Korn, E. D., Dearborn, D. G., Fales, H. M., and Sokoloski, E. A. (1973), *J. Biol. Chem. 248*, 2257.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Marchesi, V. T., and Andrews, E. P. (1971), *Science 174*, 1247.
Moore, S., Spackman, D. H., and Stein, W. H. (1958), *Anal. Biochem. 30*, 1185.
Quin, L. D. (1964), *Science 144*, 1133.
Quin, L. D. (1967), *Top. Phosphorus Chem. 4*, 23.
Rosenberg, H. (1964), *Nature (London) 203*, 229.
Shimizu, H., Kakimoto, Y., Nakajima, T., Kanazawa, A., and Sano, I. (1965), *Nature (London) 207*, 1197.
Snyder, W. R., and Law, J. H. (1970), *Lipids 5*, 800.
Spiro, R. G. (1970), *Annu. Rev. Biochem. 39*, 599.
Winterburn, P. J., and Phelps, C. F. (1972), *Nature (London) 236*, 147.
Winzler, R. J. (1955), *Methods Biochem. Anal. 2*, 210.

Comparison of the Rate Constants for General Base Catalyzed Prototropy and Racemization of the Aldimine Species Formed from 3-Hydroxypyridine-4-carboxaldehyde and Alanine†

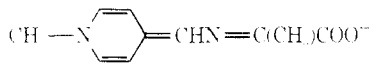
Jack E. Dixon‡ and Thomas C. Bruice*

ABSTRACT: The rate constants for the general base catalyzed racemization of the three aldimine species (SH^-, $S^\pm$, and S; Scheme II) formed from alanine and 3-hydroxypyridine-4-carboxaldehyde have been compared to the general base catalyzed rates of transamination. The two- to threefold difference in the rate constants of racemization and transamination for SH^-, $S^\pm$, and S suggests that the intermediate carbanion is protonated at the carbon originating with the pyridinecarboxaldehyde with about the same ease as protonation takes place at the carbon α to the carboxyl group. These observations are consistent with the large primary deuterium isotope effect noted by Auld and Bruice. The order of ease of proton abstraction from the α carbon by general bases is $SH^- > S^\pm > S$. The conjugated nature of the aldimine $SH^\pm$ with its protonated carboxyl residue and quaternary nitrogen greatly enhances the acidity of the (α)-C–H function of the amino acid moiety but is anti-Hammond in increasing the sensitivity of the rate of proton abstraction to the basicity of the catalyst. These features undoubtedly contribute greatly to the facility of enzymatic transamination.

Studies in this laboratory on model systems for pyridoxal transamination have been carried out in the absence of metal ions in aqueous solution. Our studies have been dictated by the fact that the influence of metal ions upon model transamination reactions (Metzler and Snell, 1952) is not relevant to the enzyme-catalyzed reactions (Jenkins and Sizer, 1957; Matsuo and Greenberg, 1958; Alexander and Greenberg, 1956; Karasek and Greenberg, 1957; Fasella *et al.*, 1962). Bruice and Topping (1963) established that the transamination of pyridoxal with phenylglycine (Scheme I) proceeded *via* imine formation, followed by imidazole general catalysis of the rate-determining prototropic shift. The establishment of proton removal by imidazole marked the first example of the observation of general catalysis in the prototropic shift of an azomethine in aqueous solution. Subsequent investigations have revealed that it is likely that a histidine residue acts as a general base for proton removal in aspartate transaminase (Peterson and Martinez-Carrion, 1970).

Because 3-hydroxypyridine-4-carboxaldehyde meets the minimum requirements for enzymic transamination (Ayling and Snell, 1968) it serves effectively in model studies. The 3-hydroxyl group of 3-hydroxypyridine-4-carboxaldehyde acts as a general catalyst for aldimine formation (French *et al.*, 1965). The rate-determining step in imine formation with 3-hydroxypyridine-4-carboxaldehyde was found to be the formation of carbinolamine, rather than its dehydration as seen with pyridine-4-carboxaldehyde (Scheme I). The overall rate of aldimine formation with 3-hydroxypyridine-4-carboxaldehyde was greater than with pyridine-4-carboxaldehyde, indicating that the 3-hydroxyl group catalyzes, in an intramolecular manner, both carbinolamine formation and dehydration. Since pyridine-4-carboxaldehyde does not undergo transamination and 3-hydroxypyridine-4-carboxaldehyde does, it is evident that the 3-hydroxyl group is involved in the interconversion of aldimine and ketimine species. Thus from considerations of the work of French *et al.* (1965) as well as Thanassi *et al.* (1965), the phenolic hydroxyl group would appear to play an essential role in both imine formation and in prototropy.

The importance of protonation at the pyridine nitrogen to activate the α hydrogen is apparent from the observation of Maley and Bruice (1968) who noted that in the reaction of *N*-methylpyridine-4-carboxaldehyde with alanine I is formed



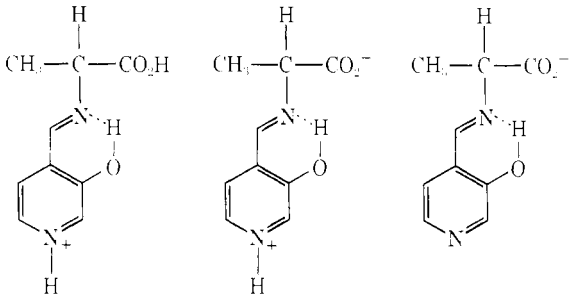
as an intermediate in the transamination reaction. Recently Abbott and Bobrik (1973) have reported the isolation and characterization of the 1,4-dihydropyridine tautomer formed

† From the Department of Chemistry, University of California, Santa Barbara, California 93106. *Received May 29, 1973*. This work was supported by a grant from the National Science Foundation.

‡ Material submitted by J. E. D. in partial fulfillment of the requirement for the Ph.D. in Chemistry, University of California (1972).

between pyridoxal and diethyl aminomalonate (see also Schirch and Slotter, 1966; Matsumoto and Matsushima, 1972). These observations in model systems parallel those of Jenkins (1961), who observed for glutamic alanine transaminase a spectral band ascribed to an intermediate 1,4-dihydropyridine tautomer.

Quantitatively, the prototropic conversion of the aldimines of alanine and 3-hydroxypyridine-4-carboxaldehyde to their isomeric ketimines has been shown to be general base catalyzed by alanine itself, as well as by water, formate, acetate, phosphate, and imidazole (Auld and Bruice, 1967). The order of reactivity of the aldimines is $SH^+ > S^+ > S$. This is as anticipated since a proton on both the pyridine nitrogen and carboxylate group should increase the acidity of the α proton on SH^+ over that of S^+ or S. The second-order rate constant for imidazole catalyzed abstraction of the α proton from SH^+ can be calculated to be $\approx 10^4$ l. mol^{-1} sec^{-1} (Auld and Bruice, 1967). This is a very large rate constant and may be compared to that for transamination of glutamic asparatic transaminase of approximately 10^7 l. mol^{-1} sec^{-1} (pH 8.0) (Hammes and Fasella, 1962). This not completely unfavorable comparison is surprising when one considers that we are comparing an enzymatic reaction with a simple bimolecular reaction. The Brønsted relationship ($\log k = \beta pK_a + C$) for the general base catalyzed isomerization of each aldimine species (SH^+, S^+, and S) has been established. Quite unexpectedly not only



SH^+, $\beta = 0.64$ S^+, $\beta = 0.41$ S, $\beta = 0.24$

is SH^+ the most reactive imine but its rate constants are also the most sensitive to changes in pK_a of the general bases employed (*i.e.*, SH^+ shows the largest Brønsted β coefficient). This finding is opposed to that predicted by the Hammond postulate (Hammond, 1955). This paper describes a search for the reasons associated with the unusual sensitivity of the general base rate constant for prototropy to the base strength of the catalyst for SH^+, S^+, and S.

Experimental Section

Materials. The preparation and purity of 3-hydroxypyridine 4-carboxaldehyde is described in a previous paper from this laboratory (Auld and Bruice, 1967). The highest quality L-alanine (Calbiochem, A grade) was used without further purification. Disodium ethylenediaminetetraacetate (Fisher) was reagent grade and used without further purification. All solutions were prepared with distilled deionized water.

Apparatus. The polarimeter used for optical rotation studies was a Perkin-Elmer Model 141 equipped with a quartz water-jacketed cell. The path length of the cell was 1 dm and the cell volume, 1 ml. All pH measurements were made on either a Radiometer Model 22 pH meter equipped with a Radiometer Model PHA 630 pH scale expander or a Radiometer Model 26 pH meter. The combined glass–calomel electrode (Radiometer GK 2021C or Radiometer EQ 125) was thermostated at 30 ± 0.1°. An Olivetti Underwood Programma 101 or Hewlett-Packard 9100A calculator was used in the computation of rate constants and in the generation of theoretical fits for experimental data.

Kinetics. All kinetic measurements were carried out at 30 ± 0.1° (μ = 1.0 with KCl). The polarimetric solutions consisted of a 1.0 M solution of L-alanine alone or 1.0 M L-alanine and 1.0 M acetate. The concentration of EDTA was 1.0×10^{-3} M in all kinetic measurements. The reactions were run under pseudo-first-order conditions where total amino acid [A_T] $\gg$ total 3-hydroxypyridine-4-carboxaldehyde ([$PyrCHO_T$]. The aldehyde concentration for the polarimetric rates was approximately 1×10^{-2} M.

A typical kinetic run on the polarimeter was carried out as follows: the pH of a solution containing 1.0 M L-alanine and 1.0 M acetate buffer was recorded. To this solution crystalline aldehyde was added and thoroughly mixed. After mixing, a stream of nitrogen was bubbled through the solution for 2 min and the solution filtered and placed in the polarimeter cell. Approximately 2 min were allowed to elapse before optical rotations were recorded so that any small air bubbles present in the solution could clear the light path. Optical rotations were recorded manually at 546 and 578 nm and duplicate runs were performed at all pH's. The rate constants for the two runs were averaged. The Olivetti 101 Programma was used to calculate all rate data from plots of $\log (\gamma_0 - \gamma_\infty)/(\gamma_t - \gamma_\infty)$ *vs.* time, where γ is the observed optical rotation.

Results[2]

A typical plot of the change in optical rotation (OR) at 546 and 578 nm accompanying the reaction of L-alanine (1.0 M) with 3-hydroxypyridine-4-carboxaldehyde in the presence of 1.0 M acetate buffer at pH 4.23 is shown in Figure 1. This change in rotation is due to both racemization and transamination of the intermediate aldimine species. Though L-amino acid was present in excess, the large specific rotation of aldimine made this measurement possible. The observed loss in optical activity is not due to racemization of unreacted

[2] Abbreviations used are: [A_T], total concentration of alanine buffer ([A_T] = [AH_2] + [AH] + [A]), where

$$AH_2 \underset{+H^+}{\overset{K_{AH_2}}{\rightleftharpoons}} AH \underset{+H^+}{\overset{K_{AH}}{\rightleftharpoons}} A$$

[$PyrCHO_T$] concentration of 3-hydroxypyridine-4-carboxaldehyde added to kinetic solution ([$PyrCHO_T$] = [$PyrCHO^+$] + [PyrCHO] + [$PyrCHO^-$]), where

$$PyrCHO^+ \underset{+H^+}{\overset{K_{PyrCHO^+}}{\rightleftharpoons}} PyrCHO \underset{+H^+}{\overset{K_{PyrCHO}}{\rightleftharpoons}} PyrCHO^-$$

[SH^+], [S^+], and [S] are concentrations of aldimine species formed from A and $PyrCHO^+$, PyrCHO, and $PyrCHO^-$, respectively. Species SH^+ possesses dissociation constants K_{SH^+}, K_{S^+}, and K_S as shown in Scheme II; k_3, k_2, and k_1 are second-order rate constants for catalysis of loss of optical rotation by buffer base (B) (*i.e.*, [B_T] = [B] + [BH] with associated acid dissociation constant K_{BH}) due to transamination and racemization of SH^+, S^-, and S, respectively; k_B' is the pH dependent third-order rate constant for reaction of $PyrCHO_T$ with A_T as catalyzed by B_T which leads to racemization plus transamination. The terms [L-S_T] and [D-S_T] represent total concentration of levo- and dextrorotatory aldimines formed on reaction of alanine with 3-hydroxypyridine-4-carboxaldehyde and [C_T^-] represents the total concentration of "carbanions" formed upon ionization of the α proton of L-S_T and D-S_T.

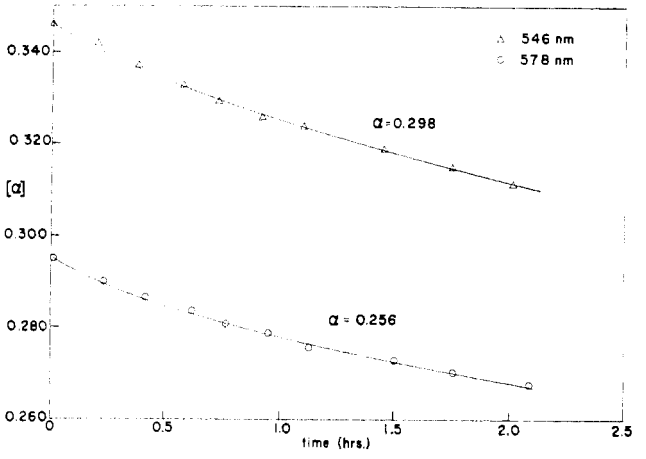
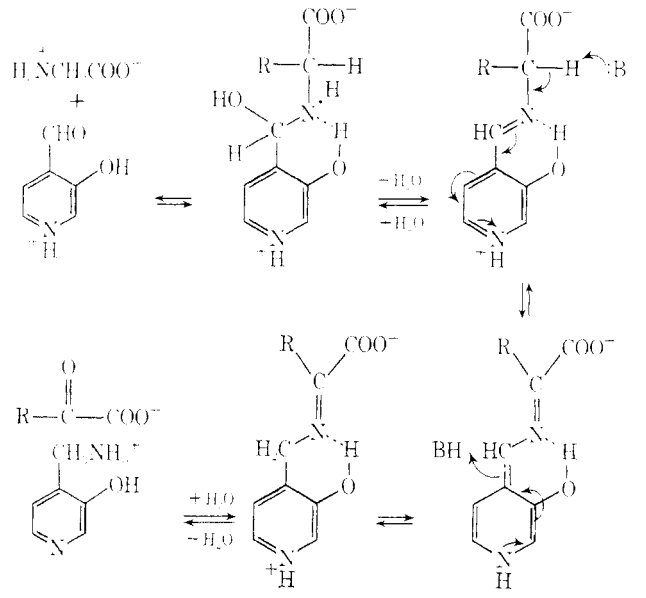


FIGURE 1: Change in optical rotation [α] at 546 nm (○) and 578 nm (△) for the reaction of 3-hydroxypyridine-4-carboxaldehyde with 1.0 M L-alanine and 1.0 M acetate at pH 4.23.

SCHEME I



L-alanine since in the absence of 3-hydroxypyridine-4-carboxaldehyde there was no change in the OR during a 24-hr period of time. The pseudo-first-order rate constants for duplicate runs at the same pH generally agreed to ±5%. Doubling the concentration of aldehyde, with all other conditions remaining constant, doubled the observed change in optical rotation but left the rate constant unaffected.

Table I lists the observed values of k_B' at various pH's for the racemization by the general bases acetate and L-alanine. Figures 2 and 3 provide experimental values for the rates of racemization (k_B') of aldimines SH^+, $S^\pm$, and S by the general bases L-alanine and acetate. The procedure for obtaining the rate of loss of optical activity was similar to that described by Auld and Bruice (1967). For reactions carried out with a buffer (1.0 M) other than alanine, the rate constant for the reaction with L-alanine was subtracted from that determined with added buffer. The pH dependence of the loss of optical activity due to racemization and transamination (k_B') is obtained from the kinetic scheme outlined in Scheme II. The general base catalyzed loss of optical activity for SH^+, $S^\pm$, and S is described by

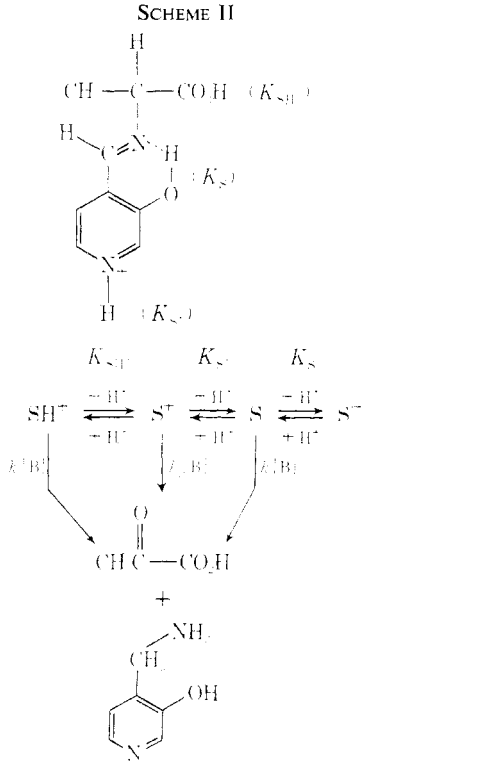
$$k_{B_T} = (k_3'[SH^+] + k_2[S^\pm] + k_1[S])[B] \qquad (1)$$

TABLE I: Observed Rates of Racemization by the General Bases L-Alanine and Acetate.[a]

L-Alanine		Acetate	
k_B' (M^{-1} $min^{-1} \times 10^{-3}$)	pH	k_B' (M^{-1} $min^{-1} \times 10^{-3}$)	pH
2.12	4.78	3.6	3.56
3.0	4.17	6.05	3.94
3.5	3.69	6.3	3.98
4.0	3.43	6.7	4.23
3.98	3.15	7.6	4.70
2.6	2.81	7.2	5.07
1.3	2.57	5.0	5.54
		4.5	5.68

[a] Each value of k_B' represents duplicate runs at 546 and 578 nm.

SCHEME II



where $k_{B_T} = k_B'[PyrCHO_T][A_T][B_T]$. Using Scheme II and eq 1 [for details of this derivation plus values of essential constants [see Auld and Bruice (1967)] it is possible to derive

$$k_B' = \frac{QR}{UVX} \qquad (2)$$

where

$$Q = k_3 a_H^3 + k_2 a_H^2 + k_1 K_s a_H$$

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

$$U = [(K_{PyrCHO}/K_B) + 1] a_H^2 + K_{PyrCHO}(K_2 + 1) a_H + K_{PyrCHO} K_{PyrCHO}$$

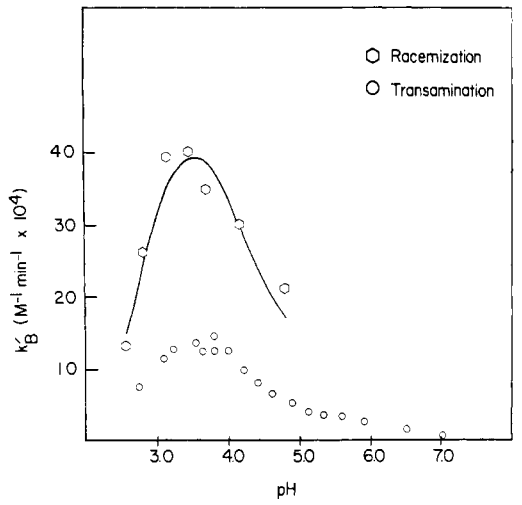


FIGURE 2: The variation of the second-order rate constant (k_B') as a function of pH for the rates of transaminaton (○) and decrease in optical rotation [this study] (○) of 3-hydroxypyridine-4-carboxaldehyde by DL-alanine (Auld and Bruice, 1967) and L-alanine, respectively. No additional buffer species present. The points are experimental and the line is theoretical.

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

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

Using experimental values of k_B' and a_H, the values of k_1, k_2, and k_3 may be varied until the best fit between calculated and experimental values are obtained. The results of fitting eq 2 to a plot of k_B' *vs.* pH are shown in Figures 2 and 3 where the lines represent the theoretically generated values of k_B'. It was noted that decreasing the values of k_1 four orders of magnitude has no effect upon the calculated values of k_B', whereas increases in k_1 alter k_B' significantly.

Discussion

Studies by Auld and Bruice (1967) suggested that in the reaction of 3-hydroxypyridine-4-carboxaldehyde with alanine conversion of aldimine to ketimine proceeds *via* a delocalized carbanion. A similar conclusion has been reached in studies of the prototropy of a number of imines by *tert*-butoxide ion in *tert*-butyl alcohol (Cram and Guthrie, 1965). Therefore, the possibility arises that the unusual order of the Brønsted β values for transamination of aldimine SH^+, S^+, and S arises from a summation of β's for proton abstraction and addition. By determining the rates of change in optical rotation (k_3', k_2, and k_1) for aldimines SH^+, S^+, and S only the proton abstraction will be monitored, thus separating the two possible steps of the reaction. Because tight isosbestic points are obtained for the changes of optical density with time for the reaction between DL-alanine and 3-hydroxypyridine-4-carboxaldehyde one would anticipate that if a two-step mechanism (eq 3) were occurring the intermediate carbanion (C_T^-) would

$$\text{L-S}_T \underset{k_b}{\overset{k_f}{\rightleftharpoons}} C_T^- \xrightarrow{k_1} \text{products} \qquad (3)$$

$$k_3 \uparrow\downarrow k_2$$

$$\text{D-S}_T$$

be in steady state concentration (Auld and Bruice, 1967). The possibility of symmetrical and unsymmetrical general catalysis in the conversion of the L-alanineimine (L-S$_T$) to C_T^- to products has been discussed elsewhere (Auld and Bruice, 1967). The rate of racemization and transamination of alanine and 3-hydroxypyridine-4-carboxaldehyde are shown in Figures 2 and 3 for the general bases alanine and acetate. The striking similarity between the pH–rate profiles for racemization and transamination is most apparent. In fact, the pH–rate profiles for racemization with the general bases acetate and alanine appear to fall approximately 0.3 log unit above the corresponding pH–rate profiles for transamination. The similar dependence upon a_H indicates that the electronic effects associated with carboxyl dissociation and loss of the proton from the pyridinium nitrogen have the same effect upon racemization as they do upon the rate of transamination. It also follows that the structural dependence of the rates of racemization are the same for the rates of transamination for all three aldimines (SH^+, S^+, and S). The fact that the $k_{racemization}$ is approximately 2–3 times larger than the $k_{transamination}$ is easily explained by eq 4, when k_b is approximately the same order of magnitude as k_f. This would imply that the intermediate carbanion is protonated at the carbon originating with 3-hydroxypyridine-4-carboxaldehyde with about the same ease as protonation takes place at the carbon α to the carboxyl group. Support for this hypothesis is evident not only from the small differences in the rates of racemization and transamination, but also from the observation that the change in rotation in going from t_0 to t_∞ is larger than that anticipated for transamination by a factor of about twofold.

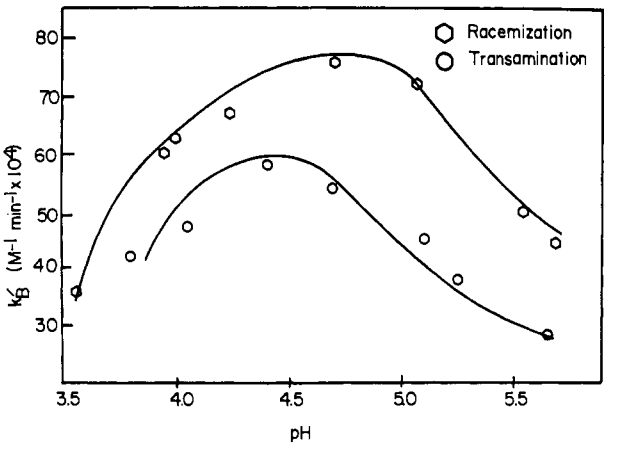


FIGURE 3: pH–second-order rate constant profile for acetate catalysis of the transamination [Auld and Bruice, 1967] (○) and decrease in optical rotation [this study] (○) of 3-hydroxypyridine-4-carboxaldehyde by DL-alanine and L-alanine, respectively. The points are experimental values, the lines are theoretical (see eq 3).

From the theoretical fitting of the pH–rate profiles in Figures 2 and 3 to eq 2 one can determine k_3', k_2, and k_1 for the general base catalyzed prototropy of aldimines SH^+, S^+, and S. It is apparent from Table II that a difference of less than one order of magnitude between $k_{racemization}$ and $k_{transamination}$ makes little difference in the rate constants k_3', k_2, and k_1 for the two processes. This observation implies that the similarity in the rates of racemization and transamination would also lead to Brønsted plots with identical values of β for racemization and transamination whether the aldimine be SH^+, S^+, or S.

The values of the Brønsted β values for proton abstraction reactions from a substrate X–H by a base B have generally been interpreted (Bruice and Benkovic, 1966) as reflecting the amount of bond breaking in the critical transition state. This assumption is based upon the Hammond postulate (Hammond, 1955) which states that the initial transition state for

TABLE II: Best Values of the Constants for the pH–Rate Constant (k_B') Profiles for General Base Catalyzed Racemization and Transamination of 3-Hydroxypyridine-4-carboxaldehyde.

General Base	pK_{BH}	$10^4k_3'$ (M^{-1} min^{-1} [b])	10^4k_2 (M^{-1} min^{-1})	10^4k_1 (M^{-1} min^{-1})
		Rates of Loss of Optical Activity[a]		
Acetate	4.61	1.60×10^7	64	0.85
Alanine	2.53	8.0×10^5	8.0	
		Rates of Transamination[c]		
Acetate	4.61	2.25×10^7	1.2×10^2	5.0×10^{-2}
Alanine	2.53	2.3×10^6	2.4×10^1	

[a] This study. [b] $k_3' = k_3/K_{SH^+}$. The value of K_{SH^+} is not known with certainty. [c] From Auld and Bruice (1967).

reactions proceeding through metastable intermediates should shift toward initial reactants in proceeding to a more stable intermediate. However, for carbon acids β may (Bordwell and Boyle, 1972) or may not (Bell, 1959; Bell and Goodall, 1966) remain constant over extended ranges of pK_a of the general base employed. If we should assume that β provides a measure of the extent of proton transfer at the transition state for the conversion of aldimines to ketimines through the intermediate carbanion, it would be anticipated that the relative carbanion stability increases in the order $SH^+ > S^+ > S$, which leads to the expectation that the Brønsted β values for racemization and transamination should be in magnitude of the opposite order to that experimentally observed. It has been recently pointed out, however, that the degree of delocalization of the electron pair of the C–H bond accompanying ionization must be considered (Bordwell *et al.*, 1970; Bordwell and Boyle, 1972) in any interpretation of β values so that where a great deal of electron delocalization takes place, β is not a good index of the amount of proton transfer in the transition state. Such a situation exists for the carbanions of the aldimines of this study where resonance delocalization of the anionic charge is anticipated to be great. Indeed, the original Braunstein–Snell mechanism, in its qualitative aspects, assumed the driving force for the prototropic shift leading from aldimine → ketimine to be charge delocalization (Snell *et al.*, 1963). This interpretation is most certainly correct as can be seen from the approximated values of the acidity of the α protons of SH^+, S^+, and S. These acidity values can be estimated from the relationship of pK_a of carbon acid to the rate of proton abstraction by H_2O (Pearson and Dillon, 1953). Employing the water rates[3] for transamination with SH^+, S^+, and S and the plot of Pearson and Dillon (1953) pK_a values of approximately 9, 12, and 14 may be interpolated for SH^+, S^+, and S, respectively. It is unlikely that inductive effects alone can explain the thermodynamic stabilities of the carbanion species. The unusual order of sensitivity of the rate constants for proton abstraction from the α position of SH^+, S^+, and S to the basicity of the general base catalyst is most likely due to the large amount of bond reorganization accompanying ionization, as previously found for nitroalkanes. Indeed, the recent isolation of the 1,4-dihydropyridine tautomer formed from diethyl aminomalonate and pyridoxal attests to the large amount of reorganization following proton abstraction (Abbott and Bobrik, 1973). These observations combined with the fact that SH^+ is the most sensitive imine species to general base catalyzed tautomerizations (transamination) most likely makes it (or an equivalent to SH^+) the enzymatically important species. It is interesting to note that the cofactor has been so designed as to take catalytic advantage of this unusual situation in the general base catalyzed ionization of carbon acids.

[3] Assuming the reasonable value of $pK_{SH^+} = 3.5$ (Auld and Bruice, 1967), the value of k_3 for H_2O catalysis may be calculated from the k_3' value of Table I.

References

Abbott, E. H., and Bobrik, M. E. (1973), *Biochemistry 12*, 846.

Alexander, N., and Greenberg, D. M. (1956), *J. Biol. Chem. 220*, 775.

Auld, D. S., and Bruice, T. C. (1967), *J. Amer. Chem. Soc. 89*, 2098, and references therein.

Ayling, J. E., and Snell, E. E. (1968), *Biochemistry 7*, 1626.

Bell, R. P. (1959), The Proton in Chemistry, Ithaca, N. Y., Cornell University Press, p 201.

Bell, R. P., and Goodall, D. M. (1966), *Proc. Roy. Soc., Ser. A 294*, 273.

Bordwell, F. G., and Boyle, W. J. (1972), *J. Amer. Chem. Soc. 94*, 3907.

Bordwell, F. G., Boyle, W. J., and Yee, K. C. (1970), *J. Amer. Chem. Soc. 92*, 5926.

Bruice, T. C., and Benkovic, S. J. (1966), Bioorganic Mechanisms, Vol. I, New York, N. Y., W. A. Benjamin, Chapter 1.

Bruice, T. C., and Topping, R. M. (1963), *J. Amer. Chem. Soc. 85*, 1480, 1488.

Cram, D. J., and Guthrie, R. D. (1965), *J. Amer. Chem. Soc. 87*, 397.

Fasella, P., Hammes, G. G., and Vallee, B. (1962), *Biochim. Biophys. Acta 65*, 142.

French, T. C., Auld, D. S., and Bruice, T. C. (1965), *Biochemistry 4*, 77.

Hammes, G. G., and Fasella, P. (1962), *J. Amer. Chem. Soc. 84*, 4644.

Hammond, G. S. (1955), *J. Amer. Chem. Soc. 77*, 334.

Jenkins, W. T. (1961), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 20*, 978.

Jenkins, W. T., and Sizer, I. W. (1957), *J. Amer. Chem. Soc. 79*, 2655.

Karasek, M. A., and Greenberg, D. M. (1957), *J. Biol. Chem. 227*, 191.

Maley, J. R., and Bruice, T. C. (1968), *J. Amer. Chem. Soc. 90*, 2843.

Matsumoto, S., and Matsushima, Y. (1972), *J. Amer. Chem. Soc. 94*, 7211.

Matsuo, Y., and Greenberg, D. M. (1958), *J. Biol. Chem. 230*, 545, 561.

Metzler, D. E., and Snell, E. E. (1952), *J. Amer. Chem. Soc. 74*, 979.

Pearson, R. G., and Dillon, R. L. (1953), *J. Amer. Chem. Soc. 75*, 2439.

Peterson, D. L., and Martinez-Carrion, M. (1970), *J. Biol. Chem. 245*, 806.

Schirch, L. V., and Slotter, R. A. (1966), *Biochemistry 5*, 3175.

Snell, E. E., Fasella, P. M., Braunstein, A., and Fanelli, A. R. (1963), Chemical and Biological Aspects of Pyridoxal Catalysis, New York, N. Y., Macmillan (see discussion following Chapter 8).

Thanassi, J. W., Butler, A. R., and Bruice, T. C. (1965), *Biochemistry 4*, 77.