetrahloride, mp 90–91° (lit.[5] mp 91°).

Kinetic runs were made in water at 25.0°. The substrate was introduced as 1 ml of 2×10^{-3} *M* methanolic solution in 100 ml of final aqueous solution, which was then analyzed spectrophotometrically at the absorption maxima of piperonal (316 nm) and nitrostyrene (372 nm). In some cases, initially alkaline reaction mixtures were quenched by dilution with hydrochloric acid to pH 1.

To determine whether vinyl hydrogen exchange takes place in aqueous solution at pH 10.5, 0.00136 g of 3,4-methylenedioxy-β-nitrostyrene-β-d_1 in 1 ml of methanol was added to 99 ml of 0.0331 *M* sodium hydroxide. After 4 min, 5.5 ml of 1.6 *M* hydrochloric acid was added, restoring the bright yellow color of nitrostyrene to the faded solution. Extraction with 15 ml of ether, drying over sodium sulfate, and evaporation yielded nitrostyrene which had retained its deuterium atom (mass spectrum). Exchange at the β position was also shown not to take place within 2 days in ethanol.

Results

The nitroalcohol **2** is an intermediate in the hydrolysis of nitrostyrene to piperonal (**3**). The kinetics were studied using **1**, **2**, and **3** as substrates. These three types of experiment will be discussed separately.

$$\underset{\mathbf{1}}{\mathrm{ArCH{=}CHNO_2}} + \mathrm{H_2O} \longrightarrow \underset{\mathbf{2}}{\mathrm{ArCH(OH)CH_2NO_2}} \longrightarrow \underset{\mathbf{3}}{\mathrm{ArCHO}} + \mathrm{CH_3NO_2}$$

Ar = 3,4-methylenedioxyphenyl

Nitroalcohol as Substrate. In alkaline solutions, the nitroalcohol **2** is rapidly cleaved to nitromethane and piperonal, the reaction going to completion at the low concentration employed. Initial slopes of the nearly linear first-order plots of nitroalcohol concentration, calculated from piperonal formed, gave rate constants k_{45}. (The notation k_{45} means the overall rate constant for the reaction consisting of steps 4 and 5 in Chart I in the Discussion.) No detectable cleavage occurred in hydrochloric acid solutions or in acetate buffers below pH 4; in acetate, phosphate, and borate buffers of pH 5.7–8.9, the rate was dependent on pH as shown in Figure 1. From this plot, the rate equation is

$$k_{45}\ (\mathrm{sec^{-1}}) = 2 \times 10^3[\mathrm{OH^-}] \qquad (3)$$

Concurrently with cleavage, the nitroalcohol **2** is transformed into nitrostyrene **1** in acid solution and into a nitrostyrene precursor at higher pH. This dehydration of **2** to **1** can be observed at pH 0–3 where cleavage does not compete. It is slow, pH independent, and

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[1] T. I. Crowell and F. A. Ramirez, *J. Amer. Chem. Soc.*, 73, 2268 (1951).

[2] T. I. Crowell and D. W. Peck, *ibid.*, 75, 1075 (1953).

[3] C. E. Bell, Jr., and T. I. Crowell, *J. Org. Chem.*, 24, 1159 (1959).

[4] T. I. Crowell and A. W. Francis, Jr., *J. Amer. Chem. Soc.*, 83, 591 (1961). At the bottom of the first column on p 591, "60%" should read "40%."

[5] J. Boileau, Thesis, University of Paris, 1953.

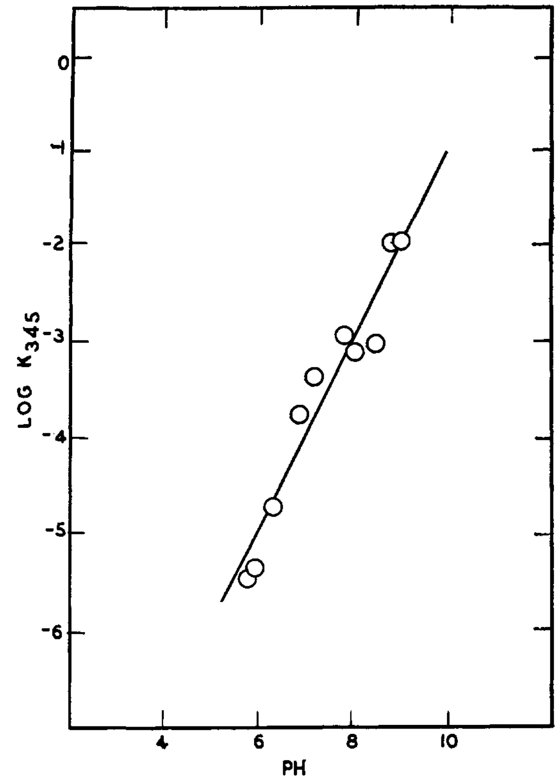

Figure 1. pH dependence of rate constant k_{45} for cleavage of nitroalcohol.

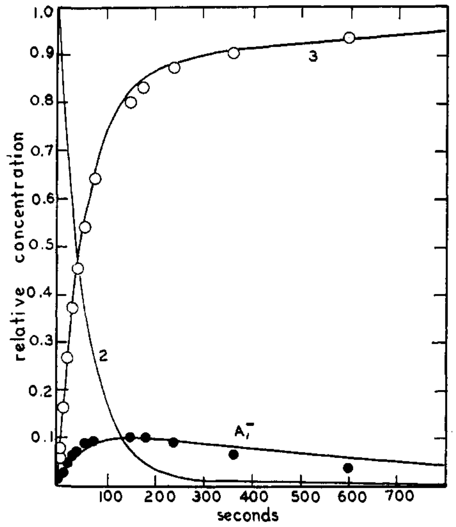

Figure 2. Calculated concentration–time plots with experimental points for 6.5×10^{-5} *M* nitroalcohol **2** in borate buffers, pH 8.9.

follows reversible first-order kinetics to equilbirum, the rate constant k_{-123} for nitrostyrene formation being 3.9×10^{-7} sec^{-1} in hydrochloric acid solutions. As expected, the dehydration of **2** resembles the hydration of **1** not only in the pH independence in acid solutions but also in that it is subject to general base catalysis in acetate buffers (Table I).

In alkaline solution, two reactions, negligible at low pH, complicate the nitroalcohol–nitrostyrene equilibrium. One is the cleavage described above. The other is the accumulation of the nitrostyrene precursor: when **2** is dissolved in a basic solution and before much cleavage can occur, the solution is acidified (quenched) with hydrochloric acid, nitrostyrene is immediately formed. Since acid does not have this effect on the nitroalcohol itself, another intermediate must be present which we assume to be $ArCH(OH)CHNO_2^-$ (A_I^-). The lifetime and the reactions of A_I^- can be studied by assuming that the amount of this intermediate nitrolate ion in a given solution is equal to the number of moles of nitrostyrene produced when the solution is quenched. The experimental points in Figure 2 show how the concentrations of the different species change after nitroalcohol is added to a borate buffer at pH 8.9. A_I^- increases to a maximum in the first 150 min. It then decreases and reverts to nitroalcohol which is continually undergoing the cleavage described at the beginning of this section. The concentration of **2** in Figure 2 is calculated by difference: $[\mathbf{2}] = [\mathbf{2}]_0 - [\mathbf{3}] - [A_I^-]$. At higher pH, the reaction is too fast for conventional measurement, but by quenching after 30 sec, when all the original nitroalcohol has reacted either by cleavage to aldehyde **3** or ionization to A_I^-, the ratio of products can be observed. This ratio, $[A_I^-]/[\mathbf{3}]$, is approximately constant at 0.06 from pH 9.7 to 12.

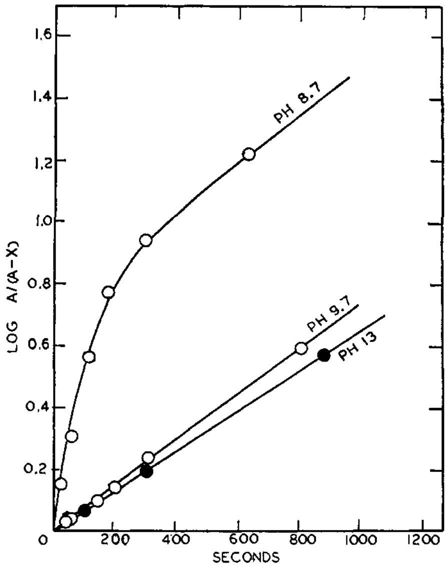

Figure 3. Reaction of nitroalcohol to form piperonal in alkaline solutions.

Table I. Rate Constants for Nitrostyrene Formation from Nitroalcohol

pH	Buffer acid	Buffer base	10^7k_{-123}, sec
1.4	HCl		3.92
3.1	HCl		3.92
4.2	HCl		4.67
3.8	0.01 *M* HOAc	0.001 *M* NaOAc	11.6
3.8	0.1	0.01	40.7
3.7	1.0	0.1	110
4.7	0.05	0.05	33.9
5.7	0.001	0.01	50.9

The rates of disappearance of the nitroanion A_I^- at various pH are shown in Figure 3. (While the curve at pH 8.9 also shows an initial period of faster piperonal production before the nitroalcohol has been converted to A_I^-, the curves at higher pH start after the few minutes required for the ionization to take place.) Evidently the first-order rate constant k_{345}, 1.6×10^{-3} sec^{-1}, for $A_I^- \rightarrow$ **3**, is pH independent from pH 8.7 to 13.

Condensation Reaction. All the experiments in the preceding section were done with the nitroalcohol concentration so low (2×10^{-5} *M*) that reversal of the final cleavage to **3** was negligible. In order to evaluate the rate of condensation of piperonal with nitromethane, reaction mixtures starting with 0.0005–0.006 *M* aldehyde and 0.001–0.025 *M* nitromethane were prepared. At these concentrations, above pH 10, A_I^- forms with no induction period and is detected as usual by observation of nitrostyrene concentration after quenching. The concentration, x, of A_I^- increases with time, finally reaching a rather small equilibrium value, x_e. For example, $x_e = 3.49 \times 10^{-5}$ *M* when the initial piperonal and nitromethane molarities, A_0 and B_0, are respectively 0.00101 and 0.00185. The equilibrium constant K_{-345}, defined as x_e/AB, is therefore 19.6.

Since A and B decrease only slightly from their initial values, the rate equation for the reversible reaction is (4) which integrates to (5) and (6).

$$dx/dt = k_{-345}A_0B_0 - k_{345}x \tag{4}$$

$$\ln(1 - x/K_{-345}A_0B_0) = -k_{345}t \tag{5}$$

$$\ln(1 - x/x_e) = -k_{345}t \tag{6}$$

Plots of eq 6 are linear, yielding at pH 12, for example, the data of Table II. The rate constant k_{-345}, obtained by multiplying k_{345} by K_{-345}, is tabulated here to correspond with the reaction actually followed, though the algebraic manipulations of eq 4–6 show k_{345}. The rate and equilibrium constants are pH independent between pH 10.9 and 12.7; at pH 9.1 the reaction is slower, presumably because nitromethane ($pK_A = 10.2$) is incompletely ionized. This change was not studied in detail, however.

Table II. Rate and Equilibrium Constants for the Reaction of Piperonal and Nitromethane, pH 12

[ArCHO], $M \times 10^3$	[CH_3NO_2], $M \times 10^3$	k_{-345}, M^{-1} sec^{-1}	K_{-345}, M^{-1}
0.51	1.85	0.0317	19.1
1.01	1.85	0.0304	19.6
1.01	3.70	0.0320	19.4
4.04	1.85		19.8
4.04	1.85		19.6
	Av	0.0314	19.5

Nitrostyrene as Substrate. The rate of hydration of **1** to **2** was previously shown to be constant from pH 1 to 5.6 and to show general base catalysis.[4] Using the series of buffers shown in Table III, the pH range was extended to 11.1. The reaction is rapid at high pH. The yellow nitrostyrene color fades within 30 sec in 0.3 *M* sodium hydroxide but is restored completely by immediate quenching with acid, while unquenched solutions undergo degradation to piperonal.[4,6] The first-order rate constant for piperonal formation as well as for the disappearance of nitrostyrene precursor from such solutions is 1.4×10^{-3} sec^{-1}, strongly implying that the same species, A_I^-, is formed from **1** as from **2** at high pH.

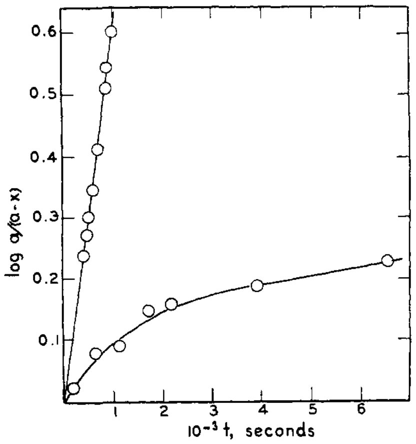

Figure 4. Piperonal formation from nitrostyrene at pH 12 in water (upper curve) and in water containing 1% methanol (lower curve).

Table III. Rate Constant for Nitrostyrene Hydrolysis as a Function of pH, Extrapolated to Zero Buffer Concentration

pH	Buffer	$10^6 k_\psi$, sec^{-1}
4.6	*p*-Toluidine + HCl	0.24
5.2	Pyridine + HCl	0.50
5.6	Pyridine + HCl	0.85
5.8	2,6-Lutidine + HCl	1.10
6.3	Pyridine + HCl	2.52
6.5	Phosphate	4.58
6.6	Phosphate	3.25
6.7	2,6-Lutidine + HCl	3.50
7.1	2,6-Lutidine + HCl	5.10
7.6	Phosphate	7.24
8.2	Phosphate	10.0
9.0	Borate	16.5
9.2	Borate	21.8
9.7	Borate	46.0
10.3	NaOH	138
11.1	NaOH	962

The presence of small amounts of methanol alters these rates very markedly. For example, 1 ml of CH_3OH in 100 ml of aqueous solution at pH 12 doubles the rate of nitrostyrene hydrolysis but causes the faded solution of nitrostyrene precursor to proceed to the cleavage products much more slowly than in pure water and with kinetics more complex than first order (Figure 4). This concentration of methanol (from the solution of **1** initially added) was present in all runs except the one referred to in the preceding paragraph. Only at high pH did it affect the rate constants for nitrostyrene consumption; in neutral or acid solutions, with or without buffers, there was no effect.

Thiosulfate ion (buffered with acetate, pH 6.6) reacts with nitrostyrene **1** with second-order kinetics, $k_1^{S_2O_3} = 2.8 \times 10^{-3}$ M^{-1} sec^{-1}. Thiocyanate is unreactive.

Discussion

The foregoing results will be interpreted in terms of the mechanism[7] shown in Chart I. The equilibrium constants K_{-3} and K_4, for ionization of the CH and OH protons of the nitroalcohol **2**, cannot be determined by conventional techniques because of the rapid hydrolysis. They are calculated as follows: during

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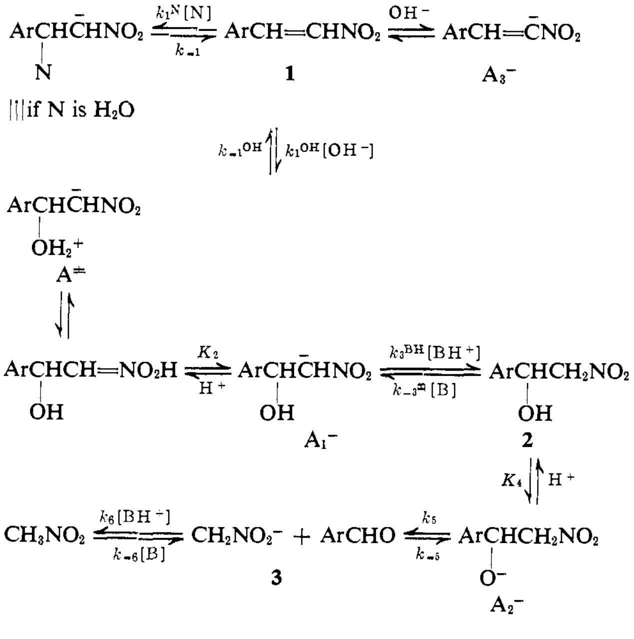
(6) L. Canonica, *Gazz. Chim. Ital.*, **79**, 192 (1949); R. Stewart, *J. Amer. Chem. Soc.*, **74**, 4531 (1952).

(7) *Cf.* M. J. Kamlet and D. J. Glover, *ibid.*, **78**, 4556 (1956); E. A. Walker and J. R. Young, *J. Chem. Soc.*, 2045 (1957).

Chart I



the reaction of **2** at pH 8.9, when the concentration of A_1^- is at a maximum (Figure 2), it is momentarily in equilibrium with its conjugate acid **2**, and the dissociation constant for the α hydrogen can be calculated.

$$K_{-3} = [H^+][A_1^-]/[2] = 1.1 \times 10^{-9}(0.10)/0.06 = 1.7 \times 10^{-9} \quad (7)$$

K_4, the dissociation constant of the OH group of the nitroalcohol, is inseparable by our experiments from the rate constant k_5, but from the pK_A of ethanol (16.5) and of 2,2,2-trifluoroethanol (12.37)[8] and the σ^* values of trifluoroethyl (0.92) and 2-nitroethyl (0.50) we estimate pK_4 to be 13.6. (The method of Branch and Calvin[9] gives pK_4 = 14.5.) This means that the hydroxyl group is mainly undissociated over the pH range of Figure 1.

The rate of cleavage of **2** to piperonal and nitromethane is proportional to the hydroxide ion concentration (eq 3). This pH dependence must be caused by very rapid equilibration of **2** with its anion A_2^-, which undergoes rate controlling though quite rapid cleavage to products. It follows that $k_{45} = k_5/K_4/K_w = 2 \times 10^3\ M^{-1}\ sec^{-1}$. Substituting the estimated value 10^{-14} for K_4 and 10^{-14} for K_w also, we obtain approximately $2 \times 10^3\ sec^{-1}$ for k_5, the rate constant for dissociation of A_2^-.

A_1^- is converted to aldehyde and nitromethide ion much more slowly than **2** or A_2^-, and with rate constant k_{345} independent of pH. Attributing the reaction to reprotonation of A_1^- followed by ionization and cleavage and noting that the pH of the more alkaline solutions is above pK_{-3}, we see that the un-ionized nitroalcohol **2** does not accumulate but can only be a low-concentration intermediate in the steady-state system

$$A_1^- \underset{k_{-3}[OH^-]}{\overset{k_3}{\rightleftharpoons}} 2 \xrightarrow{k_{45}[OH^-]} 3$$

the solution to which is

$$-\frac{d[A_1^-]}{dt} = k_{345}[A_1^-] = \frac{k_3k_{45}}{k_{-3} + k_{45}}[A_1^-]$$

A tentative value of $1.2 \times 10^2\ M^{-1}\ sec^{-1}$ for k_{-3} can be obtained by setting the high pH product ratio $[A_1^-]/[3]$, observed to be 0.06, equal to k_{-3}^{OH}/k_{45}. Using $k_{45} = 2 \times 10^3\ M^{-1}\ sec^{-1}$ and $k_{345} = 1.6 \times 10^{-3}\ sec^{-1}$, we then find $k_3^{H_2O}$ to be $1.7 \times 10^{-3}\ sec^{-1}$, which shows that the protonation of A_1^- is nearly rate controlling. The final value of K_{-3}^{OH}, calculated from k_3K_{-3}/K_w, is 2.9×10^2. Thus there is an error in the product ratio or a discrepancy of 0.3 pK unit in K_{-3}.

The curves in Figure 2 are calculated using these rate constants (the set of equilibria in Chart II was computerized). While the height of the maximum in the concentration of A_1^- necessarily agrees with experiment because it was used to calculate K_{-3}, the good approximation of the other features of both the A_1^- and the product curve to the experimental points is evidence for our interpretation. Also shown in Figure 2 is the calculated concentration of **2**, the reactant. The nitrostyrene concentration is calculated to increase gradually to a maximum of only 5/10,000 of the original nitroalcohol concentration at 6000 sec. This undetectable quantity then would decrease as the reaction proceeded beyond 99.99% completion.

Chart II. Rate Constants (sec^{-1}) for Nitrostyrene System, pH >10

$$1 \underset{6 \times 10^{-7}}{\overset{0.30[OH^-]}{\rightleftharpoons}} A_1^- \underset{290[OH^-]}{\overset{0.0017}{\rightleftharpoons}} 2 \overset{K_4 = 10^{-14}}{\rightleftharpoons} A_2^- \underset{0.2\ M^{-1}\ sec^{-1}}{\overset{2000}{\rightleftharpoons}} 3$$

Evidently the condensation reaction, observed as described in the Experimental Section, starts with a comparatively rapid equilibration of nitromethide ion and aldehyde with A_2^- which is in extremely rapid equilibrium with the nitroalcohol **2**. The rate-controlling step is ionization to A_1^-. In support of this statement, the following calculations may be made: if the ionization step -3 is rate controlling after the preequilibrium -45, the rate will be $k_{-3}^{OH}[OH^-][2]$. Defining K_{-45} as $[2][OH^-]/[CH_2NO_2^-][ArCHO]$, we have $K_{-45} = K_{-345}K_w/K_{-3}$. The overall rate constant k_{-345} would then be $k_{-3}^{OH}K_{-345}K_w/K_{-3}$. Substituting known values for all four constants yields 0.033 $M^{-1}\ sec^{-1}$, which is within 6% of the observed value 0.0314 for k_{-345} (Table II).

Using the estimated value of K_4, the equilibrium constant K_{-5} for the association of nitronate ion with piperonal to form A_2^- is found to be K_4K_{-45}/K_w or $1.1 \times 10^{-4}\ M^{-1}$, which together with k_5 yields the estimated value 0.2 $M^{-1}\ sec^{-1}$ for k_{-5}, the rate constant for nucleophilic addition of nitronate to the aromatic aldehyde. The constants are summarized in Chart II. If, for example, a solution 0.001 M in piperonal and sodium nitromethide was prepared at pH 10, the concentration of nitroalcohol **2** would reach $1.0 \times 10^{-6}\ M$, 98% of the steady-state value, in 20 sec. Hydroxide ion catalyzed ionization to A_1^- would ensue at an initial rate of $2.9 \times 10^{-8}\ M\ sec^{-1}$ and in about 20 min the system would be at equilibrium with the final A_1 concentration equal to $K_{-345}(0.001)^2$ or $2 \times 10^{-5}\ M$. In the rapidity of the condensation step in comparison with the second base-catalyzed step, our reaction resembles the aldol con-

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[8] E. T. McBee, W. F. Marzluff, and O. R. Pierce, *J. Amer. Chem. Soc.*, 74, 444 (1952); P. Ballinger and F. A. Long, *ibid.*, 81, 1050 (1959).

[9] G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, New York, N. Y., 1941, p 203.

densations studied by Noyce and Reed[10] and by Stiles, Wolf, and Hudson.[11]

Considering finally the pH-rate profile for nitrostyrene hydrolysis, we note two anomalies observed at high pH in solutions containing 1% methanol. One is the acceleration of nitrostyrene consumption as described in the Experimental Section. The other is that the rate of piperonal formation from the faded nitrostyrene solution was not first order with rate contant $k_{345} = 1.6 \times 10^{-3}$ sec^{-1} as expected for the disappearance of A_1^-, but instead followed the course shown in Figure 4. This curve can be analyzed as the sum of concurrent first-order reactions of two species initially present in the approximate ratio 3:7 and with rate constants respectively 1.6×10^{-3} sec^{-1} (k_{345}) and 3.8×10^{-5} sec^{-1} ($k_{-1}^{\mathrm{OMe}}k_{345}/k_1^{\mathrm{OMe}}[\mathrm{OCH_3^-}]$). Since this complex state of affairs was eliminated by omitting the 1% methanol used to introduce the nitrostyrene, it is clear that methoxide ion adds to the substrate much more rapidly than hydroxide (Chart I, N = OCH_3). It is in fact known[12] that methoxide ion is far more nucleophilic *in relation to its basicity* than hydroxide in displacements; apparently the same is true of additions. Conversion of the methoxy anion to piperonal probably occurs *via* nitrostyrene and A_1^-. Further details of the reaction of nitrostyrenes with alkoxide ions will be published later. The rate constants for the nucleophilic addition are $k_1^{\mathrm{OH}} = 0.30$ M^{-1} sec^{-1} and $k_1^{\mathrm{OMe}} = 7.9$ M^{-1} sec^{-1}. An alternative cause of the phenomena shown in Figure 4 is ionization of **1** by strong base to a vinyl carbanion

$$\mathbf{1} + \mathrm{OCH_3^-} \longrightarrow \mathrm{ArCH{=}\bar{C}NO_2}$$

which accumulates, playing the kinetic role postulated above for the methoxy ether. The fact that nitrostyrene does not show deuterium exchange with the solvent at pH 11.5 seems to eliminate this possibility, however.

The pH-rate profile flattens below pH 9, indicating a contribution to the rate by some other process. We assume this to be nucleophilic attack by water, with hydroxide ion catalysis which at pH 8-9 is sufficient to render the addition step rate controlling, with the same rate constant $k_1^{\mathrm{H_2O}}$ as in 0.1-0.3 *M* acetate buffers at lower pH.[4] The rate decreases from pH 7 to 5 due to the decreasing fraction of $A^\pm$ available in the anionic form A_1^- to be protonated by water molecules. Below pH 5, the anion is protonated by H_3O^+ and the rate is pH independent.

The pH-rate profile is then described by eq 8, the third term of which is obtained by a steady-state treatment of the system

$$\mathbf{1} + \mathrm{H_2O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{A_1^\pm} \underset{\mathrm{H^+}}{\overset{K_2}{\rightleftharpoons}} \mathrm{A_1^-} \overset{k_3}{\rightleftharpoons} \mathbf{2}$$

$$k_\psi = k_1^{\mathrm{OH}}[\mathrm{OH^-}] + k_1^{\mathrm{OMe}}[\mathrm{OCH_3^-}] + \frac{k_1^{\mathrm{H_2O}}(k_3^{\mathrm{H}}[\mathrm{H^+}] + k_3^{\mathrm{H_2O}})}{(k_{-1}/K_2)[\mathrm{H^+}] + k_3^{\mathrm{H}}[\mathrm{H^+}] + k_3^{\mathrm{H_2O}}} \quad (8)$$

The k_1's and $k_3^{\mathrm{H_2O}}$ are known from the kinetic studies on **1** and A_1^-, respectively. K_{12}, the equilibrium constant for the addition of hydroxide ion to **1**, is 1.02×10^{-9} M^{-1}, from $K_{123}K_{-3}$. K_2, the ionization constant of the hypothetical zwitterion $A^\pm$ (isomeric with and undoubtedly in equilibrium with the *aci*-nitrostyrene) is inseparable from k_{-1} for its dehydration to nitrostyrene; however, k_{-1}/K_2 is equal to $k_1^{\mathrm{H_2O}}/K_{12}$ or 8×10^3 *M* sec^{-1}. The rate constant k_3^{H} for protonation of A_1^- by H_3O^+ is then calculated from eq 8 at low pH, to be 180 M^{-1} sec^{-1}.

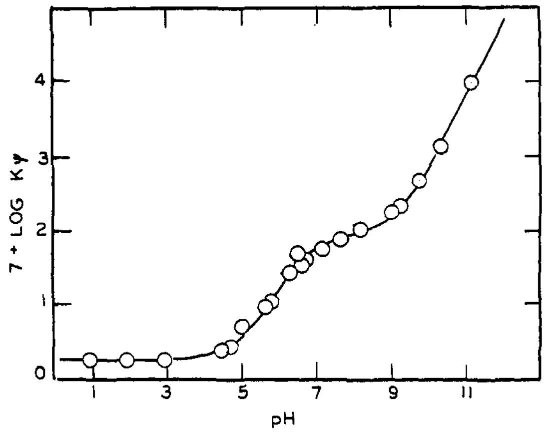

Figure 5. pH-rate profile for nitrostyrene hydrolysis.

Substitution of these numerical values into (8) gives (9), which is plotted as the curve in Figure 5, and is

$$k_\psi = \frac{0.69 \times 10^{-14}}{[\mathrm{H^+}]} + \frac{8.18 \times 10^{-6}(180[\mathrm{H^+}] + 0.0017)}{8000[\mathrm{H^+}] + 180[\mathrm{H^+}] + 0.0017} \quad (9)$$

in agreement with the experimental points shown, with an average deviation of 0.04 log unit. Note that the shoulder in the pH-rate profile between pH 5 and 9 is described without the use of any rate constants measured in this region; $k_1^{\mathrm{H_2O}}$ in particular was obtained as the limiting hydrolysis rate at high acetate concentrations at pH 4.5.[4]

The summary of rate and equilibrium constants in Table IV includes second-order rate constants k_3 for

Table IV. Summary of Rate and Equilibrium Constants for 3,4-Methylenedioxy-β-nitrostyrene in Water, 25°

Symbol	Group X	Value[a]
k_1^{X}	OH^-	3.0×10^{-1}
	H_2O	8.18×10^{-6} sec^{-1}
	OCH_3^-	7.9×10^0
	$S_2O_3^{2-}$	2.8×10^{-2}
K_{-3}		1.7×10^{-9} *M*
k_3^{X}	H_3O^+	1.8×10^2
	H_2O	1.76×10^{-3} sec^{-1}
	$ClCH_2COOH$	2.1×10^1
	CH_3COOH	4.9×10^0
	p-Toluidinium	5.5×10^0
	Pyridinium (PyH^+)	4.7×10^0
	2,6-$(Me)_2PyH^+$	1.0×10^{-1}
	$H_2PO_4^-$	2.0×10^{-2}
k_{-3}^{X}	OH^-	2.9×10^2
K_4		1×10^{-14}
k_5		2×10^3 sec^{-1}
k_{-5}		2×10^{-1}

[a] Units are M^{-1} sec^{-1} except when stated otherwise.

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protonation of A_1^- by the buffer acids which are different structural types except for chloroacetic and acetic acids. The Brønsted correlation is, therefore, not good, but its slope α is approximately 0.4.

A number of rate constants for similar reactions may be found. Using our notation and units, k_{-3}^{OH} for ionization of a methylene proton in nitroethane[13] (16.7) or in 1-*p*-methoxyphenyl-2-nitropropane[14] (**4**) (0.78 in 50% v/v H_2O–MeOH) and k_3^H for protonation of nitroethane anion[15] (15) are all considerably less than the corresponding constants for our nitroalcohol **2**, while the ionization constants K_{-3} for nitroethane, **4**, and **2** (2.5×10^{-9}, 6.3×10^{-10}, and 1.7×10^{-9}) are not very different. Evidently the 2-hydroxy group of **2** accelerates the loss or gain of a proton.

Stewart's value[16] of k_1^{OH} (0.077) for the 3-methoxy-4-hydroxy analog of **1** is less than our value of 0.3 from Table IV, as expected for a reaction between two anions. His k_{345} (1.02×10^{-3} sec^{-1}) is the same order of magnitude as ours, but note that his solutions contained 1% ethanol.

Addition of thiosulfate to acrylonitrile[17] shows $k_1^{S_2O_3} = 1.5 \times 10^{-4}$. Other k_1 values are 0.74 and 0.0032 for the respective additions[18] of barbiturate and nitroformate ions to *β*-nitrostyrene in methanol at 40°, and 530 for methoxide ion with a cyclic *β*-nitrostyrene in methanol.[19]

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Kinetics of the Reactions of Papain with Substituted α-Haloacetophenones

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Abstract: The pH–rate profile for the reaction of papain with *α*-bromo-4-hydroxy-3-nitroacetophenone (I) has been determined. Under the conditions of enzyme in excess, analysis of the pH dependence of the quantity ($k_{obsd}/[E]_0$), where k_{obsd} is the observed first-order rate constant, suggests that papain can react with I by either of two pathways. One pathway involves catalysis by an enzymatic group ($pK_1 = 3.08$), presumably functioning as a general base, of the attack of the active site sulfhydryl group ($pK_2 = 9.06$) on I. In the pH region where this pathway predominates, the reaction of papain with I clearly depends on the integrity of the active site of the enzyme, as is required for an active site titrant. The other pathway which operates at high pH appears to involve the direct nucleophilic attack of the thiolate form of the enzymatic sulfhydryl group on I. *α*-Bromo-3-hydroxy-6-nitroacetophenone (VI) reacts with the papain's active site with 1:1 stoichiometry, leading to the inactivation of the enzyme. However, because VI reacts considerably slower than I does, it is not particularly suitable for use as an active site titrant. The spectrophotometric titration curves for the *o*-nitrophenol function in papain modified by I and the *p*-nitrophenol moiety introduced in the enzyme by the reaction of VI show sigmoidal dependencies, indicating that the ionization of these groups is not significantly perturbed by that of an enzymatic ionizing group having a comparable pK_a value. However, the pK_a shifts seen for the ionization of the nitrophenol groups due to their covalent attachment to the enzyme may be interpreted in terms of the less polar environment of the active site as compared with the aqueous solution.

Recently, we reported[3] that the phenacyl halide *α*-bromo-4-hydroxy-3-nitroacetophenone (I) reacts rapidly with the active site of papain to produce an inactive modified enzyme (II), containing an *o*-nitrophenol "reporter"[4] group covalently bound to the very center of the active site. This reaction allowed us to titrate the enzyme by two different experimental approaches. In one approach the titration procedure utilized differences in the ultraviolet absorption spectra of the reagent I and the bound species II. The other approach involved monitoring the extent of the inhibition of enzymatic activity due to the reaction of I by rate assays employing *p*-nitrophenyl *N*-benzyloxycarbonylglycinate (III)[5] and ethyl *N*-benzoylargininate (IV)[6,7] as the substrates. The extent of inhibition was found to correspond to the loss of sulfhydryl groups as measured by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (V),[8] and this provided strong evidence that the sulfhydryl group at the active site of papain is the function which reacts with I to

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