

This value together with our value for the decay of benzil ketyl gives  $\epsilon = 8.0 \times 10^2 M^{-1} \text{ cm}^{-1}$  at  $\lambda_{\text{max}}$  490 nm.

As mentioned above, irradiation of **1** in *p*-xylene gave an epr spectrum apparently due to a mixture of the two semidione radicals. The total steady-state radical concentration was  $6.1 \times 10^{-6} M$  and a  $k_t$  of  $8.7 \times 10^7 M^{-1}$  was calculated on the basis of a single radical being present. Since the irradiation of **1** in *p*-xylene has been shown to yield primarily cross-coupling product (eq 8), plus smaller amounts of product derived from coupling of solvent radicals (eq 11), and disproportionation of the camphoquinone semidione radical (eq 10),<sup>16</sup>

this constant is a weighted average of several different rate constants.

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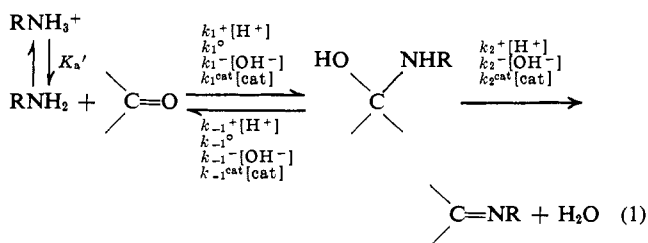
## General Base Catalysis of Thiosemicarbazone Formation<sup>1</sup>

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**Abstract:** Thiosemicarbazone formation from *p*-chlorobenzaldehyde exhibits a change in rate-determining step at about pH 4, similar to that in semicarbazone formation, with rate-determining amine attack below and rate-determining dehydration above this pH. The attack step is subject to general acid catalysis by carboxylic acids with a Brønsted coefficient  $\alpha = 0.15$ . It is also subject to base catalysis. The dehydration step provides the first reported example of general base catalysis of this class of reaction. A number of bases of different structure and charge type follow a Brønsted line of slope 0.71. The reverse reaction is formulated as an attack of hydroxide ion on the free imine which is subject to general acid catalysis. The solvent deuterium isotope effects,  $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$ , are 3.2 and 2.6 for catalysis by triethylenediamine and 3-quinuclidinol, respectively, whereas  $k_{\text{OH}^-}/k_{\text{OD}^-}$  is 1.05. The dehydration step is subject to general acid catalysis by phosphate and dimethylmalonate monoanions, as well as by the solvated proton. The isotope effect,  $K_{\text{H}_2\text{O}}/K_{\text{D}_2\text{O}}$ , on the ionization constants as acids of both thiosemicarbazide ( $\text{p}K_{\text{aH}_2\text{O}}' = 12.81$ ) and *p*-chlorobenzaldehyde thiosemicarbazone ( $\text{p}K_{\text{aH}_2\text{O}}' = 11.20$ ) is  $4.0 \pm 0.1$ .

Base catalysis of oxime formation was noted by Auwers<sup>2</sup> and was examined quantitatively by Barrett and Lapworth, who proposed that the reaction involves the attack of the hydroxylamine anion on the carbonyl group.<sup>3</sup> More recent work has shown that this catalysis, and the similar catalysis for semicarbazone and O-methyl oxime formation, involves acceleration by hydroxide ion of the dehydration of the tetrahedral intermediate that is in equilibrium with starting materials above neutral pH ( $k_2^-$ , eq 1).<sup>4</sup> In general, this type of



reaction proceeds with rate-determining attack of amine on the carbonyl compound at low pH and undergoes a

change in rate-determining step to acid- and base-catalyzed dehydration of the tetrahedral addition intermediate as the pH is increased (eq 1).<sup>5</sup> Williams and Bender observed an inverse isotope effect for catalysis of oxime formation by hydroxide ion and suggested that the reaction involves specific base catalysis.<sup>6</sup> The present investigation was carried out in an attempt to determine whether this catalysis represents specific or general base catalysis and to examine other aspects of the mechanism of thiosemicarbazone formation.

### Experimental Section

**Materials.** Reagent grade inorganic salts were used without further purification. Organic acids and bases (with the exception of reagent grade formic and acetic acids) were purified by recrystallization, distillation, or sublimation. Final purification of *p*-chlorobenzaldehyde was effected by sublimation, after recrystallization from ethanol-water. The purified aldehyde was stored in the cold in the presence of a desiccant. Thiosemicarbazide was recrystallized twice from water. Glass-distilled water was used in all experiments.

Stock solutions of *p*-chlorobenzaldehyde were prepared in ethanol and were stored at 4°. These were diluted on the day of use with water containing sufficient disodium ethylenediaminetetraacetate to give a concentration of  $10^{-4} M$  in the final reaction mixture. The ethanol concentration in the final reaction mixtures was less than 0.2%. Dilute aqueous solutions of *p*-chlorobenzaldehyde were kept under argon to prevent possible air oxidation of the al-

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(2) K. Auwers, *Chem. Ber.*, **22**, 604 (1889).

(3) E. Barrett and A. Lapworth, *J. Chem. Soc.*, **93**, 85 (1908).

(4) B. M. Anderson and W. P. Jencks, *J. Amer. Chem. Soc.*, **82**, 1773 (1960).

(5) W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).

(6) A. Williams and M. L. Bender, *J. Amer. Chem. Soc.*, **88**, 2508 (1966).

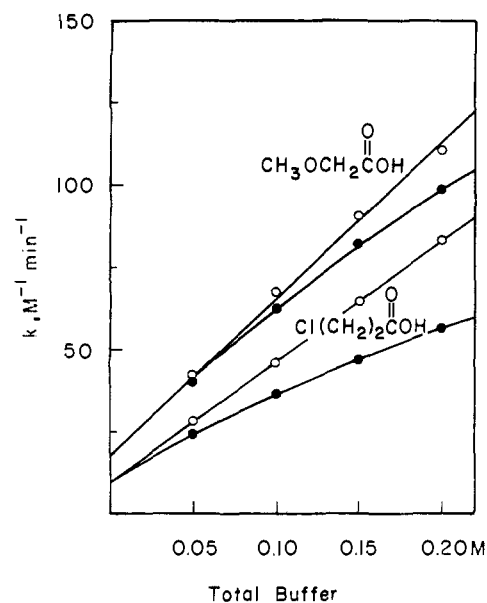


Figure 1. Catalysis of *p*-chlorobenzaldehyde thiosemicarbazone formation by methoxyacetic acid buffer, pH 3.07 (upper curves), and  $\beta$ -chloropropionic acid buffer, pH 3.76 (lower curves). Solid symbols represent observed second-order rate constants,  $k_{\text{uncor}}$ , for the over-all reaction based on the concentration of thiosemicarbazide free base. Open symbols represent values for  $k_{\text{addn}}$  after correction for the contribution of the dehydration step.

dehyde. Thiosemicarbazide solutions of concentration 0.12 *M* or less were made by dissolving the free base in water; more concentrated solutions were made by adding sufficient hydrochloric acid to convert all the base to the hydrochloride. Regeneration of the free base was effected immediately before initiating the reactions by adding the thiosemicarbazide hydrochloride to reaction mixtures containing an equivalent quantity of potassium hydroxide.

Reagent solutions used in deuterium oxide experiments were prepared by dissolving the corresponding protium compounds in glass-distilled deuterium oxide. The concentration of deuterium oxide was greater than 99% in the final reaction mixtures in all cases.

**Equilibrium Constant for Carbinolamine Formation.** The equilibrium constant was determined by measuring the decrease in absorbance at 300  $m\mu$  of a solution of *p*-chlorobenzaldehyde in the presence of varying concentrations of thiosemicarbazide. The determinations were limited to the range 0–0.13 *M* thiosemicarbazide by the solubility of this compound. The pH was maintained at 7.6 with 0.4 *M* imidazole buffer (70% free base) and the ionic strength was adjusted to 1.0 with potassium chloride. At this pH dehydration of the carbinolamine is relatively slow, so that the observed absorbance could be extrapolated to zero time to determine the initial rapid decrease in aldehyde absorbance caused by carbinolamine formation. A lag in the initial rate of the dehydration reaction, which produced nonlinearity in the extrapolation, was observed in tris(hydroxymethyl)aminomethane buffer; this phenomenon was not observed with the imidazole buffer. Extrapolated absorbance values were corrected for the contribution of the added thiosemicarbazide. Values for the equilibrium constant were obtained for each concentration of thiosemicarbazide from the equation  $K_e = \alpha / \{(1 - \alpha)[\text{thiosemicarbazide}]\}$  where  $\alpha$  = the fraction of aldehyde converted to carbinolamine, and from the negative abscissa intercept of double reciprocal plots of absorbance decrease against thiosemicarbazide concentration. The calculated values of  $\alpha$  were based on an assumed absorbance of zero for the carbinolamine addition compound at 300  $m\mu$ . This assumption was verified experimentally for the bisulfite addition compound; less than 3% of the initial absorbance remained after addition of 0.1 *M* sodium bisulfite to a solution of the aldehyde at pH 7.3.

**$pK_a'$  Determinations.** The apparent  $pK_a'$  of thiosemicarbazide cation was found to be 1.88 at ionic strength  $1.0 \pm 0.03$  (potassium chloride) by potentiometric titration at 25° of a 0.1 *M* solution of the base with 2 *N* hydrochloric acid. Appropriate corrections were made for the titration of a control solution of 1 *M* potassium chloride.

The equilibrium constants for anion formation from thiosemicarbazide and *p*-chlorobenzaldehyde thiosemicarbazone were determined spectrophotometrically in water and deuterium oxide at 25°, ionic strength = 1.0. The  $pK_a'$  of the thiosemicarbazone was determined from its absorbance at 340  $m\mu$  in a series of 3-quinuclidinol buffers and unbuffered potassium hydroxide solutions. *pD* values were calculated by adding 0.40 to the pH values measured with the glass electrode in deuterium oxide solutions.<sup>7</sup> The pH meter did not give reliable values of *pD* at the two highest deuterium ion concentrations measured; however, calculations of  $K_{\text{aH}_2\text{O}}/K_{\text{aD}_2\text{O}}$  from hydroxide and deuterium ion concentrations gave a value for the isotope effect identical with that calculated from pH and *pD* at lower deuterium ion activities. The  $pK_a'$  of thiosemicarbazide was determined in a series of unbuffered potassium hydroxide solutions in deuterium oxide and water in the presence of  $10^{-3}$  *M* ethylenediaminetetraacetate. The observed absorbance at 260  $m\mu$  (which increased with time as a result of thiosemicarbazide decomposition) was extrapolated to the time of thiosemicarbazide addition.

**Kinetic Measurements.** Reaction rates were followed using a Zeiss PMQ II spectrophotometer or a Gilford Model 2000 recording spectrophotometer. The temperature was maintained at 25° by the use of a thermostated cell compartment and the ionic strength was adjusted to 1.0 *M* with potassium chloride. Half-lives and pseudo-first-order rate constants of the acid-catalyzed reaction were determined from semilogarithmic plots of  $(A_\infty - A_t)$  at 310  $m\mu$  against time. For these reactions the final thiosemicarbazide concentration in the reaction mixtures was 0.01 *M* and the *p*-chlorobenzaldehyde concentration was approximately  $2 \times 10^{-5}$  *M*. The pH of each solution was determined after completion of the reaction. The  $pK_a'$  values of buffers were determined from the pH of the reaction mixtures and the known compositions of the added buffers. For several of the most acidic buffers it was necessary to add hydrochloric acid to the reaction mixtures to correct for hydrolysis of the cation, in order to maintain a constant pH at varying buffer concentrations.

At the highest concentrations of many of the buffers used, the dehydration step, which is subject to hydronium ion catalysis, but is not significantly catalyzed by the carboxylic acids used as catalysts for the addition step, becomes partially rate limiting. Correction of  $k_{\text{uncor}}$  for the contribution of the dehydration step gave  $k_{\text{addn}}$ , the apparent second-order rate constant for the addition step, as follows

$$k_{\text{addn}} = \frac{k_{\text{uncor}}}{1 - k_{\text{uncor}}/K_e k_2^+ a_{\text{H}^+}} \quad (2)$$

$K_e k_2^+$ , the apparent rate constant for hydronium ion catalyzed dehydration, was taken as  $1 \times 10^6 \text{ M}^{-2} \text{ min}^{-1}$ . Rate constants for catalysis by buffers,  $k'$ , were obtained from the slopes of plots of  $k_{\text{addn}}$  against the buffer concentration (Figure 1), and the catalytic constants for general acid catalysis,  $k_{\text{cat}}$ , were obtained by dividing  $k'$  by the fraction of the buffer in the acidic form. The ordinate intercepts of these plots gave the rate of hydronium ion plus water catalyzed addition at each pH. To convert this value to the rate of the over-all reaction at zero buffer concentration the relationship

$$k_{\text{over-all}} = \frac{k_{\text{addn}}}{1 + k_{\text{addn}}/K_e k_2^+ a_{\text{H}^+}} \quad (3)$$

was used. The pH dependence of the rate at zero buffer concentration ( $k_{\text{over-all}}$ ) was calculated in this way to avoid the extrapolation of curved plots to zero buffer concentration.

Above pH 5 the reaction was examined by measurements of initial rates or by determination of pseudo-first-order rate constants in more concentrated thiosemicarbazide solutions. The pseudo-first-order reaction could not be followed to completion above pH 7 because of relatively slow, base-catalyzed decomposition of thiosemicarbazide; hence, in this pH range only initial rates were measured. Initial rate measurements were made at 325  $m\mu$  (the isobestic point of the neutral and anionic forms of the product,  $pK_a' = 11.20$ ) with approximately  $7.5 \times 10^{-4}$  *M* *p*-chlorobenzaldehyde and 0.001–0.04 *M* thiosemicarbazide and were found to be linear for up to 5% of the total reaction. Blanks containing only thiosemicarbazide and buffer showed no absorbance change over the time intervals used for the initial rate measurements. The absorbance of the product at time infinity was obtained from a diluted aliquot of a

(7) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

solution in which the reaction had been allowed to proceed to completion in the presence of 0.012 *M* hydrochloric acid. The pseudo-first-order rate constant for the reaction is equal to the initial rate of absorption change divided by the absorbance of the product. Rate constants obtained by this method and by following the entire course of the reaction in a series of phosphate buffers at pH 6.9–7.0 were found to be in satisfactory agreement.

The rate constants were corrected for small variations in pH with changing buffer concentration, if necessary, according to eq 4, in

$$k_{\text{std}} = k_{\text{obsd}} + K_e k_2^- [(a_{\text{OH}^-})_{\text{std}} - (a_{\text{OH}^-})_{\text{obsd}}] + K_e k_2^+ [(a_{\text{H}^+})_{\text{std}} - (a_{\text{H}^+})_{\text{obsd}}] \quad (4)$$

which "std" refers to an arbitrary pH value within the range of the observed pH values and  $K_e k_2^+$  and  $K_e k_2^-$  are  $1.0 \times 10^8$  and  $5.4 \times 10^8 \text{ M}^{-2} \text{ min}^{-1}$ , respectively. These corrections were less than 10% of the observed rate constant in almost every case; the correction for acid catalysis was important only below pH 8.1.

**Product Analysis.** The spectrum of the reaction product at pH 2.1 and 4.9 was found to be identical with that of authentic *p*-chlorobenzaldehyde thiosemicarbazone.<sup>8</sup> The absorption of decomposition products of thiosemicarbazide precludes such a direct spectrophotometric comparison in basic solution, but the same thiosemicarbazone was isolated in 70% yield from the reaction of 0.13 *M* aldehyde and amine catalyzed by 0.10 *M* potassium hydroxide in 50% ethanol and, after two recrystallizations from ethanol–water, was found to have spectra in water and in 0.01 *N* potassium hydroxide identical with those of authentic thiosemicarbazone.

## Results

The dependence on pH of the rate constants for *p*-chlorobenzaldehyde thiosemicarbazone formation, extrapolated to zero buffer concentration, is shown in Figure 2. Corrections were made for a change in the rate-limiting step with increasing buffer concentration as described in the Experimental Section. The pH–rate curve is similar to those observed for semicarbazone formation<sup>9</sup> and for benzylideneaniline hydrolysis.<sup>10</sup> The break in the curve at about pH 4.5 is indicative of a change in rate-limiting step corresponding to a change from rate-limiting addition of the nucleophile at low pH to rate-limiting dehydration of the adduct at higher pH. The addition step proceeds through both an acid-catalyzed and a pH-independent pathway, while dehydration is predominantly acid catalyzed at low pH and base catalyzed at high pH. At zero buffer concentration the reaction follows the rate law of eq 5, in which

$$\frac{k_{\text{obsd}}}{[\text{RNH}_2]} = (k_2^+[\text{H}^+] + k_2^-K_w/[\text{H}^+]) \times \left( \frac{k_1^{\circ} + k_1^+[\text{H}^+]}{k_{-1}^{\circ} + k_{-1}^+[\text{H}^+] + k_2^+[\text{H}^+] + k_2^-K_w/[\text{H}^+]} \right) \quad (5)$$

the subscripts 1 and 2 refer to the attack and dehydration steps, respectively. At pH values below and above pH 7, eq 5 reduces to eq 6 and 7, respectively, in which

$$\frac{k_{\text{obsd}}}{[\text{RNH}_2]} = k_2^+K_e[\text{H}^+](k_1[\text{H}^+] + k_1^{\circ})/(k_1^+[\text{H}^+] + k_2^+K_e[\text{H}^+] + k_1^{\circ}) \quad (6)$$

$$\frac{k_{\text{obsd}}}{[\text{RNH}_2]} = k_2^+K_e[\text{H}^+] + k_2^-K_eK_w/[\text{H}^+] \quad (7)$$

$K_e = k_1^{\circ}/k_{-1}^{\circ} = k_1^+/k_{-1}^+$ . At thiosemicarbazide concentrations greater than 0.01 *M*,  $k_{\text{obsd}}/[\text{RNH}_2]$  was corrected for equilibrium formation of the carbinolamine

(8) P. P. T. Sah and T. C. Daniels, *Rec. Trav. Chim. Pays-Bas*, **69**, 1545 (1950).

(9) E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 4319 (1962).

(10) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 832 (1962).

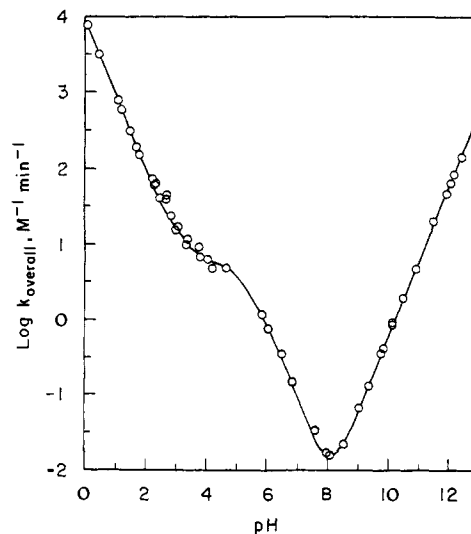


Figure 2. Dependence on pH of the logarithms of second-order rate constants, based on the concentration of thiosemicarbazide free base, for *p*-chlorobenzaldehyde thiosemicarbazone formation at 25°, ionic strength 1.0 *M*. All rate constants have been extrapolated to zero buffer concentration. The line represents the theoretical curve for the kinetic constants in Table I.

by multiplying by the factor  $(1 + K_e[\text{RNH}_2])$ . The solid line of Figure 2 was calculated from the kinetic constants in Table I. There is no evidence for a signifi-

Table I. Rate and Equilibrium Constants for Thiosemicarbazone Formation from *p*-Chlorobenzaldehyde at 25°, Ionic Strength 1.0 *M*<sup>a</sup>

$\text{p}K_a'$	1.88
$k_1^{\circ}, \text{M}^{-1} \text{min}^{-1}$	6
$k_1^+, \text{M}^{-2} \text{min}^{-1}$	$8.5 \times 10^3$ <sup>b</sup>
$k_2^+, \text{M}^{-1} \text{min}^{-1}$	$5.3 \times 10^8$ <sup>b</sup>
$k_2^-, \text{M}^{-1} \text{min}^{-1}$	$2.5 \times 10^3$ <sup>c</sup>
$K_e = k_1^{\circ}/k_{-1}^{\circ} = k_1^+/k_{-1}^+, \text{M}^{-1}$	$1.7 \pm 0.2$ <sup>d</sup>

<sup>a</sup> Constants are defined for processes shown in eq 1. These rate constants are based on hydroxide and hydronium ion concentrations. <sup>b</sup> Calculated from the observed pH and the empirical relationship  $\text{antilog}(-\text{pH}) = 0.9C_{\text{H}^+}$ . <sup>c</sup> Calculated from the stoichiometric hydroxide ion concentration. An average value of  $2.1 \times 10^3$  was obtained from the pH dependence of the base-catalyzed reaction and an empirical activity coefficient of 0.67 for hydroxide ion (J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 837 (1964)). <sup>d</sup> Limits of experimentally determined  $K_e$ .

cant water-catalyzed dehydration step ( $k_{\text{cat}} \leq 2 \times 10^{-5} \text{ M}^{-1} \text{ min}^{-1}$ ); the rate constant estimated from Brønsted plots for this step (see below) is approximately  $10^{-7} \text{ M}^{-1} \text{ min}^{-1}$ .

**General Acid Catalysis.** Table II summarizes the third-order rate constants for catalysis of the addition of thiosemicarbazide to *p*-chlorobenzaldehyde by several carboxylic acids, hydronium ion, and water. Catalytic constants were determined from plots of the observed second-order rate constant (based on the concentration of free thiosemicarbazide) against buffer concentration. Corrections for the contribution of the dehydration step to the observed rate were made when required, as described in the Experimental Section. The value of  $K_e k_2^+$  for hydronium ion catalyzed dehydration is  $1.0 \times 10^6 a_{\text{H}^+} \text{ M}^{-2} \text{ min}^{-1}$ , as determined from the pH dependence of the reaction rate in the linear portion of

Table II. Acid Catalysis of the Attack of Thiosemicarbazide on *p*-Chlorobenzaldehyde at 25°, Ionic Strength 1.0 M (KCl)

Catalyst	$pK_a'$	Concn range, <sup>a</sup> <i>M</i>	Fraction as acid	pH	$k',^b$ $M^{-2} \text{ min}^{-1}$	$k_{\text{cat}},^c$ $M^{-2} \text{ min}^{-1}$
H <sub>3</sub> O <sup>+</sup>	-1.7	0.0067-0.89 <sup>d</sup>				8500
1. Difluoroacetic acid (DFA)	1.13	0.1-0.4	0.20	1.72	330	1550
		0.1-0.4	0.30	1.48	435	
2. Cyanoacetic acid (CNA)	2.33	0.1-0.4	0.30	2.70	268	920
		0.1-0.4	0.50	2.32	475	
3. Glycine (GLY)	2.48	0.1-0.4	0.30	2.85	310	1030
		0.1-0.4	0.50	2.48	515	
4. Chloroacetic acid (CA)	2.70	0.1-0.4	0.50	2.69	390	800
		0.1-0.4	0.70	2.34	580	
5. Methoxyacetic acid (MA)	3.40	0.05-0.2	0.50	3.40	370	720
		0.05-0.2	0.70	3.07	485	
6. Formic acid (FOR)	3.56	0.053-0.5	0.40	3.75	222	
		0.053-0.5	0.60	3.38	301	560
		0.053-0.25	0.80	3.00	495	
7. $\beta$ -Chloropropionic acid ( $\beta$ -CP)	3.93	0.05-0.2	0.60	3.76	367	600
		0.05-0.2	0.80	3.35	480	
8. Acetic acid (AC)	4.65	0.01-0.04	0.50	4.66	238	470
		0.01-0.04	0.80	4.02	362	
H <sub>2</sub> O	15.7	55.5				0.11

<sup>a</sup> Total buffer concentration. <sup>b</sup> Based on total buffer concentration and corrected for the contribution of the dehydration step (see text).  
<sup>c</sup> Based on free-acid concentration. <sup>d</sup> Based on observed pH values and the empirical relationship  $\text{antilog}(-\text{pH}) = 0.9C_{\text{H}^+}$ .

the pH-rate curve between pH 6.5 and 7.5. Figure 1 shows typical results indicating the approximate magnitude of the corrections at pH 3.1 and 3.8. The corrections are significant even where the plots of  $k_{\text{uncor}}$  against buffer concentration are not obviously curved. The catalytic constants for carboxylic acids and the solvated proton follow the Brønsted relationship and give a value of  $\alpha$  of 0.15 (Figure 3). Statistical corrections

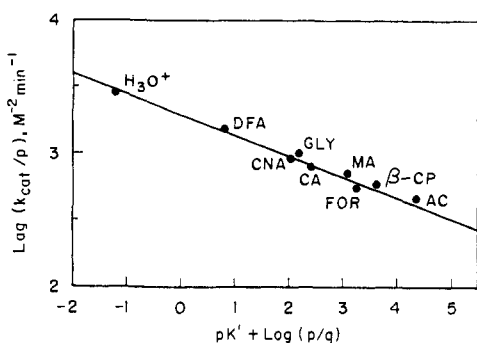


Figure 3. Brønsted plot for the general acid catalyzed addition of thiosemicarbazide to *p*-chlorobenzaldehyde. The catalysts used are given in Table II. The line has a slope of 0.15.

were applied according to the method of Bell and Evans<sup>11</sup>; for the solvated proton, the corrections,  $p = 3$  and  $q = 1$ , were used.

The dehydration step is also subject to general acid catalysis by phosphate and dimethylmalonate, but not imidazole buffers (Table III). The value of  $\alpha$ , based on these two catalysts and hydronium ion, is 0.73. Because of this large value of  $\alpha$ , general acid catalysis of this step is not significant at low pH values.

**General Base Catalysis.** The rate of thiosemicarbazone formation above pH 8, where carbinolamine dehydration is rate limiting, is dependent upon both hydroxide ion and buffer concentrations. The basic form of the buffer is the catalytically active species.

(11) R. P. Bell and P. G. Evans, *Proc. Roy. Soc. (London), Ser. A*, 291, 297 (1966).

Table III. Acid Catalysis of the Dehydration Step of *p*-Chlorobenzaldehyde Thiosemicarbazone Formation at 25°, Ionic Strength 1.0 M (KCl)

Acid	$pK_a'$	$K_e k_2^{\text{cat}},$ $M^{-2} \text{ min}^{-1}$
Hydronium ion	-1.7	$9 \times 10^5$ <sup>a</sup>
Dimethylmalonate monoanion	5.44	$11^b$
Phosphate monoanion	6.4	$2.9^c$
Imidazolium ion	7.24	$<4 \times 10^{-2}$ <sup>d</sup>

<sup>a</sup> Based on hydrogen ion concentration, from the observed pH values and the empirical relationship  $\text{antilog}(-\text{pH}) = 0.9C_{\text{H}^+}$ .  
<sup>b</sup> Based on experiments in 0.05-0.3 M phosphate buffer, 3% KH<sub>2</sub>PO<sub>4</sub>, and 0.02-0.20 M phosphate buffers, 25% and 50% KH<sub>2</sub>PO<sub>4</sub>.  
<sup>c</sup> Based on experiments in 0.02-0.20 M dimethylmalonate buffer, 20% and 5% monoanion. <sup>d</sup> Upper limit based on absence of observable acid catalysis in imidazole buffer, 0.1-0.4 M, 50% imidazolium ion.

The slopes of the lines obtained from plots of apparent second-order rate constants against total buffer concentration were determined at two or more buffer compositions. Division of these slopes by the fraction of the buffer present as the conjugate base yielded the same value for  $K_e k_2^{\text{cat}}$  for each buffer ratio. Typical plots illustrating catalysis of the reaction by tertiary amines are shown in Figure 4.

Table IV summarizes the catalytic constants,  $K_e k_2^{\text{cat}}$ , which were obtained for several tertiary amines and oxygen bases. A Brønsted plot relating the catalytic constants for these bases to their observed  $pK_a'$  values is shown in Figure 5. Statistical corrections were made as described by Bell and Evans.<sup>11</sup> The Brønsted exponent,  $\beta$ , determined from the slope of the line through the points for the bicyclic tertiary amines, triethylenediamine, 3-chloroquinuclidine, 3-quinuclidinol, and quinuclidine, is 0.71.

Catalytic constants for several of the bases examined are only approximate values because of the experimental problems described below; they are reported simply because they indicate that the constants for these compounds do not deviate markedly from the Brønsted plot for tertiary amines.

Table IV. Base Catalysis of *p*-Chlorobenzaldehyde Thiosemicarbazone Formation at 25°, Ionic Strength 1.0 M (KCl)

Base	$pK_a^a$	$p^b$	$q^b$	Concn range, <sup>c</sup> M	Fraction as base	pH	$k',^d$ $M^{-2} \text{ min}^{-1}$	$K_0 k_2^{\text{cat},e}$ $M^{-2} \text{ min}^{-1}$
Imidazole (IM)	7.24	2	1	0.05–0.30	0.86	8.10	0.012	0.014
				0.10–0.40	0.95	8.55	0.014	
Borate	8.9	3	4	0.02–0.06	0.20	8.30	0.21	1.1
				0.01–0.06	0.40	8.80	0.49	
3-Chloroquinuclidine (CQD)	9.09	1	1	0.05–0.20	0.10	8.06	0.06	0.65
				0.05–0.20	0.20	8.46	0.14	
Triethylenediamine (DABCO)	9.22	1	2	0.10–0.40	0.40	9.04	0.32	0.76
				0.10–0.40	0.60	9.42	0.48	
				0.02–0.40	0.60	9.40	0.43	
				0.05–0.40	0.80	9.78	0.57	
Hexafluoro-2-propanol (HFIP)	9.22	1	1	0.05–0.20	0.20	8.60	0.057	0.28
				0.05–0.20	0.40	9.05	0.11	
Acetohydroxamic acid (AHA)	9.32	1	1	0.10–0.40	0.20	8.71	0.095	0.48
				0.10–0.30	0.40	9.16	0.19	
Carbonate <sup>f</sup>	9.9	1	3	0.025–0.20	0.30	9.39	0.66	2.1
				0.025–0.10	0.50	9.84	0.93	
				0.05–0.20	0.60	10.30	1.3	
3-Quinuclidinol (QDL)	10.13	1	1	0.02–0.40	0.20	9.52	0.51	3.1
				0.05–0.20	0.30	9.75	1.06	
				0.02–0.40	0.40	9.95	1.19	
				0.05–0.20	0.50	10.14	1.70	
Trimethylamine (TMA)	10.20	1	1	0.10–0.40	0.30	9.85	0.72	2.4
				0.10–0.40	0.50	10.18	1.14	
Quinuclidine (QD)	11.5	1	1	0.025–0.20	0.10	10.5	2.9	27
				0.025–0.20	0.20	10.9	4.8	
Hydroxide ion	15.7	2	1	0.001–0.020		10.58–		4300 <sup>g</sup>
						12.17		

<sup>a</sup> Determined from the observed pH values of buffers or by titration at 25°, ionic strength 1 M (KCl). <sup>b</sup> These statistical factors are from the method of R. P. Bell and P. G. Evans.<sup>11</sup> <sup>c</sup> Total buffer concentration. <sup>d</sup> Based on total buffer concentration. <sup>e</sup> Based on free-base concentration. <sup>f</sup> This catalytic constant may be lower than the actual value because of reaction of carbonate-bicarbonate buffers with thiosemicarbazide (see text). <sup>g</sup> Based on the concentration of hydroxide ion.

**Imidazole.** The reported value may be in error by as much as 30% because of the relatively large magnitude of hydronium and hydroxide ion catalysis.

**Borate.** Plots of rate against buffer concentration are curved, presumably because of polymerization of the catalyst.<sup>12</sup> The reported value is taken from the

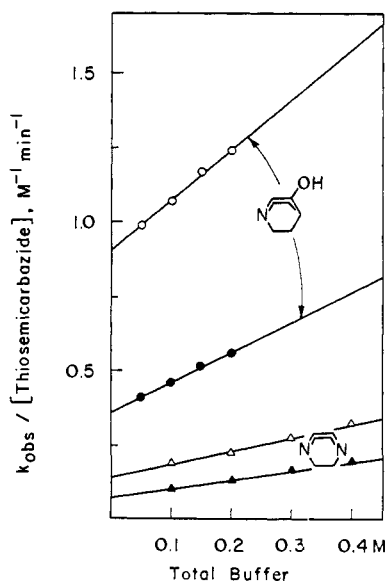


Figure 4. Catalysis of *p*-chlorobenzaldehyde thiosemicarbazone formation by amines at 25°, ionic strength 1.0 M (KCl), showing variation in the observed second-order rate constant with increasing buffer concentration in 3-quinuclidinol, 50% (○) and 30% (●) free base, and triethylenediamine, 60% (△) and 40% (▲) free base.

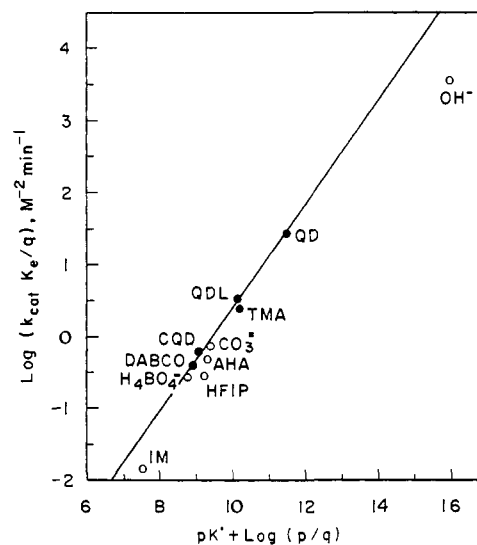


Figure 5. Brønsted plot for general base catalyzed *p*-chlorobenzaldehyde thiosemicarbazone formation under conditions where dehydration of the carbinolamine intermediate is rate limiting. The abbreviations and statistical correction factors are given in Table IV. A line of slope 0.71 has been drawn through the points.

(12) R. B. Martin, A. Parcell, and R. I. Hedrick, *J. Amer. Chem. Soc.*, 86, 2406 (1964); N. Ingri, *Acta Chem. Scand.*, 16, 439 (1962).

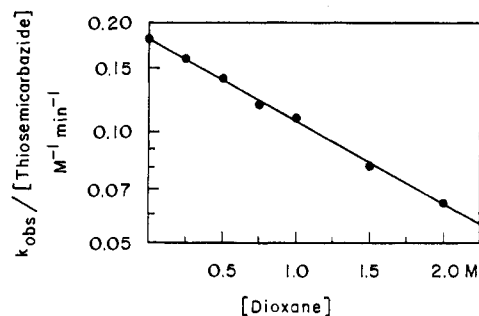


Figure 6. Effect of added dioxane upon the rate of *p*-chlorobenzaldehyde thiosemicarbazone formation at 25° in 0.05 *M* triethylenediamine buffer, 60% free base. Ionic strength was maintained at 1.0 *M* by the addition of potassium chloride. The observed pH varied from 9.33 (2 *M* dioxane) to 9.40 (no dioxane).

initial slope of plots at total buffer concentrations of less than 0.06 *M*.

**Acetohydroxamic Acid.** Analysis of this compound<sup>13</sup> indicated a contamination by hydroxylamine of 0.07% or less. It is possible that even this concentration of hydroxylamine could react with aldehyde to reduce the available aldehyde concentration and cause a slight decrease in the observed rate constant. However, no nonlinearity of the initial rate measurements, as might be expected from such a competing reaction, was observed.

**Carbonate.** Initial rate measurements showed curvature that became significant after the first minute of reaction, possibly because of carbamate formation, at buffer concentrations above 0.05 *M*. Rate constants were calculated from the linear reaction rate in the first minute of reaction. The rate increases observed in the presence of 0.2 *M* potassium sulfate at pH 9.3 and in 0.2 *M* carbonate buffer at pH 9.4 (both at ionic strength 1.0) were found to be 8% and 100%, respectively. Hence, the rate increase in carbonate buffer does not appear to be simply a specific salt effect exhibited by dianions.

**Quinuclidine.** Plots of rate against buffer concentration exhibit curvature above 0.2 *M* that is attributed to complexation of quinuclidine and aldehyde. This explanation is supported by the observation that quinuclidine is an even more effective inhibitor than *n*-butylamine<sup>14</sup> of the hydroxide ion catalyzed decomposition of ethyl benzimidate to benzonitrile. Catalytic constants were estimated from data obtained at up to 0.2 *M* buffer, at which there is no appreciable curvature.

**Salt and Solvent Effects.** Table V shows the variation with salt concentration of the third-order rate constant for the hydroxide ion catalyzed reaction (based on the apparent hydroxide ion activity calculated from the observed pH and  $pK_w = 14$ ). Both potassium chloride and tetramethylammonium chloride accelerate the reaction to approximately the same extent. Dioxane inhibits the hydroxide-catalyzed reaction at ionic strength 1.0 (Figure 6). A similar inhibition was observed in the presence of triethylenediamine, 3-quinuclidinol, and quinuclidine at free-base concentrations up to 0.4 *M* and at pH values sufficiently high that catalysis by the amines is not significant.

(13) D. S. Frear and R. C. Burrell, *Anal. Chem.*, 27, 1664 (1965).

(14) E. S. Hand and W. P. Jencks, *J. Amer. Chem. Soc.*, 84, 3505 (1962).

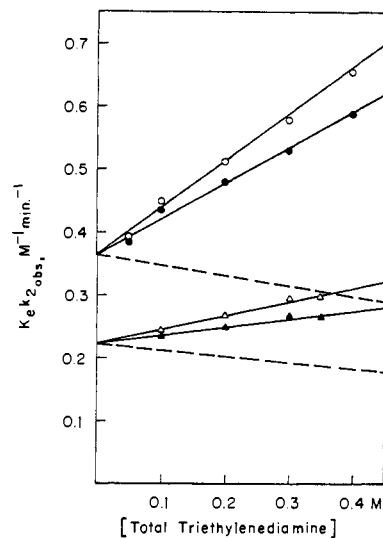


Figure 7. Catalysis of thiosemicarbazone formation by triethylenediamine in water (upper lines, pH 9.78) and deuterium oxide (lower lines, pD 10.50) at 25°, ionic strength 1.0 *M*. Closed symbols: observed rate constants. Open symbols: corrected for inhibition of the lyoxide ion catalyzed reaction by triethylenediamine. This inhibition is shown by the dashed lines.

The catalytic constants for hydroxide ion, 3-quinuclidinol, and triethylenediamine in deuterium oxide exhibit a small isotope effect for hydroxide ion catalysis

Table V. Salt Effects on the Rate of *p*-Chlorobenzaldehyde Thiosemicarbazone Formation at 25°<sup>a</sup>

Addition	Concn, <i>M</i>	pH	$k_{\text{obsd}}/[\text{TSC}]$ , $M^{-1} \text{min}^{-1}$	$K_e k_2^-$ , <sup>b</sup> $M^{-2} \text{min}^{-1}$
1. None		9.00	0.049	4400
2. KCl	0.5	9.25	0.099	5300
3. KCl	1.0	9.35	0.133	5700
4. $(\text{CH}_3)_4\text{NCl}$	0.5	9.20	0.097	5800
5. $(\text{CH}_3)_4\text{NCl}$	1.0	9.26	0.106	5500
6. $(\text{CH}_3)_4\text{NCl}$	0.5	9.32	0.118	5400
KCl	0.5			

<sup>a</sup> In 0.01 *M* triethylenediamine buffer; 60% free base. <sup>b</sup> Calculated from the equation

$$K_e k_2^- = (k_{\text{obsd}} - K_e k_{\text{cat}}[\text{triethylenediamine base}]/(\text{antilog}[14 - \text{pH}]$$

to correct for the contribution of catalysis by triethylenediamine to the observed rate.  $K_e k_{\text{cat}}$  was taken as 0.8 (See Table IV); the contribution of buffer catalysis was 10% or less of the observed rate in all experiments.

and considerably larger effects for catalysis by the tertiary amines (Table VI). Values of  $K_e k_2^-$  for the hydroxide and deuterioxide ion catalyzed reactions are those obtained after correction for partial ionization of thiosemicarbazide at high pH ( $pK_{\text{a}_{\text{H}_2\text{O}}} = 12.81$ ;  $pK_{\text{a}_{\text{D}_2\text{O}}} = 13.42$ ). The abovementioned "medium" effect of amines on the hydroxide ion catalyzed reaction requires a significant correction of the isotope effect on the triethylenediamine-catalyzed reaction. Although it does not have a large effect on the catalytic constant in water, the decrease, caused by this medium effect, in the baseline lyoxide ion catalyzed reaction causes a relatively much larger reduction in the apparent catalytic constant in deuterium oxide, because of the smaller magnitude of

Table VI. Isotope Effect on General Base Catalysis of Thiosemicarbazone Formation at 25°, Ionic Strength 1.0 M (KCl)

Concn. range, <sup>a</sup> M	Solvent	Fraction as base, $\alpha$	pH <sup>b</sup> (pD)	$pK_a'$	$k_{\text{obsd}},$ $M^{-2} \text{ min}^{-1}$	$k_{\text{cor}},^c$ $M^{-2} \text{ min}^{-1}$	$\frac{k_{\text{cor}}}{\alpha} =$ $\frac{K_a k_2^{\text{cat}}}{K_a k_2^{\text{cat}}},$ $M^{-2} \text{ min}^{-1}$	$k_H/k_D$
Triethylenediamine								
0.10-0.40	H <sub>2</sub> O	0.40	9.04	9.22	0.32	0.34	0.85	3.2
0.10-0.40		0.60	9.42		0.48	0.52	0.86	
0.02-0.40		0.60	9.40		0.43	0.48	0.80	
0.05-0.40		0.80	9.78		0.57	0.73	0.91	
0.02-0.40	D <sub>2</sub> O	0.60	10.07	9.89	0.125	0.155	0.26	
0.05-0.40		0.80	10.50		0.13	0.23	0.29	
3-Quinuclidinol								
0.02-0.40	H <sub>2</sub> O	0.20	9.52	10.13	0.51	0.54	0.27	2.6
0.05-0.20		0.30	9.75		1.06	1.13	0.38	
0.02-0.40		0.40	9.95		1.19	1.34	0.34	
0.05-0.20		0.50	10.14		1.70	1.95	0.39	
0.02-0.40	D <sub>2</sub> O	0.20	10.20	10.80	0.25	0.27	0.13	
0.02-0.40		0.40	10.62		0.46	0.54	0.13	
Lyoxide Ion								
0.005-0.020	H <sub>2</sub> O		11.51- 12.18	15.7	4300 <sup>d</sup>			1.05
0.005-0.020	D <sub>2</sub> O		12.34- 12.98	16.5	4100 <sup>d</sup>			

<sup>a</sup> Total buffer concentration. <sup>b</sup> pD values calculated from the relationship  $pD = \text{measured pH} + 0.40$ . <sup>c</sup> Corrected for retardation of the reaction caused by a "medium effect" of amines (see text). <sup>d</sup> Based on concentration of OH<sup>-</sup> or OD<sup>-</sup>.

the catalytic constant in the latter solvent (Figure 7). The solvent isotope effect on the corrected catalytic constant for triethylenediamine is 3.2 compared to an uncorrected value of 4.1. Catalysis by 3-quinuclidinol is subject to an isotope effect of 2.6; corrections for retardation by the "medium effect" with this more effective catalyst were not significant.

Thiosemicarbazide in basic solution dissociates to the anion presumably according to eq 8; the  $pK_a'$  values



for this equilibrium are 12.81 and 13.42 in water and deuterium oxide, respectively. The corresponding  $pK_a'$  values for *p*-chlorobenzaldehyde thiosemicarbazone in water and deuterium oxide are 11.20 and 11.80.

## Discussion

Two experimental points are worthy of note. First, erroneously low catalytic constants for general acid catalysis of the attack of amine on a carbonyl compound may be obtained when the dehydration step is partly rate determining, if the observed rate constants are not corrected for the contribution of the second step to the observed rate. This correction can be significant even if there is no obvious nonlinearity of the rate constants with respect to buffer concentration. The Brønsted  $\alpha$  value of 0.15 for general acid catalysis of thiosemicarbazone formation is based upon such corrected rate constants (Figures 1 and 3). This correction was not made for the rate constants from which a previously reported  $\alpha$  value of 0.25 for semicarbazone formation was derived,<sup>9</sup> and recalculation of these data suggests that the  $\alpha$  value for semicarbazone formation is not significantly different from that for thiosemicarbazone formation. Since the correction is most significant at higher pH values, near the point at which the change in rate-determining step occurs, omission of the correction will lead to an underestimation of the catalytic constants for weaker acids, with a consequent overestimation of the value of  $\alpha$ .

Second, "medium" effects resulting from the addition of general acid or base catalysts may cause a significant decrease in the base-line reaction rate at constant pH, especially for hydroxide ion catalyzed reactions. If the base-line reaction rate is large relative to the rate increase brought about by the catalyst, neglect of this effect will cause an underestimation of the catalytic constant (Figure 7). While the correction will seldom be large for a given catalytic constant, it is more likely to be significant in the estimation of deuterium isotope effects. For example, the correction is more significant for the relatively small catalysis of thiosemicarbazone formation by triethylenediamine in deuterium oxide than in water (Figure 7), so that the corrected solvent deuterium isotope effect of 3.2 for this reaction is significantly smaller than the uncorrected value of 4.1.

**The Attack Step.** The rate constants for the acid-catalyzed and water-catalyzed attack of thiosemicarbazide ( $pK = 1.88$ ) are 10- and 4-fold smaller, respectively, than those for the attack of the more basic semicarbazide ( $pK = 3.86$ ) on *p*-chlorobenzaldehyde.<sup>9</sup> The Brønsted  $\alpha$  value of 0.15 is in the range expected for a nucleophilic reagent of intermediate reactivity and basicity, but shows a negative deviation from a monotonic relationship of  $\alpha$  to basicity for a series of reactions of aldehydes with nucleophilic reagents of widely varying reactivity.<sup>15</sup> After a statistical correction of  $p = 3$ , based on H<sub>3</sub>O<sup>+</sup> as the proton donor, the rate constant for the solvated proton falls on the same Brønsted line as the carboxylic acids (Figure 3). The point for water shows a negative deviation of 100-fold, which might be interpreted in terms of a curvature of the Brønsted plot over a very wide range of catalyst acidity or might reflect the common negative deviation of the water-hydroxide ion pair from Brønsted plots (hydroxide ion is the catalyst for the water reaction in the reverse direction and, if the reactivity is "abnormal," the same negative deviation should be observed for the reaction in both directions).

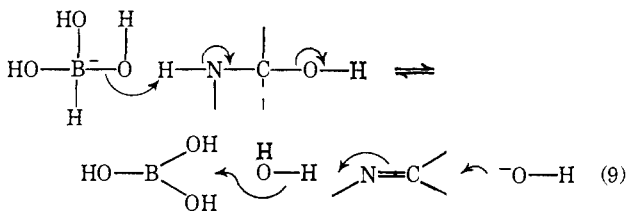
In the alkaline region, the observed rate of the reaction becomes much faster than the rate of the pH-inde-

(15) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., Inc., New York, N. Y., 1969, p 198.

pendent attack step (Figure 2). Although the rate constant for attack cannot be measured directly because dehydration is rate determining, this fact requires that the rate of attack be faster than the pH-independent reaction in this pH region; *i.e.*, the attack step must be subject to hydroxide ion catalysis with a rate constant  $\geq 5000 M^{-2} \text{ min}^{-1}$ . The attack of amides, urea, and thiourea on carbonyl compounds is also subject to base catalysis.<sup>16</sup>

**The Dehydration Step.** Below neutrality the dehydration step proceeds by an acid-catalyzed pathway. Although general acid catalysis is difficult to detect, especially at the lower pH values, because of the large value of  $\alpha$ , definite general acid catalysis is observed with phosphate and dimethylmalonate monoanions. The fact that no general acid catalysis could be detected with imidazolium cation suggests that a concerted mechanism of bifunctional acid-base catalysis may be significant for this reaction. A Brønsted  $\alpha$  value of 0.73, based on the points for phosphate, dimethylmalonate, and the solvated proton, is similar to the value of 0.77 for the dehydration step of nitron and oxime formation;<sup>17</sup> however, the value for monofunctional catalysis may be significantly larger.

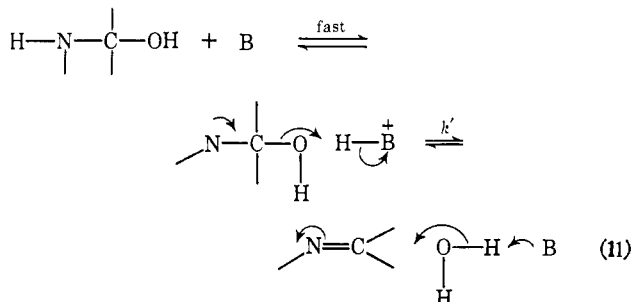
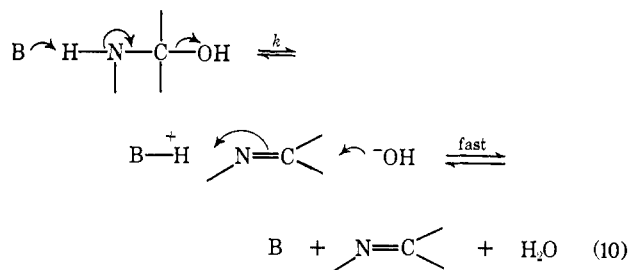
The most significant new conclusion of this work is that dehydration of the carbinolamine intermediate is catalyzed by buffer bases, as well as by hydroxide ion; *i.e.*, it is subject to general base catalysis. The reaction is highly sensitive to the base strength of the catalyst and the  $\beta$  value of 0.71 (Figure 5) indicates a large amount of proton transfer to the catalyst in the transition state. It is of interest that several different types of catalyst molecules, including borate ion, do not deviate significantly from a single Brønsted line. This suggests that transition-state stability is determined by the same factors that determine base strength and that factors, such as solvation, which might provide a larger or smaller stabilization of the transition state relative to the proton transfer equilibrium are not important. The large value of  $\beta$  would favor such a situation, as would proton transfer through an intermediate water molecule. The catalytic activity of borate could then occur according to eq 9, which avoids the necessity of invoking unusual hydration states of borate as catalysis.



Two kinetically indistinguishable mechanisms for the observed general base catalysis (neglecting intermediate water molecules) are shown in eq 10 and 11. The first mechanism involves true general base catalysis in the forward direction. In the reverse direction it involves a preliminary fast ionization step followed by attack of

(16) G. A. Crowe, Jr., and C. C. Lynch, *J. Amer. Chem. Soc.*, **71**, 3731 (1949); J. Koskikallio, *Acta Chem. Scand.*, **10**, 1267 (1956); J. Ugelstad and J. De Jonge, *Rec. Trav. Chim. Pays-Bas*, **76**, 919 (1957); K. Dušek, *Collect. Czech. Chem. Commun.*, **25**, 108 (1960); J. I. De Jong and J. De Jonge, *Rec. Trav. Chim. Pays-Bas*, **71**, 643, 661 (1952); M. Imoto and M. Kobayashi, *Bull. Chem. Soc. Jap.*, **33**, 1651 (1960).

(17) J. E. Riemann and W. P. Jencks, *J. Amer. Chem. Soc.*, **88**, 3973 (1966).



hydroxide ion on the neutral imine, which is facilitated by proton donation from the conjugate acid of the catalyst. The second mechanism involves a rapid preliminary ionization to the conjugate base of the carbinolamine, followed by an elimination of hydroxide ion which is facilitated by proton donation from the conjugate acid of the catalyst. In the reverse direction it involves true general base catalysis of the attack of water on the neutral imine.

The mechanism of eq 10, in which catalysis serves to avoid the formation of the unstable amine anion, is preferred to that of eq 11 according to the notion that catalysis of proton transfer will occur where it is most needed. An argument similar to that applied by Eigen to the hydration of acetaldehyde<sup>18</sup> provides support, but not conclusive proof, in favor of this mechanism. The  $pK$  for the ionization of the nitrogen atom of the carbinolamine to its conjugate base may be roughly estimated to be between 22 and 24.<sup>19</sup> In order to account for the observed rate of the reaction, the rate constant  $k'$  (eq 11)

(18) M. Eigen, *Discussions Faraday Soc.*, **39**, 7 (1965).

(19) If the  $pK$  for ionization of ammonia to its anion is taken as 35 and the  $pK$  of the ammonium ion = 9, an approximate  $pK$  of 26 for loss of a proton from the nitrogen of thiosemicarbazide may be calculated from the known  $pK$  of its cation, 1.88, and the assumption that the  $pK$  for anion formation is 1.3 times as sensitive to electron withdrawal by substituents as the  $pK$  for removal of a proton from the corresponding ammonium ion. (The latter assumption represents an upper limit for the substituent effect and may yield an erroneously low value of the  $pK$  for thiosemicarbazide anion formation, since the relationship was derived from studies of aromatic compounds in which resonance stabilization of the anion must be very significant.) Additional substituent effects on the acidity of the carbinolamine intermediate arise from the presence of the *p*-chlorophenyl and hydroxyl groups. A benzyl substituent has only a slight effect on amine basicity; a *p*-chlorobenzyl group might decrease the  $pK$  somewhat more, and the hydroxymethyl substituent has been shown to lower the  $pK$  of protonated amines by 2-3 units; however, this is probably largely a solvation effect (the polar effect of the hydroxymethyl group is similar to that of hydrogen) and would not be expected to be analogous for an amine anion. Hence the  $pK$  of the carbinolamine derived from thiosemicarbazide and *p*-chlorobenzaldehyde is probably between 22 and 24. The rate constant,  $k'$ , for dehydration of the hypothetical carbinolamine anion (eq 11) is related to the observed catalytic constant,  $k_2^{cat}$ , by the equation  $k' = k_2^{cat} K_{HB} / K_{HX}$  in which  $K_{HX}$  and  $K_{HB}$  are the ionization constants of carbinolamine and catalyst, respectively. [R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 87; D. Dolman and R. Stewart, *Can. J. Chem.*, **45**, 911 (1967); H. K. Hall, Jr., *J. Amer. Chem. Soc.*, **79**, 5441 (1957); R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5864 (1966); J. Hine and R. D. Weimar, Jr., *J. Amer. Chem. Soc.*, **87**, 3387 (1965).]



for general acid catalysis of the dehydration of this intermediate by triethylenediamine cation must be between  $10^{10}$  and  $10^{12} M^{-1} \text{ sec}^{-1}$ , which is equal to or greater than the limiting value for a diffusion-controlled reaction.<sup>20</sup> Furthermore, a rate-determining diffusion-controlled proton transfer to the incipient hydroxide ion in the transition state should give a value of  $\alpha = 0$  ( $\beta = 1$ ) or at least a curvature of the Brønsted plot, instead of the observed  $\beta$  value of 0.71.

Alternatively, catalysis could occur through a "one-encounter" mechanism in which the catalyst is involved in a second proton transfer before it diffuses away from the reaction "cage." In the mechanism of eq 10, for example,  $\text{BH}^+$  may donate a proton to hydroxide ion before the products separate. However, there is no indication that both proton transfers occur in a bifunctional or "concerted" manner. The fact that no catalysis was observed with phosphate dianion shows that this molecule, which contains an acidic as well as a basic group, does not exhibit a significant positive deviation from the Brønsted plot and that bifunctional acid-base catalysis does not provide appreciable transition-state stabilization relative to monofunctional base catalysis.

The observed behavior is also reasonable, in more general terms, for the attack of nucleophiles on the imine group. Most reactions of imines involve attack of the nucleophile on the fully protonated imine, which may be aided by general base catalysis. Only when the imine is strongly activated by an electron-withdrawing group, such as the thiourea, substituted glycinamide, or benzene substituent of thiosemicarbazones, Girard hydrazones,<sup>21</sup> and benzylideneanilines, respectively, and a strongly basic molecule like hydroxide ion is the nucleophilic reagent, does attack occur without prior protonation. Even with such highly activated reactants, partial proton donation by water or a general acid catalyst may be required to bring about reaction. The situation is different from reactions at the carbonyl group in that oxygen can readily accept a negative charge and is weakly basic, so that attack of strongly basic nucleophiles on the carbonyl group occurs without proton donation ( $\alpha = 0$ ) and a large degree of proton transfer occurs only with very weakly basic nucleophiles, such as hydrogen peroxide.<sup>22</sup>

**Isotope Effects.** Under conditions in which the dehydration step is rate determining, catalysis of thiosemicarbazone formation by tertiary amines exhibits a deuterium isotope effect,  $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$ , of approximately 3. Since there is no isotope effect on the equilibrium constant for the addition of semicarbazide to *p*-chlorobenzaldehyde,<sup>4</sup> this observed isotope effect must reflect

a loss of zero-point energy of the proton which is being removed by the catalyzing base in the transition state; *i.e.*, the proton is not in a stable potential well similar to that of the carbinolamine. The greater stability of hydroxide ion in water compared to deuterioxide ion in deuterium oxide<sup>23a,b</sup> may contribute a factor of between 1.0 and 2.0 to the isotope effect, depending on the extent to which C–O bond breaking has occurred in the transition state, but this does not account for the observed isotope effect. The proton being transferred may lose a significant amount of zero-point energy in the transition state if it is (a) at the top of an asymmetric potential barrier,<sup>24</sup> (b) involved in a vibration coupled with heavier atoms,<sup>25</sup> or (c) in a potential well which is broadened compared to that of the starting material.<sup>26</sup> Elimination reactions which involve the breaking of a C–H bond frequently exhibit normal isotope effects, but an elimination reaction with an asymmetrically located proton in the transition state, as indicated by a  $\beta$  value of 0.88, exhibits a primary isotope effect of 3.8.<sup>27</sup> The difference in the stretching frequencies of an N–H bond between that of a neutral substituted hydrazine ( $3300\text{--}3400 \text{ cm}^{-1}$ )<sup>28a</sup> and a protonated amine ( $2700\text{--}2900 \text{ cm}^{-1}$ )<sup>28b</sup> corresponds to an isotope effect of 1.3–1.5. If proton transfer to the catalyst is 0.7 complete in the transition state, this would correspond to an isotope effect of 1.2 to 1.3, which, combined with a large secondary effect from the leaving lyoxide group, could account for much, or conceivably all, of the observed effect. However, if potential well broadening is responsible for the observed isotope effect, it is probable that additional broadening is brought about by hydrogen bonding between the catalyst and substrate in the transition state.

The isotope effect,  $K_{\text{H}_2\text{O}}/K_{\text{D}_2\text{O}}$ , on the ionization constants as acids of both thiosemicarbazide ( $\text{p}K_{\text{a}_{\text{H}_2\text{O}}} = 12.81$ ) and *p*-chlorobenzaldehyde thiosemicarbazone ( $\text{p}K_{\text{a}_{\text{H}_2\text{O}}} = 11.20$ ) is  $4.0 \pm 0.1$ . Comparison of this value with  $K_{\text{H}_2\text{O}}/K_{\text{D}_2\text{O}} = 2.89$  observed with hydrazoic acid<sup>29</sup> suggests a trend of increasing isotope effect with decreasing acidity of nitrogen acids. A similar trend has been observed with oxygen acids over a wide range of acidity, although there is no definite evidence for such a trend in the carboxylic acid series.<sup>30</sup>

(23) (a) C. A. Bunton and V. J. Shiner, Jr., *J. Amer. Chem. Soc.*, **83**, 3207 (1961); (b) L. J. Steffa and E. R. Thornton, *J. Amer. Chem. Soc.*, **89**, 6149 (1967).

(24) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).

(25) R. P. Bell, *Discussions Faraday Soc.*, **39**, 16 (1965); A. M. Katz and W. H. Saunders, Jr., *J. Amer. Chem. Soc.*, **91**, 4469 (1969).

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