acid–dichloromethane (3 × 4 ml). The combined filtrates were evaporated in vacuo and the residue was dissolved in water for the chromatography of Leu-Ala-Gly-Val on the long column of the Beckman 120B amino acid analyzer. The color value for Leu-Ala-Gly-Val was found to be 0.71 that of valine. The resin was resuspended in 50% trifluoroacetic acid–dichloromethane (4 ml) and shaking was continued. The combined loss of Leu-Ala-Gly-Val from the resin was determined at 4 h (14.7%), 8 h (21.7%), 16 h (34.4%), 24 h (44.3%), 32 h (52.1%), 40 h (59.3%), 48 h (65.9%), and 64 h (75.8%). Figure 1 shows a plot of the peptide remaining vs. time.

Boc-Leu-Ala-Gly-Val-4-(oxymethyl)phenylacetamidomethyl-resin (100 mg, 53.8 μmol) prepared from resin **3b** was shaken with 50% trifluoroacetic acid–dichloromethane (4 ml) as described above. The combined loss of Leu-Ala-Gly-Val from the resin was monitored at 8 h (0.43%), 24 h (0.96%), 48 h (1.53%), 72 h (1.96%), and 96 h (2.26%); see Figure 1.

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Reaction of Hydroxylamine with Ethyl Acetoacetate. Details of the Addition and Cyclization Steps Studied by Flow Nuclear Magnetic Resonance[1]

Michael Cocivera,* Adan Effio, Holger E. Chen, and Shiv Vaish

Contribution from the Guelph-Waterloo Center for Graduate Work in Chemistry, University of Guelph, Guelph, Ontario, Canada. *Received April 9, 1976*

Abstract: In the pH range 6.5 to 8.5, hydroxylamine adds to the keto carbonyl of ethyl acetoacetate (EAA) to form a carbinolamine intermediate, which subsequently dehydrates to form the syn and anti oxime. The syn isomer is unstable and cyclizes to form 3-methylisoxazol-5-one (MI). The conversion of the anti isomer is much slower and presumably must isomerize before cyclizing. Because the proton NMR spectra of all of these compounds can be resolved, it is possible to measure rate constants for each step. The rapid addition step causes NMR line broadening, which can be measured while flowing the H_2O solution, after mixing, to create steady-state conditions for the transients. The dehydration and cyclization rates are measured after the flow is stopped. The pH and buffer concentration appear to have no effect on the addition step, but do affect the dehydration and cyclization rates. Since the cyclization is an intramolecular reaction that involves the expulsion of ethanol, its rate is compared with hydrolysis of the *O*-methyloxime of EAA in the presence of the oxime of acetone.

Introduction

Although the synthesis and properties of pyrazoles and isoxazoles have been the subjects of many studies,[2] a survey of the literature reveals little concerning the details of the kinetics and mechanism of their formation. Recently,[3] we have reported evidence to indicate that hydroxylamine adds to acetylacetone (ACAC) to give 3,5-dimethyl-5-hydroxylisoxazoline without the intermediacy of the oxime, i.e., cyclization of the carbinolamine intermediate occurs more rapidly than dehydration. In fact, the cyclization is sufficiently rapid to affect the proton nuclear magnetic resonance (NMR) line shapes. In the present paper, we report a study of the reaction of hydroxylamine (HA) with ethyl acetoacetate (EAA) using the NMR of flowing liquids. The acyl carbonyl of EAA is not as active as the keto carbonyl of **ACAC**, and cyclization occurs via the syn oxime to form 3-methylisoxazol-5-one (MI), i.e., dehydration of the carbinolamine (CA) is faster than cyclization. Because of the detailed information available in the NMR spectra during and after flowing, it has been possible

to measure directly the rates for the addition, dehydration, and cyclization steps under various conditions of pH and buffer concentration. This type of reaction may prove to be especially informative in view of the interest in cyclization reactions as models for enzyme catalysis.[4]

Experimental Section

The proton NMR spectra at 100 MHz were measured at 30.0 ± 0.3 °C under static and flowing conditions using a Varian HA-100-15 equipped with a flow system to be described.[5]

With the exception of 3-methylisoxazol-5-one (MI), all chemicals were obtained from suppliers. EAA was distilled and HA hydrochloride was recrystallized. MI was prepared from EAA and hydroxylamine (HA) as described in the literature[6] and had physical properties that conformed to those reported previously.[6b]

The kinetic method involved either line shape analysis of spectra obtained while flowing (for the addition step) or determination of the time dependence of spectra after the flow had been stopped (for the dehydration and cyclization steps). In both cases, the reaction mixture was made by mixing equal volumes of an aqueous EAA and an aqueous HA solution at an ionic strength of 1.06 (KCl).

The equilibrium constant K_n for the addition step, HA + EAA $\rightleftharpoons$ CA, was determined from the uv absorbance decrease measured with a Cary 118 when equal volumes of HA and EAA solutions are mixed under the same conditions as the NMR kinetic measurements. A flow system was used for this measurement. The pH of solutions was measured using 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 immediately after mixing.

Results

Sample spectra obtained under static and flowing conditions are illustrated in Figure 1. The static spectrum is due to 0.20 M EAA at pH 7.00. The chemical shifts are reported relative to DSS, and the assignment is as follows: ethyl group, CH_3 triplet at 1.39 ppm and CH_2 quartet is not shown because it lies very close to the H_2O signal; CH_3COCH_2-, CH_3 singlet at 2.45 ppm and CH_2 singlet at 3.82 ppm. Upon flowing at 20 ml/min after mixing with an equal volume of 0.40 M HA (total concentration), the CH_3 resonance of the ethyl group is unaffected whereas the CH_3- and CH_2-proton resonances of CH_3COCH_2- are replaced, respectively, by broader signals (labeled C in Figure 1) occurring at higher field (2.22 and 3.58 ppm, respectively).

The following evidence supports the view that the signal at 2.22 ppm is a coalescence to two CH_3 singlet resonances, one due to the CH_3COCH_2- group of EAA and a corresponding one due to the carbinolamine resulting from addition of HA to the keto-carbonyl of EAA. A similar conclusion applies to the CH_2 signal at 3.58 ppm also, but will not be discussed. First, for the concentrations employed, both EAA and the carbinolamine should have detectable NMR signals while flowing since K_n is 3.0 M^{-1}. Second, the forward and reverse rates for the equilibration are fast relative to the stopped-flow time scale (using a Durrum stopped-flow system) and, therefore, are sufficiently fast to coalesce the NMR signals. Third, the position of the broadened signal depends on the concentration of HA relative to EAA, moving upfield as this concentration ratio increases. Using this concentration dependence and K_n, the chemical shift between the CH_3-proton resonances of EAA and the carbinolamine was determined to be 70.1 Hz, which is close to values obtained for structurally similar carbinolamine intermediates.[3,8] Finally, the concentration dependence of the line width conforms well with this interpretation since consistent rate constants are obtained, as discussed below.

The forward k_n and reverse k_{-n} rate constants for the equilibrium were obtained from the excess line width Δ (in rad/s) using the equation for fast exchange involving two lines.[7] This equation provides the exchange rate $1/\tau$, which is equal to $k_n[HA] + k_{-n}$, in which [HA] is the equilibrium concentration of HA free base calculated using either K_n or K_n with K_a, depending upon the pH employed. Values of k_n, which are averages of at least four runs, are listed in Table I along with standard deviations. Values for k_{-n} are not listed since $K_n = k_n/k_{-n}$.

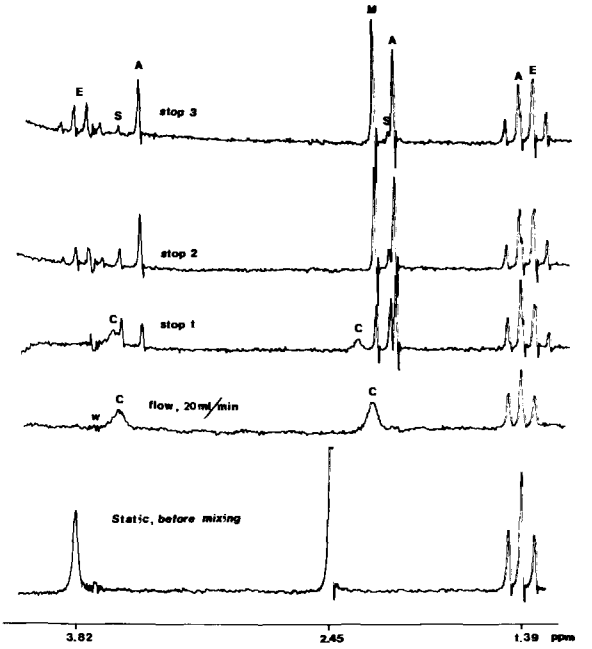


Figure 1. Slow-passage proton NMR spectra obtained without spinning at 100 MHz for aqueous solutions at 30 °C and a pH of 7.00. The labels are described in the text. The static, before mixing spectrum is due to 0.20 M EAA. The flow spectrum is obtained after mixing the EAA solution with 0.40 M HA (total). The three stop spectra are measured approximately 0, 150, and 300 s after stopping the flow with stop 3 being the last. The wiggles labeled W are an instrumental artifact.

For each run, the value for Δ (in rad/s) is obtained from the line width of the coalescence signal by subtracting out the weighted average of the line widths in the absence of exchange due to signals of each of the exchanging CH_3 groups. For the CH_3 group of EAA, this line width is obtained in the absence of HA. For the CH_3 group of CA, this line width could not be measured directly because the exchange rate could not be slowed sufficiently. Consequently, it was assumed to be the same as that for the CH_3 signal of the structurally similar carbinolamine derived from acetaldehyde.[8] To account for any changes due to field homogeneity or flow rate from one run to the next, the line widths are related to the one for the CH_3 signal of methanol, which was present for all kinetic runs.

The main aspects of the time dependence of the spectrum are illustrated in Figure 1 by the spectra labeled stop 1 through 3. For stop 1 the sweep was started immediately after stopping the flow, and the other two were obtained consecutively. Since each sweep takes about 150 s, the sweeps for stop 2 and stop 3 were started approximately 150 and 300 s after the flow was stopped. These spectra were recorded only to illustrate qualitatively the time dependence of the various signals and were not used for quantitative rate measurements. For quantitative determinations, the time dependence of each signal was determined separately by repetitive scanning as described previously.[5,8]

The three spectra obtained after stopping the flow illustrate that the broad signals (labeled C) decay and shift with time and that the new signals are themselves time dependent. The assignment is as follows: ethyl alcohol (labeled E), CH_3 triplet at 1.30 ppm and CH_2 quartet at 3.76 ppm; syn oxime of EAA (labeled S), CH_3 at 2.10 ppm and CH_2 at 3.56 ppm; anti oxime of EAA (labeled A), CH_3 at 2.07 ppm and CH_2 at 3.44 ppm

Table I. Kinetic Data for the Addition of Hydroxylamine to Ethyl Acetoacetate in H_2O at 30 °C[a]

pH[b]	[Buffer],[c] M	[NH_2OH],[d] M	k_{ds}/k_{da}[e]	$(k_{ds} + k_{da}) \times 10^3$, s^{-1} CA[f]	$(k_{ds} + k_{da}) \times 10^3$, s^{-1} Anti[g]	$k_c \times 10^3$, s^{-1} Syn[h]	$k_c \times 10^3$, s^{-1} MI[i]	$k_n \times 10^{-3}$, $M^{-1}\ s^{-1}$
6.50	Phos 0.10	0.20	1.06	22 ± 1.2	20 ± 1.5	1.9 ± 0.2	2.1 ± 0.2	9.0 ± 0.2
	0.20		1.12	26 ± 2.5	23 + 2	2.4 ± 0.1	2.6 ± 0.1	7.6 ± 0.3
	0.30		1.22	34 ± 3	31 ± 3	3.6 ± 0.3	3.8 ± 0.1	8.6 ± 0.3
	Im 0.50		0.98	27	17		1.5	12 ± 2
	0.70		1.0	21	16		1.7	16 ± 4
7.00	Phos 0.10	0.20	1.16	11 ± 1	9 ± 1	2.7 ± 0.1	3.4 ± 0.3	8.4 ± 0.2
	0.20		1.27	15 ± 1	14 ± 1	3.4 ± 0.1	3.7 ± 0.2	8.1 ± 0.1
	0.30		1.34	18 ± 1	16 ± 1.5	4.8 ± 0.2	5.2 ± 0.3	8.3 ± 0.3
	0.10	0.40						7.3 ± 0.6
	Im 0.30	0.20	0.91	11	7	3.0	3.9	10.5 ± 0.9
	0.40		0.90	15	11	3.2	3.8	10.2 ± 0.9
	0.50		1.02	12	9		3.4	10.6 ± 1.7
			1.03	10	8		6.0	10.6 ± 1.7
	0.60		1.02	15	13	3.8	4.6	10.7 ± 3.4
	0.30	0.10	1.04	8	7		6.3	9.1
7.50	Im 0.30	0.20	0.98	6	5.0		5[j]	9.2 ± 0.9
	0.50		1.13	4	4.9		5[j]	9.2 ± 1.2
	1.00		1.12	7.7	7.4		5[j]	9.4 ± 1.4
	Phos 0.30	0.20	1.35	8	7.7		6.0[j]	7.0
8.50	Bor 0.40	0.20	1.44		6.9		6.3[j]	

[a] Ionic strength is 1.06 (KCl), and EAA concentration is 0.1 M. [b] Initial pH, see text. [c] Total concentration after mixing, includes acid and base forms. [d] Sum total of acid and base forms after mixing and before reaction of NH_2OH. [e] Obtained using $(I_s + I_{MI})/I_a$ averaged over the period in which the syn isomer disappears. [f] Determined from the decay of the carbinolamine intermediate. [g] Determined from the growth of the anti oxime. [h] Determined from the decay of the syn oxime. [i] Determined from the growth of 3-methylisoxazol-5-one (MI). [j] Determined from the first-order growth of MI when the concentration of the syn isomer is approximately constant and, therefore, is ascribed to $k_{ds} + k_{da}$ rather than k_c (see text).

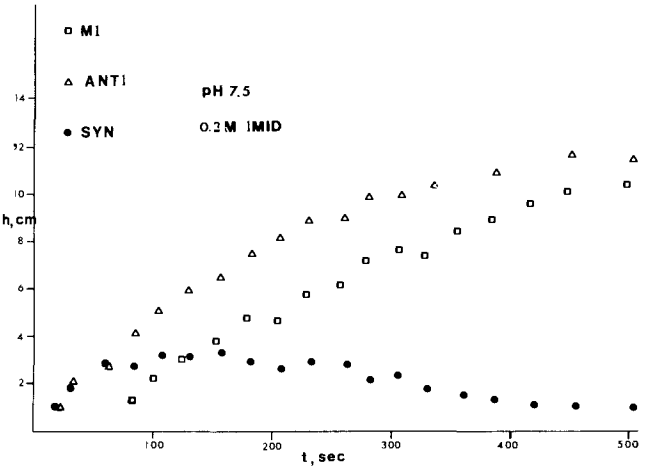
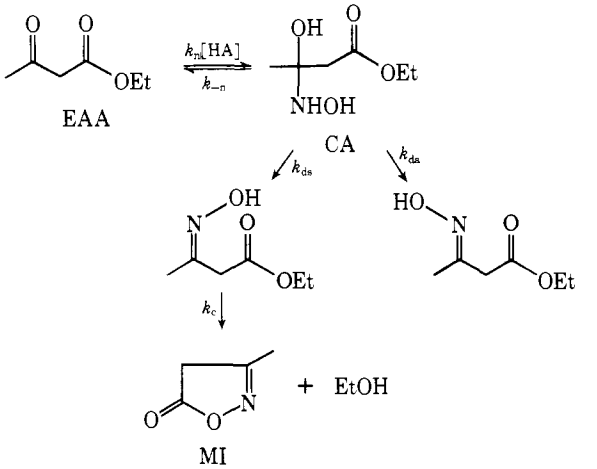


Figure 2. Time dependence of the intensities of the CH_3-proton resonances due to MI and the syn and anti oximes for a solution at 30 °C and an initial pH of 7.50 buffered by 0.50 M imidazole (total); ionic strength is 1.06 (KCl). Concentrations for EAA and HA are the same as for Figure 1.

(the triplet labeled A at 1.39 ppm is due to the CH_3 protons of the ethyl group of both isomers); MI (labeled M), CH_3 at 2.19 ppm. The resonance for the ring protons of MI is pH dependent and can be observed only at pH values lower than those used for the kinetic studies, i.e., around pH 2. Furthermore, it is observed at 3.34 ppm when CCl_4 is the solvent. As illustrated, the concentrations of MI, ethanol, and the anti oxime of EAA increase monotonically whereas the syn oxime increases and then decays to near zero during this time period. The anti oxime signals decay over a period of hours with a concomitant growth in the amount of MI. By repeated scanning of the spectrum after the flow is stopped, the time dependence of the CH_3-signal intensity for the various compounds can be obtained, and an example is illustrated in Figure 2 for MI and the syn and anti oximes at pH 7.50. Similar behavior occurs for the other pH values and buffer concentrations employed and is consistent with a mechanism involving cyclization via the syn oxime, as discussed below.

Because of the detail in the NMR spectra obtained during and after flow, it is possible to dissect the reaction of HA with EAA into several steps, each of which can be studied directly, and we propose the mechanism given in Scheme I to account

Scheme I



for our results.[9] The more rapid initial growth of MI is attributed to cyclization of the syn oxime. The slower conversion of the anti oxime to MI, which probably involves isomerization to the syn oxime, is not illustrated in the scheme. Conversion of MI to the NH form, although not indicated, may also be occurring.

The relatively slow conversion of the anti oxime to MI allows the growth rate of the anti oxime to follow first-order kinetics from which the value for $k_{da} + k_{ds}$ can be determined. Since the conversion of the syn oxime to MI is complete before the

anti oxime makes any measurable contribution, k_{ds}/k_{da} is given by $(I_s + I_{MI})/I_a$ in which I indicates CH_3 signal intensity at any time up to and including the complete disappearance of the syn isomer. This ratio is constant throughout this time period, and values along with $k_{da} + k_{ds}$ are listed in Table I. Equating k_{ds}/k_{da} to $(I_s + I_{MI})/I_a$ is valid only if MI is not formed directly from CA. The arguments in favor of this conclusion are given below.

The value for k_c is obtained on one of two ways depending on the relative rates of dehydration and cyclization. At lower pH, the dehydration rate is sufficiently fast so that an appreciable amount of the syn isomer remains after the intermediate carbinolamine (CA) has disappeared, at which point the decay of the syn isomer and the growth of MI both follow first-order kinetics. At pH 7.50 or higher, the dehydration and cyclization rates become comparable, and the amount of the syn oxime remains small and relatively constant (see Figure 2) during an appreciable period of the reaction, allowing the application of the steady-state approximation. Under these conditions, the mechanism in Scheme I requires that the growth rate of MI be the same as the decay rate of CA, as appears to be the case (see Table I and compare the values in the $(k_{ds} + k_{da})$ column with those in the k_c column). Thus, at pH 7.5 and 8.5, the first-order growth rate of MI gives $k_{ds} + k_{da}$ rather than k_c. However, because steady-state conditions appear to obtain, k_c can be estimated using k_{ds}[CA]/[Syn], which can give only an approximate range of (3 to 5) $\times 10^{-3}$ s^{-1} for the two pH values since [CA] varies appreciably in the time period over which steady-state conditions are assumed to apply. Obviously the values at pH 7.00 and 6.50 are more accurate.

Discussion

No attempt has been made to determine integrated expressions to describe the time dependence of the signals due to MI and the syn isomer because the concentration of HA is time dependent, also, making the differential equations nonlinear. Furthermore, the accuracy of each rate constant is limited by the monotonic decrease in pH during the reaction. For the dehydration step, the total decrease is 0.7 for boric acid and between 0.2 and 0.3 for phosphate and imidazole buffers. For the cyclization step, the total decrease is 0.7 for boric acid and between 0.4 and 0.5 for the other buffers. Consequently, while the initial pH is listed in Table I, the values for k_{ds}, k_{da} and k_c are necessarily averages over the above mentioned pH drops. An indication of the extent to which these values may vary in that range may be obtained by comparison of the data when the initial pH increases from 6.50 to 7.00; $k_{ds} + k_{da}$ decreases by a factor of 2, and k_c increases by 50% for the phosphate and imidazole buffers. The change is smaller for the pH change 7.00 to 7.50. Thus the error in these rate constants may be as large as 50% whereas k_n is more accurate since it is obtained at the initial pH. Nevertheless, the accuracy is sufficient to make comparisons and note trends.

As indicated in Table I, two columns of $k_{ds} + k_{da}$ are given, one for values calculated from the rate of decay of CA and the other for values calculated from the rate of growth of the anti oxime. Comparison of the two columns gives some idea of the reliability of the measurements since the two columns should be identical. Likewise, k_c is calculated from the rate of decay of the syn oxime or the growth of MI, and the two columns should be identical. The relatively good agreement in both cases is consistent with the mechanism given in Scheme I. For both the dehydration and cyclization steps, the phosphate buffer appears to catalyze the rate whereas imidazole appears to have a very small catalytic effect. However, in view of the pH change during reaction, no attempt was made to calculate the magnitude of the phosphate catalytic effect from these data. The most that can be said is that the decreasing trend in $k_{ds} + k_{da}$ and the increasing trend in k_c as the pH increases could be consistent with acid catalysis and base catalysis, respectively, by the phosphate buffer. The base catalysis of the cyclization step would indicate that deprotonation of the hydroxyl group of the oxime may be involved in the rate-determining step. This result is in contrast with those for the rapid cyclization occurring in the reaction of HA with ACAC,[3] in which no acid or base catalysis was observed in the pH range 7.3 to 8.0. In this case, however, the cyclization occurs via the carbinolamine rather than the oxime.

As mentioned above, it seems likely that MI comes solely from the syn isomer at any time up to and including the complete disappearance of this isomer, and k_{ds}/k_{da} is set equal to $(I_s + I_{MI})/I_a$. The anti isomer makes no contribution to MI during this time period. The fact that k_{ds}/k_{da} is 1.0 in the presence of imidazole but not in the presence of the phosphate or boric acid buffer can be explained in terms of a buffer effect on the relative rates of formation of the two isomers, and there is no need to postulate the formation of MI directly from CA as an additional step in the mechanism. The evidence for this conclusion is as follows. First, a buffer affect on the ratio of syn to anti oximes has been observed previously for acetaldehyde,[8] and the evidence indicates that the boric acid buffer adds to the carbinolamine. Second, the coalescence signal is very broad in the presence of boric acid, much broader than expected for the exchange between EAA and CA, indicating the occurrence of an additional exchange process that probably involves CA and a boric acid adduct of CA. The k_n values obtained for phosphate may be evidence for its addition to CA, also, as discussed below. It seems reasonable to expect that these adducts could decompose to the syn and anti isomers at different rates as observed for acetaldehyde. The ratio of about 1 obtained for imidazole appears to be independent of its concentration (unlike the phosphate buffer), and this ratio probably is the limiting value in the absence of buffer effect.

As mentioned above, the addition rate constant k_n for the phosphate buffer could possibly be used as evidence for a phosphate–CA adduct. At each pH, k_n for imidazole is larger than that for phosphate, indicating that the line width of the coalescence signal is somewhat larger in the presence of phosphate than in the presence of imidazole, for which an adduct does not appear to occur. Consequently the slight additional broadening in the presence of phosphate may be due to an additional exchange process involving CA and an adduct as in the case of boric acid.

Accepting this conclusion, the k_n values reported for imidazole can be considered to be more reliable, although the values for phosphate differ from them by only about 20%, which is almost within experimental error. At each pH, k_n is independent of imidazole concentration since the values are within experimental error. In addition, the decreasing trend in the values for imidazole as pH increases is not outside of experimental error. Thus, k_n is independent of imidazole buffer concentration and appears to be independent of pH in this pH range as has been found for the addition of HA to acetone.[5a] By arguments identical with those presented previously,[5a] we conclude that the rate-determining step in the preequilibrium is the addition of HA to EAA to form the zwitterion $CA^{\pm}$.

For a number of years, intramolecular reactions have been studied as models for reactions involving the same groups in enzyme–substrate complexes.[4] Many of these studies were concerned with intramolecular catalysis of hydrolysis of esters, most of which contained aromatic groups for various reasons. In all these cases, a determining factor has been the synthesis of reasonably stable compounds containing both the ester and catalytic group. Cyclization involving β-keto esters (and 1,3-diketo compounds) do not suffer this limitation.

A comparison of the intramolecular reaction with the intermolecular one is possible only if the electronic factors are identical. Although this is not strictly achievable in the case

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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References and Notes

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

Michael Cocivera* and Kyu W. Woo

Contribution from the Guelph-Waterloo Center for Graduate Work in Chemistry, University of Guelph, Guelph, Ontario, Canada. *Received March 3, 1976*

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