Catalytic Reactions Involving Azomethines. IX.[1] General Base Catalysis of the Transamination of 3-Hydroxypyridine-4-aldehyde by Alanine

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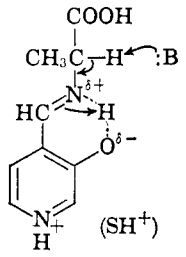
Abstract: The transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine has been studied in aqueous acetate, formate, imidazole, and phosphate buffers in the absence of metal ions at 30° (μ = 1 with KCl). The yield of the keto acid product of the transamination reaction, pyruvic acid, has been determined by polarographic means to be nearly quantitative in all buffers studied over the pH range 3 to 8. The pseudo-first-order rate constants (k_{obsd}) for transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine-d_4 in half-neutralized 1 *M* imidazole and acetate buffers were *ca.* six- to sevenfold smaller than those obtained with DL-alanine. From a knowledge of the acid dissociation constants of aldehyde, aldimine, amino acid, and buffer species and one equilibrium constant for aldimine formation (part VII), the rate constants for the general base catalyzed conversion of aldimine species into the transamination products can be calculated. The slopes (β) of the Brønsted plots for general base catalysis of aldimines SH^+, S^+, and S (Chart I) are in the reverse order of the expected stability of the intermediate carbanions. The aldimine species in half-neutralized acetate buffer racemized as fast as they underwent transamination. Therefore, the mechanism of transamination can be visualized as the general base catalyzed abstraction of the proton from the α-carbon of aldimines to form a carbanion or intimate ion pair which can be reprotonated on the α-carbon to give a racemized aldimine or protonated on the methine carbon to give a ketimine. The protonation of the methine carbon might be accomplished intramolecularly by the 3-hydroxyl group or intermolecularly by a general acid catalyst.

It has been established in papers VII[1g] and VIII[1h] in this series that DL-alanine and 3-hydroxypyridine-4-aldehyde react rapidly to form an aldimine which then undergoes a slow proton-transfer reaction to form a ketimine (H_2O, 30°; μ = 1.0 with KCl). The yields of pyruvic acid, and thus the degree of transamination, have been determined quantitatively by both polarographic means and by formation of derivatives. The slow step in the transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine has been suggested[1h] to be the conversion of aldimine to ketimine which involves abstraction of the proton from the α-carbon of the aldimine (1). The 3-hydroxyl group should



(1)

stabilize the transition state for the formation of a carbanion by placing a partial positive charge on the azomethine nitrogen and may intramolecularly protonate the methine carbon. This mechanism is in agreement with the kinetic demonstration of catalysis by amino acid, the large deuterium isotope effect obtained when DL-alanine-d_4 is substituted for DL-alanine, and the necessity of the 3-hydroxyl group for prototropy.[1h]

In order to further establish general base catalysis, it was decided to investigate the transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine in acetate, formate, imidazole, and phosphate buffers. From the results of parts VII and VIII of this series the rate constants for the conversion of each aldimine species into products can be determined,[1h] and thus the sensitivity of each aldimine species to general base catalyzed prototropy established *via* the Brønsted catalysis law. For further understanding of the general base catalyzed prototropy, the rates of racemization and transamination of the aldimines of 3-hydroxypyridine-4-aldehyde and alanine have been compared and the deuterium isotope effect determined when alanine-d_4 is substituted for alanine, employing acetate and imidazole buffers.

Experimental Section

Materials. For purity of 3-hydroxypyridine-4-aldehyde and DL-alanine see paper VII[1g] in this series. Purity of DL-alanine-d_4 (Merck Sharp and Dohme, Canada) was checked by nmr. The fully deuterated alanine is believed to be at least 95% α-deuterated DL-alanine (Calbiochem, A grade) and was used without further purification. Imidazole (Eastman, White Label) was twice crystallized from acetone–petroleum ether (bp 30–60°), dried, and stored over P_2O_5 until used. A sample of this material was subjected to a quantitative spectrographic analysis (Lucius Pitkin, Inc., N. Y.) with the following results. Present, but less than 0.001%, were silicon, silver, copper, barium, magnesium, calcium, and cadmium. Present, but less than 0.0001%, were aluminum, bismuth, lead, and iron. Absent (not detectable) were zinc, indium, tin, antimony, arsenic, phosphorus, thallium, gallium, germanium, manganese, nickel, chromium, cobalt, molybdenum, vanadium, tungsten, titanium, zirconium, sodium, strontium, potassium, and lithium. Dipotassium phosphate (Baker Analyzed Reagent) was dried at 110° for 24 hr prior to preparing buffers. Potassium chloride and potassium hydroxide were analytical reagent grade chemicals. The disodium salt of ethylenediaminetetraacetic acid was Fischer Certified Reagent. Acetic and formic acid (Baker Ana-

(1) For parts I, II, and III of this study see: (a) T. C. Bruice and R. M. Topping, *J. Am. Chem. Soc.*, **85**, 1480 (1963); (b) *ibid.*, **85**, 1488 (1963); (c) *ibid.*, **85**, 1493 (1963); for part IV: (d) T. C. French and T. C. Bruice, *Biochemistry*, **3**, 1589 (1964); part V: (e) T. C. French, D. S. Auld, and T. C. Bruice, *ibid.*, **4**, 77 (1965); part VI: (f) J. W. Thanassi, A. R. Butler, and T. C. Bruice, *ibid.*, **4**, 1463 (1965); parts VII and VIII: (g) D. S. Auld and T. C. Bruice, *J. Am. Chem. Soc.*, **89**, 2083 (1967); (h) *ibid.*, **89**, 2090 (1967).

(2) National Institutes of Health Predoctoral Fellow, 1963–1966. Part of the work to be submitted by D. S. Auld in partial fulfillment for the Ph.D. Degree, Cornell University.

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lyzed Reagent) were distilled from a glass apparatus prior to use. The water used to prepare solutions was deionized, then triply distilled from an all-glass apparatus and stored under nitrogen.

Apparatus. The spectrophotometer employed for rates was either a Zeiss M4Q III monochromator or a Beckman DU monochromator combined with a Gilford multiple-sample, absorbance recorder. The combination Beckman–Gilford spectrophotometer was equipped with dual wavelength control enabling the recording of the decreasing absorbance at 390 mμ and the increasing absorbance at 320 mμ at alternate times. The time interval between reading was *ca.* 10 min, during which time the light passed through the blank solution only. The time interval for reading a sample's optical density was 5 sec. Water of a constant temperature of 30 ± 0.1° was circulated through the cell compartment walls. The polarimeter used for optical rotation studies was a Perkin-Elmer Model 141 equipped with water-jacketed cells. The polarograph used for product analysis was a Sargent Model 1600. A water-jacketed cell was designed to be used with the latter instrument. All pH measurements were made with a Radiometer Model 22 pH meter equipped with a Radiometer Model PHA 630 Pa scale expander. The combined glass–calomel electrode (Radiometer GK 2021C) and electrode cell compartment were thermostated at 30 ± 0.1°.

Kinetics. All kinetic measurements in this paper were carried out at 30 ± 0.1° in deionized, triply distilled water at a calculated ionic strength of 1.0 (with KCl) under the pseudo-first-order conditions of $[A_T] >> [PCHO_T]$. Stock solutions of the aldehyde were 0.025 *M* and were kept frozen when not in use. No solution was kept longer than 1 week. Buffers employed were acetate, formate, imidazole, and phosphate. Normally six concentrations of buffer over a tenfold dilution at a fixed concentration of DL-alanine (1.0 *M*) were run at several pH's. Stock aldehyde solution (0.20 ml) was added to a solution of DL-alanine and buffer and a dilution made to 10 ml. The concentration of EDTA was 5.0×10^{-3} *M* in all kinetic experiments. The pH was then recorded and a portion of the solution transferred to a 2-ml ⨍ stoppered cuvette. Nitrogen was bubbled in for *ca.* 2 min, and the cells were stoppered, sealed with parafilm, and equilibrated at 30° in the cell housing of the spectrophotometer for *ca.* 10 min. Optical density was then read alternatingly at 390 and 320 mμ at a fixed time interval.

In all spectrophotometric experiments the ratio of molar concentrations of alanine to 3-hydroxypyridine-4-aldehyde was at least 2000:1 so that the concentration of DL-alanine would remain constant in the reaction and pseudo-first-order kinetics should be obtained. Pseudo-first-order rate constants were obtained from plots of $\log (OD_0 - OD_\infty)/(OD_t - OD_\infty)$ *vs.* time for 390 mμ and plots of $\log (OD_\infty - OD_0)/(OD_\infty - OD_t)$ *vs.* time for 320 mμ. Good first-order kinetics were obtained to at least 70% completion of reaction for acetate and formate and to 40–50% completion for imidazole and phosphate buffers.

For the kinetic studies carried out in the polarimeter, solutions were 1.0 *M* in acetic acid and L-alanine, 5×10^{-3} *M* in EDTA, and 10^{-2} *M* in aldehyde at pH 4.70. The blank was the equivalent solution in the absence of aldehyde. Pseudo-first-order rate constants were obtained from plots of $\log (r_0 - r_\infty)/(r_t - r_\infty)$ *vs.* time at 586, 578, and 546 mμ where *r* is an optical rotation reading.

Product Analyses. The keto acid formed in the transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine is pyruvic acid. This product was determined quantitatively by a polarographic method. Solutions 0.5 or 0.25 *M* in external buffer (acetate, formate, or imidazole), 2.5×10^{-3} *M* in EDTA, 0.5 *M* in DL-alanine, and 4×10^{-5} *M* to 4×10^{-4} *M* in pyruvic acid were prepared. The pH was 2.75 and the ionic strength 0.67. These solutions were analyzed on the polarograph at 0.02 μa/mm, $t = 2.82 \pm 0.02$ sec^{-1}, and $T = 30.0 \pm 0.1°$. A linear relationship between concentration of pyruvic acid and diffusion currents was obtained at the half-wave potential of −0.75 v. The spectrophotometric reaction mixtures were analyzed in the following manner. The remainder of the 10-ml reaction solution not used for the spectrophotometric run was placed in a black-taped, screw-cap vial, nitrogen bubbled in for 2 or 3 min, and the vial capped, wrapped with parafilm, and placed in a constant temperature bath at 30.0 ± 0.1°. After at least 99% completion of reaction a 5-ml sample was withdrawn and added to a 10-ml volumetric flask. The appropriate amounts of 3 *N* HCl and 4.0 *M* KCl were added to obtain the proper pH and ionic strength and a dilution was made to 10 ml. The final pH was 2.75 ± 0.02 and the ionic strength was 0.67. The solutions were analyzed on the polarograph under the same conditions as the knowns. In this manner each kinetic experiment reported in this paper at 1.0 and 0.5 *M* external buffer was quantitatively analyzed for pyruvate.

Results

Spectral time studies for the transamination of 3-hydroxypyridine-4-aldehyde by 1.0 *M* DL-alanine in 1.0 *M* acetate buffer at pH 4.70 and 1.0 *M* imidazole buffer at pH 6.19 are shown in Figures 1 and 2, respectively.

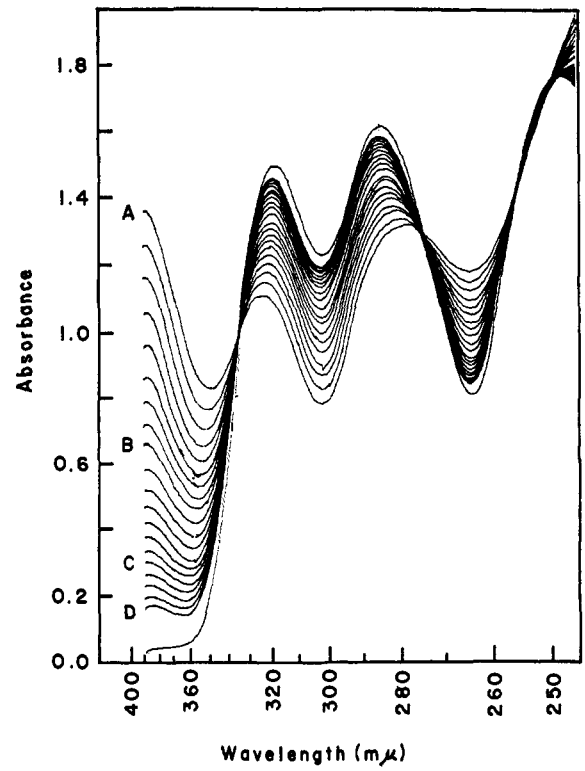


Figure 1. Spectral time study at pH 4.70 for transamination of 3-hydroxypyridine-4-aldehyde (5×10^{-4} *M*) by DL-alanine (1.0 *M*) in 1.0 *M* acetate buffer: [EDTA] = 5×10^{-3} *M*; temperature, 30°; $\mu = 1.0$. Time intervals are for A–B, 15.7 min; B–C, 24.2 min; C–D, 33.8 min.

The figures are photographs of the actual runs recorded on vellum. Tight isosbestic points are obtained at 335, 275, 256, and 250 mμ for the acetate system and at 341 and 284 mμ for the imidazole system. Since the ultraviolet absorption spectrum of the starting aldimine species and product species are different at these two pH's, changes in isosbestic points would be expected. Pseudo-first-order kinetics were normally calculated at 390 and 320 mμ. Plots of the decreasing absorbance at 390 mμ *vs.* the increasing absorbance at 320 mμ were linear to completion of reaction. Since the formation of pyruvic acid was essentially quantitative, the final ultraviolet absorption spectrum must be that of the pyridoxamine analog, 3-hydroxy-4-aminomethylpyridine (λ_{max}'s at 320 and 284 mμ, Figures 1 and 2). Acetate-buffered reactions yielded a 20–30% decrease in rate by introducing 1×10^{-3} *M* EDTA but further increases to 1×10^{-2} *M* caused no further change in rate. All kinetic studies reported in this paper were, therefore, run in the presence of 5×10^{-3} *M* EDTA as a safeguard against possible interference from metal ions. The imidazole used was checked spectrographically for trace metal ions. The results of this analysis are given in the Experimental Section.

Examples of the dependence of the observed pseudo-first-order rate constants on buffer dilutions for imid-

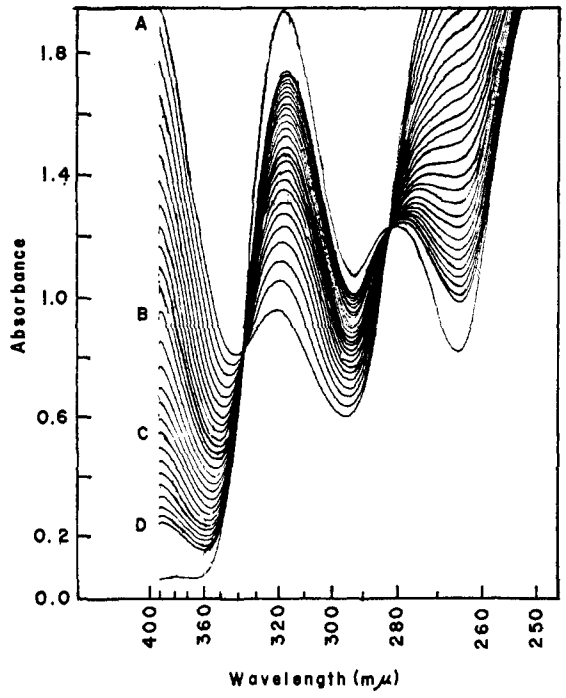


Figure 2. Spectral time study at pH 6.19 for the transamination of 3-hydroxypyridine-4-aldehyde (5×10^{-4} *M*) by DL-alanine (1.0 *M*) in 1.0 *M* imidazole buffer: [EDTA] = 5×10^{-3} *M*; temperature 30°; μ = 1.0. Time intervals are for A–B, 15.7 min; B–C, 23.8 min; C–D, 33.8 min.

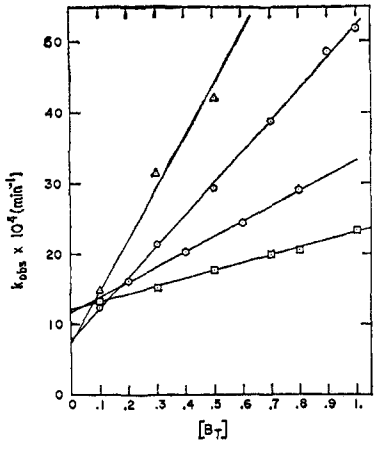


Figure 3. Plots of the pseudo-first-order rate constants (k_{obsd}) *vs.* [B_T] for the general base catalyzed transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine: phosphate, △, pH 6.40; acetate, ○, pH 5.02; formate, ○, pH 4.25; imidazole, □, pH 8.05.

azole, acetate, formate, and phosphate at 1.0 *M* DL-alanine are shown in Figure 3. The relationship between the rate of disappearance of aldehyde and the concentration of buffer ([B_T]) is shown in eq 2 and 3 (for abbreviations see Chart I of the previous paper).

$$v = d[PCHO_T]/dt = k_B[S_T][B_T] + k_{AH}[S_T][A_T] + k_{H_2O}[S_T] \quad (2)$$

$$v = (k_B K_{pH}[B_T] + k_{AH}K_{pH}[A_T] + k_{H_2O}K_{pH})[PCHO_T][A_T] \quad (3)$$

Since

$$K_{pH} = [S_T]/[PCHO_T][A_T] \quad (4)$$

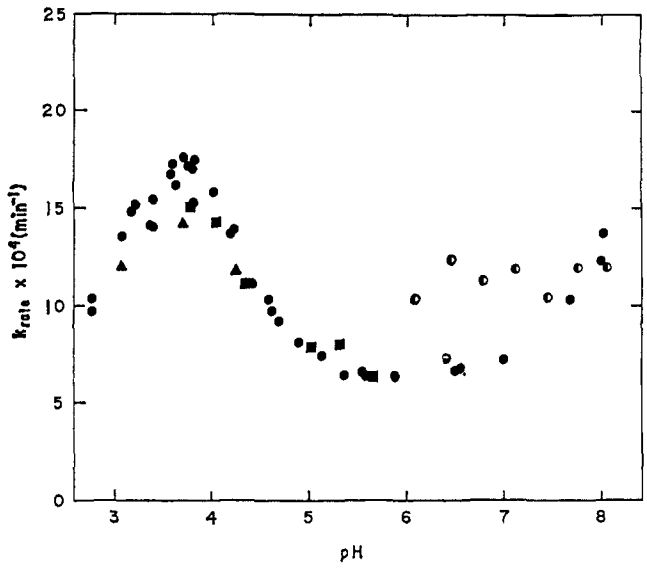


Figure 4. pH–pseudo-first-order rate constant (k_{rate}) profile at 1.0 *M* DL-alanine for the transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine. All rate constants calculated at 390 mμ. Buffered with alanine$^{1}_{h}$ only (k_{rate} = observed rate constant at 1.0 *M* DL-alanine), ●. For the following buffers $k_{rate} = k_Y$ (see eq 5); formate buffer dilution intercepts, ▲; acetate buffer dilution intercepts, ■; imidazole buffer dilution intercepts, ◐; phosphate buffer dilution intercepts, ◒.

The total concentration of aldehyde species, aldimine species, and amino acid species in solution are denoted as [$PCHO_T$], [S_T], and [A_T], respectively. If the rates of transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine are carried out at fixed pH and DL-alanine concentration (1.0 *M*) but at various buffer concentrations the expression for k_{obsd} will be

$$k_{obsd} = (k_B'[B_T] + k_Y)[A_T] \quad (5)$$

where $k_B' = k_B K_{pH}$ and k_Y is the pseudo-first-order rate constant for transamination at 1.0 *M* DL-alanine in the absence of any buffer other than DL-alanine.

The slopes (k_B') and intercepts (k_Y) of plots of k_{obsd} *vs.* [B_T] at [A_T] = 1.0 *M* were calculated with the aid of a least-squares program. The errors in the slopes and intercepts were calculated from the standard error of estimate, standard deviations, and the Student t distribution at the 70% confidence level. The linear dependence of the pseudo-first-order rate constants (k_{obsd}) on total buffer concentration indicates that there are no detectable terms second order in buffer species, in contrast with the pyridoxal–phenylglycine system investigated by Bruice and Topping.[1a] The intercept values (k_Y) for the acetate, formate, and phosphate buffers were identical within experimental error with the determined rate constants obtained for reaction mixtures buffered only with 1.0 *M* DL-alanine (Figure 4). Although values of k_Y for imidazole were similar to the rate constants for the kinetic runs buffered only with DL-alanine (1.0 *M*) above pH 7.1, the imidazole intercepts were consistently high by *ca.* 50–75% in the pH region 6.1–7.1. The errors in the intercept of the least squares lines for the plots of k_{obsd} *vs.* concentration of imidazole in this pH region are 5–6%. For these pH's there appeared to be slight downward curvature in the plots which might account for the high values of k_Y. Substitution of imidazolium ion for potassium ion in the buffer dilution may have caused this effect.

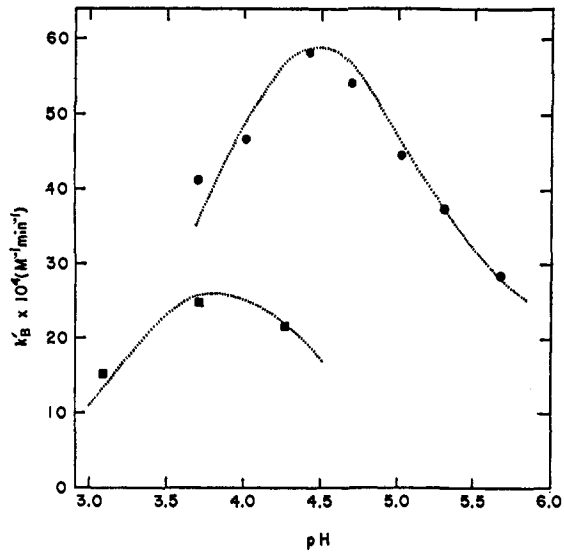


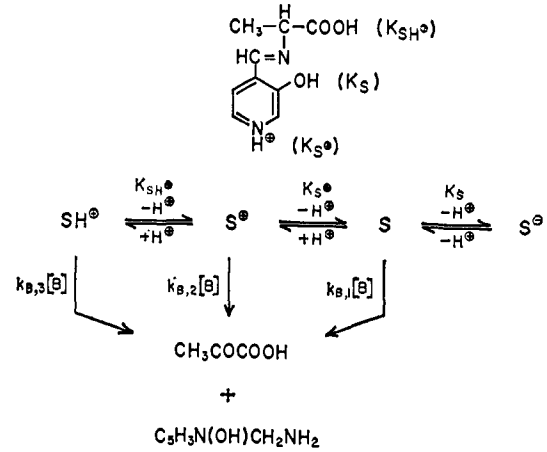
Figure 5. pH–second-order rate constant profile for acetate catalysis, ●, and formate catalysis, ■, of the transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine. The theoretical lines are calculated from eq 7 with the aid of the constants in Table I.

A derivation of an equation for the pH dependence of (k_B') is obtained by using the same kinetic scheme employed for water and alanine catalysis.[1h] For general base catalyzed prototropy of aldimines SH^+, S^+, and S (Chart I) the rate constant expression is

$$k_{B_T} = (k_{B,3}[SH^+] + k_{B,2}[S^+] + k_{B,1}[S])[B] \quad (6)$$

where $k_{B_T} = k_B'[PCHO_T][A_T][B_T]$ and [B] is the contration of free base species. Using Chart I of the pre-

Chart I



vious paper as a guide, eq 7 can be derived in the same manner as was shown previously for the water and DL-alanine catalysis.[1h] The fitting of eq 7 to provide a

$$k_B' = QR/UVX \quad (7)$$

where

$$Q = k_{B,3}'a_H^3 + k_{B,2}a_H^2 + k_{B,1}K_{S^+}a_H$$

$$R = KK_{PCHO^+}K_{PCHO}K_{AH}K_{AH_2}K_{BH}/K_{S^+}K_S$$

$$U = \left(\frac{K_{PCHO^+}}{K_B} + 1\right)a_H^2 + K_{PCHO^+}(K_Z + 1)a_H + K_{PCHO}K_{PCHO^+}$$

$$V = a_H^2 + K_{AH_1}a_H + K_{AH_2}K_{AH}$$

$$X = K_{BH} + a_H$$

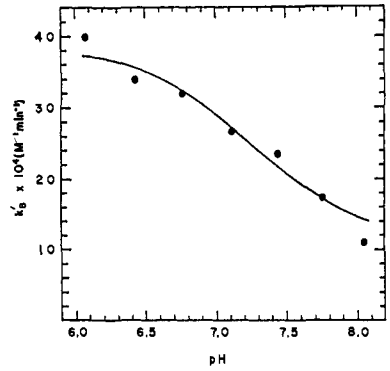


Figure 6. pH–second-order rate constant profile for imidazole catalysis of the transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine. The theoretical line is calculated from eq 7 with the aid of the constants in Table I.

pH–rate profile for k_B' was carried out with the aid of an IBM 1620 digital computer. The order of criteria for best fit was: (a) the sum of the calculated rate constants that differed from the observed rate constants by more than three times the experimental error at the 70% confidence level was made a minimum; (b) the sum that differed by less than twice the experimental error was made a maximum; and (c) the sum of the absolute value of (observed rate constant − calculated rate constants)/observed error was made a minimum. The values of K, pK_S, and pK_{AH} determined previously[1g] were held constant throughout the fitting at 25.80 M^{-1}, 9.17, and 9.62, respectively. The pK_a of the conjugate acid of the buffer (pK_{BH}) was initially taken as that value of pH obtained at half-neutralization of the buffer. The values of all other constants except rate constants were set near the best values obtained from the theoretical fitting of the pH–rate constant profiles for water and DL-alanine catalysis of prototropy[1h] ($pK_{S^+} = 5.25$, $pK_{PCHO^+} = 3.30$, $pK_{PCHO} = 6.55$, $pK_B = 4.22$, $K_Z = 0.50$, and $K_{AH_2} = 2.45$). The rate constants $k_{B,3}'$, $k_{B,2}$, and $k_{B,1}$ (Chart I) were then allowed to vary over wide ranges, holding all other constants fixed at their initial values until the order of magnitude of the rate constants were obtained. All constants in (7) were then allowed to vary by an iteration procedure except K, pK_S, and pK_{AH}. The latter were not varied since they cannot affect the shape of the profile. The iteration program used was described in part VIII of this series.[1h] The results of the fitting of eq 7 to plots of k_B' *vs.* pH for acetate, formate, and imidazole are shown in Figures 5 and 6 and Table I.

Determination of Pyruvic Acid. Analysis for pyruvic acid was carried out with the aid of a polarograph on each spectrophotometric kinetic reaction between DL-alanine and 3-hydroxypyridine-4-aldehyde in 1.0 and 0.5 M buffers at better than 99% completion of reaction. All buffer-catalyzed reactions that were analyzed provided high yields of pyruvic acid. The amount of pyruvic acid formed was not pH dependent. Thus, the average yields at seven pH's (from pH 6.0 to 8.0) for 1.0 M imidazole and seven pH's (from pH 3.7 to 5.6) for 1.0 M acetate were 99 ± 4 and 90 ± 4%, respectively. The average yields for all pH's for 0.5 M imidazole and 0.5 M acetate were 91 ± 5 and 88 ± 5%, respectively. Analyses were carried out at three pH's (from pH 3.0 to 4.3) for formate buffers at 1.0

Table I. Best Values of the Constants for the pH–Rate Constant (k_B') Profiles for the General Base Catalyzed Transamination of 3-Hydroxypyridine-4-aldehyde by DL-Alanine

Base	M^{-2} min^{-1} × 10^4 $k'_{B,3}$	M^{-1} min^{-1} × 10^4 $k_{B,2}$	$k_{B,1}$	pK_{PCHO}^+	pK_{PCHO}	pK_B	$pK_{AH_2}^+$	pK_S^+	K_Z	pK_{BH}	Error test[a] N1	N2	N3	NG3
Imidazole	1.50×10^9	390	1.20	3.26	6.55	4.18	2.43	5.25	0.45	7.09	4	2	0	1
Formate	2.00×10^6	15	...	3.30	6.53_5	4.15	2.34	5.25	0.55	3.49	2	1	0	0
Acetate	1.60×10^7	64	0.85	3.39	6.53_5	4.18	2.56	5.25	0.40	4.61	2	3	1	1

[a] The number of points in the theoretical calculations within one (N1), two (N2), three (N3), and greater than three times (NG3) the observed error at the 70% confidence level are designated in the fashion: error tests N1, N2, N3 and NG3.

and 0.4 M, the average yields of pyruvic acid being 83 ± 2 and 83 ± 7%, respectively. There was no second reduction wave at −0.94 v indicative of a side reaction observed without buffer[1h] in the pH region 4.6 to 5.6 for 5×10^{-4} M 3-hydroxypyridine-4-aldehyde and amino acid at 1.0 M in acetate buffers, although this half-wave was present when the aldehyde concentration was raised to 0.02 M.

Deuterium Isotope Effect. The rates of transamination as measured at 390 mμ were compared for DL-alanine-d_4 and DL-alanine at 1.0 M under identical conditions in *ca.* half-neutralized 1 M acetate and imidazole buffers. The optical densities extrapolated to zero time were essentially the same for the reactions carried out with both light and heavy alanine, indicating the concentrations of deuterated and undeuterated aldimines to be equivalent if their extinction coefficients are equivalent. The kinetic isotope effect (k_{obsd}^H/k_{obsd}^D) was 6.06 for the acetate buffer and 6.93 for the imidazole buffer. Since under these conditions most of the transamination reaction must be proceeding through the buffer-catalyzed pathway (see Table II), the observed deuterium isotope effects pertain mainly to this pathway.

Table II. Deuterium Isotope Effect on the Rates of Buffer Catalysis of the Transamination of 3-Hydroxypyridine-4-aldehyde by DL-Alanine

Buffer	pH	$k_{obsd} \times 10^4$ min^{-1}
1.0 M imidazole + $CH_3CH(NH_3^+)CO_2^-$	7.05	39.0
1.0 M imidazole + $CD_3CD(NH_3^+)CO_2^-$	7.05	5.62
1.0 M $CH_3CH(NH_3^+)CO_2^-$	6.99	7.3
1.0 M acetate + $CH_3CH(NH_3^+)CO_2^-$	4.70	58.6
1.0 M acetate + $CD_3CD(NH_3^+)CO_2^-$	4.70	9.62
1.0 M $CH_3CH(NH_3^+)CO_2^-$	4.62	9.8

Rates by Optical Rotation Measurements. The loss of optical activity during the reaction of L-alanine (1.0 M) with 3-hydroxypyridine-4-aldehyde (10^{-2} M) in 1.0 M acetate buffer (pH 4.70) was followed at 546 578, and 589 mμ. Though amino acid was present in excess the very large specific rotations of aldimine made the measurements possible. The average pseudo-first-order rate constant measured at the three wavelengths was 101 ± 4 × 10^{-4} min^{-1}. The observed loss in optical activity cannot be due to racemization of unreacted L-alanine under the reaction conditions since the optical rotation of a solution of 1.0 M L-alanine in the absence of 3-hydroxypyridine-4-aldehyde did not change during a 24-hr period of time. Doubling the concentration of aldehyde, all other conditions remaining constant, doubled the change in optical rotation observed, but left the rate unaffected. Thus, it would appear that the change in optical rotation is due to removal of a proton from the α-carbon of the aldimine since doubling the total initial concentration of aldehyde ([$PCHO_i$]) should double the equilibrium concentration of aldimine ([S_e]), all other conditions remaining constant (eq 8 and 9). The optical rotation at

$$K_{pH}[A_T] = [S_e]/([PCHO_i] - [S_e]) \quad (8)$$

$$K_{pH}[A_T]\{([PCHO_i]/[S_e]) - 1\} = 1 \quad (9)$$

t_∞ was found to be slightly less than that of the amino acid solution employed prior to addition of aldehyde. This result would be anticipated if the rate of loss of optical activity is slightly greater than the rate of transamination and only *ca.* 2% of the amino acid is reacted.

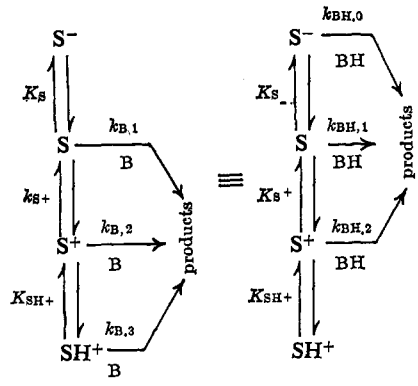
Discussion

Thanassi, Butler, and Bruice[1f] found that imidazole quantitatively directed the reaction of glutamic acid and 3-hydroxypyridine-4-aldehyde to transamination at pH 7.1 whereas the water-catalyzed reaction at pH 5.7 produced only a 10% yield of α-ketoglutarate. In the present study of the reaction between DL-alanine and 3-hydroxypyridine-4-aldehyde the yields of pyruvic acid are nearly quantitative in imidazole, acetate, and formate buffers. Since yields of pyruvic acid in the reaction buffered only by DL-alanine[1h] fell as low as 55% in the pH region near neutrality, the buffers apparently catalyze the transamination reaction, but not the reactions leading to side products.

It was previously suggested[1h] that the zwitterion form of DL-alanine and water acted as general bases in the conversion of aldimines SH^+ and S^+ and aldimines SH^+, S^+, and S, respectively to products (eq 1 and Chart I) in the reaction of 3-hydroxypyridine-4-aldehyde with DL-alanine in solutions buffered only with DL-alanine. Using the same kinetic scheme (Chart I) good fits of the observed rate constants (k_B') to the theoretically derived pH–rate profiles are obtained for buffer catalysis by the base species free imidazole, acetate, and formate on aldimines SH^+, S^+, and S (Figures 5 and 6). In Chart I, B represents H_2O, $HCOO^-$, CH_3COO^-, Im, and $CH_3CH(NH_3)^+)CO_2^-$. Phosphate also readily catalyzed the transamination reaction, but no attempt was made to determine a pH profile in its presence since both the mono- and dianion species present at neutrality could catalyze the reaction, thus adding additional complications to the fitting of the pH profile. The value of all equilibrium and dissociation constants determined (Table I) agrees closely with those required for the pH–rate profiles for water and alanine catalysis.[1h] The evidence for catalytic reactions involving aldi-

mine species (S) lies mainly in the need for rate constants associated with this species for the fit of the imidazole and water pH–rate profiles to the experimental rate constants.

Chart II



The kinetically equivalent scheme shown in Chart II is for general acid catalysis of the conversion of aldimines S^+, S, and S^- into products by BH. Employing the constants in Table I and Chart II as a guide, the kinetically equivalent rate constants for general acid catalysis given in Table III can be calculated.

Table III.[a] Calculated Rate Constants for General Acid Catalysis of the Conversion of Aldimines S^+, S, and S^- into Products

General acid	$10^4\ M^{-1}\ min^{-1}$ $k_{BH,2}$	$k_{BH,1}$	$k_{BH,0}$
ImH^+	1.22×10^2	5.64	1.45×10^2
CH_3CO_2H	3.94×10^2	2.80×10^2	3.10×10^4
HCO_2H	6.50×10^2	8.43×10^2	...

[a] See Chart II for definition of rate constants.

The effect on the rate of transamination caused by dissociation of the carboxyl group in the aldimine (1) is of interest since in the enzymatic transamination reaction the aldimines of pyridoxal 5′-phosphate and amino acids could be bound to the enzyme so that the carboxylate anion of the amino acid is adjacent to a center of positive charge as an ε-amino group of lysine. Since the carboxyl pK_a of DL-alanine (pK_{AH_2}) is *ca.* 2.5, pK_{SH^+} would not be expected to be much greater than 1 pK_a unit above pK_{AH_2}. Assuming pK_{SH^+} to be 3.5, the rate constant $k_{B,3}$ can be calculated (eq 7). From inspection of the ratios $k_{B,3}/k_{B,2}$ and $k_{B,2}/k_{B,1}$ (Table IV) it can readily be seen that the ionization of

Table IV. Ratio of General Base Constants for the Catalysis of the Conversion of Aldimines into Products

Catalyst	$k_{B,3}/k_{B,2}$	$k_{B,2}/k_{B,1}$
Imidazole	1200	325
Acetate	78	75
Formate	41	...

the carboxyl group decreases the rate of the prototropic shift to an extent comparable to dissociation of the protonated pyridine nitrogen. The decrease in rate of transamination upon dissociation of the carboxyl group would be expected since the undissociated carboxyl

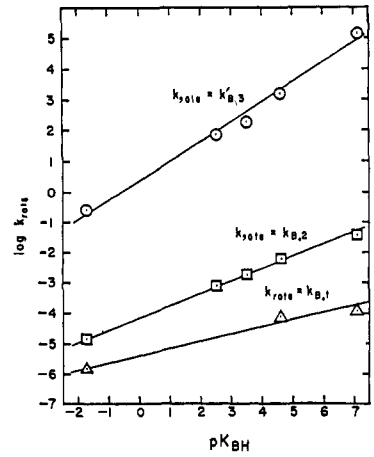


Figure 7. Brønsted plots for each aldimine species SH^+, O; S^+,□; S, Δ, undergoing transamination. In the order of their increasing pK_a the catalysts are H_2O, $CH_3CH(NH_3^+)CO_2^-$, formate, acetate, and free imidazole.

group has the most positive inductive effect.[4] Thus, the dissociated carboxyl group would provide less of a stabilizing influence on the transition state for breaking the C–H bond at the α-carbon.

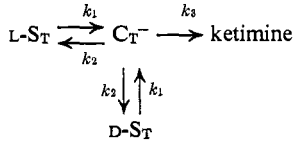
The pseudo-first-order rate constants (k_{obsd}) for transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine-d_4 in half-neutralized 1.0 *M* imidazole and acetate buffers were *ca.* six- to sevenfold smaller than those obtained with DL-alanine under the same conditions (Table II). These values may be compared to those of 2.2 and 9.0, respectively, for the spontaneous and amino acid catalyzed reactions.[1h] Isotope effects of between 7 and 10.2 have been reported for the base-catalyzed bromination of acetone-d_6.[5] These values exceed the theoretical kinetic isotope effect for breaking a CH/CD bond based on a model considering only the vibrational zero point energy of the initial state and must reflect secondary isotope effects of the deuteriums not involved in the reaction (ref 5b, p 172). The deuterium kinetic isotope effect of 9.0 obtained for the amino acid catalyzed reaction must also reflect secondary isotope effects. Both steric and inductive effects of $-CD_3$ as compared to $-CH_3$ are of little importance in the determination of the isotope effects.[6] The kinetic isotope effect of 2.2 for the water catalysis of prototropy is within the range (2.0–2.7) obtained for the rates of ionization in water of substituted malonic esters.[7] The isotope effects determined in the present study refer to experiments carried out on mixtures of aldimines. It is anticipated that each aldimine species will exhibit individual kinetic deuterium isotope effects. If one might draw upon the work on ketone enolization,[7] the values of k^H/k^D should be related to the Brønsted β constant (Figure 7) so that the order of the values of k^H/k^D should be $SH^+ > S^+ > S$. Establishment of the order of k^H/k^D for imine prototropy remains to be done. Nevertheless, the large deuterium isotope effects ob-

(4) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).
(5) (a) O. Reitz and J. Kopp, *Z. Physik. Chem.* (Leipzig), **184A**, 429 (1939); (b) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 201; (c) O. Reitz, *Z. Physik. Chem.* (Leipzig), **179A**, 119 (1937).
(6) (a) K. Mislow, R. Graeve, A. J. Gordon, and G. H. Wahl, Jr., *J. Am. Chem. Soc.*, **86**, 1733 (1964); (b) H. C. Brown and G. J. McDonald, *ibid.*, **88**, 2514, 2520 (1966).
(7) R. P. Bell, *Discussions Faraday Soc.*, 18 (1965).

tained demonstrate the kinetic importance of the breaking of the C–H bond in the conversion of aldimine into ketimine.

The rate constants for the water-catalyzed prototropy of aldimines SH^+, S^+, and S are comparable to the rate constants of water-catalyzed carbanion formation from pseudo-acids such as monochloroacetone and acetylacetone.[8] The largest rate constants for both aldimine prototropy ($SH^+ > S^+ > S$) and ketone bromination were obtained for the pseudo-acids which could form the most stable carbanions.

The rate constant for the loss of optical activity of the aldimines of 3-hydroxypyridine-4-aldehyde and L-alanine in half-neutralized 1 *M* acetate buffer was 101×10^{-4} min^{-1}. This value may be compared to that of 59×10^{-4} min^{-1} also determined in half-neutralized 1 *M* acetate buffer for the rate of transamination of 3-hydroxypyridine-4-aldehyde by DL-alanine obtained spectrophotometrically at 390 mμ. L-Alanine does not racemize under the reaction conditions in the absence of aldehyde. Under the experimental conditions employed the results support



(10)

where $k_3 \cong k_2$ and C_T^- = carbanions produced from aldimine species.

Symmetrical and unsymmetrical general catalysis can be visualized for (10). For symmetrical catalysis the formation of the carbanion (C_T^-) is general base catalyzed by B and the conversion of carbanion to starting aldimine or product ketimine is general acid catalyzed by BH so that

$$v = k_1k_3/(k_2 + k_3)[B][S_T] \quad (11)$$

The same kinetic expression is obtained if the reaction of B with S_T yields ion pairs (C_T^- BH^+) which then partition to starting material and products. In this case the conversion of the ion pairs to product need not involve BH as a general acid catalyst.

If B converts aldimines to the carbanions then by microscopic reversibility, BH must catalyze the retrograde process but not necessarily conversion of carbanions to products. If BH does not catalyze conversion of C_T^- to products and the intermediates partitioned are not ion pairs, the mechanism is unsymmetrical.

$$v = k_1k_3/(k_2[BH] + k_3)[B][S_T] \quad (12)$$

For either the intimate ion pair mechanism or the unsymmetrical mechanism the 3-hydroxyl group could serve as the general acid to convert carbanion to products. The rate expression 12 would predict a nonlinear dependence of the observed pseudo-first-order rate constant (k_{obsd}) on the total concentration of buffer (B_T) unless $k_3 >> k_2[BH]$. The latter condition is not true at pH 4.7, as can be seen from the observed rates of racemization at this pH (10). Also, k_{obsd} is linearly dependent on B_T for all buffers (Figure 3) with the exception of imidazole below pH 7.0, where a very slight curvature is obtained.

(8) (a) R. G. Pearson and R. L. Dillon, *J. Am. Chem. Soc.*, **75**, 2439 (1953); (b) D. J. Cram, "Fundamentals of Carbanion Chemistry," Academic Press Inc., New York, N. Y., 1965, p 10.

With a knowledge of the general base catalytic rate constants for transamination ($k_{B,3}'$, $k_{B,2}$, $k_{B,1}$) of aldimines SH^+, S^+, and S, Brønsted plots (13) for each active aldimine species may be constructed (Figure 7).

$$\log k_{rate} = \beta_{exptl}pK_a + C \quad (13)$$

In (13) $k_{rate} = k_{B,3}'$, $k_{B,2}$, or $k_{B,1}$ for aldimines SH^+, S^+, and S and K_a refers to the acid dissociation constant of the conjugate acid (BH) of the catalytic base (B). The values of β_{exptl} are 0.64 for SH^+, 0.41 for S^+, and 0.24 for S. Since the conversion of aldimines into ketimine appears to be a two-step process, the values of β_{exptl} would be combinations of the β's and α's associated with the individual rate steps for formation and conversion of intermediate carbanion into reactants and products. For a reaction displaying symmetrical catalysis of the conversion of an intermediate into reactants and products as in (11), k_{rate} would be given by the expression

$$k_{rate} = CK_a^{-\beta_{exptl}} = C_1K_a^{-\beta_1}C_3K_a^{-\alpha_3}/(C_2K_a^{-\alpha_2} + C_3K_a^{-\alpha_3}) \quad (14)$$

From (14) it follows

$$\log k_{rate} = \beta_1pK_a - \log\left(1 + \frac{C_2}{C_3}K_a^{-\alpha_2+\alpha_3}\right) + \log C_1 \quad (15)$$

In (14) and (15) β's refer to the slopes of the log k_{rate} *vs.* pK_a for general base catalyzed reactions and are positive while α's refer to the slopes of log k_{rate} *vs.* pK_a for general acid catalysis and are negative. If $(C_2/C_3)K_a^{\alpha_3-\alpha_2} << 1$ (*i.e.*, $k_3 >> k_2$) then (15) reduces to

$$\log k_{rate} = \beta_1pK_a + \log C_1 \quad (16)$$

The experimental slope of the Brønsted plot would, therefore, pertain to the general base catalyzed abstraction of the proton from the α-carbon of the aldimine.

If $k_2 >> k_3$ then (15) reduces to

$$\log k_{rate} = (\beta_1 + \alpha_3 - \alpha_2)pK_a + \log (C_1C_3/C_2) \quad (17)$$

and since $\beta_1 = \alpha_2 + 1$ and $\beta_4 = \alpha_3 + 1$

$$\log k_{rate} = \beta_4pK_a + \log (C_1C_3/C_2) \quad (18)$$

The experimental slope of the Brønsted plot would, therefore, be for the abstraction of the proton from the methine carbon of the ketimine in the over-all retrograde process. In a similar fashion it can be shown that $\beta_{exptl} = \beta_1 = \beta_4$ for the case where $k_2 = k_3$.

For proton abstraction from a substrate X–H by a base B, the values of β change abruptly from 1.0 (transition state reached when proton transfer is complete) to 0.0 (a diffusion-controlled reaction with little or no proton transfer in the transition state) if X^- does not undergo electronic rearrangement. If X^- undergoes electronic rearrangement (*i.e.*, a pseudo-base) there is a gradual change of β from 1.0 to 0.0. For these cases the value of β remains a constant over extended ranges of pK_a of BH.[9] The interpretation of β in the range between 1.0 and 0.0 is not clear. Presumably β is an index of the per cent transfer of H^+ from X^- to B at the transition state. This statement finds support both from rate equilibrium relationships[10] and the rather parallel

(9) M. Eigen, *Angew. Chem. Intern. Ed. Engl.*, **3**, 1 (1964).

(10) J. E. Leffer and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1963, p 241.

change of k^H/k^D for H_2O general base catalyzed bromination of ketones and β for general base catalysis of the same process by a series of bases.[7] The value of k^H/k^D is generally conceded to indicate the position of the proton in the transition state although theoretical considerations indicate that this is not an infallible criterion.[11] Assuming that β does provide a measure of the extent of proton transfer at the transition state, one would have to assume that the lengthening of the C–H bond in the transition state for conversion of aldimines ($\beta_{exptl} = \beta_1$, eq 16) or the conversion of ketimines ($\beta_{exptl} = \beta_4$, eq 18) to their respective carbanions increases in the order $S < S^+ < SH^+$. According to the Hammond postulate[12] the initial transition state for reactions proceeding through metastable intermediates (in our case carbanions), should shift toward initial reactants in proceeding to more stable intermediates. An example of this type of behavior is to be found in the general base catalyzed enolization of ketones where β_{exptl} decreases as the stability of the intermediate carbanion increases (ref 5b, p 172). The order of β_{exptl} for the conversion of aldimines or ketimines to carbanions is inverse to that for the conversion of ketones to carbanions. This result is clearly not in accord with the Hammond postulate.

Regardless of the reason for the order of the observed slopes of the Brønsted plots, the results of the racemization study indicate that the abstraction of the proton from the α-carbon by a base is not concerted with the protonation of the methine carbon. This observation is in accord with the recent findings of Cram and Guthrie[13] that *t*-butoxide ion and *t*-butyl alcohol do not act in concert in the prototropy of imines.

Summary of Catalytic Reactions Involving Azomethines

The following is a summary of catalytic reactions involving azomethines, parts I–IX.[1] It is provided to accentuate the important points concerning the transamination reaction in the absence of metal ions developed through these studies. The first step in the transamination reaction involves the formation of an aldimine of the pyridine-4-aldehyde and amino acid. The reaction of pyridine-4-aldehyde itself with ten amino acids was investigated (part IV).[1d] These studies revealed that in the vicinity of the basic pK_a' of the amino acid (pK_{AH}), carbinolamine is formed in a preequilibrium step followed by specific acid catalyzed and spontaneous dehydration of carbinolamine to yield aldimine. In the pH range $pK_{AH} \pm 0.6$ no catalysis of carbinolamine dehydration by amino acid was detectable. From equilibrium studies the apparent equilibrium constant for aldimine formation was found to be dependent on pK_{AH} of the amino acid as predicted from eq 29.

In parts V[1e] and VII[1g] the kinetics of aldimine formation as well as the pH dependence of the equilibrium for aldimine formation with 3-hydroxypyridine-4-aldehyde were investigated. A comparison of the reactions of 3-hydroxypyridine-4-aldehyde and pyridine-4-aldehyde with glutamic acid, glycine, alanine, and valine established the 3-hydroxyl group to be a catalyst for aldimine formation. Thus, the rate-determining step with 3-hydroxypyridine-4-aldehyde was found to be the formation of carbinolamine (which could not be detected kinetically) rather than its dehydration as noted with pyridine-4-aldehyde. The over-all rate of aldimine formation with 3-hydroxypyridine-4-aldehyde was found to be much greater than with pyridine-4-aldehyde, necessitating the assumption that *the 3-hydroxyl group catalyzes in an intramolecular manner both carbinolamine formation and dehydration.* The role of the 3-hydroxy group as an intramolecular catalyst of aldimine formation has recently been corroborated in the studies of Reeves.[14] From comparison of the dependence of aldimine formation on pH for 3-hydroxypyridine-4-aldehyde and pyridine-4-aldehyde an additional role of the hydroxyl group becomes evident. Because of the shift of the pK_a of the hydroxyl group from 6.5 to *ca.* 9.3 in going from aldehyde to aldimine, protons are not liberated in the course of the reaction at neutrality. Comparison of eq 19 and 20 reveals that the 3-hydroxyl group acts as a proton sink at neutral pH, allowing a measurable concentration of aldimine to be present at physiological pH with 3-hydroxypyridine-4-aldehyde but not with pyridine-4-aldehyde. *Thus, the 3-hydroxyl group assures the presence of aldimine at physiological pH. A third function of the 3-hydroxyl group is in the catalysis of the prototropic shift* converting aldimine to ketimine. This has been verified with pyridine-4-aldehyde which does not undergo the transamination reaction at any pH at 30° in aqueous medium. It has also been established that *association of the carboxyl group of aldimine has as great a kinetic effect on the prototropic rearrangement as protonation of the pyridine nitrogen.*

Following formation of aldimine, the over-all rate-determining prototropic shift occurs leading to the completion of the mechanistically interesting portions of the transamination reaction. Prior to the interests of Banks, Diamantis, and Vernon[15] and Bruice, *et al.*,[1] little attention was given to the mechanism of removal of the α proton. In the study of the imidazole catalysis of the transamination reaction between α-phenylglycine and pyridoxal,[1a,b] the rate of reaction was found to be dependent upon the product of free imidazole and imidazolium ion concentration. Other

(11) A. V. Willi and M. Wolfsberg, *Chem. Ind.* (London), 2097 (1964).
(12) G. S. Hammond, *J. Am. Chem. Soc.*, 77, 334 (1955).
(13) D. J. Cram and R. D. Guthrie, *ibid.*, 87, 397 (1965).
(14) R. L. Reeves, *J. Org. Chem.*, 30, 3129 (1965).
(15) B. E. C. Banks, A. A. Diamantis, and C. A. Vernon, *J. Chem. Soc.*, 4235 (1961).

bases such as morpholine and carbonate were ineffective as catalysts when reactants were of *ca.* 10^{-4} *M* and from the Linweaver–Burk kinetics obtained, it was suggested that a preequilibrium complex of aldimine with imidazole and imidazolium ion occurred followed by an intracomplex general catalysis. It was thus established that *the rate-determining prototropic shift could be catalyzed via general base and/or acid catalyzed mechanisms.* Imidazole catalysis of the prototropic shift in the reaction of glutamic acid and 3-hydroxypyridine-4-aldehyde was subsequently established[1f] to be quite effective. In the absence of imidazole, only a portion of the product arose from the transamination reaction while in the presence of imidazole catalyst not only was the appearance of products accelerated but the reaction was directed quantitatively to transamination (*i.e., general bases catalyze the transamination reaction but not competing side reactions leading to other products*). For the reaction of glutamic acid with 3-hydroxypyridine-4-aldehyde glutamic acid itself was not found to be a catalyst. Catalysis of the quantitative prototropic conversion of the aldimines of alanine and 3-hydroxypyridine-4-aldehyde to its isomeric ketimine has been shown to be catalyzed by alanine itself as well as by water, formate, acetate, phosphate, and imidazole (this study). *Carbanion intermediates have been established,* deuterium isotope effects determined, and *the Brønsted relationship for the general base catalyzed isomerization of each aldimine species established.* Unlike the reaction of α-phenylglycine with pyridoxal, the reactions of glutamic acid[1f] and alanine (ref 1h and this study) with 3-hydroxypyridine-4-aldehyde are dependent on the first power of the catalyst concentration and give no evidence of catalyst–substrate complex formation.[16]

Acknowledgment. This work was supported by a grant from the National Science Foundation.

(16) NOTE ADDED IN PROOF. Studies in progress (J. R. Maley and T. C. Bruice) have established that N-methylpyridine-4-aldehyde undergoes a transamination reaction with alanine in basic media. This shows that the 3-hydroxyl group is not required for transamination if a positive charge can be maintained on the pyridine nitrogen at basic pH. That the observed reaction is transamination and not decarboxylation has been shown by both polarographic product analysis and the lack of reaction of the imine of 2-amino-2-methylpropionic acid.

Aminolysis of Phenyl Acetates in Aqueous Solutions. VII.[1] Observations on the Influence of Salts, Amine Structure, and Base Strength

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Abstract: The rate of aminolysis of phenyl acetate (PA) by various amines can be predicted from $v = [PA]\cdot[amine](k_{OH}\text{-}[OH^-] + k_n + k_{gb}[amine] + k_{ga}[amine\text{-}H^+])$. The only reported case for a specific acid term in the aminolysis of an acetylated phenol could not be substantiated. The effect of changing salts at $\mu = 1.0$ for KCl, $(CH_3)_4NCl$, $(n\text{-}C_3H_7)_4NCl$, and LiCl has been investigated for a series of amines of varying structure. The effect of salt type on k_n, k_{gb}, k_{ga}, and k_{OH} is discussed in terms of preferential salting in or out of ground and transition states. In addition the possibility that $Li(OH_2)_3^+$ might act as a general acid catalyst for the aminolysis reaction is considered. The values of ρ for the aminolysis of substituted phenyl acetates by aziridine and two azetidines have been determined and shown not to differ appreciably from values of ρ obtained for amines not exhibiting steric acceleration. The aminolysis of PA has been extended to substituted hydrazines and trifluoroethylamine. The reaction of PA with morpholine was reinvestigated. The α effect already observed in the hydrazinolysis of PA decreased with N-methyl substitution and disappeared completely on N,N-dimethyl substitution. A critical evaluation of existing postulations regarding the origin of the α effect is offered. Morpholine appears to be the first secondary amine to exhibit a k_{gb} term in the aminolysis of a phenyl acetate. Brønsted equations for k_n, k_{gb}, k_{ga}, and k_{OH} are derived, and the effects of variation of the structure of the amine are discussed. Plausible mechanisms associated with each rate term which are consistent with the observed salt effects, deuterium solvent isotope effects, ρ values, and Brønsted β values are postulated.

Under the experimental conditions in which amine and its conjugate acid are in great excess over substrate, the values of the pseudo-first-order rate constants (k_{obsd}) for ester aminolysis have been found to be correlated by[1,4]

$$k_{obsd} = k_n[N_f] + k_{gb}[N_f]^2 + k_{ga}[N_f][NH^+] + k_{OH}[N_f][OH^-] \quad (1)$$

where $[N_f]$ and $[NH^+]$ represent concentrations of amine and its conjugate acid, respectively, k_n, k_{gb}, k_{ga}, and k_{OH} represent rate constants for unassisted

(1) For previous studies in this series see: (a) T. C. Bruice and M. F. Mayahi, *J. Am. Chem. Soc.*, **82**, 3067 (1960); (b) T. C. Bruice and J. J. Bruno, *ibid.*, **83**, 3494 (1961); (c) T. C. Bruice and S. J. Benkovic, *ibid.*, **85**, 1 (1963); (d) *ibid.*, **86**, 418 (1964); (e) T. C. Bruice and R. G. Willis, *ibid.*, **87**, 531 (1965); (f) L. R. Fedor, T. C. Bruice, K. L. Kirk, and J. Meinwald, *ibid.*, **88**, 108 (1966).

(2) Postdoctoral Fellow, University of California, Santa Barbara, Calif.

(3) Postdoctoral Fellow, Cornell University, Ithaca, N. Y.

(4) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).