merged. Equilibration was speeded by agitating manually a Pyrex-enclosed magnet in bulb G by means of a horseshoe magnet outside bulb G. The mercury level was kept adjusted at the etched line at F during this equilibration. Two thermoregulators were used so that equilibration of each sample of water and methyl halide could be carried out at two temperatures.

When the pressures had been determined, the total number of moles of methyl halide present and the number of moles present in the vapor above the solution at equilibrium could be calculated. The perfect gas law was used in the calculations. For methyl bromide the correction afforded by the use of the second virial coefficient is about 0.5%. For methyl chloride and fluoride the correction would be even smaller percentage-wise. Since the second virial coefficient of methyl iodide is apparently not available, it is not thought necessary to make this small correction. It could only change the magnitude of the results and not the isotope effect in any case. Therefore, both the pressure of the methyl halide at equilibrium and the concentration of methyl halide in solution could be calculated. The equilibrium constant in mm. M^{-1} is the ratio of these two quantities. The pressure is in mm. at 0°. The densities of light and heavy water were also necessary.[19]

After a run, the water and methyl halide were removed by distillation into a bulb immersed in liquid nitrogen. Since a small amount of mercury had condensed into bulb G in each run, there was a residue of mercury plus (probably) mercury halides in bulb G. However, the consistency of results (*e.g.*, methyl chloride runs 1 and 19 in Table III) suggests that no appreciable error was incurred from this source. Further, the vapor pressure of pure water, measured in the same way as the actual runs were performed except that no methyl halide was introduced, was nearly the same as the results recorded in the literature.

Our results agree quite well with those of Glew and Moelwyn-Hughes.[4] We have noted some inconsistencies in the Glew and Moelwyn-Hughes paper between equilibrium constants calculated from the equation they give and the experimentally observed constants recorded in their tables. These are probably attributable to typographical errors.

A sample calculation for run 28, methyl iodide–H_2O follows.[3] Methyl iodide initial pressure was 151.15 mm., or 150.11 mm. after a −1.04 mm. correction for expansion of mercury from 40.34 to 0°. Since the total volume of the cell in absence of water was 138.9 ml., this was 10.6655×10^{-4} mole. The volume of the cell corrected for 15.0230 g. water was 123.8 since the density of water at 40.34° is 0.99211 g. ml.$^{-1}$. The total pressure at equilibrium was 182.05 mm. After corrections of −1.25 mm. for expansion of mercury from 40.34 to 0° and −55.94 for the vapor pressure of water at 40.34°, the methyl iodide in the gas phase is 124.86 mm. (p_2) or 7.9070×10^{-4} mole. Therefore the concentration in solution (c_2) is 1.8217×10^{-2} M and the equilibrium constant is p_2/c_2 or 6854 mm. M^{-1}.

Table III gives equilibrium constants from all the runs. Table I gives thermodynamic quantities (for a standard state of 1 atm.) derived from the data in Table *III*. The value of $\Delta H°$ was computed by multiplication of the least squares straight-line slope of log (p_2/c_2) *vs.* $(1/T)$ by $2.303R$. The least-squares slope was calculated from the formula for a in the equation $y = ax + b$

$$a = (n\Sigma xy - \Sigma x\Sigma y)/(n\Sigma x^2 - (\Sigma x)^2)$$

where $x = 1/T$ and $y = \log(p_2/c_2)$. The squares of the deviations in y were thus minimized. This $\Delta H°$ is therefore only an approximation, since it is known from the work of Glew and Moelwyn-Hughes[4] that the plot is not linear. However, the results at three temperatures for methyl chloride in light and heavy water were plotted[3] as log-(p_2/c_2) *vs.* $1/T$ and it was found that when the three points for H_2O were fitted with a flexible curve, this same curve almost exactly fitted the points for D_2O when merely shifted along the $1/T$ axis. This is an interesting observation since it has been found that H_2O at one temperature has a viscosity like D_2O at a temperature 8.5° higher.[20] The curves for methyl chloride in H_2O and D_2O are also superimposable by a shift in the vertical direction. Therefore, it is probable that the two curves for each methyl halide have the same shape, and that the differences in thermodynamic quantities for reaction 1 are comparable between light and heavy water even though it has been assumed in the calculations that $\Delta H°$ is independent of temperature. The results for both methyl chloride and bromide at 50° were approximately corrected for hydrolysis, but hydrolysis contributed so much to the result for methyl bromide that the figures at 50° are very crude.

The standard deviations are given after the computed thermodynamic quantities in Table I. They were computed from the standard deviations σ_y and σ_a for the least-squares plot where

$$\sigma_y^2 = \Sigma(y_{\text{obs}} - y_{\text{calc}})^2/(n - 2) \text{ and } \sigma_a^2 = n\sigma_y^2/(n\Sigma x^2 - (\Sigma x)^2)$$

$\Delta F°$ was computed from the formula $\Delta F° = -RT \ln K$ where K is p_2/c_2, p_2 is the pressure in atm., R is 1.987 cal. mole^{-1} deg.$^{-1}$, and 0°C. is 273.15°K. $\Delta S°$ was then computed from $\Delta F° = \Delta H° - T\Delta S°$.

Acknowledgment.—We are grateful to Prof. R. F. W. Bader and Prof. W. H. Stockmayer for suggestions and discussion.

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[19] N. A. Lange, "Handbook of Chemistry," ed. 8, Handbook Publishers, Inc., Sandusky, Ohio, 1952, p. 1219; R. Schrader and K. Wirtz, *Z. Naturforsch.*, **6A**, 220 (1951).

[20] W. N. Baker, *J. Chem. Phys.*, **4**, 294 (1936); K. Wirtz, *Angew. Chem.*, **A59**, 138 (1947).

[CONTRIBUTION NO. 136 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

Nucleophilic Catalysis of Semicarbazone Formation by Anilines[1]

BY EUGENE H. CORDES AND WILLIAM P. JENCKS

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The following lines of evidence demonstrate that catalysis of semicarbazone formation by aniline and several of its ring-substituted derivatives proceeds *via* the rate-determining formation of a Schiff base between substrate and catalyst, followed by a rapid attack of semicarbazide on the Schiff base to yield the semicarbazone. (1) Catalysis by anilinium ions is very much more efficient than catalysis by other acids of comparable acid strength. (2) The rate of Schiff base formation accounts quantitatively for the rate of aniline-catalyzed semicarbazone formation. (3) Schiff bases react rapidly and quantitatively with dilute aqueous solutions of semicarbazide to yield the semicarbazone. (4) Except in very dilute solutions, the rate of aniline-catalyzed semicarbazone formation is independent of semicarbazide concentration. (5) The rate of aniline-catalyzed semicarbazone formation is the same as the rate of aniline-catalyzed oxime formation.

In a study of general acid catalysis of semicarbazone formation by a number of acids of varying structure and acid strength,[2] aniline and several of its ring-substituted derivatives were found to be 10–1000 times more effective catalysts than predicted from their pK_a values. Such abnormal catalytic ability suggested that anilines may function *via* a mechanism other than classical general acid catalysis. Evidence presented in

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[1] Presented at the 138th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1960 (Abs. 46P).

[2] E. H. Cordes and W. P. Jencks, unpublished experiments.

this paper supports the reaction sequence shown in eq. 1 and 2 as the pathway of aniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation. In this scheme aniline functions as a nucleophilic catalyst *via* the rate-limiting formation of a Schiff base. In a subsequent fast step the Schiff

$$\rangle C{=}O + ArNH_2 \longrightarrow \rangle C{=}N{-}Ar + H_2O \quad \text{slow} \tag{1}$$

$$\rangle C{=}N{-}Ar + NH_2NHCONH_2 \longrightarrow \rangle C{=}NNHCONH_2 + ArNH_2 \quad \text{fast} \tag{2}$$

base reacts with semicarbazide to yield the semicarbazone. In addition to demonstrating nucleophilic catalysis of reactions involving substrates at the oxidation level of the carbonyl group, the system provides a convenient method for the study of the mechanism of Schiff base formation in aqueous solution.

Experimental

Materials.—The following Schiff bases were prepared from the appropriately substituted benzaldehydes and anilines in 50% ethanol and recrystallized from absolute ethanol: *p*-chloro-N-*p*'-chlorobenzylideneaniline (m.p. 109–111°), N-*p*-chlorobenzylideneaniline (m.p. 60–62°), N-*p*-hydroxybenzylideneaniline (m.p. 90–91°), N-*p*-methylbenzylideneaniline (m.p. 44–45°), N-benzylideneaniline (m.p. 50–52°). Other reagents, except for reagent grade inorganic salts, were recrystallized or redistilled before use. Solutions of *p*-chlorobenzaldehyde were made up in 15% ethanol containing 10^{-4} *M* ethylenediaminetetraacetic acid to retard metal-catalyzed decomposition, and were stored at −15°. Such solutions were found to be stable for at least several days at this temperature. Solutions of semicarbazide and other nitrogen bases were prepared just prior to use. Glass-distilled water was used throughout.

Ultraviolet measurements were made on a Cary model 14 recording spectrophotometer or a Zeiss PMQ II spectrophotometer. Infrared measurements were made in CaF_2 cells, 0.05 mm. path length, with a Perkin–Elmer model 21 spectrophotometer.

Kinetic measurements were carried out at 25° as previously described.[3,4] Second-order rate constants were calculated from the slope of plots of pseudo-first-order rate constants against the concentration of the second reactant. Catalytic constants were obtained from the slope of plots of the first- or second-order rate constants against the concentration of the catalyst. In several cases, the complete reaction mixtures were shown to yield reproducible kinetic values over periods of time much longer than those required for the completion of the actual kinetic runs. The decomposition of the Schiff bases listed above in dilute solutions of semicarbazide to yield the free aldehyde and the corresponding semicarbazone was studied as follows: 2.9 ml. of an aqueous solution of semicarbazide was added to 0.1 ml. of 1×10^{-3} *M* Schiff base in absolute ethanol. Within 10 seconds after mixing the reactants, the solutions were read at the absorption maximum of the semicarbazone. Under these conditions no correction is necessary for the slow formation of semicarbazone resulting from reaction of excess semicarbazide with aldehyde liberated by the partial hydrolysis of the Schiff base (see Results section). The ionic strength of all reactions was maintained at 0.50 by the addition of KCl. The *p*H values were measured with the glass electrode and the Radiometer PHM-4b *p*H meter. All reactions were run in water containing 1 to 3% ethanol.

Analysis of Products.—An authentic sample of *p*-chlorobenzaldehyde semicarbazone (m.p. 229–232°) was prepared in 95% yield by direct condensation of semicarbazide hydrochloride and *p*-chlorobenzaldehyde in 50% ethanol at room temperature and recrystallization from absolute ethanol. The condensation of 420 mg. of *p*-chlorobenzaldehyde with excess semicarbazide hydrochloride in the presence of 300 mg. of *p*-chloroaniline yielded 517 mg. (97%) of semicarbazone with m.p. and mixed m.p. of 229–232°. The ultraviolet spectra of the two products were identical. The latter reaction proceeded approximately 70% *via* the *p*-chloroaniline-catalyzed reaction path as judged from previous kinetic experiments.

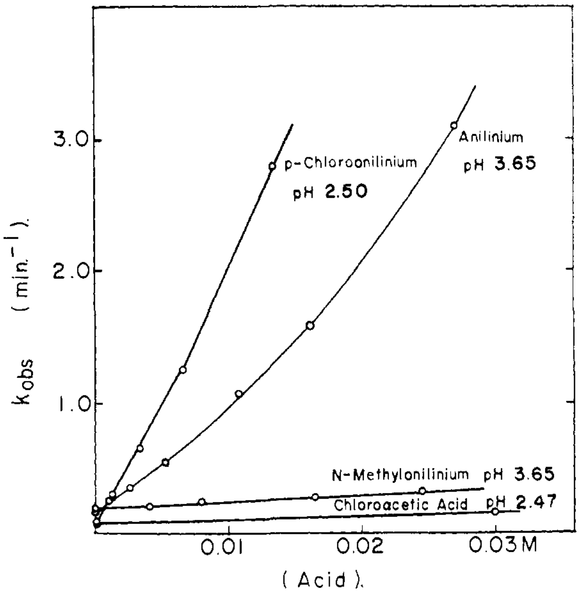

Fig. 1.—The rate of *p*-chlorobenzaldehyde semicarbazone formation as a function of the concentration of several acids at 25°: glycolate buffer, 0.02 *M*, *p*H 3.65; chloroacetate buffer, 0.02 *M*, *p*H 2.50; *p*-chlorobenzaldehyde 3.3×10^{-5} *M;* semicarbazide 0.0025 *M;* ionic strength maintained at 0.50 with KCl; followed at 284 mμ.

Results

The observed pseudo-first-order rate constants for *p*-chlorobenzaldehyde semicarbazone formation under acidic conditions are shown in Fig. 1 as a function of the concentration of several acids. The exceptionally effective catalysis by primary anilinium ions is in sharp contrast to the behavior of other ammonium ions of varying structure. For example, under similar conditions the catalytic constants for the conjugate acids of the secondary amines, imidazole, morpholine and N-methylaniline, are close to those predicted from a Brönsted plot with a slope of 0.2, which was obtained with a larger series of acids of varying structure.[2] The catalytic constants for *p*-OCH_3, *p*-CH_3, *p*-Cl, *o*-Cl, *o*-OCH_3 and H substituted anilinium ions lie one to three orders of magnitude above the line in the Brönsted plot. Some representative experimental data are given in Table I. The rate of aniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation increases more rapidly than the first power of the anilinium ion concentration (Fig. 1), suggesting that the catalyzed reaction is itself subject to general acid catalysis by a second molecule of catalyst.

In dilute aqueous solutions of semicarbazide, substituted N-benzylideneanilines react rapidly and quantitatively to form the aldehyde (the reverse of eq. 1) and the corresponding semicarbazone (eq. 2). The variation in the fraction of N-*p*-chlorobenzylideneaniline which partitions to the semicarbazone is shown in Fig. 2 as a function of semicarbazide concentration at *p*H 3.60 and

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(3) W. P. Jencks, *J. Am. Chem. Soc.*, **81**, 475 (1959).

(4) B. M. Anderson and W. P. Jencks, *ibid.*, **82**, 1773 (1960).

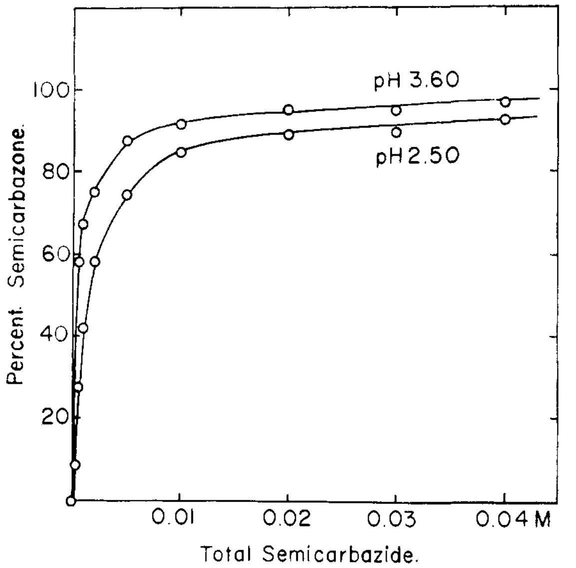

Fig. 2.—The partitioning of N-*p*-chlorobenzylideneaniline between water and semicarbazide as a function of semicarbazide concentration at 25°: glycolate buffer, 0.05 *M*, *p*H 3.60; chloroacetate buffer, 0.05 *M*, *p*H 2.50; N-*p*-chlorobenzylideneaniline 3.3 × 10^{-5} *M*; ionic strength maintained at 0.50 with KCl.

2.50. At semicarbazide concentrations above 0.01 *M*, the fraction of the Schiff base yielding the semicarbazone approaches 1. The decomposition of the *p*-NO_2, *p*-Cl, *p*-CH_3, *p*-OCH_3, *p*-OH, and H_2N-benzylideneanilines, substituted in the benzaldehyde portion of the molecule, is complete in less than 10 seconds under the conditions of these experiments. Under the same conditions, the half-time for the formation of the semicarbazone from the free aldehyde is more than 1.5 minutes (see the intercepts in Fig. 1). Formation of the semicarbazone from the Schiff base, therefore, does not involve the free aldehyde as an intermediate and must involve a direct attack of semicarbazide on the Schiff base. The partitioning of the Schiff bases listed above does not vary greatly with *p*H. Furthermore, the partitioning of N-*p*-chlorobenzylideneaniline was studied as a function of buffer strength and found not to vary significantly in the range 0.1 to 0.8 *M* cyanoacetate buffer. These results indicate that the reactions of water and semicarbazide with the Schiff base depend on the acidity and on general acid catalysis in a similar, but not identical, manner. Assuming that both water and semicarbazide attack predominantly the protonated Schiff base, it has been calculated that semicarbazide is some 2 × 10^5 times more reactive toward this substrate than water. Plots of the fraction of Schiff base yielding the semicarbazone, α, as a function of semicarbazide concentration were constructed for all the Schiff bases in order to allow the estimation of α at intermediate semicarbazide concentrations in subsequent experiments.

TABLE I

CATALYSIS OF *p*-CHLOROBENZALDEHYDE SEMICARBAZONE FORMATION BY VARIOUS RING-SUBSTITUTED ANILINES AT 25°[a]

Aniline	pK_a	Total concn., *M*	k_{obs}, min.$^{-1}$	k_{cat}, min.$^{-1}$	*p*H
o-Cl–	2.62	....	0.105		2.63[b]
		0.0010	.143	0.038	
p-Cl–	3.81	....	.099		2.60[c]
		.0035	.660	.561	
o-CH_3O–	4.49	....	.105		2.64[b]
		.0025	.192	.087	
p-CH_3O–	5.29	....	.105		2.69[b]
		.0025	.210	.105	
p-CH_3–	5.07	....	.151		3.48[d]
		.0020	.258	.107	
H–	4.56	....	.076		2.64[c]
		.0040	.266	.190	

[a] In the presence of 0.0025 *M* total semicarbazide; ionic strength maintained at 0.50 with KCl. [b] Chloroacetate buffer, 0.04 *M*. [c] Chloroacetate buffer, 0.02 *M*. [d] Glycolate buffer, 0.02 *M*.

The reaction of hydroxylamine with Schiff bases was also studied. While hydroxylamine reacts rapidly with N-*p*-chlorobenzylideneaniline to yield the oxime and the aldehyde, the fraction of Schiff base reacting with hydroxylamine decreases rapidly with decreasing *p*H at a given hydroxylamine concentration. Furthermore, at *p*H 2.30 the fraction of the Schiff base yielding the oxime was found to decrease as much as twofold in going from 0.1 to 0.8 *M* cyanoacetate buffer. These results suggest that the reactions of water and hydroxylamine with N-*p*-chlorobenzylideneaniline do not have a similar dependence on the concentration of hydronium ion or on general acid catalysis. These results are not unexpected, since the addition of hydroxylamine to the carbonyl group, in contrast to that of semicarbazide, shows only a small acid-catalyzed reaction and is not subject to general acid catalysis.[3] The addition of water to acetaldehyde, on the other hand, appears to depend almost completely on specific and general acid catalysis.[5]

The rate of aniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation in 0.04 to 0.12 *M* semicarbazide at *p*H 6.25 is independent of semicarbazide concentration (Table II). In each case, the rate of the aniline-catalyzed reaction is obtained by subtracting the rate of semicarbazone formation in an aniline-free control from that in the complete reaction mixture. At these concentrations of semicarbazide, the partitioning of N-*p*-chlorobenzylideneaniline is essentially 100% to the semicarbazone (α = 1).

The rate of *p*-chloroaniline-catalyzed semicarbazone formation was studied at *p*H 2.60 in the presence of lower semicarbazide concentrations (Fig. 3). A sixfold increase in total semicarbazide concentration, from 0.0025 to 0.015 *M*, results in a 75% increase in the rate of the *p*-chloroaniline-catalyzed reaction. α for *p*-chloro-N-*p*′-chlorobenzylideneaniline and the ratio of α and k_{cat} have been plotted in Fig. 3 as a function of semicarbazide concentration. The semicarbazide-independence of the ratio of α to k_{cat} demonstrates that the effect of semicarbazide concentration on the rate of the *p*-chloroaniline-catalyzed reaction is accounted for quantitatively by its

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[5] R. P. Bell and W. P. Higginson, *Proc. Roy. Soc.* (*London*), **A197**, 141 (1949).

TABLE II

RATE OF ANILINE-CATALYZED *p*-CHLOROBENZALDEHYDE SEMICARBAZONE FORMATION AS A FUNCTION OF SEMICARBAZIDE CONCENTRATION IN 0.05 *M* PHOSPHATE BUFFER, *p*H 6.25, AT 25°[a]

Semicarbazide, *M*	Aniline, *M*	k_{obs}, min.$^{-1}$	k_{cat}, min.$^{-1}$
0.04	0.00	0.234	
.04	.04	.693	0.46
.06	.00	.346	
.06	.04	.865	.52
.08	.00	.395	
.08	.04	.915	.52
.12	.00	.452	
.12	.04	.945	.49

[a] Ionic strength maintained at 0.50 by the addition of KCl.

effect on the partitioning of the Schiff base. The rate of *p*-chloroaniline-catalyzed semicarbazone formation is otherwise completely independent of semicarbazide concentration.

The rates of aniline-catalyzed semicarbazone and oxime formation under similar conditions were studied at two *p*H values (Table III). Under the conditions of these experiments, the partitioning of the Schiff base is nearly 100% to the semicarbazone or oxime, so that variations in α with *p*H cannot complicate the kinetics. The data in Table II show that, within experimental error, the rate of aniline-catalyzed semicarbazone formation is equal to that of aniline-catalyzed oxime formation.

TABLE III

THE EQUIVALENCE OF THE RATE OF ANILINE-CATALYZED SEMICARBAZONE AND OXIME FORMATION AT 25°[a]

Aniline,[b] *M*	Semicarbazide,[b] *M*	Hydroxylamine,[b] *M*	*p*H	k_{obs}, min.$^{-1}$	k_{cat}, min.$^{-1}$
..	0.01	..	5.14	0.392	
0.02	.01	..	5.15	3.05	2.60
..	..	0.01	5.12	0.505	
.02	..	.01	5.11	2.96	2.46
..	.01	..	4.77	0.65	
.008	.01	..	4.76	2.07	1.42
..	..	.01	4.76	0.56	
.008	..	.01	4.76	1.81	1.25

[a] *p*H maintained with 0.05 *M* acetate buffer; ionic strength maintained at 0.50 by the addition of KCl. [b] Total concentrations.

The equilibrium constant for N-*p*-chlorobenzylideneaniline formation in water at *p*H 4.56 and 25° was measured spectrophotometrically at 320 mμ by the method of Bloch-Chaudé.[6] Four aniline concentrations, ranging from 0.01 to 0.08 *M* free aniline, were used and a mean value of 4.6 M^{-1} was obtained.

The rate of hydrolysis of N-*p*-chlorobenzylideneaniline was measured in water in the *p*H range 5.10 to 14 and the pseudo-first-order rate constant in this *p*H region was found[7] to follow eq. 3.

$$k_{-1} = 3.4 \times 10^6 (\mathrm{H^+}) + 0.137 (\mathrm{OH^-}) + 0.0079 \quad (3)$$

The hydrolysis of this Schiff base, therefore, shows acid-catalyzed, base-catalyzed and uncatalyzed reactions similar to those observed by Holleck, Melkonian and Kastening.[8] The break in the *p*H profile in the acid-catalyzed region observed by Willi[9] for the hydrolysis of benzylideneaniline in 50% methanol apparently occurs at a *p*H at which the rate of hydrolysis is too fast to measure in the present case.[7]

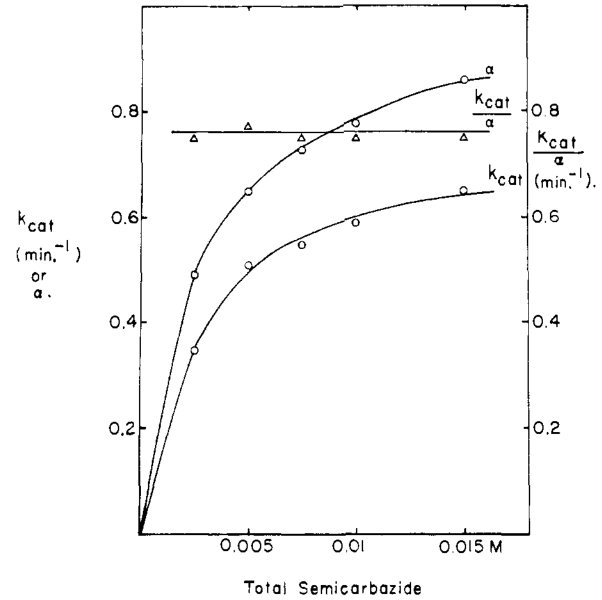

Fig. 3.—The rate of *p*-chloroaniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation as a function of semicarbazide concentration at 25°: *p*-chlorobenzaldehyde 3.3 × 10^{-5} *M;* total *p*-chloroaniline 0.0025 *M;* 0.04 *M* chloroacetate buffer, *p*H 2.61; ionic strength maintained at 0.50 with KCl.

The rate of N-*p*-chlorobenzylideneaniline formation cannot be measured directly in aqueous solution and has, therefore, been calculated and compared with the observed rate of aniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation as follows: At a given *p*H value, the pseudo-first-order rate constant for the formation of this Schiff base is given by

$$k_1 = K_{eq} \times k_{-1}[\text{aniline}]_{\text{free base}} \quad (4)$$

where k_{-1} is the first-order rate constant for the hydrolysis of the Schiff base at the given *p*H and K_{eq} is the equilibrium constant for the formation of the Schiff base. At *p*H 6.34, k_{-1} was found to be 1.78 min.$^{-1}$ and K_{eq} is 4.6 M^{-1}. At this *p*H and in the presence of 0.004 *M* free aniline, k_1 is calculated to be 0.033 min.$^{-1}$ from eq. 4. Under these conditions, the observed first-order rate constant for aniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation is 0.043 min.$^{-1}$. Under more acid conditions, the rate of Schiff base hydrolysis is too fast to allow a direct measurement of k_{-1}, but this quantity may be calculated from eq. 3. Using eq. 3 and 4, k_1 for Schiff base formation at *p*H 5.10 in the presence of 0.003 *M* free aniline is calculated to be 0.37 min.$^{-1}$; the observed pseudo-first-order rate constant for aniline-cata-

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(6) O. Bloch-Chaudé, *Compt. rend.*, **239**, 804 (1954).

(7) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 832 (1962).

(8) B. Kastening, L. Holleck and G. A. Melkonian, *Z. Elektrochem.*, **60**, 130 (1956).

(9) A. V. Willi, *Helv. Chim. Acta*, **39**, 1193 (1956).

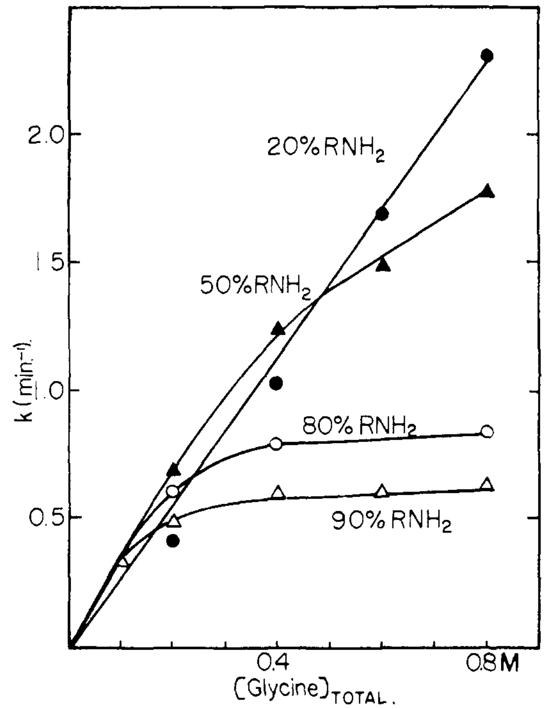

Fig. 4.—Catalysis by glycine buffers of the reaction between benzaldehyde and methoxyamine at 25°: followed at 271 mμ; methoxyamine 0.09 *M*, benzaldehyde 6 × 10^{-5} *M*, ionic strength maintained at 1.0 with KCl.

lyzed *p*-chlorobenzaldehyde semicarbazone formation under the same conditions is 0.30 min.$^{-1}$. These results indicate that the rate of aniline-catalyzed semicarbazone formation may be quantitatively accounted for, within the error of the experimental measurements and calculations, by the rate of Schiff base formation.

Glycine, a more basic primary amine, is also a very effective catalyst, at a higher *p*H, of the related reaction of benzaldehyde with methoxyamine (Fig. 4). With increasing *p*H the rate at low catalyst concentrations increases, but at high concentrations the rate becomes independent of glycine concentration. These results suggest a catalytic mechanism involving reaction of aldehyde with the free amino group of glycine to give the Schiff base, followed by an acid-catalyzed reaction of Schiff base with methoxyamine; at high concentrations of glycine anion the aldehyde is completely converted to Schiff base and the rate becomes independent of glycine concentration. This mechanism is further supported by the following observations: (1) addition of glycine anion to benzaldehyde in 1:1 dioxane–D_2O results in a decrease in the infrared carbonyl absorption band at 1700 cm.$^{-1}$ to less than 10% of its original absorbance and the appearance of C=N absorption at 1652 cm.$^{-1}$, with approximately one-half the intensity of the original C=O band; and (2) addition of glycine to an aqueous solution of benzaldehyde results in a very rapid shift of the absorption maximum from 250 to 248 mμ, accompanied by a 22% increase in extinction coefficient. The equilibrium constant for Schiff base formation was estimated to be 45 ± 5 M^{-1} from measurements of the increase in absorption at 250 mμ at intermediate glycine concentrations; this is of the same order as found by Zuman for similar reactions.[10]

Discussion

Nature of the Aniline Catalysis.—The exceptionally effective catalysis of semicarbazone formation by aniline and substituted anilines compared to other catalysts suggests that anilines do not function primarily as classical general acid catalysts. Three independent lines of evidence establish that aniline catalysis is nucleophilic, rather than classical general acid catalysis, and that it proceeds through a rate-limiting formation of Schiff base followed by the rapid formation of semicarbazone (eq. 1 and 2). First, the rate of Schiff base formation accounts quantitatively for the rate of aniline-catalyzed semicarbazone formation. Furthermore, the formation of semicarbazone from the Schiff base in dilute aqueous solutions of semicarbazide (eq. 2) is instantaneous and quantitative under the conditions of these experiments. Second, except for an effect on the partitioning of the Schiff base at low semicarbazide concentrations, the rate of aniline-catalyzed semicarbazone formation is independent of semicarbazide concentration. The activated complex for the rate-limiting step of the aniline-catalyzed reaction, therefore, does not contain semicarbazide. Third, the rate of aniline-catalyzed semicarbazone formation is the same as the rate of aniline-catalyzed oxime formation, demonstrating that the same rate-limiting step must be common to the two reactions.

Numerous examples are known of nucleophilic catalysis of reactions involving substrates at the oxidation level of saturated carbon and the carboxyl group, such as the iodide ion-catalyzed solvolysis of methyl bromide[11] and imidazole-catalyzed ester hydrolysis and acyl transfer reactions.[12] Nucleophilic catalysis of reactions involving substrates at the oxidation level of the carbonyl group is less well known. The most direct evidence for catalysis of this type has been obtained by Crowell and Peck in the case of *n*-butylammonium acetate-catalyzed carbon–carbon condensation of piperonal with nitromethane.[13] These workers demonstrated that the rate of piperonylidenebutylamine formation and the rate of attack of nitromethane on the Schiff base account for the over-all rate of 3,4-methylenedioxy-β-nitrostyrene appearance. Evidence has been obtained by Schroeder, *et al.*, which suggests that the aniline-catalyzed Michael condensation leading to the formation of warfarin may involve the formation of a Schiff base as a reactive intermediate.[14] Several other amine-catalyzed reactions, including secondary amine catalysis of the Knoevenagel reaction,[15] the transfer of N-glycosyl groups to aniline,[16] and the

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Mannich reaction[17] may involve the formation of the $\gt C{=}\overset{+}{N}\lt$ structure. The primary and secondary amine-catalyzed dealdolization of diacetone alcohol,[18] the primary amine-catalyzed decarboxylation of β-keto acids,[19] and the Amadori rearrangement[20] all appear to involve the formation of the $\gt C{=}\overset{+}{N}\lt$ structure, but this group functions as an intramolecular electron sink in these reactions and the nature of the catalytic process is, therefore, different from that in the present case.

Nucleophilic catalysis of reactions involving carbon–nitrogen condensations have, as a second step, a "*trans*-Schiffization" reaction. Such transfer reactions have been demonstrated for several aromatic Schiff bases in non-aqueous solvents[21] and for a pyridoxal derivative formed in the course of a more complex reaction.[22] Jenkins and Sizer have reported that glutamic–aspartic transaminase behaves spectrally as an acid–base indicator, pK_a 6.2,[23] in a manner similar to pyridoxal phosphate imines rather than pyridoxal phosphate itself,[24] suggesting that enzyme-bound pyridoxal phosphate is present in the form of a Schiff base and, consequently, that a "*trans*-Schiffization" is involved in the catalytic process of this enzyme.[23,25]

Efficient nucleophilic catalysis of the over-all reaction between nucleophilic reagents and the carbonyl group generally requires that the following conditions be met: (1) the catalyst must be more reactive toward the substrate than the final acceptor; (2) the catalyst–substrate intermediate must be more susceptible to attack by the final acceptor than the original substrate; (3) the equilibrium constant for the formation of the catalyst–substrate intermediate must be smaller than that for the formation of the final product. The first two conditions ensure that the rate of the catalyzed reaction will be greater than that of the uncatalyzed reaction; the third condition guarantees that the reaction product will not be changed by the addition of catalyst.

In the case of aniline-catalyzed *p*-chlorobenzaldehyde semicarbazone formation, these conditions are fulfilled as follows: (1) aniline and its ring-substituted derivatives are considerably more reactive than semicarbazide toward *p*-chlorobenzaldehyde; (2) the rate of attack of semicarbazide on Schiff bases is several orders of magnitude more rapid than the rate of attack of semicarbazide on the free aldehydes. This large rate difference is probably principally the result of the much greater basicity of Schiff bases compared to aldehydes, despite the fact that the protonated aldehyde would be expected to be much more reactive than the protonated Schiff base; (3) the most striking feature of aniline catalysis of semicarbazone formation is the gross disparity in the equilibrium constants for the formation of N-*p*-chlorobenzylideneaniline and *p*-chlorobenzaldehyde semicarbazone. The equilibrium constant for the formation of the semicarbazone is approximately $1 \times 10^6\ M^{-1}$,[26] or about 2×10^5 greater than that for the formation of the Schiff base, despite the fact that the formation of the latter proceeds more rapidly. Thus, in dilute aqueous solutions no appreciable concentration of the Schiff base will exist at equilibrium, in contrast to the situation in the presence of semicarbazide, in which no appreciable concentration of the free aldehyde exists at equilibrium. It may be noted that a molecule acting as a nucleophilic catalyst must have unusual properties, since most factors which tend to increase the kinetic reactivity of a reactant tend to increase the thermodynamic stability of the product. The thermodynamic instability of the Schiff base relative to the starting materials may partly reflect the loss of conjugation between the ring electrons and the unshared electron pair of the nitrogen atom of the aniline molecule, due to rehybridization of the orbitals of the nitrogen atom.

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