Flow Nuclear Magnetic Resonance Measurement of Nucleophilic Addition to Pyruvate Anion[1]

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The rate of addition of nucleophiles such as hydroxylamine, methoxyamine, hydrazine, and semicarbazide to the conjugate base of pyruvic acid in water has been determined by line-shape analysis of proton nuclear magnetic resonance signals measured while flowing the reaction mixture. Under these conditions a steady-state concentration of the carbinolamine intermediate is maintained and the signal due to this intermediate is detected either separately or as a coalescence signal. The rate constant for the addition of the nucleophile to form the carbinolamine is found to correlate with K, the equilibrium constant, and not with K_a for the nucleophile. The rate of dehydration of the carbinolamine is found to agree with data found previously. The signals of the syn and anti isomers of the Schiff base products are detected, and the ratios, which may correspond to kinetic control, are reported.

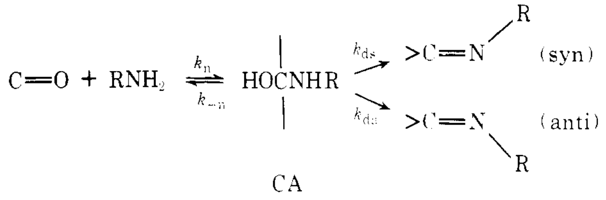
The elaboration of details of the mechanism of the addition of nucleophiles to carbonyl compounds has been of interest to us. The proton nuclear magnetic resonance spectroscopy (NMR) of flowing liquids has been particularly useful in the study of the nucleophilic addition step as well as the dehydration step (and any other subsequent steps). The application of this technique to the study of addition to pyruvic acid (PA) was of interest to us because PA has a somewhat different structure than those studied previously and could, perhaps, provide additional information concerning the preequilibrium addition step. Furthermore, PA plays a key role in many biological pathways, and there may be a possible analogy of our reaction system with some enzyme-catalyzed reactions, e.g., the amino transferase.[2] We wish, therefore, to report the results of a study of the preequilibrium as well as the dehydration step for the reaction of various nucleophiles with PA. This system has been studied previously[3,4] by UV spectroscopy, and the accepted mechanism may be formulated as shown in Scheme I.[5] The detection by our technique of the carbinolamine intermediate CA enabled us to measure the rate constants for the forward and reverse steps k_n and k_{-n} of the equilibrium and those (k_{ds} and k_{da}) for the dehydration of CA to the syn and anti Schiff bases. The values of k_n and k_{-n} are found to be independent of buffer concentration and pH over a limited pH range for all the nucleophiles studied. The values for k_n which ranged from 49 $M^{-1}\,s^{-1}$ for semicarbazide (SC) to 63 000 $M^{-1}\,s^{-1}$ for hydroxylamine (HA) correlate with the equilibrium constant K_n for the addition, which was determined using UV spectroscopy with flowing liquids, and not with the pK_a values for the conjugate acids of the nucleophiles. Hydroxylamine, methoxyamine (MA), and hydrazine (HY) have nearly similar values of k_{-n} whereas the value for SC is smaller by at least two orders of magnitudes. The rate for the dehydration step is catalyzed by buffer and hydronium ion. The values in the absence of buffer, obtained by extrapolation, are in reasonable agreement with those reported previously.[3,4]

Experimental Section

The ^{1}H NMR spectra at 100 MHz were measured at 30.0 ± 0.3 °C under static and flowing conditions without spinning using a suitably modified Varian HA 100-15 spectrometer equipped with a flow system described earlier.[6] All chemicals were obtained from suppliers and the pyruvic acid was distilled before using. Glass distilled water was used for the solutions, which were prepared with an ionic strength of 1.3 (KCl). The pH was measured using a Radiometer PHM 63 digital pH meter equipped with a glass electrode and is reported to ±0.02 unit. The pH of the reaction mixtures was measured as a function of time at 10-s intervals immediately after mixing and was constant to within 0.1 pH unit.

The equilibrium constants for the equilibrium (Scheme I) were measured using UV and flowing liquids in a high-pressure mixing chamber as reported earlier.[6] The discrete rate constants for the forward k_n and reverse k_{-n} steps of the equilibrium were determined by analysis of the NMR line shape for spectra obtained while flowing, as described below. The rate constants for the dehydration step were obtained by stopped flow experiments and an example, obtained by repetitive sweeping of the product oxime CH_3 signals (syn and anti isomers) for the reaction of PA with HA, is illustrated in Figure 1.

Scheme I



The pK_a of each buffer and nucleophile at an ionic strength of 1.3 M KCl was determined potentiometrically at 30 °C and is reported as pK_a'.

Results and Discussion

Figure 2 illustrates the types of 100-MHz proton resonance spectra observed at 30 °C for static solutions (Figures 2A and 2D) and while flowing at 20 mL/min after mixing a solution containing pyruvic acid with one containing a nucleophile. Figure 2A is due to a solution (buffered at 7.5) containing 0.2 M PA prior to mixing; Figure 2B is due to a reaction mixture buffered at pH 7.5 containing 0.1 M PA and 0.2 M SC initially; Figure 2C is due to a reaction mixture buffered at pH 7.5 containing 0.1 M pyruvic acid and 0.2 M hydroxylamine. Figure 2D is obtained for a static solution at the end of the reaction with semicarbazide. The three signals in Figure 2A are assigned to the CH_3 proton resonances of methanol (lowest field), which is used as a reference, the pyruvate anion (middle signal), and the hydrate of pyruvate anion (highest field). According to the relative intensities for these two signals only 10% of the pyruvate anion exists as the hydrate. This value is similar to previously reported results.[7] Upon mixing with SC a new signal at higher field is observed in addition to that due to the pyruvate anion CH_3 group. For several reasons, this signal is assigned to the CH_3 proton resonance of the carbinolamine intermediate arising from the addition of SC. First, it cannot be due to the hydrate of pyruvate anion because its intensity and chemical shift do not coincide with that observed in the absence of semicarbazide. Furthermore the small signal due to the hydrate can also be observed. Second it is a transient signal whose rate of decay is identical to the rate of growth observed for the syn and anti isomers of the semicarbazone whose CH_3 proton resonances are at lower field (Figure 2D). Third, this transient signal has a chemical shift close to

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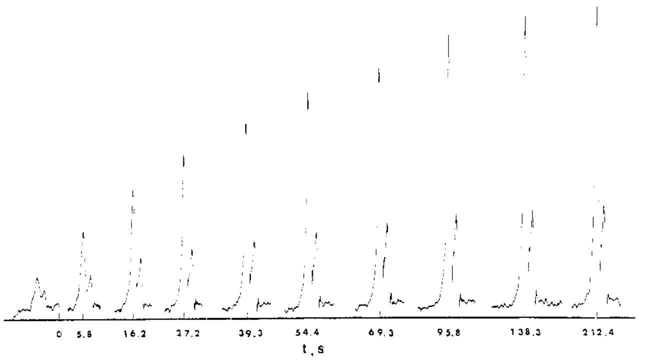


Figure 1. Time dependence for the CH_3-proton resonances of the syn and anti oximes obtained by repetitive scanning of these resonances after the flow had been stopped for a solution at pH 7.5 containing 0.1 M pyruvate anion and 0.2 M NH_2OH initially.

the hydrate signal and, therefore, is probably due to a CH_3 group bound to a tetrahedral carbon atom. Fourth, the intensity ratio, transient/pyruvate anion, depends upon the concentration of semicarbazide and corresponds to an equilibrium constant of 11 M^{-1}, which is very close to 12 M^{-1} measured using flow UV and 10 M^{-1} measured previously.[3] Based on extrapolation of the UV absorbance to infinite nucleophile concentration, Jencks[3] concluded that the intermediates arising from addition of SC, HA, and MA have very small absorbances, i.e., less than 10% of the original absorbance. As illustrated in Figure 2C, the spectrum observed for the NH_2OH reaction mixtures differs markedly from the semicarbazide case in that only one broad signal is observed. The line width and chemical shift of this signal are dependent upon the NH_2OH initial concentration. When the initial concentration of pyruvate ion is held constant, the line width of this signal increases and it moves upfield as the initial concentration of NH_2OH is increased, indicating that this signal is a coalescence of two signals and, therefore, that CH_3 proton exchange between two sites is occurring.[8] In view of the semicarbazide result, this exchange probably is due to the rapid equilibrium between pyruvate anion and its carbinolamine resulting from addition of NH_2OH. Additional support for this exchange process is that the chemical shift for the CH_3 proton resonance of this carbinolamine (δ 1.58) is close to that observed for the one formed by addition of semicarbazide (δ 1.61). This chemical shift was calculated from the NH_2OH concentration dependence of the chemical shift of the broad line using the nucleophilic addition equilibrium constant K_n determined by flow UV. The other two nucleophiles, hydrazine and *O*-methyhydroxylamine, that have been studied give flow NMR spectra similar to that observed for NH_2OH.

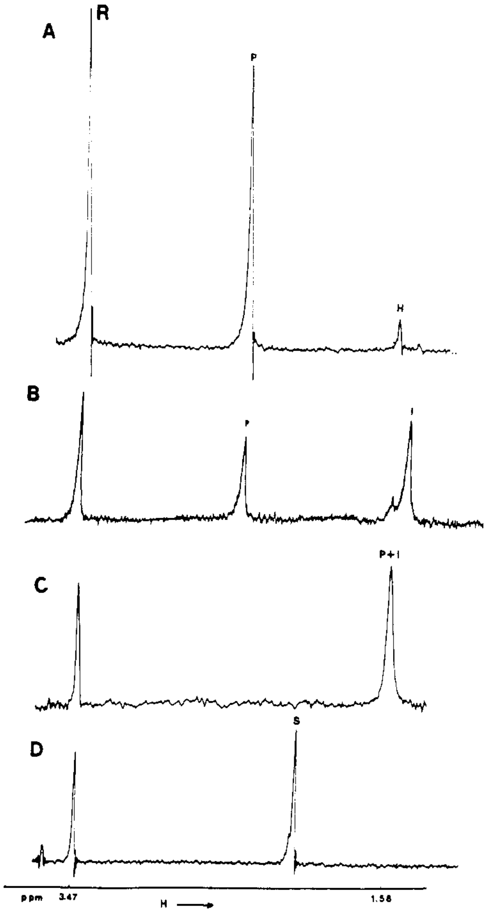


Figure 2. Partial NMR spectra (of CH_3 region only) obtained under static and flowing (20 mL/min) conditions obtained at 30 °C and 100 MHz without spinning. (A) Solution at pH 7.5 containing 0.2 M PA and methanol (labeled R), which acts as intensity and line width reference. The assignment is: P, CH_3 of PA anion; H, CH_3 of PA anion hydrate. (B) Solution at pH 7.5 containing methanol, 0.1 M PA, and 0.2 M semicarbazide initially after mixing; spectrum obtained while flowing. The line labeled I is assigned to the CH_3 resonance of the carbinolamine intermediate. (C) Spectrum obtained while flowing a solution buffered at pH 7.5 containing 0.1 M PA and 0.2 M NH_2OH. Signal labeled P + I is a coalescence of the signals due to the CH_3 protons of PA anion and the carbinolamine intermediate. (D) Static spectrum obtained after the CH_3 signals due to P and I had disappeared for the solution similar to that described in B. Signals labeled S are due to the CH_3 resonances of the syn and anti isomers of the semicarbazone. The resonance due to the anti isomer is a small shoulder to the left of the dominant signal.

Upon stopping the flow after mixing, the resultant spectra are time dependent in the case of each nucleophile; the pyruvate CH_3 resonance or the coalescence signal decays and the signals due to the Schiff base grow. An example of the time dependence of the growth of the syn and anti oximes is illustrated in Figure 1, which was obtained by repetitive scanning of these two signals immediately after stopping the flow. Data of this type and the line width data discussed above were used to obtain information about the rate constants for various steps in Scheme I. The line width data provide values for k_n and k_{-n}, the rate constants for the forward and reverse steps of the rapid preequilibrium, and the time dependence of the various signals provides values for k_{ds} and k_{da}, the rate constants for the dehydration of the carbinolamine CA to form the syn and anti oximes, respectively. The interconversion of syn and anti isomers was not studied since the intensity of the signals due to these compounds is time independent over the time range that they have been monitored (about 2 hours), indicating several possibilities: (a) that these isomers are formed under thermodynamic control, (b) that they are formed under kinetic control and equilibration is fast, (c) that they are formed under kinetic control and equilibration is very slow. The present results give no indication about which of these possibilities obtains; however, in the case of acetaldehyde,[9] the isomers appear to form according to (c). Before presenting and discussing the data from the study of the time dependence, the line width data will be considered.

Methyl-Proton Exchange. Because the flow rate of the liquid is fast relative to the rate of dehydration of CA and is slow relative to the forward and reverse rates of the preequilibrium, a steady-state concentration of CA is maintained while the solution is flowing, and CA is in equilibrium with the pyruvate anion and the nucleophile. For the nucleophiles, semicarbazide and *O*-methylhydroxylamine, the reaction was studied at pH values that are at least 1 pH unit larger than the pK_a of their conjugate acids, and therefore, the concentration for CA could be calculated using the initial concentrations of

Table I. Kinetic Data for the Addition of Semicarbazide to Pyruvate at 30 °C and μ = 1.3 (KCl)

pH	[Buffer],[a] (total), M	SC[b] (total), M	k_n, $M^{-1} s^{-1}$	k_{-n}, s^{-1}	k_d(anti)[c] × 10^3, s^{-1}	k_d(syn)[c] × 10^3, s^{-1}	k_d(int)[c] × 10^3, s^{-1}	k_{da}/k_{ds}[d]
7.50	Phos							
	0.30	0.20	49.5 ± 0.3		13.7 ± 1.8	15.3 ± 3.5	16.2 ± 0.1	2.9
		0.40	48.6 ± 0.4	4.0 ± 0.1	9.1 ± 0.8	8.1 ± 0.8	8.4 ± 0.5	
		0.80	48.9 ± 0.9	4.1 ± 0.1	10.9 ± 0.6	11.0 ± 0.4	10.3 ± 0.4	3.0
	0.10	0.20	47.5 ± 1.2		6.7 ± 0.1	7.2 ± 0.3	6.9 ± 0.5	
	0.20		49.2 ± 0.8		15.8 ± 3.8		9.3 ± 0.7	
	0.30		48.7 ± 1.1		12.4 ± 0.7	13.7 ± 0.7	11.6 ± 0.4	3.1
7.00	0.30	0.20	47.3 ± 0.8					
		0.40	48.5 ± 0.4	4.0 ± 0.1	33.8 ± 1.9		28.6 ± 1.4	
		0.80	49.3 ± 0.6	4.1 ± 0.7	29.2 ± 0.8		28.8 ± 0.9	
	0.10	0.20	49.0 ± 0.1		18.9 ± 1.3	19.6 ± 1.8	21.24 ± 0.5	
	0.20		46.9 ± 1.3		25.4 ± 2.0	27.0 ± 0.8		
	0.30		48.7 ± 1.1	3.7 ± 0.6	29.4 ± 0.9		31.3 ± 1.6	3.2

[a] Approximate total concentration after mixing (includes all degrees of protonation). The exact values deviated by no more than ±10% from those indicated in the table. [b] Approximate total initial concentration after mixing (includes all degrees of protonation). The exact values, which were used in all calculations, deviated by no more than ±10% from those indicated in the table. The initial concentration of pyruvate anion after mixing was approximately 0.1 M in all runs. [c] Calculated from the decay of the coalescence signal (int) and growth of the syn and anti oxime signals. $k_d = k_{ds} + k_{da}$, first order rate constant calculated as described in text. [d] Obtained from the ratio of the anti to syn oxime signals after completion of the dehydration step.

the starting materials and K_n, the equilibrium constant for addition determined by flow UV. For NH_2OH and NH_2NH_2, the pH of some of the solutions was close to the pK_a of their conjugate acids, and therefore, the pK_a of CA as well as the nucleophile had to be included in the calculation of the equilibrium concentration of CA and CAH^+, the conjugate acid of CA, as will be described in another paper.[10] For this purpose, pK_a values of CAH^+ derived from hydroxylamine and hydrazine were estimated to be 5.29 and 7.20, respectively, using pK_a values for the cations of N-methylhydroxylamine[11a] and N-methylhydrazine[11b] and adjusting for the substituent effects of the hydroxyl and carboxylate groups by using[12] σ_I = 0.25 and −0.17, respectively, and ρ_I = −3.4. From this equilibrium concentration of CA (plus CAH^+ when appropriate), the proton fraction of the intermediate P_I and pyruvate anion P_P can be calculated, and the average lifetime τ for proton exchange is calculated according to either the equation for slow exchange or the one for fast exchange between two sites.[8]

Slow Exchange: Semicarbazide. Because the CH_3 signals due to pyruvate anion and CA are resolved and do not shift as the concentration of semicarbazide is increased, the exchange rate resulting from the equilibrium is slow relative to $1/\delta$, the reciprocal of the chemical shift between the two signals,[8] and therefore, $1/\tau_P = \Delta_P$ and $1/\tau_{CA} = \Delta_{CA}$ in which $\Delta = \pi(\Delta\nu_e - \Delta\nu_0)$, $\Delta\nu$ is the width at half-height of the appropriate signal, and the subscripts e and 0 denote exchange and no exchange, respectively. For pyruvate, $\Delta\nu_0$ is obtained when semicarbazide is absent. The intermediate CA is assumed to have the same value. The rate constants, which are listed in Table I, are related to exchange lifetimes by the following equations: $1/\tau_{CA} = k_{-n}$ and $1/\tau_p = k_n[N]$ in which [N] is the equilibrium concentration of the semicarbazide free base. Each number listed is an average of at least three measurements. Although the precision of the values appears very satisfactory, it may be somewhat fortuitous since Δ does not always make the major contribution to the total line width, i.e., $\Delta\nu_0$ is about 2.3 Hz and $\Delta\nu_e$ varies from 3 to 6 Hz as the concentration of semicarbazide increases. At any rate since $\Delta\nu$ can be measured to within 0.3 Hz, the rate constants are probably accurate to within about 40% at the low concentrations, and the accuracy improves as the concentration of semicarbazide increases. The fact that the ratio, k_n/k_{-n}, is about 12 M^{-1}, the same value as obtained for the equilibrium constant K_n determined by flow UV, for all the data in the table indicates that the accuracy is probably no worse than the above mentioned since k_n and k_{-n} are obtained independently. As indicated in the table, k_n is independent of phosphate buffer concentration of semicarbazide. In addition, it remains constant as the pH is increased from 7.00 to 7.50. This absence of any acid or base catalysis is also observed for the other nucleophiles, and the mechanistic implications will be discussed below.

Fast Exchange: NH_2OH, CH_3ONH_2, NH_2NH_2. As mentioned above, when the nucleophile is NH_2OH, CH_3ONH_2, or NH_2NH_2 the preequilibrium of Scheme I is sufficiently fast to make the methyl-proton exchange rate fast relative to $1/\delta$. Consequently, the exchange lifetime is calculated using the equation $\Delta = P_P P_I \delta^2 \tau$, which was derived for exchange between two sites.[8] In this equation, $\Delta = \pi(\Delta\nu_e - (\Delta\nu_0)_P P_P - (\Delta\nu_0)_I P_I)$ and $1/\tau = k_n[N] + k_{-n}$, in which [N] is the equilibrium concentration of nucleophile free base. Thus, using the value for τ and K_n, k_n can be obtained. At pH values for which the concentration of CAH^+ is not negligible, its proton fraction was included, i.e., $P_I = P_{CA} + P_{CAH^+}$. In other words, the exchange is considered to occur between the methyl protons of pyruvate anion and those of CA and CAH^+. This description seems reasonable since the equilibration between CA and CAH^+, which involves only a proton transfer, is expected to be faster than the nucleophile addition, as suggested in another paper.[10] For the three nucleophiles studied under these conditions, Δ makes a major contribution to the line width of the coalescence signal, and the values for k_n listed in Tables II, III, and IV for NH_2OH, CH_3ONH_2, and NH_2NH_2, respectively, are probably more accurate than the semicarbazide results. The values for k_{-n} are also listed in the tables; however, they are not determined independently as they are calculated using k_n and K_n. As in the case of semicarbazide, k_n for the addition of thase nucleophiles to the pyruvate anion is independent of pH and the concentration of buffers employed. This absence of catalysis in this pH range has been observed previously,[6a] and the suggested description is illustrated in Scheme II, which is similar to one discussed by Jencks.[5] In this scheme N, >C=O, and >C=N represent nucleophile free base, pyruvate anion, and Schiff base, respectively, and $CA^{\pm}$, CAH^+, and CA represent three possible

Table II. Rate Constants for Addition k_n and Dehydration k_d Steps at 30 °C and μ = 1.3 (KCl) for Hydroxylamine

pH	[Buffer][a]	$[NH_2OH]$[b] (total), M	$k_n \times 10^{-4}$, $M^{-1} s^{-1}$	k_d[c](anti) × 10^3, s^{-1}	k_d[c](syn) × 10^3, s^{-1}	k_d[c](int) × 10^3, s^{-1}	k_{da}/k_{ds}[d]
7.50	Phos						
	0.10	0.20	6.3 ± 0.2	10.7 ± 0.1	9.8 ± 0.1		3.2
		0.40	6.2 ± 0.9	9.7 ± 0.4	9.2 ± 0.5	10.5 ± 0.7	3.3
		0.50	6.5 ± 0.9	8.6 ± 0.4	8.6 ± 0.3	8.9 ± 0.4	3.1
	0.10	0.40	6.4 ± 0.7	8.6 ± 0.3	8.5 ± 0.2	8.8 ± 0.3	3.1
	0.30		6.4 ± 0.3	11.0 ± 0.6	11.1 ± 0.7	10.9 ± 0.5	3.3
7.00	Phos						
	0.10	0.40	6.5 ± 0.1	23.1 ± 0.8			
		0.50	6.1 ± 0.9	21.9 ± 0.5			
	0.10	0.40	6.5 ± 0.1	23.3 ± 0.2			
	0.20		6.4 ± 0.1	26.2 ± 0.4	27.9 ± 0.4		
	0.30		6.2 ± 0.2	29.8 ± 0.1	28.1 ± 0.2	28.5 ± 0.8	3.3
6.50	Phos						
	0.30	0.40	5.9 ± 0.4	42.5 ± 0.6	44.3 ± 0.4	43.8 ± 0.8	3.1

[a] Approximate total concentration after mixing (includes all degrees of protonation). The exact values deviated by no more than ±10% from those indicated in the table. [b] Approximate total initial concentration after mixing (includes all degrees of protonation). The exact values, which were used in all calculations, deviated by no more than ±10% from those indicated in the table. The initial concentration of pyruvate anion after mixing was approximately 0.1 M in all runs. [c] Calculated from the decay of the coalescence signal (int) and growth of the syn and anti oxime signals. $k_d = k_{ds} + k_{da}$; first order rate constant calculated as described in text. [d] Obtained from the ratio of the anti to syn oxime signals after completion of the dehydration step.

Table III. Kinetic Data for the Addition of CH_3ONH_2 to Pyruvate at 30 °C and an Ionic Strength of 1.3 (KCl)

pH	[Buffer][a] (total), M	$[CH_3ONH_2]$[a] (total), M	$k_n \times 10^{-4}$, $M^{-1} s^{-1}$	$k_{-n} \times 10^{-3}$, s^{-1}	k_d(prod)[b] × 10^3, s^{-1}	k_d[b](int) × 10^3, s^{-1}
6.50	Phos					
	0.10	0.05	3.8 ± 0.1	1.0 ± 0.1	20.3 ± 0.1	18.8 ± 1.8
		0.20			19.5 ± 0.1	20.0 ± 0.4
		0.40	3.7 ± 0.1	1.0 ± 0.1	15.1 ± 3.0	13.1 ± 0.9
	0.20	0.10	4.1 ± 0.2	1.1 ± 0.1	17.7 ± 2.9	17.3 ± 2.1
		0.20	3.9 ± 0.4	1.0 ± 0.1	13.2 ± 3.9	12.3 ± 0.2
	0.30	0.05	4.1 ± 0.3	1.1 ± 0.1	20.3 ± 0.7	18.8 ± 1.8
		0.15	3.8 ± 0.2	1.0 ± 0.1	17.7 ± 1.2	17.9 ± 2.6
		0.20	3.8 ± 0.1	1.0 ± 0.1	20.7 ± 0.9	27.5 ± 4.8
7.00	0.10	0.20	3.8 ± 0.2	1.0 ± 0.1	4.7 ± 0.1	4.6 ± 0.2
	0.30		3.7 ± 0.2	1.0 ± 0.1	4.8 ± 0.2	4.8 ± 0.3
	0.20	0.20	3.7 ± 0.1	1.0 ± 0.1	4.6 ± 0.1	4.9 ± 0.2
		0.40	3.9 ± 0.2	1.1 ± 0.1	4.8 ± 0.1	4.8 ± 0.6

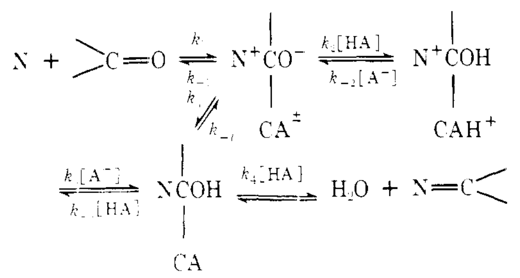
[a] Concentrations after mixing as described in Table I. [b] Obtained from growth of the product signal and decay of the coalescence signal (int). Syn and anti signals are not resolved.

Table IV. Rate Constants for Addition k_n and Dehydration k_d Steps at 30 °C and μ = 1.3 (KCl) for Hydrazine

pH	[Buffer][a] (total), M	$[NH_2NH_2]$[a] (total), M	$k_n \times 10^{-3}$, $M^{-1} s^{-1}$	$k_{-n} \times 10^{-2}$, s^{-1}	k_d(prod)[b] × 10^2, s^{-1}	k_d(int)[b] × 10^2, s^{-1}	k_{ds}/k_{da}
8.5	Dabco						
	0.10	0.40	14.3 ± 0.9	6.2 ± 0.1	4.3 ± 0.3	4.6 ± 0.4	
	0.30		14.0 ± 1.1	6.0 ± 0.1	6.5 ± 0.3		
	0.60		13.9 ± 1.7	5.9 ± 0.7	9.2 ± 0.3		
	0.30	0.10	14.8 ± 1.3	6.4 ± 0.1	6.1 ± 0.4		
		0.20	14.5 ± 0.8	6.3 ± 0.1	6.4 ± 0.3		3.0
9.0	0.10	0.40	15.3 ± 1.2	6.4 ± 0.1	3.6 ± 0.1	3.8 ± 0.2	3.4
	0.30		15.8 ± 1.4	6.6 ± 0.1	5.5 ± 0.3	5.8 ± 0.4	
	0.60		14.9 ± 0.9	6.2 ± 0.1	9.5 ± 0.8		
	0.30	0.10	15.2 ± 1.4	6.3 ± 0.1	5.2 ± 0.2		
		0.20	15.7 ± 1.9	6.5 ± 0.8	6.2 ± 0.7		

[a] Concentration after mixing, as described in Table I. [b] Obtained from product signal growth and decay of the coalescence signal (int).

Scheme II



intermediates of which CA (and CAH^+ at lower pH) is believed to exist in appreciable quantity. According to this scheme, the addition reaction involves three distinct steps: nucleophilic addition to form $CA^{\pm}$, protonation of $CA^{\pm}$ to form CAH^+, and deprotonation of CAH^+ to form CA. As can be seen, two steps in this scheme that are independent of acid and base catalysis are the addition step to form $CA^{\pm}$ and direct conversion of $CA^{\pm}$ to CA. Consequently our results for all four nucleophiles are consistent with this path in the pH range studied. This conclusion is consistent with rates of proton exchange found previously for acid–base reactions. Rate constants for proton transfer between nitrogen acids and bases are commonly between 10^7 and 10^9 M^{-1} s^{-1}.[14a] The analogous reaction for phenol-phenoxide ion is equally fast.[14b] Since the concentration of buffer is at least 0.1 M and that for the nucleophile is usually 0.1 M or larger, the overall rate constant for methoxyamine addition via the intermolecular proton transfer steps is estimated to have an upper limit of 2×10^3 M^{-1} s^{-1}, which is one order of magnitude lower than the observed value.[15,16]

Our results provide some information concerning the nature of the proton switch step. A proton switch mechanism involving the OH proton has been suggested as a possibility for the conversion of $CA^{\pm}$ directly to CA in the case of addition of NH_2OH to *p*-chlorobenzaldehyde under conditions in which no buffer is present.[17] Although this mechanism requires no acid–base catalysis and could apparently apply to the present reaction for NH_2OH (and also perhaps for NH_2NH_2 and semicarbazide since the adjacent nitrogens have available protons), this mechanism cannot apply when CH_3ONH_2 is the nucleophile since the oxygen contains no transferable protons. Consequently, we see no need to invoke this type of switch mechanism for the present system since there is no reason to suspect CH_3ONH_2 acts in a different manner than do the other nucleophiles, i.e., its rate of addition is comparable to that for NH_2OH and NH_2NH_2. The absence of buffer effect also precludes a preassociation mechanism.[18]

Apparently, the only data available for comparison purposes concern the addition of semicarbazide[4] in the pH range 3.5 to 5.5. In this pH range, it was suggested that a transition from rate-determining dehydration to rate-determining addition occurs and that this transition is complete at pH 4.0. This conclusion is based on the fact that a plot of the observed rate constant vs. pH exhibits a break, which is commonly considered diagnostic of a transition from one type of rate-determining step to another.[5] Two addition rate constants (one uncatalyzed, the other hydronium ion catalyzed) were obtained by a fit of this pH dependence (in absence of buffer) according to a mechanism similar to Scheme I using the steady-state approximation for CA. Since our results do not indicate a hydronium ion dependence for k_n', the mechanism for addition at lower pH is apparently different than the one proposed for pH range of the present study.

Table V lists the average values of k_n for each nucleophile as well as their pK_a values and K_n. For all of the nucleophiles, k_n does not correlate with K_a. On the other hand, k_n correlates well with K_n for all nucleophiles except semicarbazide, i.e., a plot of log k_n against log K_n is linear with only the point for semicarbazide deviating from the line. The reason for this deviation is unclear; to correlate, k_n for semicarbazide would have to be two powers of ten larger. Also listed in Table V are the average values for k_{-n}, which is equal to the reciprocal of τ_{-n}, the average lifetime for the break down of the carbinolamine intermediate into starting materials. Thus, the lifetime for this intermediate is about the same for all the nucleophiles except semicarbazide. The factors affecting the lifetime of the intermediate are not clear at this time and will have to await the accumulation of more data on other systems.

Table V. Values for pK_a', K_n, and the Addition Rate Constant k_n for each Nucleophile

Nucleophile	pK_a'	K_n,[a] M^{-1}	k_n, M^{-1} s^{-1}	k_{-n}, s^{-1}
Semicarbazide	3.76	12.0	49	4.1
Methoxyamine	4.65	36.5	38000	1040
Hydroxylamine	6.08	54.3	63000	1160
Hydrazine	8.24	24	14500	604

[a] Determined by flow UV spectroscopy.

Dehydration of Carbinolamine. Because of the values of K_n, the concentration of each nucleophile is such that the equilibrium concentrations of pyruvate anion and carbinolamine intermediate are appreciable. Consequently, while the decay of the pyruvate anion and CA is pseudo-first-order, the rate constant obtained from such a treatment is not the dehydration rate constant k_d. However, k_d may be obtained from the time dependence of the coalescence signal and Schiff base signals in the following manner. Since the intermediate is detected, the rate-determining step is dehydration, and rate $= k_d[CA]$ may be converted to rate $= k_d[PA]_tK_n[N]/(K_n[N] + 1)$ in which $[PA]_t$ is the total concentration of pyruvate anion and [N] is the equilibrium concentration of nucleophile free base.[19,20] If [N] is constant this rate expression may be integrated to give $\ln [PA]_t = k_d\beta t + C$ in which $\beta = K_n[N]/(K_n[N] + 1)$. However, under the present conditions [N] is not constant. Nevertheless this type of expression was used to describe the time dependence of the above-mentioned signals by allowing β to be a variable also. Thus, at each t, [N] was calculated using the appropriate equilibrium constants and taking into account the amount of PA and nucleophile that had been converted to product, and k_d was evaluated as the slope of a least-squares fit of $\ln [PA]_t$ vs. βt. To determine the accuracy of this technique, the time dependence of the concentration of PA was simulated by an incremental method using the above-mentioned rate expression with a specific rate constant similar to those found in our study. Using this simulated time dependence, the rate constant was then calculated according to the least-squares approach mentioned above and compared with the "real" value that had been specified. The deviation of the least-squares value was found to be largest when the concentration of nucleophile is equal to that of PA. Thus, for an initial concentration of 0.1 M PA, the least-squares value was larger than the real value by 25, 10, and 6% for initial concentrations of 0.1, 0.2, and 0.3 M nucleophile, respectively. PA and nucleophile have identical concentrations for only two solutions, and most of the data are for initial nucleophile concentrations of 0.2 M or greater. Therefore, the rate constants listed in Tables I, II, III, and IV are not corrected since the correction is 10% or less. Values labeled "int" were calculated from the time dependence of the coalescence signal; others are calculated from syn or anti Schiff base signals as specified. As indicated, values of k_d calculated from the time dependence of the various signals for a given solution

Table VI. Kinetic Parameters for Buffer Catalysis of the Dehydration Step in the Reaction of NH_2OH, Semicarbazide, and Hydrazine with Pyruvate Anion

Nucleophile	Catalyst	pK_a	pH	Concn range (total), M	Fraction acid[a]	Slope[b] × 10^2 M^{-1} s^{-1}	$k_4^0 \times 10^3$, s^{-1}	k_4^{GA}, M^{-1} s^{-1}	k_4^{GB}, M^{-1} s^{-1}
NH_2OH	Phosphate	6.37	7.5	0.10–0.30	0.069	2.40	7.0	0.10[c]	0.02[c]
			7.0	0.10–0.30	0.190	3.33	20.0		
			6.5	0.30	0.430	5.21	28[d]		
Semicarbazide	Phosphate	6.37	7.5	0.10–0.30	0.069	3.53	5.0	0.10[c]	0.03[c]
			7.0	0.10–0.30	0.190	4.40	16.8		
NH_2NH_2	Dabco	9.24	9.0	0.1–0.60	0.63	12.54	24.0	0.058[c]	0.24[c]
			8.5	0.1–0.60	0.85	8.52	28.0		

[a] Fraction of buffer in the acid form. [b] Obtained by least-squares fit of k_d vs. the total concentration of catalyst. [c] Obtained from a plot of slope vs. fraction acid. [d] Assuming k_4^{GA} and k_4^{GB} have the indicated values.

are within experimental error. In addition, k_d appears to be independent of nucleophile concentration but dependent on buffer concentration except for the nucleophile CH_3ONH_2 as discussed below. For NH_2NH_2, NH_2OH, and semicarbazide, the CH_3 proton resonances to the syn and anti isomers can be resolved. The ratio of these resonances for each nucleophile is independent of time over the time period studied. Consequently this ratio is assumed to be k_{da}/k_{ds}, the ratio of the first-order rate constants for the formation of these isomers from CA,[21,22] and this ratio, which is listed in Tables I, II, and IV, appears to be independent of the nature and concentration of the nucleophile as well as the pH in the range studied. Whether this ratio represents kinetic or thermodynamic control of product formation cannot be decided from our results.

As mentioned above the rate of dehydration of CA depends on the concentration of buffer for all nucleophiles except methoxyamine. The plots of k_d vs. buffer concentration are linear, and the slope which was calculated by least-squares fit is listed in Table VI, along with the buffer concentration range. The general acid k_4^{GA} and general base k_4^{GB} (Scheme II) catalytic rate constants that are listed in this table are calculated in the manner described previously,[6a] assuming a linear relationship between the slope and the fraction of buffer existing in its acid form. According to the results, the phosphate buffer has the same general acid catalytic effect as well as the same general base catalytic effect on the dehydration step of CA when NH_2OH and semicarbazide are the nucleophiles. In addition, Dabco appears to be more effective as a general base catalyst than as a general acid for dehydration of CA when NH_2NH_2 is the nucleophile.

The intercept of the least-squares plot k_4^0, which is also listed in Table VI, appears to be linearly related to the hydronium ion concentration for the nucleophiles, NH_2OH and semicarbazide, although the data are quite limited. The value for pH 6.5, calculated using k_4^{GA} and k_4^{GB}, is based on only one buffer concentration and, therefore, should be considered approximate. For NH_2NH_2, k_4^0 is not linear in hydronium ion concentration, indicating, perhaps, that hydroxide ion may be acting as a catalyst or, perhaps, that an uncatalyzed dehydration process is occurring in this case. Although the data from previous studies were obtained under somewhat different conditions than the present study,[3,4] they appear to be in reasonable agreement with the present results. For semicarbazide, $K_n k_4^0$, which has a value of 0.12 M^{-1} s^{-1} from a previous study,[4] agrees fairly well with the present value of 0.2 M^{-1} s^{-1}. From an earlier work by Jencks,[3] k_4^0 has the following values at 25 °C and an ionic strength of 0.3: 0.007, 0.004, 0.01, 0.08 s^{-1} for NH_2OH (pH 7.0), CH_3ONH_2 (pH 6.5), NH_2NH_2 (pH 8.5), and semicarbazide (pH 7.0), respectively. The difference between these and the values in Tables III and VI could be due to the difference in ionic strength and temperature.

We thank the referee for suggesting the method for estimating $K_\pm$, K_z, and pK_a values for the carbinolamine cations.

Registry No.—Semicarbazide, 57-56-1; pyruvate, 57-60-3; hydroxylamine, 7803-49-8; CH_3ONH_2, 67-62-9; hydrazine, 302-01-2.

References and Notes

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(20) When the concentration of CAH^+ is significant, this expression must be modified. Thus, if CAH^+ is also considered to dehydrate, rate = $(B/Q) \cdot [PA]_t K_n[N]/(1 + K_n[N])$ in which $B = k_d + k_d^+[H^+]/K_a$ and $Q = 1 + [H^+]/K_a$ in which K_a is the acid dissociation constant for CAH^+ and k_d and k_d^+ are dehydration rate constants for CA and CAH^+, respectively.

(21) This assumption is based on the observation that equilibration of syn and anti oximes is slow in this pH range for acetaldehyde (see ref 9) and for diethylacetonyl phosphonate (unpublished results). The assignment of the signals is based on the results of Karabatsos; see ref 22.

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