Kinetics of the Pyridoxal-Catalyzed Dealdolation and β Elimination of Some Aromatic β-Hydroxy α-Amino Acids

Joseph A. Marcello[1] and Arthur E. Martell*

Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received August 10, 1981*

Abstract: The rates of dealdolation and β elimination of a number of para-substituted phenylserines have been determined by proton NMR. Electron-withdrawing substituents on the amino acid side chain enhance the dealdolation rate. In the substituted phenylserines β elimination and dealdolation were found to occur as parallel reactions. The metal ion–pyridroxal catalyzed systems undergo reaction more rapidly than the metal-free pyridoxal-catalyzed systems. A reaction mechanism for the parallel dealdolation and β-elimination reactions is proposed. In the case of one of the amino acids, phenylthreonine, the rate constants for the metal-free Schiff base were resolved into the specific rate constants for the individual solution components, and the variations obtained for the specific rate constants are interpreted in terms of the proposed reaction mechanism.

Threonine is converted to glycine and acetaldehyde, and serine is converted to glycine and formaldehyde, respectively, in pyridoxal-activated enzyme systems.[2-4] The reaction has been shown to take place with pyridoxal and metal ions in model systems, albeit at slower rates.[5-9] The reaction also occurs in β-hydroxyamino acids having no hydrogen at the α position.[10]

One of the major questions concerning the dealdolation of β-hydroxyamino acids has to do with the factors that favor dealdolation over β elimination and vice versa. A number of β-hydroxy amino acids undergo β elimination and/or dealdolation reactions[5,7-9,11-13] in model systems. In fact, a study by Martell and Tatsumoto[14] has shown that phenylserine is capable of undergoing both dealdolation and β-elimination reactions simultaneously in both pyridoxal and pyridoxal–metal ion catalyzed model systems.

In an earlier paper[15] it was shown that the dealdolation reaction proceeds through the direct cleavage of the α, β carbon–carbon bond of the Schiff base. It was further demonstrated that the dealdolation rate is enhanced by the presence of electron-donating groups at the β-carbon of the amino acid moiety of the Schiff base. This paper reports on the detailed kinetic measurements of the pyridoxal and pyridoxal–metal ion catalyzed dealdolation and β-elimination reations of a series of para-substituted phenylserines. The rate constants of these model system reactions have been determined, and a probable reaction mechanism is proposed. The effects of the para substituents on the dealdolation and β-elimination reactions are discussed.

Experimental Section

Materials. Pyridoxal hydrochloride was obtained from Mann Laboratories as Mann Analyzed grade and used without further purification. The deuterium oxide used as a solvent for this study was obtained from Aldrich Chemical Co. and was specified as 99.8% deuterium. The aluminum(III) solutions were prepared by dissolving hydrated $Al_2(SO_4)_3$ in D_2O and evaporating to dryness. This procedure was repeated several times to remove any residual H_2O. The standard Al(III) solutions were prepared from the deuterated material by dilution to the appropriate concentration. The standard Zn(II) solutions were prepared from hydrated $Zn(NO_3)_2$ by a procedure similar to that employed for the Al(III) solutions. The Al(III) and Zn(II) solutions were standardized by conventional chelatometric titrations.[16] The standard Ga(III) solution was prepared by dissolving a specific amount of gallium metal in DCl (20%) and diluting to the appropriate volume. The ionic strengths of the samples used in the measurements were maintained at unity with reagent-grade KCl. All of the solutions were initially 0.100 M in both pyridoxal hydrochloride and amino acid. In the 1:1:1 systems (pyridoxal–amino acid–metal ion) the concentration of the metal ion was 0.100 M and in the 2:2:1 systems it was 0.050 M.

threo-p-**Chlorophenylserine** was synthesized as described by Holland et al.[17] *p*-**Nitrophenylserine** was synthesized by modification of the method used to prepare the *p*-chlorophenylserines. *p*-**Aminophenylserine** was prepared by the hydrogenation of the *p*-nitrophenylserine at low pressure with 5% palladium on charcoal as catalyst.

N-**Benzylphenylthreonine.** The following procedure is a modification of that employed by Oh-Hashi and Harada[18] for the synthesis of β-hydroxyvaline. Sodium β-phenyl-β-methylglycidate[19] was placed in a 50-mL round-bottom flask with water (10 mL), benzylamine (15 mL), and 10 M sodium hydroxide (2 mL), and the reaction mixture was refluxed at 130 °C for a period of 36 h. The solution was then cooled, and the excess benzylamine was removed by extraction with ether. The aqueous solution that remained was diluted to 100 mL and concentrated 12 M HCl was added dropwise. When the pH was reduced to about 8, the precipitate that separated out of the solution was removed and more HCl was added to the filtrate. When the pH reached 4, the gummy precipitate that formed was removed and dissolved in hot water, and the resulting solution was allowed to cool slowly. After several hours a precipitate formed from the solution. Both of the precipitates were dried, and ^1H and ^{13}C NMR spectra were run on these compounds to determine their identities. The substance that precipitated at pH 4 was found to be the desired product, *N*-benzylphenylthreonine, while the substance that had precipitated at the higher pH was found to be 2-hydroxy-3-(α-benzylamino)-3-phenylbutanoic acid, which formed when the epoxide ring opened at the β-carbon position rather than at the desired α-carbon position. A total of 3.0 g of the desired product, *N*-benzylphenylthreonine, was obtained.

DL-**Phenylthreonine.** *N*-Benzylphenylthreonine (3 g) was placed in 50% ethanol–water and hydrogenated at room temperature and pressure with a Pd on charcoal catalyst. Complete hydrogenation required 6 h. The Pd–charcoal catalyst was filtered off and the filtrate was concentrated under reduced pressure. The gummy residue was taken up in a minimum amount of hot ethanol–water and was filtered hot. After the

(1) Abstracted in part from a dissertation submitted by Joseph A. Marcello to the faculty of Texas A & M University in partial fulfillment of the requirements for the Ph.D. degree.
(2) Meister, A. "Biochemistry of the Amino Acids"; Academic Press: New York, 1957.
(3) Gilbert, J. B. *J. Am. Chem. Soc.* **1957**, *79*, 2242.
(4) Karasek, M. A.; Greenberg, G. M. *J. Biol. Chem.* **1957**, *191*, 22.
(5) Metzler, D. E.; Longenecker, J. B.; Snell, E. E. *J. Am. Chem. Soc.* **1954**, *76*, 639.
(6) Abbott, E. H.; Martell, A. E. *J. Chem. Soc., Chem. Commun.* **1968**, *23*, 1501.
(7) Longenecker, J. B.; Snell, E. E. *J. Biol. Chem.* **1957**, *225*, 409.
(8) Metzler, D. E.; snell, E. E. *J. Am. Chem. Soc.* **1953**, *75*, 2786.
(9) Marcello, J. A.; Martell, A. E.; Abbott, E. H. *J. Chem. Soc., Chem. Commun.* **1975**, 16.
(10) Longenecker, J. B.; Ikawa, M.; Snell, E. E. *J. Biol. Chem.* **1957**, *227*, 663.
(11) Longenecker, J. B.; Snell, E. E. *J. Am. Chem. Soc.* **1957**, *79*, 142.
(12) Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *198*, 353.
(13) Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *198*, 363.
(14) Tatsumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1978**, *100*, 5549.
(15) Marcello, J. A.; Martell, A. E., *J. Am. Chem. Soc.* **1982**, *104*, 1087.
(16) Schwarzenbach, G. "Complexometric Titrations"; Interscience: New York, 1957.
(17) Holland, D. O.; Jenkins, P. A.; Nayler, J. H. C. *J. Chem. Soc.* **1953**, 273.
(18) Oh-Hashi, J.; Harada, K. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 22,87.
(19) Allen, D. F. H.; van Allen, J. "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III; p 213.

0002-7863/82/1504-3441$01.25/0

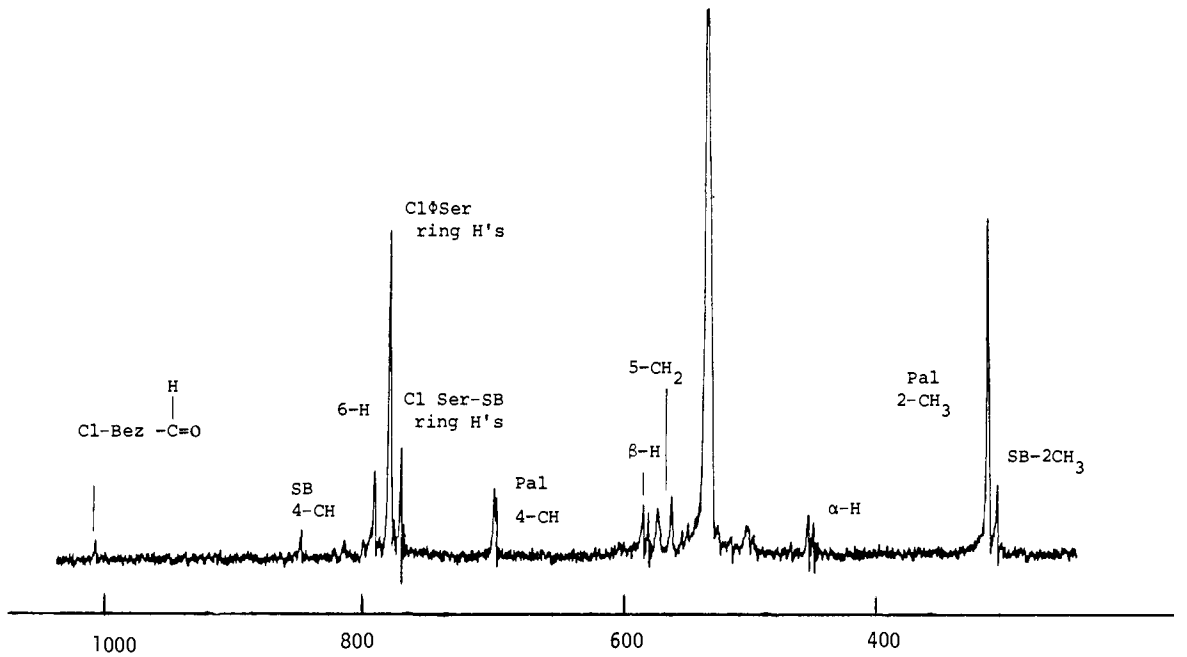


Figure 1. 100-MHz spectrum of an equimolar solution of pyridoxal and *p*-chlorohenylserine at pD 6.79 taken 1 h after mixing. Resonances are in Hz relative to HMDS. ClΦSer = *p*-chlorophenylserine; Cl–BEz = *p*-chlorobenzaldehyde. All Schiff base resonances indicated by SB; all others apply to amino acid or pyridoxal.

solution was cooled, the product precipitated out and was obtained; mp 213–214 °C dec. An NMR spectrum of the product gave the expected resonances.

Sodium *p*-Tolylglycidate. The epoxide salt was prepared by the general method described by Darzens[20] for the synthesis of other epoxides. To a solution of *p*-tolualdehyde (76.8 g) and ethyl chloroacetate (78.4 g) in a thoroughly dried three-necked flask equipped with an overhead stirrer and low temperature thermometer was added dropwise a solution of 15.3 g of sodium in 250 mL of ethanol. The reaction was maintained at a temperature of −15 to −10 °C by means of an external alcohol–ice–salt bath until all of the sodium ethoxide was added. The reaction was then stirred for 12 h at 0 °C and for 6 h more at room temperature. Two reflux columns were then attached to the side arms of the reaction flask and the reaction mixture was slowly heated to 90 °C, was refluxed at this temperature for 8 h, and then was allowed to cool. After the mixture had cooled to room temperature, 150 mL of ice water was added. The contents of the flask were then transferred to a large beaker, and glacial acetic acid was added to pH 5.

The brown solution was extracted with ether, and the organic layer was washed 3 times with 250 mL of water and then washed with 250 mL of 5% acetic acid. The ether extract was dried over sodium sulfate and the ether was removed under reduced pressure. The solution containing the glycidic ester was placed in a beaker, and a solution of 10.34 g of sodium in 200 mL of ethanol was added. Ten milliliters of cold water was then added, and the solution was cooled in an ice bath. After the sodium *p*-tolylglycidate was filtered, it was washed with 200 mL of absolute alcohol followed by 100 mL of dry ether. The product was air-dried for several hours, and a total of 66.9 g (76%) was collected. The identity of the material was confirmed by NMR.

The fractions of Schiff base in the experimental solution were determined from the sum of the resonances of 6-H, 4-CH, and 2-CH_3 groups. (Changes in the integrals of the individual groups paralleled the changes in the totals.)

2-(α-Benzylamino)-3-hydroxy-3-(*p*-methylphenyl)propanoic Acid (*N*-Benzyl-*p*-methylphenylserine). This compound was prepared by modification of the method described above for *N*-benzylphenylserine. Sodium *p*-tolylglycidate (15 g), water (10 mL), benzylamine (20 mL), and 10 M sodium hydroxide (2 mL) were treated as described above. The gummy residue obtained at pH 4 was placed in 50 mL of hot water and the pH was adjusted to ~7 with a few drops of 10 M sodium hydroxide. Absolute ethanol was added until the solution started to become cloudy (~35 mL). The solution was allowed to cool overnight under refrigeration. A yield of 6.9 g of the product, *N*-benzyl-*p*-methylphenylserine, was obtained.

***p*-Methylphenylserine.** The amino acid was obtained from the *N*-benzyl derivative by the method described above for DL-phenylthreonine. Hydrogenation of *N*-benzyl-*p*-methylphenylserine (6.9 g) required 30 h. A total of 3.9 g of the amino acid was obtained.

Procedures. The NMR spectra were obtained with a Varian HA-100 nuclear magnetic resonance spectrophotometer and were measured at the ambient probe temperature (30 ± 2 °C). The chemical shifts are given in hertz relative to an internal capillary of hexamethyldisiloxane (HMDS), the proton resonance of which was used as an internal locking signal. In this paper, pD is the negative logarithm of the deuterium ion concentration. The pD values were obtained by adding 0.40 to the apparent pH,[21] which was measured with a Corning Model 101 digitial pH meter equipped with a Beckman miniature combination electrode. The pD was adjusted with NaOD. The potentiometric apparatus was calibrated with standard buffer solutions, and readings were converted to -log $[H^+]$ through the use of appropriate activity coefficients. The concentrations of individual species in solution were obtained by integration of the appropriate resonances.

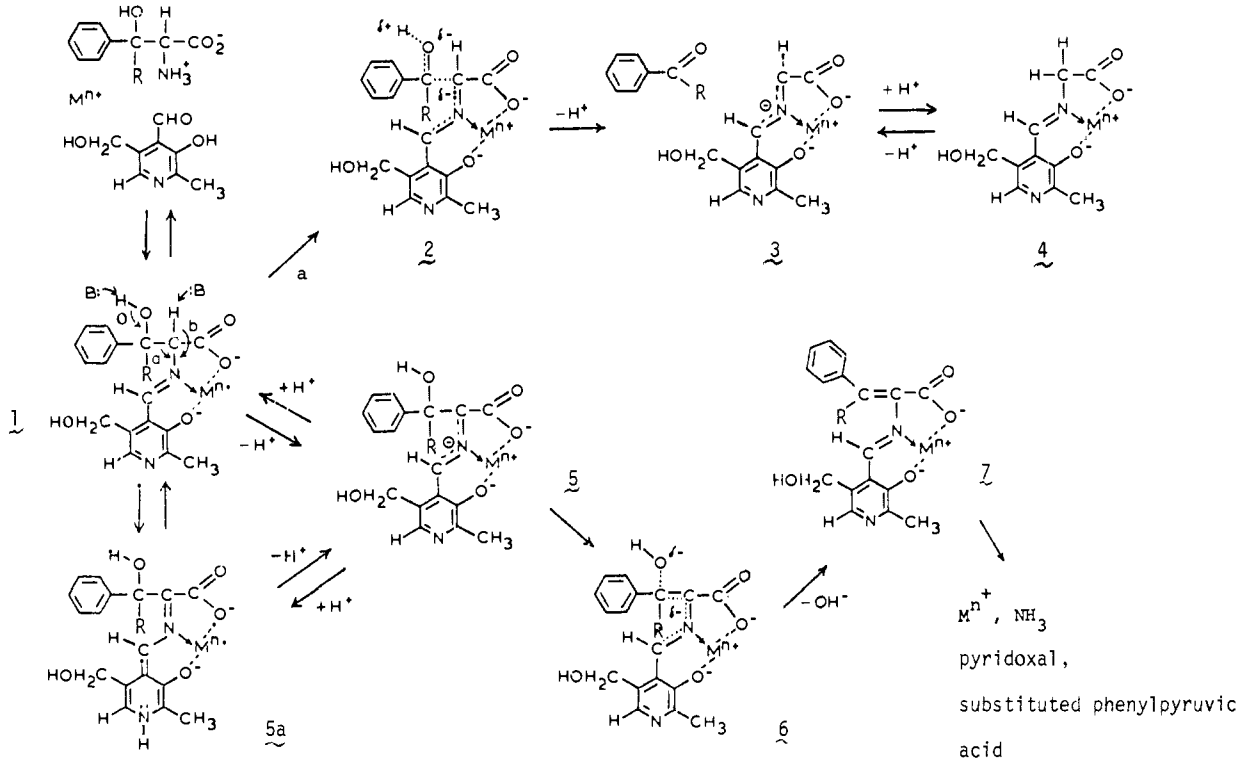
Results and Discussion

NMR Spectra for the Schiff Bases. *p*-Chlorophenylserine Schiff Base Systems. The NMR spectra of the pyridoxal–*p*-chlorophenylserine Schiff base and of the pyridoxal–*p*-chlorophenylserine–Al(III) Schiff base complexes were determined, and the appropriate resonances were assigned. The 100-MHz spectrum of pyridoxal–*p*-chlorophenylserine taken 1 h after mixing at pD 6.79 is shown in Figure 1. The α-proton resonance appears as a doublet at 424 Hz and the β-proton resonance is found at 561 Hz. The *p*-chlorophenylserine ring protons appear as a sharp singlet at 774 Hz. The resonances of the ring protons of *p*-chlorobenzaldehyde appear between 820 and 780 Hz as indicated. The aldehydic proton resonance can be seen at 1017 Hz. Since *p*-chlorobenzaldehyde is insoluble in D_2O, a mixed solvent system of 50% CH_3OH–D_2O was used for quantitative study. Reactions of the pyridoxal–*p*-chlorophenylserine Schiff bases were studied between pD 4.0 and 11.0 in the mixed solvent system. At pD 6.73 and above, one is able to detect the formation of two products in the reaction mixture, namely, *p*-chlorobenzaldehyde and *p*-chlorophenylpyruvic acid. This result indicates that both dealdolation and β elimination occur in these Schiff base systems. This is not unexpected since Tatsumoto and Martell[14] had observed both β elimination and dealdolation in pyridoxal–phenylserine Schiff base systems. The proposed mechanism for these reactions is shown in Scheme I. The pyridoxal–*p*-chlorophenylserine Schiff base systems as well as other para-substituted phenylserine Schiff

(20) Darzens, G. *C. R. Hebd. Seances Acad. Sci.* **1905**, *141*, 766.

(21) Glascoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

Scheme I. Proposed Mechanism for Parallel Dealdolation and β Elimination



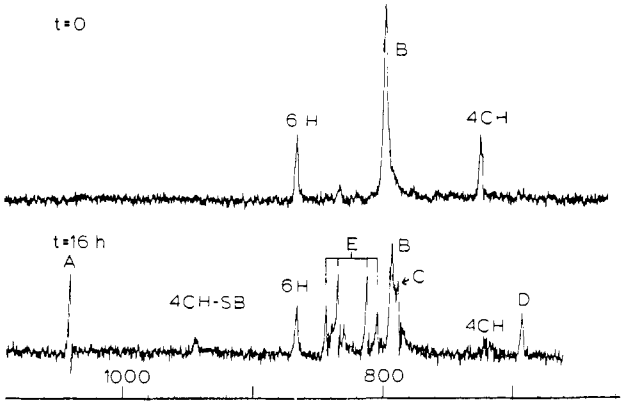


Figure 2. 100-MHz spectra of equimolar amounts of pyridoxal and *p*-chlorophenylserine taken immediately after mixing and at 16 h at pD 6.73 (50% MeOH-D_2O). Resonances in Hz relative to HMDS. A = *p*-chlorobenzaldehyde CHO; β = *p*-chlorophenylserine ring protons; C = *p*-chlorophenylpyruvic acid Schiff base ring protons; D = *p*-chlorophenylpyruvic acid Schiff base —CH═. All other resonances not labeled SB are those of pyridoxal.

base systems were chosen as models in order to determine the effects of these substitutions on the rates of dealdolation and β elimination. The NMR spectra of equimolar amounts of pyridoxal and *p*-chlorophenylserine taken immediately after mixing and at 16 h after mixing at pD 6.73 are shown in Figure 2. In these spectra the product of dealdolation, *p*-chlorobenzaldehyde, and the product of β elimination, *p*-chlorophenylpyruvic acid Schiff base, can be seen.

Both the 1:1:1 (pyridoxal–*p*-chlorophenylserine–Al(III)) and the 2:2:1 Schiff base chelate systems were studied. The 1:1:1 system was studied at low pD, below pD 4.40. Even though the extent of Schiff base formation at these low pD's was less than 8%, both dealdolation and β elimination were observed.

The 2:2:1 Schiff base chelate systems were studied between pD 7.5 and 10.5. The solutions below pD 7.5 were turbid, and it was not possible to obtain spectral data of quality sufficient to provide quantitative kinetic data. In the pD range 7.5–10.5 no resonances assignable to the free forms of either pyridoxal or *p*-chlorophenylserine were obtained, indicating that the Schiff base was completely formed in a rapid preequilibrium. It was apparent from the spectra in this pD range that dealdolation is favored over β elimination, since at any given time the concentration of *p*-chlorobenzaldehyde was greater than the concentration of the reaction product of β elimination.

***p*-Methylphenylserine Schiff Base Systems.** The NMR spectra of pyridoxal–DL-*p*-methylphenylserine and of pyridoxal–DL-*p*-methylphenylserine–Al(III) Schiff base complexes were determined and the resonances assigned. The metal-free systems were studied between pD 4.0 and 10.5. A sample NMR spectrum taken at pD 9.84, shown in Figure 3, indicates the formation of *p*-methylphenylpyruvic acid. Unlike the model system containing the pyridoxal–*p*-chlorophenylserine Schiff base for which dealdolation was favored, β elimination would appear to be favored when *p*-methylphenylserine is used. The appearance of a resonance corresponding to the aldehyde proton of *p*-methylbenzaldehyde at about 1010 Hz relative to HMDS is quite slow and accounts for only a small fraction of the total initial *p*-methylphenylserine concentration. For example, 3 days after mixing at pD 8.71, the extent of formation of *p*-tolylaldehyde amounted to about 12% of the initial molar concentration of amino acid in solution.

The pyridoxal–*p*-methylphenylserine–Al(III) Schiff base systems were studied between pD 4.5 and 11.0. As in the case of the metal-free systems, β elimination was observed as well as dealdolation. Here also, the extent of dealdolation accounted for a small fraction of the total reaction product.

***p*-Aminophenylserine Schiff Base Systems.** The metal-free pyridoxal–*p*-aminophenylserine Schiff base was studied between pD 8.0 and 10.5. The Al(III) chelate system was studied between pD 8.5 and 11.0. In both the metal-free and metal-catalyzed systems, dealdolation was readily observed.

***p*-Nitrophenylserine Schiff Base Systems.** Since *p*-nitrophenylserine was solubly only at high pD, NMR spectra of the pyridoxal–*p*-nitrophenylserine–Al(III) Schiff base chelates were measured in the pD range 9.0–10.5. The dealdolation of *p*-nitrophenylserine to *p*-nitrobenzaldehyde was readily observed under these conditions. In this system, as well as the *p*-aminophenylserine Schiff base system described above, it appears that dealdolation is very highly favored over β elimination. Even after a significant amount of dealdolation product had accumulated,

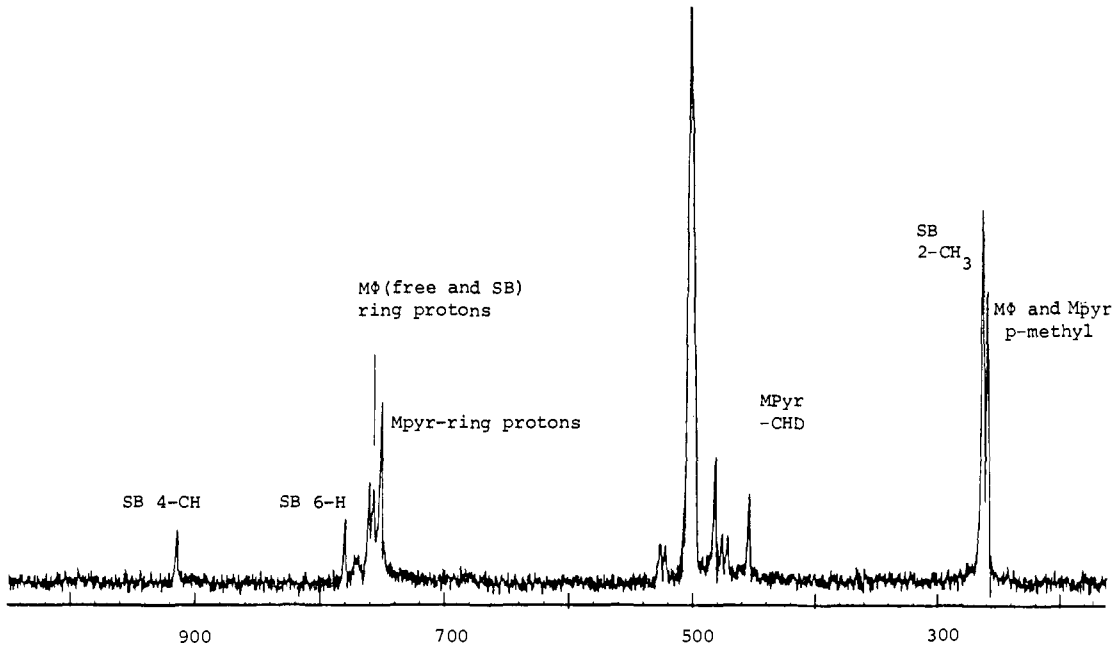


Figure 3. 100-MHz spectrum of an equimolar solution of *p*-methylphenylserine and pyridoxal at pD 9.84 taken 24 h after mixing. Resonances are in Hz relative to HMDS. MΦ = *p*-methylphenylserine; MPyr = *p*-methylphenylpyruvic acid Schiff base.

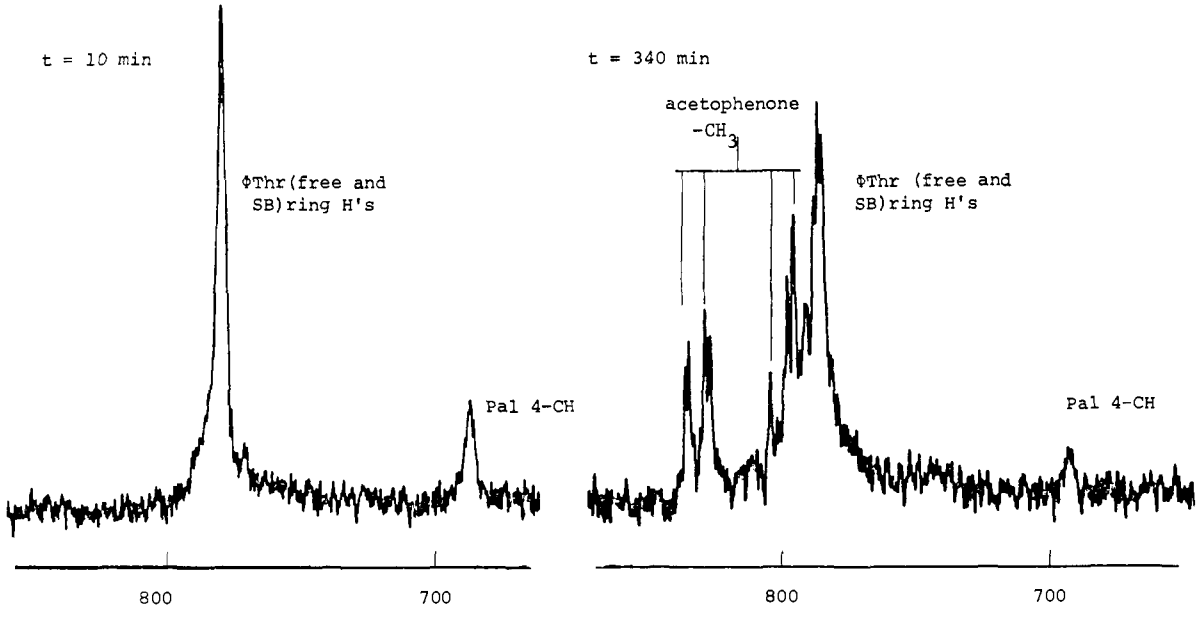


Figure 4. 100-MHz spectra of a solution of equimolar amounts of pyridoxal and phenylthreonine, ΦThr, at pD 8.24 showing the formation of the dealdolation product, acetophenone. Resonances are in Hz relative to HMDS.

only a very small proportion of the β-elimination product was detected. For this reason no attempt was made to calculate β-elimination rate constants for these Schiff bases.

Phenylthreonine Schiff Base Systems. Phenylthreonine was selected for study to determine the effect of an electron-donating (methyl) group at the β-carbon position on the rate of dealdolation, for comparison with phenylserine.

The NMR spectra of the pyridoxal–DL-phenylthreonine Schiff bases were measured over the pD range 4.5–10.5. Below pD 6.10 the NMR spectra for the 1:1 system exhibited resonances entirely attributable to free pyridoxal and phenylthreonine. As the pD was increased, resonances resulting from the formation of the Schiff base were observed. Evidence for dealdolation of the pyridoxal–phenylthreonine Schiff base to yield acetophenone can be seen in the NMR spectra in Figure 4. The changes in the resonances of the ring protons as a function of time provide kinetic data for this chemical transformation.

The pyridoxal–phenylthreonine–Al(III) Schiff base chelates were also studied. The resonances associated with the 2:2:1 complex were detectable at pD values as low as 4.5, even when the reactants were mixed in 1:1 molar ratios. Since it was not possible to form the 1:1:1 Schiff base–metal chelate in the absence of the bis(Schiff base) chelate at any pD where there was an appreciable amount of Schiff base, only the 2:2:1 system was studied with the Al(III) ion. Between pD values 6.8 and 8.0 the various samples that were prepared were turbid, and it was not possible to obtain quantitative data under these conditions because of the broadening of all the NMR resonances. Between pD 8.0 and 9.5 the solutions were sufficiently clear so that quantitative data could be obtained. At pD 9.53 and above the solutions were again somewhat turbid; however, the characteristic odor of acetophenone was detected immediately upon mixing of these samples at high pD.

Treatment of Kinetic Data. The determination of reaction rates was based upon measurements of integrated NMR resonances reflecting concentrations of the reaction products *p*-chlorobenzaldehyde, *p*-nitrobenzaldehyde, *p*-tolylaldehyde, or *p*-methylphenylpyruvic acid as well as the disappearance of the amino acids,

Table I. The Observed Rate Constants for the Pyridoxal-*p*-Chlorophenylserine-Al(III) Schiff Base Chelates

pD	$k^{M'}_{obsd}$, M^{-2} s^{-1}	$k^{M}_{obsd,2}$, s^{-1}	$k^{M}_{obsd,3}$, s^{-1}	$k_{obsd,2}/k_{obsd,3}$
3.60	$1.9 \pm 0.1 \times 10^{-3}$			
3.90	$3.1 \pm 0.1 \times 10^{-3}$			
4.30	$3.4 \pm 0.1 \times 10^{-3}$			
7.02		$0.98 \pm 0.1 \times 10^{-5}$	$2.5 \pm 0.1 \times 10^{-5}$	3.92
7.63		$3.3 \pm 0.1 \times 10^{-4}$	$7.7 \pm 0.1 \times 10^{-5}$	4.28
9.41		$6.5 \pm 0.1 \times 10^{-4}$	$1.5 \pm 0.1 \times 10^{-5}$	4.33
10.31		$9.2 \pm 0.2 \times 10^{-4}$	$2.1 \pm 0.1 \times 10^{-5}$	4.38

p-chlorophenylserine, *p*-nitrophenylserine, *p*-aminophenylserine, *p*-phenylthreonine, or *p*-methylphenylserine.

As mentioned above, both dealdolation and β elimination were found to occur simultaneously in some of the substituted phenylserine–pyridoxal systems. For the metal-free systems the rate equations are based on reaction scheme:

$$\text{Pal} + \text{XPhSer} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{SB} \xrightarrow{k_2} \text{XBzal} + \text{gly-SB}$$
$$\xrightarrow{k_3} \text{XPhpyr} + \text{Pal} + \text{NH}_3 \quad (1)$$

where XPhSer is the para-substituted phenylserine, XBal is the corresponding para-substituted benzaldehyde, and XPhpyr is the corresponding para-substituted phenylpyruvic acid. In the absence of an appreciable amount of Schiff base in the metal-free system, the following rate equation is obtained:

$$-\frac{d[\text{XPhSer}]}{dt} = \left(k_1 - \frac{k_1 k_{-1}}{k_{-1} + k_2 + k_3}\right)[\text{Pal}][\text{XPhSer}]$$
$$= k^{1}_{obsd}[\text{Pal}][\text{XPhSer}] \quad (2)$$

Similarly, for the metal–Schiff base 1:1:1 systems at low pD:

$$-\frac{d[\text{XPhSer}]}{dt} = \left(k_1 - \frac{k_1 k_{-1}}{k_{-1} + k_2 + k_3}\right)[\text{Pal}][\text{XPhSer}][M^{n+}]$$
$$= k^{M'}_{obsd}[\text{Pal}][\text{XPhSer}][M^{n+}] \quad (3)$$

The steady-state assumption is no longer applicable to Schiff base formation, where the extent of Schiff base formation is appreciable. Under such conditions, the changes in the concentration of the reactive Schiff base can be monitored by NMR. Under these conditions the following equations are employed:

$$d[\text{XBzal}]/dt = k_{obsd,2}[\text{SB}] \text{ or } k^{M}_{obsd,2}[\text{M-SB}] \quad (4)$$

$$d[\text{XPhpyr}]/dt = k_{obsd,3}[\text{SB}] \text{ or } k^{M}_{obsd,3}[\text{M-SB}] \quad (5)$$

Since no byproducts were observed, the reaction stoichiometry is given by

$$[\text{XPhSer}]_0 = [\text{XPhSer}] + [\text{XBzal}] + [\text{XPhpyr}] + [\text{SB}] \text{ or } ([\text{M-SB}]) \quad (6)$$

The values of the first-order rate constants $k_{obsd,2}$ and $k_{obsd,3}$ were then determined by a plot of $d[\text{XBzal}]/dt$ vs. [SB] and $d[\text{XPhpyr}]/dt$ vs. [SB] in the metal-free and metal-containing systems.

It should be noted that the rate constants determined in the lower pD range where no appreciable Schiff base concentration was detected are second order for the metal-free system and third order in the metal ion–Schiff base chelate system, in accordance with eq 2 and 3. Where the concentration of Schiff base is appreciable, the rate constants for the β-elimination and dealdolation reactions are first order with respect to Schiff base concentration, in accordance with eq 4 and 5.

Reaction Kinetics of Para-Substituted Phenylserine Schiff Bases. *p*-Chlorophenylserine. The pyridoxal–*p*-chlorophenylserine Schiff base and the Al(III) catalyzed Schiff base systems were studied and the rate constants determined. The values of the observed rate constants for the dealdolation reaction, $k_{obsd,2}$ and of the observed rate constant for the β-elimination reaction, $k_{obsd,3}$ are given in Table I. There are a number of factors that influence the rates of reaction in the metal-free system. The degree of protonation of the Schiff base and the actual concentration will affect the reaction rate. The diprotonated and monoprotonated forms of the Schiff base are the reactive intermediates in both dealdolation and β elimination, since protonation at the azomethine nitrogen favors the electron flow required for both reactions, as shown in Scheme I. In *p*-chlorophenylserine the benzene ring favors β elimination by conjugation and stabilization of transition state **6**, which leads to the formation of the β-eliminated Schiff base **7**. At the same time, the benzene ring in *p*-chlorophenylserine favors dealdolation by the conjugation and stabilization of transition state **2**, which leads to the formation of *p*-chlorobenzaldehyde and the glycine Schiff base **4**.

Even though the benzene ring stabilizes both the dealdolation and β-elimination intermediates, it is apparent from a comparison of the observed rate constants $k_{obsd,3}$ and $k_{obsd,2}$ that dealdolation is favored over β elimination. This may be due to the fact that the β-hydroxyl group is not a good leaving group. It has been reported that the choice of the leaving group is critical in determining the rates of β-elimination reactions.[7,22-24] These studies have shown that of the amino acids for which pyridoxal-catalyzed β elimination has been observed, the hydroxyl group is the poorest leaving group. Another factor that must be considered is that the electron-withdrawing *p*-chloro-substituent should increase the acidity of the β-hydroxyl proton somewhat and increase the probability of dissociation of this proton, thus favoring dealdolation. Perhaps still more important is the fact than an electron-withdrawing substitutent on the benzene ring would tend to impede the removal of the negative leaving group and would therefore interfere with the second rate-limiting step in the β-elimination reaction. This effect would result in mitigation of the competing β-elimination pathway and thus result in a net increase in the observed dealdolation rate. Yet another factor to consider is that since the pK_a of the azomethine nitrogen was found to be 10.25 and the pK_a of the pyridine nitrogen was found to be 6.40, the monoprotonated Schiff base is the predominate species under the reaction conditions employed. A Schiff base intermediate similar to **5** that is protonated at the azomethine nitrogen, but not at the pyridine nitrogen would upon deprotonation of the α-proton form an intermediate whose electrons are more localized in the region of the azomethine nitrogen. This would increase the likelihood of reprotonation at the α-carbon resulting in a net racemization reaction. Racemization for which the rate maximum occurs at pH 9[22] would compete with the β-elimination reaction.

The values of the observed rate constants k^{M}_{obsd2}, $k^{M}_{obsd,3}$ are given in Table I. The Al(III) ion enhances both the dealdolation and β-elimination reactions as indicated by the increase in the values of the observed rate constants. For example, in the metal-free system at pD 9.48 $k_{obsd,2}$ is 1.7×10^{-4} s^{-1} compared to a $k^{M}_{obsd,2}$ of 6.5×10^{-4} s^{-1}, while $k_{obsd,3}$ is 7.6×10^{-5} s^{-1} in the metal-free system and $k^{M}_{obsd,3}$ is 1.5×10^{-4} s^{-1}. This is expected since the electropositive Al(III) ion is more effective than a proton at the azomethine nitrogen in promoting the flow of electrons toward the azomethine nitrogen, which is required for dealdolation (**1** → **2** → **3** → **4**) and for β elimination (**1** → **5** → **6** → **7**). It

(22) Olivard, J.; Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *199*, 669.
(23) Tatsumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1977**, *99*, 6082.
(24) Tatsumoto, K. Ph.D. Dissertation, Texas A&M University, 1978.

Table II. Observed Rate Constants for Al(III) Catalyzed Dealdolation (Pyridoxal–Amino Acid–Al(III)) 2:2:1 Systems

amino acid	pD	$k^{M}_{obsd,2}$, s^{-1}
p-aminophenylserine	8.26	$3.4 \pm 0.1 \times 10^{-4}$
	9.32	$3.6 \pm 0.1 \times 10^{-4}$
	10.04	$4.6 \pm 0.1 \times 10^{-4}$
p-nitrophenylserine	9.38	$8.0 \pm 0.2 \times 10^{-4}$
	9.79	$8.8 \pm 0.2 \times 10^{-4}$

Table III. Observed Rate Constants for the Al(III) Ion–Pyridoxal Catalyzed Dealdolation of Para-Substituted Phenylserines

amino acid	$k^{M}_{obsd,2}$, s^{-1} [a]
phenylserine	3.2×10^{-4} [b]
p-aminophenylserine	3.6×10^{-4}
p-chlorophenylserine	6.7×10^{-4}
p-nitrophenylserine	8.0×10^{-4}
p-methylphenylserine	10^{-6}

[a] All pD's = 9.3 ± 0.1. [b] Reference 87; all other values are from this work.

is apparent that in the presence of Al(III) ion the dealdolation reaction is promoted to a greater extent than β elimination. In the metal-free system $k_{obsd,2}/k_{obsd,3}$ is between 2 and 2.5, whereas in the Al(III)–pyridoxal catalyzed systems $k^{M}_{obsd,2}/k^{M}_{obsd,3}$ is greater than 4. Although Al(III) ion may favor α deprotonation, a necessary first step in β elimination, over the metal-free case, the electron flow required for the subsequent loss of the β-hydroxyl group is opposed to the electrostatic influence on the metal ion. Thus metal ions, especially those of high ionic charge, increase dealdolation to a greater extent than β elimination.

***p*-Methylphenylserine.** In both the metal-free pyridoxal–*p*-methylphenylserine Schiff base system and in the 2:2:1 (pyridoxal–*p*-methylphenylserine–Al(III)) Schiff base chelate system, the rates of dealdolation and β elimination were found to be slow. Even at pD greater than 9 in the presence of Al(III) ion, detectable amounts of either *p*-tolylaldehyde or *p*-methylphenylpyruvic acid were not observed until the reaction had proceeded for several hours. At pD 9.28 in the Al(III) ion catalyzed Schiff base chelate system, the $k^{M}_{obsd,2}$ for dealdolation and $k^{M}_{obsd,3}$ of β elimination are on the order of 10^{-6} s^{-1}. Apparently the electron-donating inductive effect of the methyl group at the para position of the benzene ring hinders the stabilization of transition states (**2** and **6** in Scheme I) and the dissociation of the α-proton of the amino acid moiety, to form intermediate **5**. It would, however, assist in the dissociation of the hydroxide ion leaving group, which has also been shown to be rate determining in pyridoxal-catalyzed elimination reactions.[23,24] The observed effect is much greater than could possibly have been predicted, and on the basis of what is presently known about these systems, the low reactivity of the *p*-tolyserine Schiff base must be considered to be anomalous.

Other Para-Substituted Phenylserines. The values of the observed rate constants for the Al(III) catalyzed dealdolation of pyridoxal–*p*-aminophenylserine and of the pyridoxal–*p*-nitrophenylserine Schiff base complexes are listed in Table II. The values for k^{M}_{obsd} were determined over a small pD range because of the fact that the complex was insoluble outside the range employed. The values of k^{M}_{obsd} are greater for the chelate of the *p*-nitrophenylserine Schiff base than that of *p*-aminophenylserine. This is in accord with the reasoning employed above, that an electron-withdrawing substituent at the para position of the benzene ring tends to favor dealdolation. Since the nitro group is the most effective substituent of those employed, it is expected that the dealdolation reaction should proceed at rates greater than those observed for analogous compounds without electron-withdrawing substituents on the aromatic ring.

A comparison of the values of k^{M}_{obsd} for the various para-substituted phenylserines is given in Table III. The values of the observed rate constants for the Al(III) ion–pyridoxal Schiff base chelates support the arguments advanced above in the analysis of the *p*-chloroalanine reaction rates. If phenylserine is used as a reference compound, then one finds that the values for the observed dealdolation rate constants are in the order *p*-nitrophenylserine > *p*-chlorophenylserine > *p*-aminophenylserine > phenylserine > *p*-methylphenylserine. The *p*-methylphenylserine substrate is the only amino acid in the group that has an electron-donating substituent at the para position and thus is has the lowest ability to stabilize intermediate **2** in the dealdolation reaction sequence. The other substituted amino acids have *p*-phenyl substituents that have electron-withdrawing effects, and thus they are better able to stabilize the intermediate in the dealdolation reaction sequence and slow down the elimination of electronegative β substituents. The resonance stabilization of intermediate **2** in *p*-nitrophenylserine, *p*-chlorophenylserine, and *p*-aminophenylserine would also stabilize the transition state for β elimination (formula **6**), so that the use of resonance or conjugation alone for interpretations of the observed rates involves competitive effects for which it is difficult to draw definite conclusions.

Table IV. Observed Rate Constants for Pyridoxal–Al(III) Catalyzed Dealdolation of Phenylthreonine (2:2:1 System)

pD	k^{M}_{obsd}, s^{-1}
5.92	$0.58 \pm 0.1 \times 10^{-4}$
8.15	$1.4 \pm 0.1 \times 10^{-3}$
8.89	$2.6 \pm 0.1 \times 10^{-3}$
9.53	$2.8 \pm 0.1 \times 10^{-3}$
9.70	$2.9 \pm 0.2 \times 10^{-3}$
10.31	$3.1 \pm 0.2 \times 10^{-3}$

Effect of β-Methyl Substitution. In the course of an earlier study[15] it was found that an electron-donating group at the β-carbon of the amino acid moiety of the Schiff base tends to increase the rates of dealdolation reactions. It was also shown that a phenyl group at the β-carbon[24] or a *p*-substituted phenyl group at the β-carbon increases the rate of dealdolation. Phenylthreonine, which has both a methyl group and a phenyl group at the β-carbon, was chosen as a substrate for further study in order to observe what the overall effects on the rate of dealdolation would be on an amino acid containing both of these β-carbon substituents. The values of the observed rate constants for the dealdolation of the pyridoxal–phenylthreonine and of the 2:2:1 (pyridoxal–phenylthreonine–Al(III)) Schiff base chelate are given in Table IV. The values obtained for k^{M}_{obsd} are somewhat less reliable at and above pD 9.7 because at this pD and above the solutions were slightly turbid and thus it was not possible to determine the concentrations of species as accurately as was possible at the lower pD values. At pD values above 10.3 the rate of dealdolation reaction was so fast that by the time the reactants could be mixed and the first spectra taken, the reaction had proceeded to over 50% completion. Thus it is not possible to obtain precise quantitative data above pD 10.3.

It would appear from the values obtained from the k^{M}_{obsd} for the pyridoxal–Al(III) ion catalyzed dealdolation of phenylthreonine that the combined influence of the methyl group and the phenyl group at the β-carbon in phenylthreonine are as effective, or more effective, in promoting dealdolation as the two methyl groups at the β-carbon in β-hydroxyvaline. For example, at pD 9.70 the value of k^{M}_{obsd} is 2.9×10^{-3} s^{-1} for the phenylthreonine system while k^{M}_{obsd} at pD 9.78 has a value of 2.5×10^{-3} s^{-1} in the β-hydroxyvaline Schiff base chelate system.

Specific Rate Constants of Protonated Schiff Bases (Metal-Free Systems). The value of the observed rate constant, k_{obsd}, for the dealdolation of the metal-free pyridoxal–phenylthreonine system is dependent upon the sum of the rates of the individual reactive Schiff base species. As pointed out above, the reactive forms of the Schiff base are the monoprotonated and the diprotonated Schiff bases. The catalytic effect of hydroxide ion on the monoprotonated form of the Schiff base must also be taken into account. The values of k_{obsd} may be considered to be the summation of the individual rate constants associated with the three forms of the Schiff base, namely, the diprotonated form (H_2SB), the monoprotonated form (HSB), and the deprotonated form (SB). Thus, the conditional rate constant k_{obsd} may be expressed as

$$k_{obsd}[SB_T] = k^{I}[H_2SB] + k^{II}[HSB] + k^{III}[SB] \quad (7)$$

where $[SB_T]$ is the total concentration of Schiff base species. Since the total concentration of Schiff base varies with pH, it is more convenient to express eq 7 in the following way:

$$k_{obsd} = k^{I}\frac{[H_2SB]}{[SB_T]} + k^{II}\frac{[HSB]}{[SB_T]} + k^{III}\frac{[SB]}{[SB_T]}$$

The mole fraction of each species can be determined as a function of pH with the aid of the appropriate pK_a values of the Schiff base. The completely deprotonated form of the Schiff base should have little, if any, inductive effect toward the dealdolation reaction, since it does not have protons coordinated to either the azomethine nitrogen or the pyridine nitrogen. Thus, the contribution of the deprotonated Schiff base toward the overall dealdolation reaction can be neglected in determining the specific rate constants. The conditional rate constant may now be expressed by

$$k_{obsd} = k^{I}\frac{[H_2SB]}{[SB_T]} + k^{II}\frac{[HSB]}{[SB_T]} \quad (8)$$

Another factor that must be considered in the dealdolation reaction sequence is the effect of hydroxide ion concentration on the overall reaction rate. In both the metal-free and metal ion–pyridoxal model systems studied, the value of k_{obsd} increases with increased alkalinity. The hydroxide ion can aid in the removal of the β-hydroxyl proton of the amino acid moiety and thus should promote the shift of electrons shown in Scheme I (formulas **1** and **2**). Therefore, at higher pH values where the hydroxide ion becomes significant, allowance for the contribution of the hydroxide ion should be incorporated in the rate law. Thus, the specific rate constants may be expressed by

$$k_{obsd} = k^{I}\frac{[H_2SB]}{[SB_T]} + k^{II}\frac{[HSB]}{[SB_T]} + k^{IV}\frac{[HSB][OH^-]}{[SB_T]} \quad (9)$$

It should be noted that there is no specific rate constant corresponding to $k^{V}[SB][OH^-]/[SB_T]$ since the contribution of the hydroxide ion on the deprotonated Schiff base is neglected for the reason stated above. Also the effect of hydroxide ion on the diprotonated species is neglected because at pH values where the hydroxide ion becomes significant the concentration of the diprotonated Schiff base is negligible. The values of k^{I}, k^{II}, and k^{IV} are obtained by the use of regression analysis[25] of the sum of the squares of the errors, U, by minimizing U, where U is defined as

$$U = \sum\{k_{obsd}[SB_T] - k^{I}[H_2SB] - k^{II}[HSB] - k^{IV}[HSB][OH^-]\}^2 \quad (10)$$

The values of k_{obsd} are found to be adequately represented by eq 10. The mole fraction of each species in solution is determined from the species distribution obtained from the protonation constants, which were determined spectrophotometrically. The constants found for the phenylthreonine Schiff base are pK_1 = 6.35 and pK_2 = 10.20. The specific rate constants for the dealdolation reaction pyridoxal–phenylthreonine system are k^{I} = 1.42×10^{-3} s^{-1}, k^{II} = 9.8×10^{-4} s^{-1}, and k^{IV} = 14.30 M^{-1} s^{-1}. These specific rate constants were used to determine calculated values for k_{obsd}. The experimental and calculated values for k_{obsd} are given in Table V. The small differences between the observed and calculated values indicate that the model represented eq 10 is a reasonable one.

Table V. Observed Rate Constants for Pyridoxal-Catalyzed Dealdolation of Phenylthreonine

pD	k_{obsd}^{exptl}, s^{-1}	k_{obsd}^{calcd}, s^{-1}	% difference[a]
8.24	$1.02 \pm 0.05 \times 10^{-3}$	1.00×10^{-3}	2.0
9.19	$1.16 \pm 0.05 \times 10^{-3}$	1.08×10^{-3}	7.0
9.52	$1.20 \pm 0.05 \times 10^{-3}$	1.18×10^{-3}	1.6
9.90	$1.37 \pm 0.05 \times 10^{-3}$	1.42×10^{-3}	3.6
10.43	$2.10 \pm 0.05 \times 10^{-3}$	2.06×10^{-3}	2.0

[a] $\sigma = \Sigma n_i(k_{obsd}^{calcd} - k_{obsd}^{exptl})^2/(n-1) = 9.8 \times 10^{-9}$.

On the basis of the values obtained for the specific rate constants, it would appear that the diprotonated form of the Schiff base is intrinsically more reactive. However, since the second protonation constant has a value of 6.35, the contribution of this species to the overall rate constant is minimal in the pD range where there is an appreciable amount of Schiff base species. The contribution of the hydroxide term to the overall reaction rate is also found to be significant. It should be pointed out that the k^{III} and $k^{IV}[OH^-]$ terms are kinetically indistinguishable, since they are related to each other by the first protonation constant of the Schiff base. However, it would seem highly unlikely that an unprotonated Schiff base would be an effective catalyst for the dealdolation of the Schiff base species, since as is apparent from the reaction scheme, the flow of electrons needed to carry out the dealdolation would be promoted by a neutral azomethine nitrogen to a much lesser extent than by a positive (protonated) azomethine group.

The values of the monoprotonated and diprotonated specific rate constants in the phenylthreonine system are greater than the specific rate constants obtained for the β-hydroxyvaline system.[15] This result indicates that the combined effects of the methyl and phenyl group at the β-carbon may be more significant than the effects of two alkyl groups at the β-carbon. Thus, the stabilization of intermediate **2** by the phenyl group in the phenylthreonine Schiff base is an important factor in promoting dealdolation in pyridoxal-catalyzed systems.

Acknowledgment. This research was supported by Grant AM-11694 from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health.

Registry No. Pyridoxal hydrochloride, 58-56-0; *threo*-*p*-chlorophenylserine, 37925-17-0; *p*-nitrophenylserine, 10098-38-1; *p*-aminophenylserine, 81520-50-5; *N*-benzylphenylthreonine, 81476-86-0; sodium β-phenyl-β-methylglycidate, 25957-43-1; DL-phenylthreonine, 81476-87-1; sodium *p*-tolylglycidate, 81476-88-2; *p*-tolualdehyde, 104-87-0; ethyl chloroacetate, 105-39-5; *N*-benzyl-*p*-methylphenylserine, 81476-89-3; *p*-methylphenylserine, 52773-84-9.

(25) Brookes, C. J.; Betteley, I. G.; Loxston, S. M. "Mathematics and Statistics for Chemists"; Wiley: New York, 1966; Chapter 15.