$K_{nc} = K_nK_c$, 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.

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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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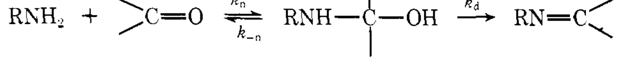
Received March 16, 1976

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



$$RNH_2 + {>}C{=}O \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} RNH{-}\overset{|}{\underset{|}{C}}{-}OH \xrightarrow{k_d} RN{=}C{<}$$

CA

(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

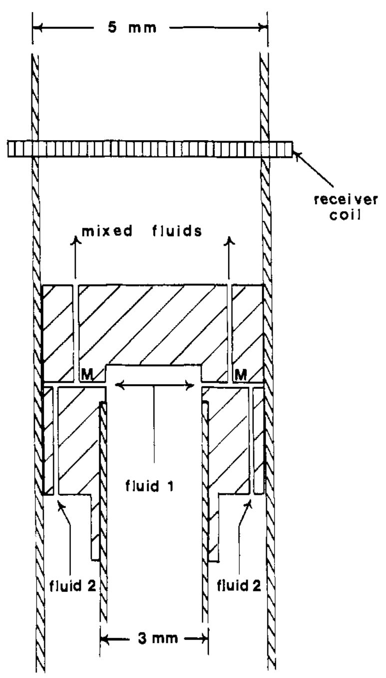


Figure 1. Half-section of the mixing chamber made of Teflon, while only two mixing sites (labeled M) are illustrated, the chamber contains six. The chamber is held in position by a dimple (not illustrated) on the inner (3 mm) tube.

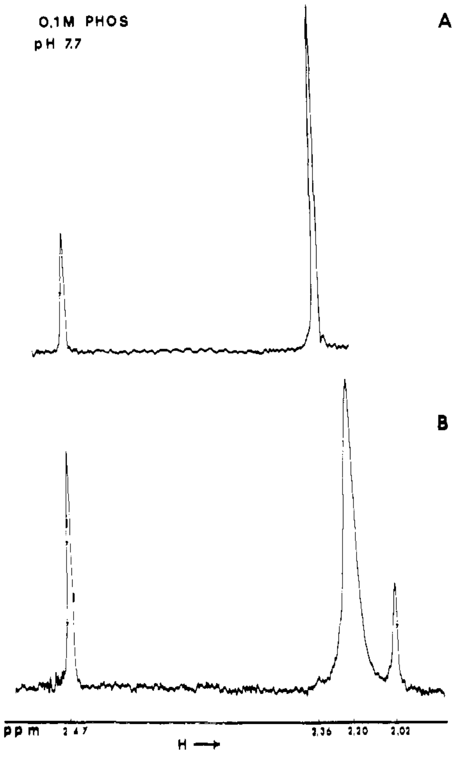
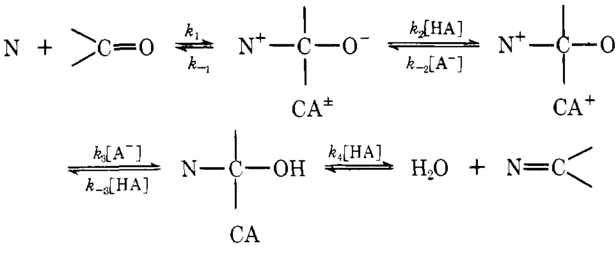


Figure 2. Proton nuclear magnetic resonance spectra at 100 MHz for H_2O solutions at pH 7.70, 30 °C, and an ionic strength of 1.90 (KCl). (A) Acetone solution before mixing. Solution contains 0.1 M acetone plus methanol and 0.10 phosphate buffer. (B) Spectrum of the flowing liquid (20 ml/min) after mixing the acetone solution with the hydroxylamine solution. The solution contains 0.10 M phosphate buffer and initial concentrations of 0.1 and 0.2 M for acetone and hydroxylamine, respectively. The gain for this spectrum is larger than that for spectrum A.

for the reaction of hydroxylamine (HA) with acetone (AC). This system was chosen because it has been studied previously[3] and comparisons could be made.

Because the flow maintains steady-state concentrations of the transients, time-independent slow-passage spectra can be obtained, and rate constants for the forward and reverse reactions of the equilibrium can be measured using NMR line shape analysis. The rate data are consistent with the following reaction, which is similar to one discussed by Jencks:[4]



$$\mathrm{N} + {>}\mathrm{C{=}O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\mathrm{CA}^{\pm}}{\mathrm{N^+{-}C{-}O^-}} \underset{k_{-2}[\mathrm{A^-}]}{\overset{k_2[\mathrm{HA}]}{\rightleftharpoons}} \underset{\mathrm{CA}^{+}}{\mathrm{N^+{-}C{-}OH}} \underset{k_{-3}[\mathrm{HA}]}{\overset{k_3[\mathrm{A^-}]}{\rightleftharpoons}} \underset{\mathrm{CA}}{\mathrm{N{-}C{-}OH}} \overset{k_4[\mathrm{HA}]}{\rightleftharpoons} \mathrm{H_2O} + \mathrm{N{=}C}{<} \quad (2)$$

In this equation, N, >C═O, and >C═N represent NH_2OH free base, acetone, and the oxime, respectively, and $CA^{\pm}$, CA^{+}, and CA represent three possible intermediates of which only CA could be detected by our technique. However, because it was possible to measure the rate constants for the forward and reverse steps of the overall equilibrium given in eq 1, information concerning the other equilibria can be obtained from the lack of buffer catalysis. Although values for these rate constants have been reported earlier,[3a] they were not measured directly but were obtained by a fit of the pH dependence (in the absence of buffer) of the rate constant for oxime formation to a mechanism given in eq 1. This fit was possible only below pH 4, a region in which the dehydration step is not rate determining and steady-state conditions obtain for CA. This approach seems useful and has been applied in a number of more recent studies. In contrast, our technique permits a direct determination of these rate constants under conditions in which the curve-fitting approach gives no information about them, i.e., when the dehydration step is rate determining and steady-state conditions do not obtain for CA unless flowing is used. Furthermore, because they can be measured in the presence of buffer, the buffer effect as well as the pH effect can be ascertained. Thus, data obtained by our technique complement the data obtained by the curve-fitting approach. In this paper, we report the results of measurements at a number of pH values and concentrations for NH_2OH and for phosphate and imidazole buffers.

Experimental Section

Flow NMR measurements were made using a suitably modified Varian HA 100-15 spectrometer of which a detailed description has been presented earlier.[5] Equal volumes of two solutions (each containing a reactant) are mixed in a high-pressure mixing chamber and either flowed continuously through the measuring coils of the probe to obtain time-independent slow-passage spectra, or flowed and stopped to obtain time-dependent spectra. The design of the mixing system is illustrated in Figure 1 since it differs from the one described previously.[5] The drawing in Figure 1 represents a half-section of a mixing chamber designed to fit inside a 5-mm NMR tube. As indicated, both fluids are delivered to the Teflon mixing chamber via the 5-mm tube and a concentric 3-mm tube. Because the mixing points (labeled M in Figure 1) force the two liquids to collide head-on in very small bore holes, the mixing is very efficient. This chamber has six mixing points. The compactness of this mixing system allows the receiver coil to be placed very close to the site of mixing.

Chemicals were from commercial sources and were purified to make spectroscopic properties consistent with those previously reported. All solutions for rate measurements were prepared at 30.1 °C and an ionic strength of 1.9 (KCl). The pH 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. The pK_a of each buffer at an ionic strength of 1.9 is denoted as pK_a' and was measured by potentiometric titration.

The equilibrium constant K_n for the overall equilibrium of eq 1 was measured using uv and flowing liquids with the mixing system discussed above.

Rate constants for the forward k_n and reverse k_{-n} steps of this overall equilibrium were determined from the slow passage spectra obtained after mixing while flowing. Under these conditions, steady-state concentrations are maintained for AC, HA, and CA, and the equilibrium is established. This equilibrium is sufficiently rapid on the NMR time scale to coalesce the CH_3 signals due to AC and CA (Figure 2B), indicating that $\delta\tau < 1$, and τ, the average exchange lifetime, may be calculated according to the equation[6] $\Delta = P_1P_2\delta^2\tau$. In this equation, P_1 and P_2 are the fractions of the exchanging protons calculated from the equilibrium concentrations of AC and CA, δ is the chemical shift between them, and $\Delta = (1/T_2)_c - P_1/(T_2)_1 - P_2/(T_2)_2$ in which $1/T_2$ is the half-width at half-height (in rad/s), the subscripts 1 and 2 denote the AC and CA signals in the absence of exchange, and c denotes the coalesced signal. k_n is obtained from τ using the relations, $K_n = k_n/k_{-n}$ and $1/\tau = k_n[N] + k_{-n}$. Each value of k_n reported in Table I is an average of at least five runs.

For these calculations at high pH, the equilibrium concentrations of AC, HA, and CA were calculated using K_n. At lower pH in which NH_2OH is appreciably protonated, these concentrations were obtained from K_n and K_a by an iterative method using a program written for a Hewlett-Packard HP-65 calculator. Because the exchange rate could not be slowed sufficiently to resolve the two signals, the value for δ (397.5 rad/s) was determined from the NH_2OH concentration dependence of the chemical shift of the coalescence signal using K_n. For AC, $1/T_2$ was measured in the absence of NH_2OH. For CA, the exchange rate could not be slowed sufficiently to obtain $1/T_2$ directly. However, it is reasonable to expect a value similar to that obtained for the CH_3-proton resonance of the structurally similar carbinolamine obtained from the addition of HA to acetaldehyde.[2] To adjust for any changes in magnetic field homogeneity and flow rate from one run to the next, $1/T_2$ in the absence of exchange was obtained relative to the value for the CH_3-proton resonance of methanol, an internal reference in all runs.

The rate constant for the dehydration step k_d was obtained from the time dependence of the spectrum after the flow had been stopped. Figure 3 illustrates an example that was obtained by repetitive sweeping of the product oxime CH_3 signal, which follows a first-order rate of growth. The value for k_d can be obtained from this rate or the first-order rate of decay of CA since they are identical. For the latter rate, the concentration of CA at any time was calculated as described above, taking the concentration of oxime into account. Each value of k_d reported in Table I as well as all others is an average of at least four runs.

Results and Discussion

Samples of the rate constants at two pH values and various buffer and NH_2OH concentrations are listed along with standard deviations in Table I for solutions containing 0.10 M AC. The dependence of k_d on the total and buffer concentration indicates that these species catalyze the dehydration step. Plots of k_d vs. [catalyst] have been fit by linear regression, and the slopes and $k_4{}^0$ are given in Table II for the data in Table I as well as data for all of the other runs. In all, the correlation is better than 0.98. Each fit involves at least four concentrations in the range indicated. Assuming a linear relation between the slopes and the acid fraction of the catalyst, the general acid $k_4{}^{GA}$ and general base $k_4{}^{GB}$ rate constants were obtained by extrapolation. Values are not listed for NH_2OH since it does not appear to catalyze the dehydration step (see Table I). The values for the intercepts $k_4{}^0$ can be compared with only two values obtained under somewhat different conditions at pH 7.7: 0.03 s^{-1} obtained at 25 °C by Jencks[3a] and 0.09 s^{-1} obtained by Hine[3c] at 35 °C. The reasonably good agreement with our value of 0.08 s^{-1} indicates that the flow NMR technique can be reliable as a tool for kinetic studies.

Listed in Table I are some equilibrium concentrations of CA at a flow rate of 20 ml/min. Even at this flow rate, a small amount of oxime has been formed as can be seen in Figure 2B, and the listed values have been adjusted for this amount of dehydration. The initial concentration of AC is always 0.10 M. Samples of the width at half-height (in Hz) of the coalescence signal are also listed. These values are converted to Δ and used to calculate k_n as described above. Since K_n is 1.0 M^{-1}, which is identical with the value determined previously,[3a] k_{-n} has the same magnitude as k_n and, therefore, is not tabulated.

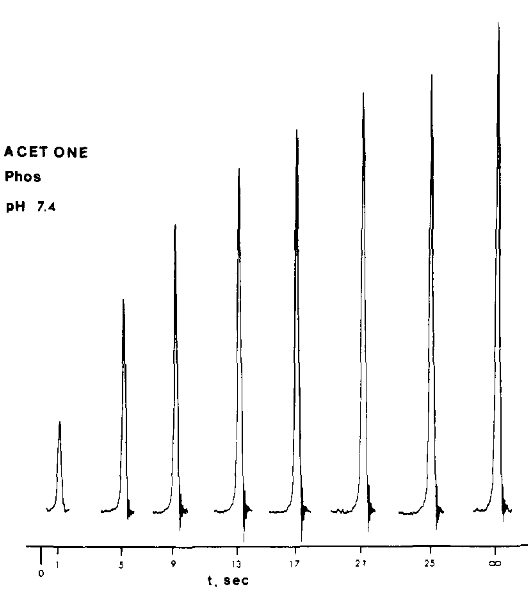


Figure 3. Time dependence of the CH_3-proton resonance of oxime after mixing and stopping flow for a solution at pH 7.40 buffered by 0.40 M phosphate containing 0.10 M acetone and 0.20 M hydroxylamine initially.

The calculation of k_n is valid only if we are correct in our assignment of the broad signal as a coalescence of the CH_3-proton signals due to AC to CA. The supporting evidence for this conclusion is as follows. First, the line width of the signal is larger than that for the methanol (and the acetone) signal, which gives a measure of the field inhomogeneity. Second the position of this signal (relative to the methanol signal) and its line width (see Table I) depend on the concentration of NH_2OH free base. Increasing the concentration causes an upfield shift. Third, the value of 397.5 rad/s determined for δ conforms very well to the chemical shift measured for the CH_3-proton resonance due to a structurally similar tetrahedral intermediate generated by the addition of NH_2OH to acetaldehyde.[2]

The values listed in Table I illustrate that k_n is independent of the concentration of the buffers as well as the total concentration of hydroxylamine. This statement also applies to pH 7.70 and 7.10, for which k_n is not listed. Thus, in the pH range 8.00 to 7.10, the addition of NH_2OH to acetone is not general acid or base catalyzed. Furthermore, because k_n is independent of pH in this range, the addition step is not catalyzed by hydronium or hydroxide ion. This pH independence can be seen in Table II, which lists k_n values that are averages obtained from four to 12 concentrations in the range indicated. This pH independence of k_n and k_{-n} appears to hold over a wider pH range since our value agrees well with a value of 1×10^3 M^{-1} s^{-1} obtained previously[3a] by fitting the pH dependence (in the range 1 to 4 at 25 °C) of the rate constant for oxime formation to the mechanism given by eq 1.

These results permit an elaboration of the details of the addition step according to the mechanism give by eq 2. As can be seen, the only step in the sequence on the way to the formation of CA that is independent of acid and base catalysis is the addition step, i.e., the formation of $CA^{\pm}$. Thus, our results are consistent with a rate-determining formation of $CA^{\pm}$ in the pH range 8.00 to 7.10. 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[7] 10^7 and 10^9 M^{-1} s^{-1}.

Table I. Sample Rate Constants for Addition k_n and Dehydration k_d Steps at 30 °C and $\mu = 1.9$ (KCl)

pH	[Buffer] (total), M	$[NH_2OH]$ [a] (total), M	CA,[b] M	$\Delta\nu$,[c] HZ	$k_n \times 10^{-3}$, $M^{-1}\ s^{-1}$	$k_d \times 10^2$,[d] s^{-1}
8.00	Phos					
	0.20	0.20	0.015	6.0	1.4 ± 0.1	9.1 ± 0.3
		0.30	0.021	6.1	1.65 ± 0.07	6.8 ± 0.1
		0.40	0.026	7.3	1.4 ± 0.1	5.9 ± 0.4
		0.50	0.030	7.1	1.49 ± 0.09	5.8 ± 0.4
7.40	Phos					
	0.20	0.20	0.012	4.8	2.0 ± 0.2	26.8 ± 0.4
		0.30	0.016	5.4	2.0 ± 0.2	22 ± 2
		0.40	0.020	5.8	1.9 ± 0.1	22 ± 2
		0.50	0.023	6.0	1.9 ± 0.1	20.0 ± 0.7
	0.30	0.20	0.012	4.7	2.1 ± 0.1	30.0 ± 0.7
	0.40	0.20	0.012	4.9	1.9 ± 0.3	33.4 ± 1.4
	Imid					
	0.10	0.20	0.014	4.3	2.5 ± 0.25	13.3 ± 0.7
	0.20		0.014	4.1	2.7 ± 0.2	15.8 ± 0.4
	0.30		0.013	4.1	2.7 ± 0.2	17 ± 1
	0.40		0.013	4.7	2.1 ± 0.25	19 ± 1

[a] Initial concentration after mixing with an equal volume of 0.10 M acetone solution. [b] Equilibrium concentration while flowing at 20 ml/min. [c] Sample width at half-height of the coalescence signal while flowing. Line width in the absence of exchange varies but is around 2.0 Hz. [d] Calculated using $k_d = k(K_n[NH_2OH] + 1)/K_n[NH_2OH]$ in which k is the pseudo-first-order rate constant obtained from the time dependence for CA or from the time dependence for the oxime NMR signal. Since $[NH_2OH]$ decreases during the dehydration step, an average value is used.

Table II. Kinetic Parameters Including Catalysis for Reaction of NH_2OH with Acetone

Catalyst	pK_a'	pH	Concn range (total), M	Fraction as acid	Slope,[a] $M^{-1}\ s^{-1}$	k_4^0, s^{-1}	k_4^{GA}, $M^{-1}\ s^{-1}$	k_4^{GB}, $M^{-1}\ s^{-1}$	$k_n \times 10^{-3}$, $M^{-1}\ s^{-1}$
Phosphate	6.37	8.00	0.10–0.40[b]	0.023	0.215	0.04			1.6 ± 0.2
		7.70	0.10–0.40[b]	0.045	0.234	0.08	3.7	0.1	2.1 ± 0.1
		7.40	0.10–0.40[b]	0.085	0.428	0.17			2.1 ± 0.2
Imidazole	7.24	7.40	0.10–0.40[b]	0.41	0.196	0.12		0.36	2.5 ± 0.3
		7.10	0.10–0.40[b]	0.58	0.129	0.23			2.1 ± 0.1
NH_2OH	6.15	8.00	0.20–0.50	0.014					1.5 ± 0.1
		7.70	0.20–0.50	0.0274					1.9 ± 0.2
		7.40	0.20–0.50	0.053					2.1 ± 0.2

[a] Obtained by least-squares fit of k_d vs. the total concentration of catalyst. [b] Solution contains 0.20 M NH_2OH initially.

The analogous reaction for phenol–phenoxide is equally as fast.[8] Since the concentration of buffer and hydroxylamine is always greater than 0.2 M while flowing, it seems safe to conclude that the proton-transfer steps involving $CA^{\pm}$ and CA^+ are very rapid.

A proton switch mechanism has been suggested as a possibility for the conversion of $CA^{\pm}$ to CA for the addition of NH_2OH to *p*-chlorobenzaldehyde under conditions in which no buffer is present.[9] Although this mechanism requires no acid–base catalysis and, therefore, could apply to the acetone reaction, the mechanism given in eq 2 should be at least competitive with this switch mechanism in view of the significant concentration of buffer and NH_2OH present in our solutions and the large rate constants for the proton transfer steps. Consequently, the conclusion made above still seems acceptable. The absence of buffer effect also precludes a preassociation mechanism.[4]

In summary, the flow NMR technique has several advantages for studying nucleophilic addition reactions. First, intermediates with appropriate lifetimes can be detected and identified. Second, flow allows steady-state conditions to obtain and, therefore, permits the direct measurement of the forward and reverse rates of rapid equilibrium involving transient species under conditions of relatively high concentrations of catalysts. Third, in the case of carbonyl compounds, the addition step can be studied in a pH range not possible before, i.e., a range in which the dehydration (rather than the addition) step is rate determining. This range is usually around 7 or greater, a common range for many enzyme-catalyzed reactions. Finally, this technique can be applied to a wide variety of equilibria involving transient species, and other examples will be presented.

Acknowledgment. This work is supported by the donors of the Petroleum Research Fund, administered by the American Chemical Society. We thank Professor J. Hine for clarifying a point concerning the calculation of k_d.

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