(11) I. Ugi and C. Steinbrückner, *Chem. Ber.*, **94**, 2802–2814 (1961).
(12) Another approach, "amine capture", using 4-methyloxy-3-acyloxysalicylaldehyde has been developed by Kemp et al., ref 13.
(13) D. S. Kemp, D. Roberts, C. Hoyng, J. Grattan, F. Vellacio, and J. Reczek in "Peptides: Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, pp 295–305.
(14) R. Urban, G. Eberle, D. Marquarding, D. Rehn, H. Rehn, and I. Ugi, *Angew. Chem.*, **88**, 644–646 (1976).
(15) R. Urban and I. Ugi, *Angew. Chem., Int. Ed. Engl.*, **14**, 61–62 (1975).
(16) R. Geiger, G. Jäger, W. König, and A. Volk, *Z. Naturforsch. B*, **24**, 999–1004 (1969).
(17) I. Ugi, W. Betz, U. Fetzer, and K. Offermann, *Chem. Ber.*, **94**, 2814–2816 (1961).
(18) I. Ugi and K. Offermann, *Chem. Ber.*, **97**, 2996–3007 (1964).
(19) H.-J. Prätorius, J. Flossdorf, and M.-R. Kula, *Chem. Ber.*, **108**, 3079–3090 (1975).
(20) Ugi and Steinbrückner (ref 11) reported a synthesis of this tripeptide.
(21) P. Sieber, B. Kamber, A. Hartmann, A. Jöhl, B. Riniker, and W. Rittel, *Helv. Chim. Acta*, **57**, 2617–2621 (1974); B. Kamber, A. Hartmann, A. Jöhl, F. Märki, B. Riniker, W. Rittel, and P. Sieber in "Peptides: Chemistry, Structure, and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, pp 477–485.
(22) The use of tetramethylurea as a solvent for 4CC (I. Ugi, personal communication) has not been investigated in this work.
(23) H. R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2348–2351 (1926).
(24) J. H. Billman and E. O'Mahony, *J. Am. Chem. Soc.*, **61**, 2340–2341 (1939).
(25) M. Passerini, G. Ragni, and L. Simone, *Gazz. Chim. Ital.*, **61**, 964–969 (1931).
(26) M. Waki and J. Meienhofer, *J. Org. Chem.*, **42**, 2019–2020 (1977). Prepared from For-L-Leu-OBu-*t* by treatment with phosgene in $CH_2Cl_2$–*N*-methylmorpholine at −30 °C (ref 28).
(27) D. Marquarding, P. Hoffmann, H. Heitzer, and I. Ugi, *J. Am. Chem. Soc.*, **92**, 1969–1971 (1970).
(28) H. Immer, V. Nelson, W. Robinson, and M. Götz, *Justus Liebigs Ann. Chem.*, 1789–1796 (1973).
(29) H. L. Maia, B. Ridge, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 98–105 (1973).
(30) P. Hoffmann, D. Marquarding, and I. Ugi, unpublished. Quoted in ref 8.
(31) A. Patchornik, B. Amit, and R. B. Woodward, *J. Am. Chem. Soc.*, **92**, 6333–6335 (1970).
(32) F. Weygand, W. Steglich, and J. Bjarnason, *Chem. Ber.*, **101**, 3642–3648 (1968).
(33) L. Wackerle and I. Ugi, *Synthesis*, 598–599 (1975).
(34) Y. Wolman, *Synthesis*, 732 (1975).
(35) This compound was readily prepared by the reaction of 3-formylindole (1 equiv) with triethylamine (3 equiv) and Boc-azide (2 equiv) in tetrahydrofuran–water (1:1 v/v for 15 h) at 45–50 °C in a yield of 78% after purification by silica gel column chromatography using $CHCl_3$ as an eluent, mp 124.5–125.5 °C (lit. mp 124–125 °C,[33] mp 121–123 °C[34]), $R_f$ 0.92 (A). Anal. ($C_{14}H_{15}NO_3$) C, H, N (ref 49).
(36) 4,4′-Dimethyloxybenzophenone, which is insoluble in methanol, ethanol, 1-butanol, and 2,2,2-trifluoroethanol, did not provide any product even when dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol.
(37) The diastereoisomer Z-Gly-D-Ala-Leu-Gly-OBu-*t* (**46**) and the corresponding acids (**47** and **48**) were also prepared.
(38) Peptides which contain tryptophan or other photosensitive residues would not be amenable to photolysis under the conditions used (ref 39).
(39) S. S. Wang, *J. Org. Chem.*, **41**, 3258–3261 (1976).
(40) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164–2167 (1967).
(41) R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc. C*, 1911–1916 (1969).
(42) Compound **27** (5.23 mg) dissolved in electrolyte (1 mL, composed of 25% DMF in 0.2 M pyridine and 0.1 M HOAc, pH 5.19) was added in five equal portions over 4 min to an electrolytic cell containing 10 mL of preequilibrated electrolyte. Controlled potential electrolysis was conducted for 3 h at −1.15 V and 20 °C. TLC showed complete cleavage.
(43) Half-wave potentials of compound **27** in volts relative to the saturated calomel electrode were −0.78 (at pH 0.2 in 1 M HCl), −0.81 (pH 1, 0.1 M HCl), −0.87 (pH 2.25, 0.01 M HCl + 0.09 M KCl), −1.07 (pH 4.72, 0.1 M acetate buffer), and −1.19 (pH 7.0, 0.1 M phosphate buffer).
(44) Examination of these aldehydes had been suggested to us by Dr. C. Birr, Heidelberg, in a private communication.
(45) K. Kuromizu and J. Meienhofer, *J. Am. Chem. Soc.*, **96**, 4978–4981 (1974).
(46) R. Charles, B. Felbush, and E. Gil-Av in "Peptides 1974", Y. Wolman, Ed., Wiley, New York, N.Y., 1975, pp 93–96; see also E. Gil-Av, *ibid.*, pp 247–256.
(47) The route of preparation of **45**, i.e., by carbodiimide–*N*-hydroxysuccinimide mediated condensation of Z-Gly-Ala-OH and H-Leu-Gly-OBu-*t*, did not exclude the possibility of some low degree of racemization (see ref 48).
(48) W. König and R. Geiger, *Chem. Ber.*, **103**, 2024–2033 (1970).
(49) NMR and IR spectral data agreed with the expected values.
(50) M. T. Bogert and A. Stull in "Organic Syntheses", Collect. Vol. I, Wiley, New York, N.Y., 1941, pp 220–221.
(51) IR spectral data agreed with expected values.
(52) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359–3363 (1960).
(53) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067–1068 (1955).
(54) J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Am. Chem. Soc.*, **74**, 3822–3825 (1952).
(55) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525–2528 (1961).
(56) R. Roeske, *Chem. Ind. (London)*, 1121–1122 (1959).
(57) E. Wünsch in Houben-Weyl, "Methoden der Organischen Chemie", Vol. 15, Part 1, E. Müller, Ed., Georg Thieme Verlag, Stuttgart, 1975, p 397.
(58) N. Izumiya, M. Muraoka, and H. Aoyagi, *Bull. Chem. Soc. Jpn.*, **44**, 3391–3395 (1971).
(59) S. Goldschmidt and K. K. Gupta, *Chem. Ber.*, **98**, 2831–2836 (1965).
(60) B. F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, **73**, 3508–3510 (1951).
(61) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **117**, 189–202 (1937).
(62) R. Willstätter and E. Waldschmidt-Leitz, *Ber. Dtsch. Chem. Ges. B*, **54**, 113–138 (1921); H. Wieland, *ibid.*, **45**, 484–493 (1912).
(63) F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch. B*, **21**, 426–428 (1966).

# Catalysis of the β Elimination of *O*-Phosphoserine and β-Chloroalanine by Pyridoxal and Zinc(II) Ion

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**Abstract:** The β-elimination reaction of *O*-phosphoserine and β-chloroalanine was investigated by proton NMR in deuterium oxide media at 30 ± 2 °C in the presence of pyridoxal and zinc ions. The reaction rate constants and relevant equilibrium constants were obtained for these systems. Catalysis by pyridoxal alone showed an increase in rate as pH was increased with a rate maximum in the region of pH ~9. Metal ion–pyridoxal catalysis was observed when zinc(II) was added to the amino acid–pyridoxal system. The observed catalysis is dependent on β-substituent electronegativity, and the rate-determining steps involve α-proton abstraction as well as β-substitution dissociation from the amino acid moiety. A new intermediate in the β-elimination reaction has been detected.

β elimination of electronegative substituents from α-amino acids is an interesting example of pyridoxal and metal ion catalysis. Gregerman and Christensen[1] have reported β elimination of chloride from β-chloroalanine, and Longenecker and Snell[2] have reported β elimination of phosphate from *O*-phosphothreonine and *O*-phosphoserine. The nonenzymatic reactions promoted by metal ions have mechanistic similarities to the corresponding enzymatic reactions. The mechanism involves the initial labilization of the α-hydrogen atom of the α-amino acid moiety following the condensation of α-amino acid and pyridoxal to form an aldimine Schiff base, **1**. β elimination then occurs when the β substituent is a reasonably

Table I. Proton Dissociation Constants

| Amino acid | $pK_1$ | $pK_2$ | $pK_3$ | $pK_4$ |
|---|---|---|---|---|
| Pyridoxal,[a] $H_2L^+$ | 4.20 | 8.66 | 13.0 | |
| β-Chloroalanine, $H_2L^+$ | 1.95 | 8.18 | | |
| O-Phosphoserine,[b] $H_3L$ | 2.07 | 5.62 | 9.71 | |
| β-Chloroalanine Schiff base, $H_3L^+$ | | 6.3 | 9.2 | |
| O-Phosphoserine Schiff base, $H_4L$ | | | 6.1 | 9.0 |

[a] D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **77**, 2431 (1955). [b] A. E. Martell and R. M. Smith, "Critical Stability Constants", Vol. 1, Plenum Press, New York, N.Y., 1974, p 29.

good leaving group. The resulting unsaturated aldimine complex subsequently undergoes hydrolysis to the keto acid, pyridoxal, and ammonia. The purpose of the present work is to undertake a detailed kinetic study of the reaction sequences of β elimination as an aid in determining the reaction mechanism and possibly contributing to the understanding of the nature of the corresponding enzymatic reactions.

In general, previous workers[1-5] have followed the nonenzymic β-elimination reactions by spectrophotometric measurement of recovered products, concurrent with product analysis and other physical methods. In this paper, the use of NMR measurements is employed for both kinetic and equilibrium determinations of β-chloroalanine and O-phosphoserine under β-elimination conditions in the presence of pyridoxal and zinc(II) ion.

## Experimental Section

Pyridoxal hydrochloride was obtained from Mann Laboratories as Mann Analyzed grade and was used without further purification. The amino acids, β-chloroalanine and O-phosphoserine, were obtained from Cyclo Chemical and Calbiochem, respectively. The NaOD and $D_2O$ were obtained from Diaprep Corporation. The purity of $D_2O$ was 99.7% and NaOD was diluted to the appropriate concentration under dry nitrogen. A stock solution of the zinc(II) ion was prepared from the nitrate salt. The zinc(II) solution was standardized by conventional chelatometric titration.[6]

The pH was measured with a Beckman research pH meter fitted with a combination glass electrode, and pH values were adjusted with NaOD. The pH was calibrated before and after each kinetic run by the use of buffers in accordance with standard procedures. The pH values thus obtained were converted to hydrogen ion concentrations through the use of appropriate activity coefficients. In the case of $D_2O$ solutions, the deuterium ion concentration was computed by adding 0.40 to the observed reading of the meter. NMR spectra were obtained with a Varian HA-100 nuclear magnetic resonance spectrometer. The temperature of the reaction was maintained at 30 ± 2 °C, the ambient temperature of the NMR probe. The ionic strength was maintained at $\mu$ = 1.0 with potassium nitrate. In binary metal-free pyridoxal-amino acid systems the analytical concentrations of amino acid and pyridoxal were 0.20 M. In ternary metal-pyridoxal-amino acid systems the concentrations of amino acid and pyridoxal were 0.20 M, and the concentrations of metal ions were set at 0.20 and 0.10 M for stoichiometric concentration ratios of 1:1:1 and 2:2:1, respectively. The chemical shifts are reported in hertz with respect to the resonance of tetramethylsilane ($Me_4Si$) in a coaxial tube.

All kinetic runs were carried out in homogeneous systems. The fraction of Schiff base in the experimental solutions was determined by (the sum of) the resonances of the 6-H, 4-CH, and 2-$CH_3$ groups. The fractions of Schiff base used in equilibrium calculations were obtained from the initial NMR measurements in the kinetic runs.

## Results and Discussion

**Equilibria.** The proton dissociation constants, $pK_a$, for β-chloroalanine, pyridoxal, and Schiff base are listed in Table I. The results correspond closely to the reported constants[7-9] for similar pyridoxal-amino acid Schiff base systems. The equilibrium constants, $K_f$, employed for expressing Schiff base equilibria were those used by Leussing[8] and are defined as:

$$\text{pyridoxal}^- + \text{amino acids}^- + jH^+ \overset{K_{fj}}{\rightleftharpoons} \text{Schiff base } H_j^{j-2}$$

$$K_{fj} = \frac{[\text{Schiff base } H_j^{j-2}]}{[\text{pyridoxal}^-][\text{amino acid}^-][H^+]^j}$$

Conditional constants, $K_{cond}$, were calculated from the values of $K_{fj}$ for comparison with those reported by Murakami[5] and Metzler[10] for similar Schiff bases. This comparison is given in Table II. The conditional constants are defined as:

$$K_{cond} = \frac{[\text{Schiff base}_T]}{[\text{pyridoxal}_T][\text{amino acid}_T]}$$

The agreement between the values listed in Table II is quite satisfactory considering the different conditions. The species distribution calculated from the formation constants in Tables I and II showed an increase in Schiff base formation with increase of pH, verifying the trend observed qualitatively in the NMR measurements.

**NMR Study of Reaction Products and Intermediates.** Autodechlorination and metal-catalyzed dechlorination of β-chloroalanine in the absence of pyridoxal were investigated over a pH range of 4.5–9.5 at 25, 30, and 37 °C by monitoring the free chloride anion through the use of an ion specific chloride electrode. Neither autodechlorination nor metal-catalyzed dechlorination was observed under these conditions. Higher temperatures were considered impractical and were not investigated in view of the fact that Gregerman and Christensen[1] reported rapid spontaneous degradation of β-chloroalanine at 100 °C. In the present work, spontaneous dephosphorylation of O-phosphoserine, as well as metal-catalyzed dephosphorylation, was also investigated at 30 °C in the pH 4.5–9.5 range, under which conditions no detectable reaction was observed.

At pH values less than 4 in the presence of metal ions, and at pH values less than 6 in the absence of metal ions, the NMR spectrum of an equimolar solution of pyridoxal and β-chloroalanine consists of resonances attributable entirely to the components, as shown in Figure 1 for pH 4.9 with no metal ions present. The pyridoxal resonances have been assigned, discussed in detail, and are well known.[11] The β-chloroalanine resonances consist of a triplet near 460 Hz (the α proton) and a multiplet near 420 Hz (the β proton). The O-phosphoserine resonances are assigned as a triplet near 460 Hz (the α proton) and a multiplet near 440 Hz (the β proton). As the pD is raised

Table II. Formation Constants of Schiff Bases

| Amino acid | Log $K_{cond}$ | Log $K_{f_0}$ | Log $K_{f_1}$ | Log $K_{f_2}$ |
|---|---|---|---|---|
| O-Phosphothreonine | 1.70[a] | | | |
| Threonine | 1.48[a] | | | |
| Alanine | 1.61[b] | 1.53[c] | 12.30[c] ± 0.01 | 18.63[c] ± 0.08 |
| β-Chloroalanine | 1.66 | 0.54 | 9.7 ± 0.1 | 16.0 ± 0.2 |
| O-Phosphoserine | 1.27 | 0.52 | 9.6 ± 0.1 | 15.6 ± 0.2 |

[a] Reference 5. [b] Reference 12. [c] Reference 10.

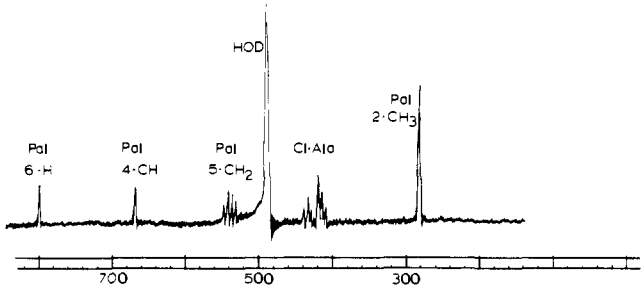



Figure 1. The 100-MHz NMR spectrum of 0.20 M pyridoxal and 0.20 M β-chloroalanine at pD 4.9; frequencies are reported in hertz with respect to $Me_4Si$.

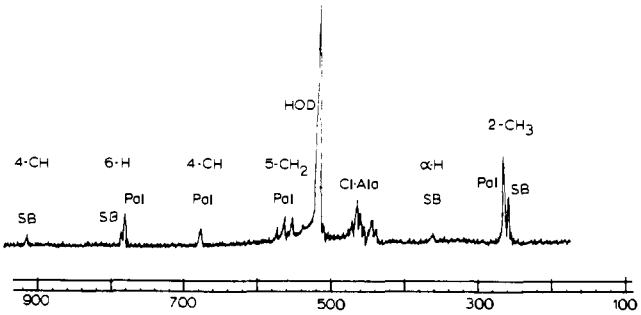



Figure 2. The 100-MHz NMR spectrum of 0.20 M pyridoxal and 0.20 M β-chloroalanine at pD 8.7 showing the formation of pyridoxylidene-chloroalanine; frequencies are reported in hertz with respect to $Me_4Si$.

above 6, resonances of the Schiff base, **1**, became apparent. These are labeled SB in Figure 2. The 5-$CH_2$ resonances of these Schiff bases are obscured by the intense resonance of HOD. All resonances shift to higher field with increasing basicity. The 4-CH resonance of the Schiff base species initially formed is located near 900 Hz.

At high pD values a new peak near 820 Hz appears after the reaction has proceeded for some time (Figure 3), and this phenomenon is observed for both amino acid systems. Appearance of this new peak correlates with the parallel disappearance of the 4-CH Schiff base resonance near ~900 Hz. With no noticeable change in 6-H resonances of pyridoxal, this new peak is assigned as the 4-CH resonance of a new Schiff base intermediate which seems to be stable for a considerable length of time, as shown in Figure 3.

The probable structure of this intermediate is discussed below. The products of β elimination resulting from the hydrolysis of the Schiff base intermediate were identified at low and neutral pH by detection of resonances near 250 Hz for the keto form and at 160 Hz for the diol form of pyruvic acid, as well as the familiar resonances of pyridoxal.

Thus, it is seen that NMR data are in agreement with and generally support the β-elimination mechanism outlined in Scheme I, through the observation of the disappearance of reactants, formation of the intermediate Schiff base **1**, and the appearance of reaction products. Formulas **1** and **2** are the familiar tautomeric forms of the monoprotonated Schiff base suggested by Snell and coworkers[3,12] as a general intermediate in several vitamin $B_6$ catalyzed reactions. Intermediates **3** and **4** were not formed in sufficient quantities to be detected by NMR, but are required for the essential steps in the elimination of a proton (in **3**) from the α position, followed by loss of the electronegative substituent (in **4**), to give the final intermediate that may yield reaction products on hydrolysis.

The new intermediate has a spectrum illustrated by Figure 3, and is characterized by a 4-CH resonance that is considerably upfield (~75 Hz) from that observed for the 4-CH resonance of the pyridoxal Schiff base, which is usually observed

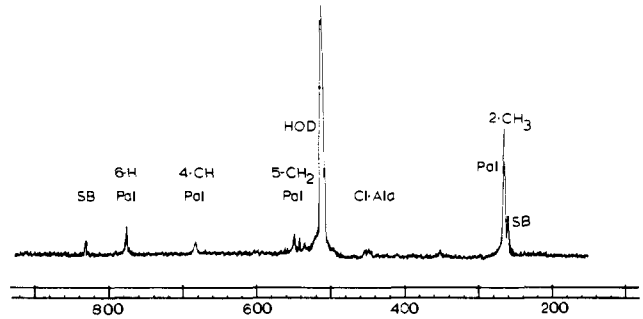
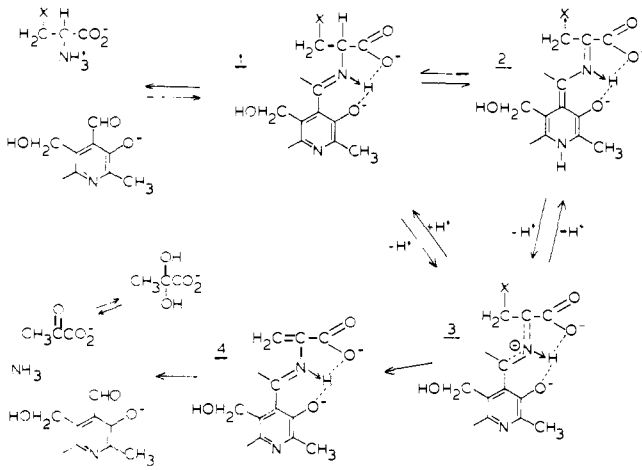
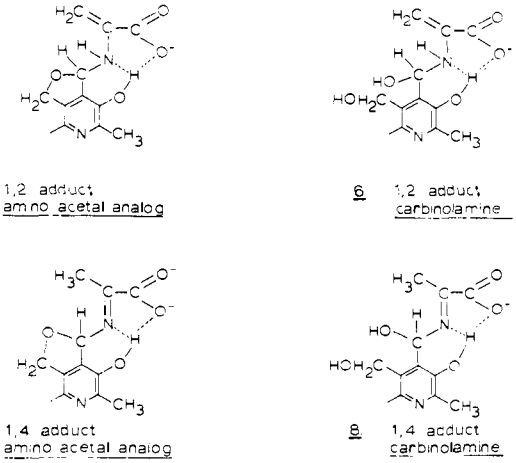



Figure 3. The 100-MHz NMR spectrum of 0.20 M pyridoxal and 0.20 M β-chloroalanine at pD 8.7 after 24 h showing the resonance of the new intermediate at 835; frequencies are reported in hertz with respect to $Me_4Si$.

Scheme I



around 900 Hz. Since this shift indicates a change in the chemical environment of the 4-CH position in the direction that would result from addition to and saturation of the azomethine linkage, it would seem that the intermediate is formed by addition of water or the adjacent hydroxyl group across the conjugated double bond system of **4**. Such addition reactions could take place to form 1,2 or 1,4 adducts, as indicated by formulas **5–8**.




There is considerable evidence in favor of 1,4 addition and the elimination of 1,2 addition as a possibility for this intermediate. The azomethine proton of the 4-CH group is in the deshielding region of the conjugated aromatic system in **1**, while the proton of the corresponding saturated species is not, so that an upfield shift of 200–300 Hz would be expected for

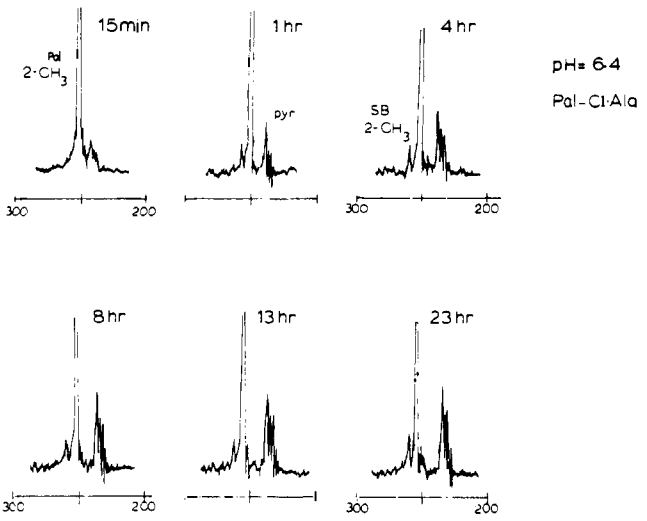



Figure 4. The 100-MHz NMR spectrum of 0.20 M pyridoxal and 0.20 M β-chloroalanine at pD 6.8 showing the formation of pyruvic acid; frequencies are reported in hertz with respect to $Me_4Si$.

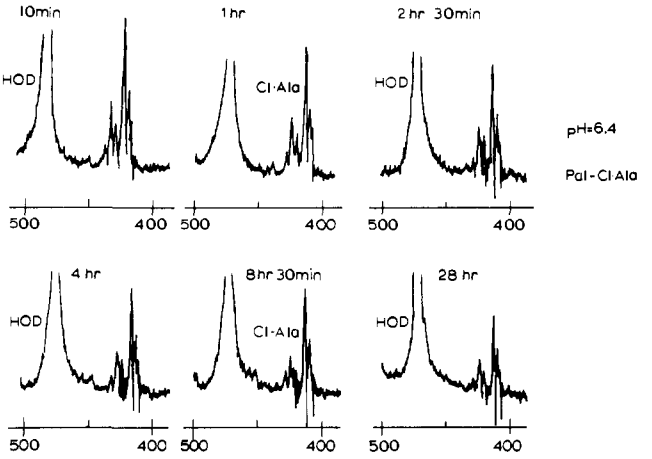



Figure 5. The 100-MHz NMR spectrum of 0.20 M pyridoxal and 0.20 M β-chloroalanine at pD 6.8 showing the disappearance of β-chloroalanine; frequencies are reported in hertz with respect to $Me_3Si$.

the 1,2 adduct, **5**. Matsushima and Hino[13] have assigned a resonance at about 600 Hz to the proton on the carbinol carbon, and Abbott and Martell[14] have assigned a resonance at about 600 Hz to the 4-CH group of the cyclic (saturated) thiazolidine formed by addition of a sulfhydroxyl group across the azomethine linkage, to form a 1,2 adduct. Thus, for 1,2 addition one would expect a chemical shift to much higher field than the observed value of 75 Hz. Also, the 1,2-adducts **5** and **6** should exhibit ethylenic resonances at about 600 and 650 Hz, but these resonances were completely absent in the observed spectra. Thus, on the basis of the NMR data, it is possible to rule out **5** or **6** for the observed intermediate.

In the 1,4 adducts (**7** and **8**) conjugation of the azomethine double bond with the carboxyl carbonyl would be expected to have a deshielding effect on the 4-CH proton, in agreement with the observed resonance at ~820 Hz. The choice between the aminoacetal adduct, **7**, and the carbinolimine, **8**, can be made on the basis of the observed 5-$CH_2$ resonance. Cyclization would have coupling effects on both the 4-CH and 5-$CH_2$ resonances, resulting in multiplets for each group. Since singlet peaks were obtained for both groups, it is clear that the new intermediate corresponds to formula **8**.

**Kinetic Treatment**. The rate measurements were based on the disappearance of the amino acids, β-chloroalanine and *O*-phosphoserine, as well as the appearance of products: mainly pyruvic acid in the keto and hydrate forms. The latter was detected only in acidic media. For the metal-free systems the rate equations are based on the following reaction scheme:

$$\mathrm{PAL} + \mathrm{X}\cdot\mathrm{Ala} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{SB} \xrightarrow{k_2} \mathrm{Pyr} + \mathrm{PAL} + \mathrm{NH_3} \quad (1)$$

For the metal-Schiff base (1:1) systems:

$$\mathrm{PAL} + \mathrm{X}\cdot\mathrm{Ala} + \mathrm{Zn^{2+}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{SB} \xrightarrow{k_2} \mathrm{Pyr} + \mathrm{PAL} + \mathrm{NH_3} + \mathrm{Zn^{2+}} \quad (2)$$

where Pyr = pyruvic acid; PAL = pyridoxal; SB = Schiff base; and X·Ala = amino acid with electronegative leaving group X, either chloride or phosphate. The following rate equation is obtained for the metal-free system in the absence of an appreciable amount of Schiff base:

$$\frac{d[\mathrm{X}\cdot\mathrm{Ala}]}{dt} = [\mathrm{PAL}][\mathrm{X}\cdot\mathrm{Ala}]\left(\frac{k_{-1}k_1}{k_{-1}+k_2} - k_1\right) = k_{\mathrm{obsd}}[\mathrm{PAL}][\mathrm{X}\cdot\mathrm{Ala}] \quad (3)$$

Similarly, for the metal-Schiff base (1:1) systems:

$$\frac{d[\mathrm{X}\cdot\mathrm{Ala}]}{dt} = [\mathrm{Zn^{2+}}][\mathrm{PAL}][\mathrm{X}\cdot\mathrm{Ala}]\left(\frac{k_{-1}k_1}{k_{-1}+k_2} - k_1\right) = k_{\mathrm{obsd}}[\mathrm{PAL}][\mathrm{X}\cdot\mathrm{Ala}][\mathrm{Zn^{2+}}] \quad (4)$$

All the observed Schiff base complexes reported in this research involved 1:1:1 molar ratios of metal ion, pyridoxal, and amino acid. When the steady-state assumption is not applicable to Schiff base formation (i.e., when the accumulation of Schiff base in solution is appreciable), the following equation is obtained:

$$\frac{d[\mathrm{Pyr}]}{dt} = k'_{\mathrm{obsd}}[\mathrm{SB}] \quad (5)$$

Thus, the kinetics of reaction are related directly to the concentration of Schiff base in solution. The reaction stoichiometry, assuming no by-products, gives the following relationship:

$$[\mathrm{Pyr}] = [\mathrm{X}\cdot\mathrm{Ala_0}] - [\mathrm{X}\cdot\mathrm{Ala}] - [\mathrm{SB}] \quad (6)$$

where $[X\cdot Ala_0]$ is the concentration of initial amino acid. The value of the first-order rate $k'_{obsd}$ is then found by a plot of $d[Pyr]/dt$ vs. [SB]. Since [X·Ala] and [SB] are both found directly from NMR integrations, the slope of [Pyr] vs. time and the corresponding $k'_{obsd}$ values are readily obtained.

In acidic media, the pyruvic acid resonance was observed as the keto or diol form up to about 1 half-life of the elimination reaction. It was possible to use the pyruvic acid resonances as a measure of the progress of the elimination reaction since H–D exchange of Pyr was relatively slow in neutral and weakly acid solution. At high pH, H–D exchange was too rapid, and the pyruvic acid resonance was therefore not used as the main indicator of reaction rate. This was also the case for the metal ion catalysis runs. No diol resonance was observed when the new intermediate peak appeared. The $CH_2D$ resonance of the amino acid moiety, corresponding to formula **8**, appeared when expected at about 250 Hz, near the $CH_3$ resonance of pyruvic acid. This peak then disappeared with time as the result of further H–D exchange.

**Reaction Kinetics of Metal-Free Systems**. Figure 4 presents 100-MHz NMR spectra showing the variation with time of the resonances of the Schiff base of β-chloroalanine and of the keto form of pyruvic acid. Figure 5 is a 100-MHz NMR spectrum showing the decrease of intensity with time of β-chloroalanine resonances used for determination of rate constants for β elimination. Figure 6 is an example of a plot of species vs. time for the β-chloroalanine–pyridoxal system. The

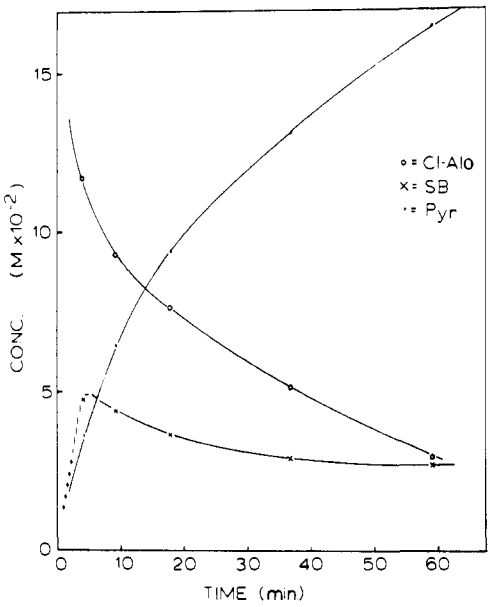



**Figure 6.** Plot of species vs. time of the chloroalanine-pyridoxal system illustrating variation of intermediate Schiff base concentration.

results are summarized in Table III. The rate constants determined in the lower pD range are second order, as expected, in accordance with eq 3. At higher pD the concentration of Schiff base becomes appreciable so that eq 5 applies, and first-order rate constants for the Schiff base elimination reaction are obtained. The rate constants thus determined show considerable pH dependence. Due to precipitation during the kinetic run for pD 6.0, the concentration of initial reactants was recalculated from the normalized integration of NMR spectra. Although precipitation also occurred for pD 6.8, the correction necessary for the rate determination was minimal due to the smaller extent of precipitation. The high reaction rate and the broadening of the resonances that occur at high pD made quantitative integration of the NMR resonances somewhat more difficult. In spite of these problems, however, the rate constant obtained at pD 9.3 is still considered accurate to two significant figures.

The kinetic results for the *O*-phosphoserine-pyridoxal system are summarized in Table IV. Because of the precipitation during the kinetic run, the rate constant for pD 6.3 was determined with the use of a correction similar to that used for β-chloroalanine at pD 6.0. For high pD (8.95 and 10.05) the considerations that apply are similar to those expressed above for β-chloroalaniine at pD 9.3.

The factors that influence the rates in the absence of metal ion are (1) the amount of the Schiff base formation and the degree of protonation of the Schiff base, (2) the ability to labilize the α proton of the amino acid moiety, and (3) the electronegativity of the substrate. Calculations based on formation constants determined in this study and reported by others[7-9] demonstrate that the fraction of Schiff base in these systems never exceeds 10% of the total substrate below pH 6, and increases at higher pH. This low degree of formation of the Schiff base must be partly responsible for the observed low reaction rate of acidic media for *O*-phosphoserine as well as β-chloroalanine. Also, this low reaction rate for pH values below 6 may be related to the fact that under these conditions the pyridine ring of the Schiff base is protonated.

The labilization of the α proton of the amino acid moiety is promoted by the ability of Schiff base to accommodate the resulting lone pair through the extended conjugated π-bond system. In addition this dissociation is promoted by both the metal ion and the inductive effect of the heterocyclic nitrogen atom. The latter effect is greatly amplified upon protonation

**Table III.** Observed Rate Constants for β Elimination from the β-Chloroalanine Schiff Base (Pyridoxal-Amino Acid, 1:1)

| pD | $k_{obsd}$, $s^{-1}$ $M^{-1}$ | $k'_{obsd}$, $s^{-1}$ |
|---|---|---|
| 4.9 | $2.1 \times 10^{-4}$ | |
| 6.0 | $2.7 \times 10^{-4}$ | |
| 6.8 | | $1.6 \times 10^{-3}$ |
| 8.0 | | $3.0 \times 10^{-3}$ |
| 8.7 | | $2.8 \times 10^{-3}$ |
| 9.3 | | $2.3 \times 10^{-3}$ |

**Table IV.** Observed Rate Constant for β Elimination from the *O*-Phosphoserine Schiff Base (Pyridoxal-Amino Acid, 1:1)

| pD | $k_{obsd}$, $s^{-1}$ $M^{-1}$ | $k'_{obsd}$, $s^{-1}$ |
|---|---|---|
| 4.08 | $1.3 \times 10^{-5}$ | |
| 5.28 | $1.4 \times 10^{-5}$ | |
| 6.3 | $3.1 \times 10^{-5}$ | |
| 8.2 | | $1.6 \times 10^{-3}$ |
| 8.95 | | $2.0 \times 10^{-3}$ |
| 10.05 | | $9.2 \times 10^{-4}$ |

of the pyridine ring. These inductive effects favoring α-proton dissociation are counterbalanced in part through the formation of the dihydropyridine intermediate **2**, which would render the generated lone pair of electrons in the α position less available for the withdrawal of the electronegative leaving group. It seems possible that these two opposing effects may very well largely cancel each other, and that the low rate below pH 6 would be due mainly to the lower degree of Schiff base formation. The testing of this hypothesis would require examination of the first-order elimination rates below pH 6 as a function of pH. Such experiments are not possible with the amino acids being studied in this investigation.

In the intermediate pH range, it is seen that the first-order rate constants increase with pH, probably because of a change in the nature of the reactive Schiff base species. The observed rate may be considered the summation of the rates of the individual species differing in degree of protonation. Thus:

$$k'_{obsd}[SB_T] = k_2'[HSB^+] + k_2''[SB] + k_2'''[SB^-]$$

The concentrations of the three Schiff base species will vary with pH, in accordance with the protonation constants listed in Table I. As the pH increases from 6.8 to 8.0, the proportion of SB/HSB increases, indicating that β elimination takes place more rapidly in the neutral Schiff base species than in the more protonated form in which the pyridine ring is protonated (i.e., $k_2'' > k_2'$). Since the latter species is a much stronger promoter of α-proton dissociation of the α-amino acid moiety of the Schiff base, the observed increase in the rate seems to support the above hypothesis.

The decrease in the first-order rate constant at still higher pH may be accounted for by the deprotonation of the Schiff base at the azomethine nitrogen, resulting in the formation of a much higher proportion of the $SB^-$ species. In the latter form, the catalytic effect on α-proton dissociation of the α-amino acid moiety is minimal. Thus, it seems that the initial deprotonation step (**1** → **2** → **3**) is also rate determining in the β-elimination reaction.

The final condition for β elimination is that the leaving group be highly electronegative; otherwise other pyridoxal-catalyzed reactions such as C-C cleavage for threonine[15,16] would take place. This condition (high electronegativity) is met by the chloride and phosphate ions formed from the substrates employed. The difference in the rate between β-chloroalanine and *O*-phosphoserine elimination is therefore probably due to the above difference in the electronegativities of the chloride and

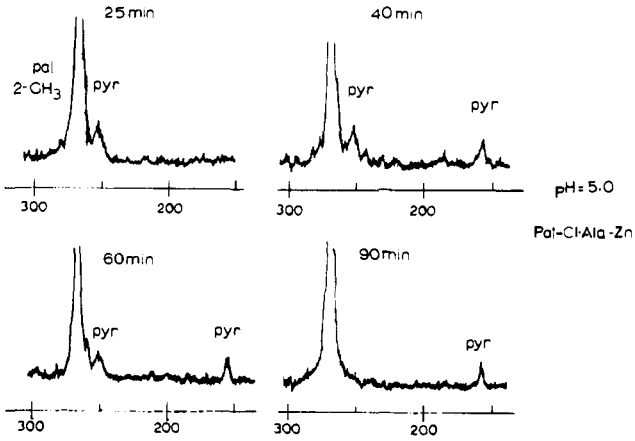
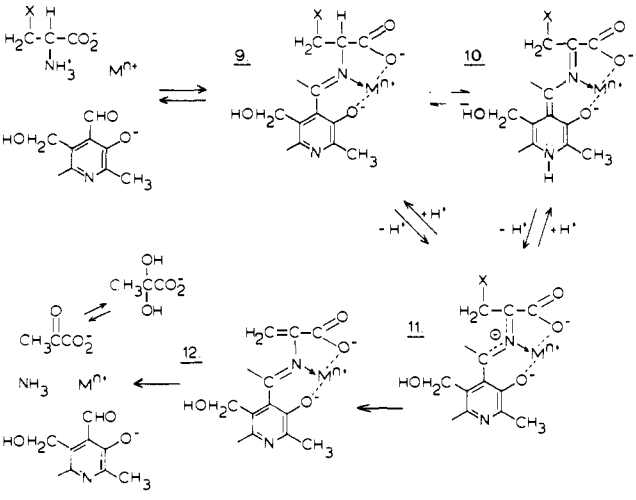



Figure 7. The 100-MHz NMR spectrum of 0.02 M pyridoxal, 0.20 M β-chloroalanine, and 0.20 M zinc metal ion at pD 5.4 showing the formation of pyruvic acid in the keto and diol forms; frequencies are reported in hertz with respect to $Me_4Si$.

Scheme II



phosphate leaving groups. This difference in rate for different leaving groups indicates that the second step in the elimination process, reaction **3** → **4**, is also rate determining.

**Zinc(II) Catalysis.** The proposed reaction mechanism for β elimination in the presence of zinc(II) ion is illustrated in Scheme II. A 1:1:1 stoichiometric ratio of pyridoxal, amino acid, and metal ion was investigated under conditions similar to those given above for the metal-free systems. The observed extent of keto-enol tautomerism of the pyruvic acid formed, as well as the degree of hydration of the keto form to the diol,[17,18] were greatly enhanced by the presence of the metal ions, as indicated in Figure 7. The β-elimination rates were calculated entirely on the basis of the disappearance of the α-amino acid moieties of the chelate Schiff bases. As expected, the observed rates of β elimination were much greater for the metal-catalyzed systems than for the metal-free systems.

When no Schiff base was observed, or when the proportion of Schiff base was less than 5%, the kinetic data were interpreted with the aid of eq 4, which was based on reaction scheme 2, and third-order rate constants were determined. When the accumulation of the Schiff base was greater than 5%, the concentrations were sufficiently accurately known to base the reaction kinetics on the disappearance of Schiff base, and first-order rate constants were therefore reported.

The results obtained for Zn(II) catalysis of β elimination are summarized in Table V. A larger rate increase is observed for *O*-phosphoserine at pD ~4.1. Under these conditions the

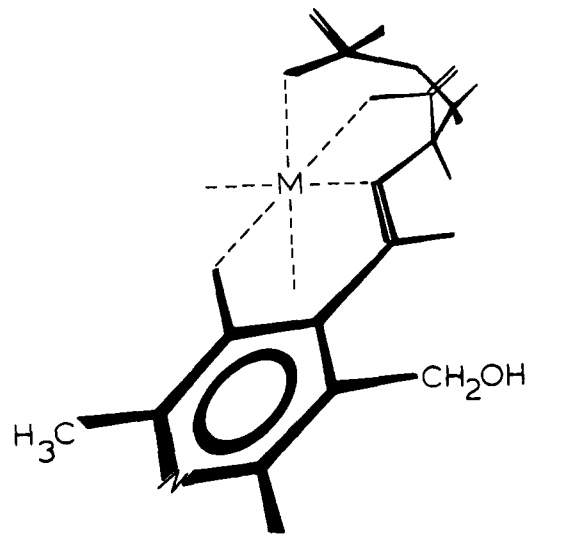



Figure 8. Structural formula showing possible tetradentate coordination of octahedral metal ion by phosphoserine Schiff base.

Table V. Observed Rate Constants for Zinc(II)-Catalyzed β Elimination (Pyridoxal-Amino Acid-Zinc(II), 1:1:1)

| Amino acid | pD | $k_{obsd}$, $M^{-2}$ $s^{-1}$ | $k'_{obsd}$, $s^{-1}$ |
|---|---|---|---|
| β-Chloroalanine | 4.2 | | $1.2 \times 10^{-3}$ |
| | 5.0 | | $2.0 \times 10^{-3}$ |
| | 5.4 | | $1.4 \times 10^{-3}$ |
| *O*-Phosphoserine | 3.44 | $1.1 \times 10^{-3}$ | |
| | 4.06 | $2.2 \times 10^{-3}$ | |
| | 4.34 | $4.0 \times 10^{-3}$ | |
| | 4.56 | $4.1 \times 10^{-3}$ | |

rate for the metal-free Schiff system is $1.3 \times 10^{-5}$ $M^{-1}$ $s^{-1}$ while at pD 4.06 the metal-catalyzed rate constant is $4.3 \times 10^{-4}$ $M^{-1}$ $s^{-1}$. This considerable rate enhancement is also due in part to the electron-withdrawing effect of the metal ion, and its labilizing effect on the α proton of the amino acid moiety.

It is possible to bind a metal ion to the phenolate oxygen, carboxylate oxygen, and the azomethine nitrogen, and still bring the phosphate group over to the vicinity of the metal ion. Although the resulting phosphate-metal ion distance is somewhat longer than normal (Figure 8), the resulting electrostatic energy would serve to stabilize the bond. The metal ion, having an electron-withdrawing effect, enhances the electron shift toward the phosphate, thus increasing the tendency toward β elimination. Although the additional coordination may weaken slightly the labilization of the α hydrogen of the amino acid moiety, the electron withdrawal effect on the phosphate group would greatly outweigh the slight loss of α-proton labilization.

Solutions containing 2:2:1 molar ratios of pyridoxal-amino acid-metal ion were also studied under conditions similar to those employed for the 1:1:1 systems. Measurement of the NMR spectra of the 2:2:1 systems failed to show the formation of the isomeric species characteristic of bis-Schiff base complexes,[19] indicating that, at the low pH values of these system, no 2:2:1 complexes were formed. Therefore, the rate constants were calculated on the basis of the formation of only 1:1:1 complexes of the type indicated by formula **9**. The results are listed in Table VI for zinc catalysis of β elimination. As with the metal-free systems, the observed increase in the rate constants may be accounted for by decreasing protonation with increasing pH of the metal-Schiff base complexes. The increased observed rate of the 2:2:1 mixtures relative to the 1:1:1

**Table VI.** Observed Rate Constants for Zinc(II)-Catalyzed $\beta$ Elimination (Pyridoxal-Amino Acid-Zn(II), 2:2:1)

| Amino acid | pD | $k_{obsd}$, $M^{-2}$ $s^{-1}$ | $k'_{obsd}$, $s^{-1}$ |
|---|---|---|---|
| $\beta$-Chloroalanine | 4.35 | $1.0 \times 10^{-2}$ | |
| | 5.52 | | $2.5 \times 10^{-3}$ |
| | 5.9 | | $2.7 \times 10^{-3}$ |
| *O*-Phosphoserine | 4.11 | $2.1 \times 10^{-3}$ | |
| | 5.23 | | $2.0 \times 10^{-3}$ |

systems is ascribed to a higher equilibrium concentration of the intermediate Schiff base.

Conditions favorable to $\beta$ elimination are met by the electronegative leaving groups for $\beta$-chloroalanine and by the metal-coordinated phosphate anion in *O*-phosphoserine. Precipitation at higher pH regions prevented quantitative rate measurements for $\beta$-chloroalanine and *O*-phosphoserine at neutral and high pH; the latter had an extensive precipitation range beginning as low as pH 5. Although Schiff base solutions redissolved as the pH was increased beyond pH 8–8.5, NMR measurement of these solutions was rendered very difficult because of turbidity and/or possibly an increase in viscosity. Thus, rate constants were not determined at higher pH.

$\beta$ elimination proceeds very rapidly in the presence of the zinc(II) ion above pH 8. On the basis of the behavior of both metal-free and metal complex systems, it may be concluded that the pyridyl-deprotonated Schiff base is the more reactive species in the $\beta$-elimination reaction. As indicated in Scheme II, labilization of the $\alpha$ proton of the amino acid moiety is a necessary step, but the electronegativity of the leaving groups seems to be the rate-determining factor for the $\beta$-elimination reaction of vitamin $B_6$ catalyzed systems.

The experimental results described above are in general agreement with the general concepts of Gregerman and Christensen[1] and of Longenecker and Snell,[2] and have considerably extended our knowledge of the $\beta$-elimination reaction in model systems. The reaction rates obtained in the present work are independent of the use of buffers[1,2] that have affected previous work, and all possible solution species are accounted for. The choice of leaving group is apparently very critical in determining whether $\beta$ elimination will predominate over other vitamin $B_6$ catalyzed reactions such as transamination and racemization.

The strong metal catalysis of $\beta$ elimination raises some interesting questions about the nature of this reaction, since the electron shift for the breaking of the bond between the $\beta$ carbon atom and the electronegative leaving substituent is opposed to the electronic effect of the metal ion. Thus, the relative effects of Al(III) and Zn(II) catalysis are interesting. As shown in other studies now in progress,[20] Al(III) is more effective than Zn(II) for chloroalanine, but less effective than Zn(II) for elimination of phosphate from phosphoserine. This observation brings up questions of the relative influences of the metal ion on $\alpha$-proton dissociation and electronegative group removal. An important consideration is the probable binding of the phosphate moiety by Al(III), as indicated by Figure 8.

Further investigations of this reaction with related amino acids and different metal ions are currently underway to determine their relative catalytic effects, and their importance in achieving reaction selectivity. A greater variety of leaving groups will also be employed.

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## References and Notes

(1) R. I. Gregerman and H. N. Christensen, *J. Biol. Chem.*, **220**, 765 (1956).
(2) J. B. Longenecker and E. E. Snell, *J. Biol. Chem.*, **225**, 409 (1957).
(3) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).
(4) D. E. Metzler, J. B. Longenecker, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 639 (1954).
(5) Y. Murakami, H. Kondo, and A. E. Martell, *J. Am. Chem. Soc.*, **95**, 7138 (1973).
(6) G. Schwarzenbach, "Complexometric Titration", Interscience, New York, N.Y., 1957.
(7) K. Nagano and D. E. Metzler, *J. Am. Chem. Soc.*, **89**, 2891 (1967).
(8) W. L. Felty, C. G. Ekstrom, and D. L. Leussing, *J. Am. Chem. Soc.*, **92**, 3006 (1970).
(9) W. L. Felty and D. L. Leussing, *J. Inorg. Nucl. Chem.*, **36**, 617 (1974).
(10) D. E. Metzler, *J. Am. Chem. Soc.*, **79**, 485 (1957).
(11) O. A. Gansow and R. H. Holm, *J. Am. Chem. Soc.*, **90**, 5629 (1968); **91**, 573 (1969).
(12) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).
(13) Y. Matsushima and T. Hino, *Chem. Pharm. Bull.*, **16**, 2277 (1968).
(14) E. H. Abbott and A. E. Martell, *J. Am. Chem. Soc.*, **92**, 1754 (1970).
(15) J. B. Longenecker and E. E. Snell, *J. Am. Chem. Soc.*, **79**, 142 (1957).
(16) J. A. Marcello, A. E. Martell, and E. H. Abbott, *Chem. Commun.*, 16 (1976).
(17) M. Eigen, K. Kustin, and H. Strehlow, *Z. Phys. Chem. (Frankfurt am Main)*, **31**, 140 (1961).
(18) M. Becker, *Ber. Bunsenges. Phys. Chem.*, **68**, 669 (1964).
(19) E. H. Abbott and A. E. Martell, *J. Am. Chem. Soc.*, **92**, 5845 (1970).
(20) K. Tatsumoto and A. E. Martell, unpublished results.