of EAA plus HA, it seems reasonable to compare this cyclization reaction with the hydrolysis of the *O*-methyloxime of EAA in the presence of the oxime of acetone. A solution of these two compounds at pH 6.5 was monitored by NMR for 14 days without observation of signals due to ethanol. We estimate that 5–10% reaction could have been detected under these conditions. Thus, an upper limit for the second-order rate constant for this hydrolysis is about 4×10^{-7} M^{-1} s^{-1}, and the lower limit for the effective molarity[10] of the hydroxyl group is about 10^4 M.

Comparison of the cyclization rates for EAA and ACAC[3] indicates that the electrophilic reactivity of the carbonyl group is important in determining the rate of cyclization. Since the cyclization for ACAC occurs via its carbinolamine, its rate must be compared with the one for the analogous step for EAA. A rate constant of about 2×10^{-3} s^{-1} at pH 7.0 is estimated to be the upper limit for this step since it does not compete effectively with the dehydration of CA. For ACAC, the addition and cyclization steps are sufficiently rapid to affect the NMR line shapes, and rates for the two steps cannot be determined separately. However, if the forward and reverse rates for the addition of HA to ACAC are assumed to be identical with those for EAA, the rate constant for the cyclization step is estimated to be 10^3 s^{-1}, assuming steady state for the carbinolamine intermediate and using a value of 2.3×10^3 M^{-1} s^{-1} for the rate constant for the combined addition and cyclization steps.[3] Thus the cyclization step for ACAC is 10^6 faster than the one for EAA. This difference probably reflects the difference in electrophilic reactivity of keto- vs. acyl-carbonyl group.

Recently,[11] the reaction of hydroxylamine or *N*-methylhydroxylamine with ethyl cinnamate has been reported to involve the formation of the isoxazolone via the intermediate, *O*-cinnamoylhydroxylamine, i.e., addition of the oxygen of HA to the acyl carbonyl precedes cyclization. Since there is no evidence for this path for EAA, it would appear that the nature of the electrophile dictates the mode of attack for HA, i.e., nitrogen or oxygen. Studies of the reaction of HA with a variety of electrophiles should help in determining the factors that control the mode of reaction.

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Flow Nuclear Magnetic Resonance Study of the Addition, Cyclization, and Dehydration Steps for the Reaction of Hydroxylamine with Acetylacetone[1]

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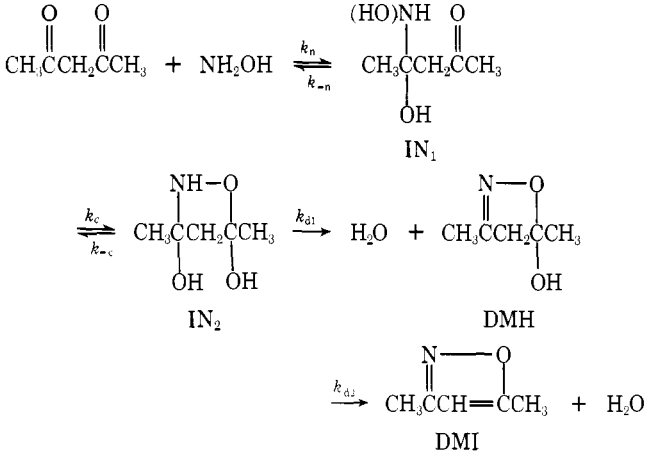
Abstract: The nuclear magnetic resonance spectrum of a flowing solution measured after mixing equal volumes of aqueous solutions of acetylacetone and NH_2OH indicates that addition and cyclization occur rapidly without the intermediacy of the oxime. That is, the dehydration of the tetrahedral intermediate is slow relative to the cyclization step. The equilibrium constant for the equilibrium A + N $\rightleftharpoons$ cyclic is 25 M^{-1}, and a value of 2.3×10^3 M^{-1} s^{-1} was obtained for the forward rate constant by means of NMR line shape analysis. This value is independent of buffer concentration and pH in the range 7.3 to 8.0. In addition, the dehydration steps were studied in this pH range as well as in the range 3.0 to 1.5. In the latter range, the rate-determining step is no longer the dehydration step as it is in the higher pH range. Furthermore, the second-order rate is acid catalyzed in the lower pH range, indicating a different mechanism for the addition and cyclization in this range.

Introduction

The synthesis and properties of isoxazoles and pyrazoles have been the subjects of studies by many workers over the years.[2] However, a survey of the literature reveals that very little information exists concerning the detailed mechanism for the formation of these compounds from β-diketones. In this paper, we report the details of the reaction of hydroxylamine (HA) with acetylacetone (ACAC) in aqueous solution as studied by means of the proton nuclear magnetic resonance (NMR) spectroscopy of flowing liquids. By means of this technique, it has been possible to detect, characterize, and study the decay of an intermediate that is generated during the reaction. The evidence suggests strongly that the mechanism given in Scheme I occurs at around pH 7. According to this mechanism, the rapid equilibria occur prior to the first dehydration step (k_{d1}). These equilibria are sufficiently fast on the NMR time scale to cause line broadening of the CH_3-proton resonances due to ACAC and the intermediate IN_2, indicating

Scheme I



proton exchange occurs. The rate for this proton exchange can be measured under various conditions of pH and buffer concentration because the NMR spectrum is time independent while the liquid is flowing and equilibration is rapid compared with the flow rate. Upon stopping the flow, the spectrum becomes time dependent, and k_{d1} and k_{d2} can be determined. Rate constants for these steps are reported and discussed.

Experimental Section

The details of the NMR flow system are reported elsewhere and will not be repeated here.[3]

Except for the 3,5-dimethylisoxazole (DMI) and 3,5-dimethyl-5-hydroxylisoxazoline (DMH), chemicals were from commercial sources and were purified by literature methods such that mp's and spectroscopic properties agree with those reported previously. DMI was obtained in liquid form after completion of the reaction: bp (uncor) 142–144 °C; reported, 142 °C.[4] Mass spectra, CHN, ir,[5] uv, and NMR[6] data are consistent with the DMI structure. DMH was isolated as a liquid from the reaction solution at pH 7 since k_{d1} is much greater than k_{d2} at this pH. While the mass spectra and CHN are consistent with either DMH or the monooxime of ACAC, the other physical properties are consistent with only the DMH structure. For example, the mono-*O*-methyloxime of ACAC and $(CH_3)_2C(OH)CH_2COCH_3$ both exhibit the expected CO absorption band in the ir and uv spectral regions whereas no carbonyl absorption was found for the isolated material. In addition, the NMR spectrum of this material is more consistent with DMH than with the monooxime. The three NMR signals that are observed in water with relative intensities 3:3:2 have chemical shifts 2.00, 1.64, and 3.07 ppm, respectively, that are very close to those (2.01, 1.68, and 2.96) measured for DMH in $CDCl_3$.[7] Furthermore, the 0.84 Hz coupling between the CH_3 resonance at 2.00 ppm and the CH_2 resonance is consistent with only the DMH structure. This conclusion is based on the fact that this coupling is not observed with the mono-*O*-methyloxime of ACAC whereas it is observed for 2-bromo-4-bromomethyl-4-isopropyl-3-methyl-2-cyclopentenone.[8] For these reasons, we assign the DMH structure to this material.

Kinetic data were obtained at 30 ± 0.3 °C using buffered aqueous solutions at an ionic strength of 1.6 (KCl). The pH of each solution was measured with a Radiometer PHM 63 digital pH meter and is reported to ±0.02 unit. The pH of the reaction mixture was measured as a function of time at 5-s intervals starting immediately after mixing. The NMR spectra were obtained before, during, and after flowing. The spectra obtained during flowing provided the line shape data for calculation of the proton exchange rates. The spectra that were obtained at various intervals after the flow was stopped provided the time dependence of the signal intensities used for determination of decay and growth rates. While the exchange broadened signal was also observed in these spectra, the line shape was complicated by the superposition of a product signal, which grows relatively rapidly in some cases. Consequently, reliable proton exchange data could be obtained only from the flowing spectra since the product signal is negligible under these conditions. For calculation of the exchange rate, the line width in the absence of exchange is required along with the line width

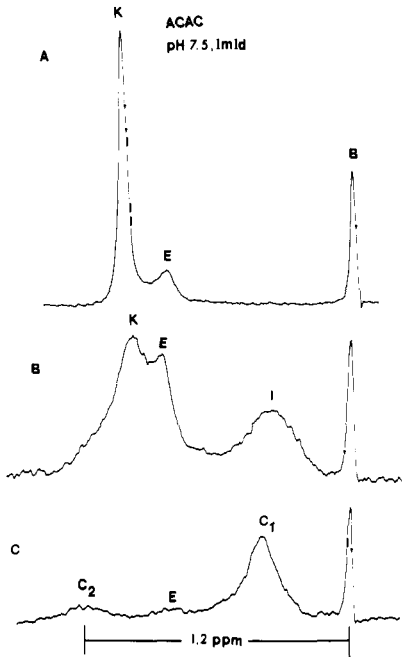


Figure 1. Slow passage 100 MHz proton nuclear magnetic resonance spectra of H_2O solutions flowing at 20 ml/min at 30 °C and at pH 7.50 buffered with 0.3 M imidazole. (A) 0.50 M acetylacetone plus 0.10 M *tert*-butyl alcohol. (B) After mixing the solution above with an equal volume of 0.15 M hydroxylamine. (C) After mixing 0.20 M acetylacetone with an equal volume of 0.50 M hydroxylamine.

of the exchange broadened signal.[9] For the CH_3-proton resonance of ACAC the line width was measured at various flow rates prior to mixing. To monitor any changes in magnetic field homogeneity, the CH_3-proton resonance of *tert*-butyl alcohol at 1.23 ppm relative to DSS was used as a reference since this alcohol could be present in the solution without affecting the reaction. Thus, the line width for the CH_3-proton resonance of ACAC could be adjusted for any changes in field homogeneity. In practice the adjustment was usually less than 20% of the line width in the absence of exchange. For the intermediate IN_2, the CH_3-proton resonance could not be obtained in the absence of exchange. In this case, we assumed that this line width was the same as the one for the CH_3-proton resonance of the carbinolamine intermediate arising from the addition of hydroxylamine to acetaldehyde.[10] The exchange reaction for acetaldehyde is sufficiently fast so that the carbinolamine CH_3-proton resonance is not exchange broadened.[10] Again, the CH_3-proton resonance of *tert*-butyl alcohol is used as a line width reference.

The pK_a for each buffer at an ionic strength of 1.6 (KCl) was measured at 30.0 °C by a potentiometric titration. The equilibrium constant K_{nc} for the equilibrium ACAC + $NH_2OH \rightleftharpoons IN_2$ was determined spectrophotometrically while flowing using our flow system[3] with a Cary 15. Although IN_1 would be expected to absorb in the same spectral region as ACAC, no correction for its presence is necessary since its concentration is small (see Discussion), and its extinction coefficient is expected to be much smaller than the one for ACAC. The latter conclusion is based on the fact that the extinction coefficient at 274 nm is 1571 for ACAC whereas it is only 25 for $(CH_3)_2C(OH)CH_2COCH_3$, a compound somewhat similar to IN_1 in structure.

Results

Proton Exchange Process. Figure 1 illustrates the sort of CW spectra obtained at 30 °C before mixing while flowing at 20 ml/min (Figure 1A) and after mixing while flowing at 20 ml/min (Figure 1B and 1C). All spectra contain the CH_3-proton resonance (labeled B) of *tert*-butyl alcohol at 1.23 ppm, and the H_2O-proton resonance is used as the lock signal. The field strength increases from left to right. The spectrum in Figure 1A was obtained for an aqueous solution of 0.5 M ACac that was buffered at pH 7.5 using 0.3 M imidazole. The signal labeled K is assigned to the CH_3 proton of the keto form of

Table I. Line Width at Half-Height in Hz and Ratio of Areas for the CH_3-Proton Resonances of ACAC and the Intermediate while Flowing at 20 ml/min after Mixing Equal Volumes of ACAC and HA Solutions

pH	[Phos], M (total)	[ACAC],[a] M	[NH_2OH],[a] M (total)	$\Delta\nu$[b], Hz ACAC	$\Delta\nu$[b], Hz INT	Ratio of areas[c] Exptl	Ratio of areas[c] Calcd IN_1[d]	Ratio of areas[c] Calcd IN_2[d]
8.00	0.30	0.20	0.075	22.0	30.0	2.4 ± 0.1	5.9	2.5
7.50	0.30	0.10	0.075	36.6	31.3	1.3 ± 0.1	3.6	1.3
	0.30	0.15	0.075	22.7	32.6	1.8 ± 0.1	4.8	1.9
		0.20	0.075	17.2	32.9	2.5 ± 0.2	5.9	2.5
		0.25	0.075	15.2	30.2	3.1 ± 0.2	7.1	3.1
	0.10	0.15	0.075	25.0	26.5	1.9 ± 0.05	4.8	1.9
	0.20			22.5	27.1	2.0 ± 0.1	4.8	1.9
	0.40			22.9	31.0	1.9 ± 0.1	4.8	1.9
	0.50			22.0	32.2	1.9 ± 0.1	4.8	1.9
	0.30	0.10	0.2	15.7[e]				
		0.10	0.3	11.1[e]				
		0.10	0.4	8.4[e]				
		0.10	0.5	7.0[e]				
7.30	0.30	0.10	0.075	34.3	29.8	1.5 ± 0.1	3.6	1.3
	0.30	0.10	0.20	16.6[e]				
			0.30	10.6[e]				
			0.40	8.8[e]				

[a] Initial concentration after mixing. [b] Line width at half-height for CH_3 signal due to ACAC and the one due to the intermediate. The line width in the absence of exchange varies from one run to the next but is about 5 Hz for ACAC and 2 Hz for the intermediate (see text). [c] Ratio of areas (ACAC/intermediate) of the CH_3-proton signals. [d] Calculated using $K_{nc} = 25$ and assuming the intermediate signal is due to the CH_3 proton of IN_1 or IN_2 as indicated. [e] The two CH_3-proton signals are coalesced under these conditions.

ACAC, and the CH_2-proton resonance, which occurs at 3.83 ppm (relative to DSS), is not shown. The signal labeled E is assigned to the CH_3-proton resonance of the enol form of ACAC. Both K and E are broader than B, indicating proton exchange between the keto and enol forms. This exchange is catalyzed by the buffers (and by HA as discussed below). Phosphate has a stronger catalytic effect than does imidazole as evidenced by the coalescence of K and E in the presence of phosphate. The spectrum of the hydroxylamine solution is not illustrated since the hydroxylamine proton resonances are merged with the H_2O signal because of rapid exchange processes. Figure 1B is obtained at higher gain after mixing equal volumes of the above mentioned solution and 0.15 M HA. While the line widths B and E are unaffected, K is broadened and moved upfield slightly. In addition, a new broad signal (labeled I) is observed and is assigned to the CH_3-proton resonance of the intermediate. The fact that K and I are resolved and broad indicates that the exchange rate is slow relative to the chemical shift between them.[9] The resonance due to the CH_2 proton of ACAC or the intermediate is not observed at about 3.8 ppm, presumably because each is broad and weak. Figure 1C illustrates the spectrum under conditions in which the exchange is fast relative to the chemical shift. This spectrum is obtained after mixing 0.2 M ACAC with 0.5 M HA. The two exchange broadened signals labeled C_1 and C_2 are each a coalescence of signals due to the CH_3 proton and CH_2 proton, respectively, for the keto form of ACAC and the intermediate. These signals are weighted averages of the signals due to ACAC and the intermediate. In addition, E is now broader than in previous spectra and has moved upfield, indicating that HA is catalyzing CH_3 exchange between enol and intermediate. The line widths used for calculating the exchange lifetimes were obtained from these two types of spectra, i.e., Figure 1B for slow exchange and Figure 1C for fast exchange. For the slow exchange rates,[9] the reciprocal of the average lifetime τ for the CH_3 protons in a given magnetic environment is obtained directly from the excess broadening, $\Delta = \pi(\Delta\nu_e - \Delta\nu_0)$; $\Delta\nu_e$ and $\Delta\nu_0$ are the widths at half-height for the CH_3 signal during and in the absence of exchange, respectively. When necessary, as in the case for imidizole at pH 7.5, Δ is corrected for the overlap of K with E. For phosphate, this

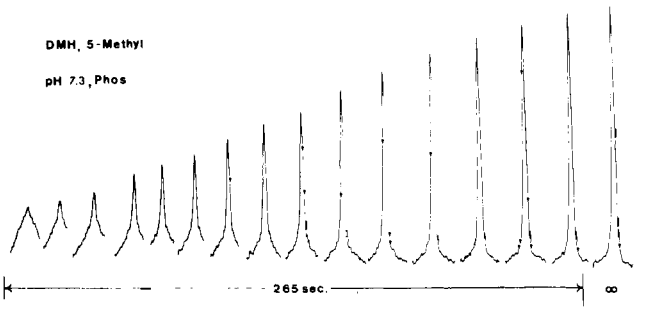


Figure 2. Time dependence for the growth of the 5-CH_3-proton resonance of DMH obtained by repeated scanning after the flow had been stopped. The solution, which was buffered at pH 7.30 using phosphate, was made by mixing 0.20 M ACAC with an equal volume of 0.40 M hydroxylamine.

correction was not necessary since K and E are coalesced, and the width of the coalescence signal was used as $\Delta\nu_0$, which was around 5 Hz and varied somewhat from one run to the next. To account for the variation, $\Delta\nu_0$ was obtained relative to the value for the CH_3-proton resonance of *tert*-butyl alcohol. The exchange rate could not be slowed sufficiently to measure $\Delta\nu_0$ for I. In this case, $\Delta\nu_0$ was assumed to be equal to the value (about 2.0 Hz) obtained for a structurally similar intermediate detected in the reaction of HA with acetaldehyde.[10] Sample values for $\Delta\nu_e$ for ACAC and intermediate are given in Table I for various concentrations and pH values. Each value listed is an average of five or more determinations. In addition, the ratio of the concentration of ACAC to that of the intermediate at equilibrium is related to the ratio of the areas of the two signals given in the slow exchange spectra. These ratios are reported in Table I, also.

For the fast exchange rates, the excess broadening of the CH_3-proton resonance in spectra of the type given in Figure 1C is $\Delta = \pi(\Delta\nu_e - P_K\Delta\nu_{0K} - P_I\Delta\nu_{0I})$. P is the proton fraction, which was calculated (using an iterative method when necessary)[3b] with a value of 25 M^{-1} for K_{nc} (determined spectrophotometrically) and a value of 6.15 for the pK_a (ionic strength 1.6) of hydroxylamine. For calculation of the exchange lifetime τ from Δ, the exchange narrowing equation derived for two

Table II. Kinetic Data for the Addition of NH_2OH to ACAC at 30 °C and an Ionic Strength of 1.6 (KCl)

pH	[Buffer], M (total)	[ACAC],[a] M	[NH_2OH],[a] M (total)	$k_{nc} \times 10^{-3}$, $M^{-1}\,s^{-1}$	k_{nc}/k_{-nc}[b]	k_{d1}[c] $\times 10^3$, s^{-1}	k_2[d] $\times 10^{-2}$, $M^{-1}\,s^{-1}$	k_{d2}[e] $\times 10^3$, s^{-1}
8.00	Phos[f] 0.30	0.10	0.075	2.3	26			
	0.50	0.10	0.25			2.7		
7.50	Phos[f] 0.30	0.10	0.075	2.3	26	3.6		
		0.15	0.075	2.3	26	3.6		
		0.20	0.075	2.1	21	3.5		
		0.25	0.075	2.1	21	4.4		
		0.10	0.10			4.3		
		0.10	0.20	4.5[j]				
		0.10	0.30	3.5[j]		4.2		
		0.10	0.40	2.8[j]				
		0.10	0.50	2.2[j]		4.7		
	0.10	0.15	0.075	2.3	29	2.1		
	0.20	0.15	0.075	2.2	24	2.4		
	0.30	0.15	0.075	2.3	26	3.6		
	0.40	0.15	0.075	2.3	26	4.3		
	0.50	0.15	0.075	2.3	26	4.6		
	Imid[g] 0.20	0.15	0.075	2.2	31			
	0.30	0.15	0.075	2.3	31			
	0.40	0.15	0.075	2.4	30			
	0.50	0.15	0.075	2.5	30			
	0.30	0.25	0.075	2.4	30			
7.30	Phos[f] 0.30	0.10	0.075	2.2	24	4.4		
		0.10	0.10			4.0		
		0.10	0.20	4.7[j]		5.8		
		0.10	0.30	3.8[j]		6.3		
		0.10	0.40	3.0[j]		5.1		
3.0	Form[h] 3.5	0.10	0.25				4.5	
	3.0	0.10	0.25				5.4	
	2.5	0.10	0.25				4.8	
	3.0	0.10	0.15				5.8	
		0.10	0.20				5.5	
		0.10	0.25				5.4	
		0.10	0.30				4.4	
	KHP[i] 0.10[k]	0.10	0.25					2.0
2.5	KHP[i] 0.10[k]	0.10	0.25					5.1
2.0	Form[h] 0.5[k]	0.10	0.20					7.5
		0.10	0.30					7.1
1.5	Form[h] 0.2[k]	0.10	0.25					14.2
	0.3[k]	0.10	0.25					14.2

[a] Initial concentrations after mixing equal volumes of ACAC and HA solutions. [b] k_{nc} is obtained from the ACAC, and k_{-nc} is obtained from the intermediate signal when the two signals are resolved. When these signals are coalesced, k_{nc} is obtained as described in the text using $K_{nc} = 25\ M^{-1}$. These represent averages of several runs, and the standard deviations are about ±15% for k_{nc} and k_{-nc}. [c] Obtained from the rate of growth of DMH since it is identical with the decay rate of the intermediate. [d] Second-order rate = k_2[ACAC][HA] in which [HA] is the concentration of hydroxylamine free base. Values obtained from the decay of ACAC are very close to those obtained from the growth of DMH, and the average of these determinations is reported, the standard deviations are ±15%. [e] Values obtained from the first-order growth of DMI are very close to those obtained from the first-order decay of DMH, and the listed values are averages of these determinations. The standard deviations are about ±8%. [f] Phosphate buffer. [g] Imidazole buffer. [h] Formic acid buffer. [i] Potassium hydrogen phthalate buffer. [j] Obtained form the coalescence signal using $K_{nc} = 25\ M^{-1}$. [k] There is a pH drop on mixing the solutions. However, the pH is constant during the formation of DMI at the values indicated.

sites is used.[9] In this calculation, a value of 65 Hz was used for the chemical shift between K and I, as measured from spectra obtained under conditions of slow exchange such as the one in Figure 1B.

Decay and Growth Rates. The decay of the ACAC and intermediate as well as the growth of the products was studied at various pH and buffer concentrations by measuring the time dependence of the intensity for each signal of the reaction mixture after the flow was stopped. Figure 2 gives an example of this type of measurement for the growth of the 5-CH_3-proton resonance of DMH in a reaction solution consisting of 0.10 M ACAC (after mixing), 0.30 M phosphate, and 0.20 M hydroxylamine at pH 7.30. Since the intermediate can be detected under these conditions, the dehydration of IN_2 to form DMH is the rate-determining step, and k_{d1} can be obtained directly from the first-order decay of IN_2 or the first-order growth of DMH. Values for k_{d1} in the pH range 7.30 to 8.00 are obtained in this manner and are listed in Table II. For each buffer, the pH, which was monitored at 5-s intervals after mixing, decreased by less than 0.1 unit during the reaction.

The rate of formation of DMH from ACAC has also been measured at pH 3.0 using a formic acid buffer. Under these conditions, the signal due to the intermediate is not detected, and the CH_3-proton resonance due to ACAC is not exchange broadened and does not exhibit any change in chemical shift compared with the case when hydroxylamine is not present. Therefore, this addition–cyclization reaction appears to have a pH behavior similar to that found for many reactions involving addition to carbonyl compounds, i.e., lowering the pH causes a change in the rate-limiting step from dehydration to addition.[11] Consequently in this pH range, the disappearance of ACAC and the growth of DMH follow second-order kinetics (first order each in ACAC and hydroxylamine free base), and Table II lists the rate constants. Only data for buffer concentrations of 2.5 M or larger are listed because the pH is not time independent during this step at lower concentrations.

On the other hand, the pH is time independent for the second dehydration step, and k_{d2} values based on the disappearance of DMH or the growth of DMI are listed in Table II. At pH 3.0, this step is slow and was not measured.

Discussion

The calculation of k_{nc} and k_{-nc} from Δ for the resolved broadened lines in Figure 1B does not require the identification of the intermediate, as described above. On the other hand, when the exchange rate is fast enough to coalesce these signals (as in Figure 1C), the equation used for calculating τ involves the proton fractions of the two exchanging species, making the identity of the intermediate necessary. The identification of this transient is based on various information including: (1) a value of 25 M^{-1} for the equilibrium constant K_{nc} (ACAC + N $\rightleftharpoons$ ACACN); (2) the ACAC and NH_2OH concentration effect on the relative areas of the two exchange broadened signals; (3) the ACAC and NH_2OH concentration effect on the relative excess broadening of these two signals; (4) the structure of the product derived from the transient. In determining the value for K_{nc}, IN_1 was considered as a possible structure for ACACN. The extinction coefficient for this compound was assumed to be similar to the one for $(CH_3)_2C(OH)CH_2COCH_3$, which has a value of 25 at 274 nm. This small value has little effect on the value for K_{nc} since the extinction coefficient for ACAC is 1571 at 274 nm. Thus, the accuracy of the value for K_{nc} is independent of our assignment of either IN_1 or IN_2 as the transient species. Based on this value, we conclude that the ratio of signal areas, ACAC-CH_3:transient CH_3, is consistent only if the transient signal is due predominantly to IN_2. The experimental ratio and the values calculated, using K_{nc} and allowing either IN_1 or IN_2 to be the predominant species, are given in Table I for various ratios of ACAC to NH_2OH concentrations. These values were calculated using the reasonable assumption that both CH_3 groups of IN_2 could contribute to the transient resonance whereas only one could contribute in the case of IN_1. An alternate possibility of the addition of two molecules of NH_2OH to ACAC is ruled out for several reasons. First K_{nc} depends on only the first power of the concentration of NH_2OH. Second, even at the highest concentration of NH_2OH, the dioxime makes up only about 5% of the product, the remainder being DMH. Third, when methoxyamine is used as the nucleophile, the monooxime is the main product (about 95%, then slowly forms the dioxime).

The product derived from the decay of IN_2 also supports our conclusion. Since the conversion of DMH to DMI depends on pH and buffer concentration, it has been possible to slow this reaction sufficiently to isolate DMH and characterize it (see Eperimental Section). Finally, it is converted to DMI, which has been isolated and found to have properties identical with those reported earlier (see Experimental Section). Thus, the structure of DMH seems to be confirmed. The fact that the transient decays to DMH and no anti oxime is observed also supports the proposed mechanism since the anti oxime is observed for the reaction of NH_2OH with ethyl acetoacetate, a reaction that generates the isoxazolone via the syn oxime.[12] The conversion of the anti oxime to the isoxazolone is very slow. Consequently in the case of ACAC, if the anti oxime were formed, it should be sufficiently stable to be detected, and the absence of a measurable amount of anti oxime supports the mechanism involving the formation of DMI from IN_2.

The excess line width Δ for the exchange broadened lines (such as Figure 1B) also is consistent with the interpretation given above. Because the K and I resonances are resolved, the exchange rate is slow relative to the chemical shift between these two resonances. Therefore, the average lifetime τ is given by the relation $\Delta = 1/\tau$. For the K resonance, $1/\tau = k_{nc}[NH_2OH]$. For the I resonance, $1/\tau = k_{-nc}$, and k_{nc}/k_{-nc} should have the same value as K_{nc} if our analysis is correct. Values for k_{nc} and k_{nc}/k_{-nc} are listed in Table II for various ACAC: NH_2OH concentration ratios. The total concentration of NH_2OH is listed, i.e., free base plus its conjugate acid. The good agreement between k_{nc}/k_{-nc} and K_{nc} provides additional support for the mechanism proposed above, since k_{nc} and k_{-nc} are obtained without involving P in the calculation.

In the region of fast exchange, τ is equal to $\tau_{nc} + \tau_{-nc}$, and τ_{nc} may be determined since P_K/τ_{nc} is equal to P_I/τ_{-nc}. Values for k_{nc}, which is obtained using the relation $1/\tau_{nc} = k_{nc}[HA]$ are listed in Table II. While k_{nc} is independent of buffer concentration, it decreases as the total hydroxylamine concentration increases. The effect is identical whether phosphate or imidazole is the buffer. Consequently only the phosphate data are tabulated. This concentration dependence might be due to catalysis of an additional exchange process involving the enol. The effect of hydroxylamine on the line width of the enol can be seen in Figures 1B and 1C, which illustrate that the line width of the E signal increases upon increasing the total concentration of HA. We suggest that this line broadening is due to exchange between the enol and the species responsible for the coalescence signal C_1, i.e., ACAC and/or IN_2. This process would be reflected in a decrease in k_{nc} as the HA concentration is increased because it would broaden the coalescence signal, making τ appear larger.[9] In addition, it is likely that the 3- and 5-methyl groups of IN_2 do not have exactly the same chemical shift, which could make a contribution to the line width also. The deviation in k_{nc} due to this contribution would become larger as line C_1 became narrower and, therefore, may account in part for the decrease in k_{nc} at higher HA concentrations. The alternate possibility that a metal ion impurity is responsible has been ruled out because the value for k_{nc} is identical when doubly distilled water is used or when EDTA is added to the solution. At any rate, the exchange process involving the enol makes the rate data in the coalescence region less reliable than those obtained in the region of slow exchange.

It has been possible to measure the rate of approach to equilibrium using a Durrum stopped-flow system and thereby determine k_{nc} and k_{-nc} for comparison with the values determined by the NMR method. This rate was measured at two pH values, 6.50 and 7.50, under conditions that are identical with those used for the NMR measurements except that the concentrations are lower (0.0025 M ACAC, and 0.05 and 0.10 M HA for each pH). Doubling the concentration of HA had no effect on k_{nc}, which has the same value, 1.5×10^3 M^{-1}, at both pH values. This value is in good agreement with those listed in Table II for the NMR measurements in the region of slow exchange. While this agreement illustrates that the kinetics can be studied equally well by either method, the capability of the NMR technique for identification of transients, as discussed above, provides an advantge not available to the optical technique. Furthermore, we have demonstrated that the NMR technique can be used to measure exchange rates that are too fast for the conventional stopped-flow optical technique.[3b,12]

The data in Table II illustrate that k_{nc} is independent of pH and buffer concentration in the pH range 7.30 to 8.00. A similar absence of catalysis in this pH range has been observed for addition reactions involving hydroxylamine and acetone[3b] or ethyl acetoacetate.[12] In these cases, since the exchange process involves only an addition step that is not complicated by cyclization, it was possible to conclude that the rate-determining step for the addition is the formation of the zwitterion of the carbinolamine. For ACAC, the absence of catalysis indicates that the rate-determining step does not involve protonation or deprotonation, also. However, the rate-determining step may occur on the way to forming either IN_1 or IN_2. We would speculate that it occurs in the cyclization step. This conclusion is based on the following arguments. Although

$K_{nc} = K_n K_c$, the determination of the exact values for K_n and K_c is not possible. However, the standard deviation for the area ratios given in Table I permits the estimation of an upper limit for K_n, namely about 5 M^{-1}, i.e, IN_1 could be as much as 16% of the total concentration of intermediate without exceeding the standard deviation. For ethyl acetoacetate (EAA),[12] K_n is 3 M^{-1}, and, therefore, it might not be unreasonable to assume that ACAC and EAA behave similarly in the addition step and have comparable values for k_n and k_{-n}. Then it is possible to estimate k_c and k_{-c} by assuming steady-state conditions for IN_1, and values of 10^3 s^{-1} and 1.3×10^2 s^{-1}, respectively, are obtained, making cyclization slower than addition.

In the pH range 7.30 to 8.00, the rate of dehydration of IN_2 can be measured, and k_{d1} can be obtained directly. The phosphate buffer appears to act as an acid catalyst for this step since k_{d1} increases as the pH decreases while its concentration is fixed. Hydroxylamine and imidazole have little effect on k_{d1}. The second dehydration step to form DMI is very slow in this pH range and has been studied only at lower pH as discussed below.

In the pH range, 1.5 to 3.0, the formation of DMH does not appear to involve dehydration as the rate-determining step, and the disappearance of ACAC follows second-order kinetics, first order each in ACAC and HA free base as discussed above. Values for the second-order rate constant k_2 are listed in Table II only for pH 3.0 because the decay rate for ACAC becomes too fast to measure by repetitive scans at lower pH, indicating that the reaction is acid catalyzed in this pH range. Whether this reaction follows the mechanism given in Scheme I or a different one in which the dehydration of the carbinolamine is faster than cyclization cannot be decided on the basis of the available information. Although the anti oxime is not detected, the latter mechanism cannot be ruled out since it is possible that the anti isomer converts to the syn isomer and subsequently to DMH at rates too fast for our technique. An attempt to clarify this point using the *O*-methyloxime of ACAC was inconclusive because the relative signal intensities for the two isomers are time independent over a interval of about 1 h, indicating either the interconversion is slow or equilibration has been attained. The NMR lines are narrow; however, the interconversion lifetime could be as short as 0.5 s without measurable effect on the line width. At any rate, the mechanism for the addition–cyclization in the pH range 7.3 to 8.0 probably is different from that in the range 3.0 and lower since acid catalysis is important only in the latter range. In contrast for acetone, the formation of the carbinolamine appears to follow the same mechanism at low (1 to 4) and high (7 to 8) pH.[3b]

The conversion of DMH to DMI follows first-order kinetics and is apparently acid catalyzed. During and after the formation of DMI, the pH of the solution is constant, and k_{d2} is listed in Table I.

References and Notes

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Flow Nuclear Magnetic Resonance Study of the Rapid Addition of NH_2OH to Acetone and the Rate-Determining Dehydration of the Carbinolamine

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Abstract: The nucleophilic addition of NH_2OH to acetone in H_2O has been studied using the NMR spectroscopy of flowing liquids. While the liquid is flowing the spectrum in the CH_3-proton region consists of a narrow signal due to the CH_3 protons of a small amount of oxime and a broad signal which is due to the coalescence of two signals, one due to the tetrahedral intermediate and the other due to acetone. The line width of this broad signal can be related to the rate constant for the addition step, and values have been obtained. We find that the value for the rate constant is independent of buffer concentration and pH in the range 8.00 to 7.10, and we conclude that the rate-determining step for the addition is the formation of the intermediate N^+-C-O^-. The rate of growth of the oxime signal has been measured also.

Introduction

The addition of nitrogen nucleophiles to carbonyl compounds has been studied by a number of workers,[1] and the accumulated evidence supports the mechanism

$$RNH_2 + \rangle C{=}O \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} RNH{-}\underset{\text{CA}}{\underset{|}{C}}{-}OH \xrightarrow{k_d} RN{=}C\langle \qquad (1)$$

According to this mechanism, addition of the nucleophile results in the formation of a tetrahedral intermediate that subsequently dehydrates. Recently we have reported the use of the NMR spectroscopy of flowing liquids to detect the tetrahedral intermediate and study its decay kinetics in a reaction involving acetaldehyde and hydroxylamine.[2] In that study it was possible to measure the rate of formation of each of the oxime isomers; however, it was not possible to measure the rates for the preequilibrium step. In this paper, we wish to report the results of a study of the preequilibrium as well as the dehydration step