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Vitamin B_6 Catalyzed Reactions of α-Amino and α-Keto Acids: Model Systems

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Introduction

Medical and Biochemical Significance. Not only is the B_6 group of vitamins, pyridoxine and related compounds, essential for growth and maintenance of many life processes, but its deficiency has been suggested as the cause of many types of illness and disease.[1] A few examples of disorders thought to be vitamin B_6 related are mental illness, anemia, bronchial asthma, carpal tunnel syndrome,[2] gastric and peptic ulcers, and Chinese restaurant syndrome.[2] The ubiquitous application of vitamin B_6 in medicine is matched by the wide variety of enzyme systems requiring vitamin B_6 as a cofactor. It is the essential coenzyme for enzymes involved in biosynthesis, metabolism, and regulatory functions. The number of vitamin B_6 dependent enzymes that have been discovered and identified is large and still growing. Recent advances in the determination of crystal structures of several pyridoxal-dependent enzymes[3] are providing information about functional groups at active sites and are stimulating detailed mechanistic studies of the various types of reactions involved. Although several reviews of vitamin B_6 model systems have appeared,[4-11] a unified treatment of the reactions catalyzed by the coenzyme is currently not available. It is the purpose of this paper to describe the pathways of the reactions catalyzed by vitamin B_6 in its various forms in a systematic way and to point out those that are most in need of further investigation.

Reactions of Model Systems. In view of the large number of vitamin B_6 dependent enzymes, it is not surprising that pyridoxal phosphate and related coenzyme derivatives catalyze a large variety of chemical reactions and are among the most versatile of the catalysts available for homogeneous systems. The following twelve types of reactions have been identified for model systems:

A. Aldimines (from pyridoxal or pyridoxal analogues with α-amino acids)
   1. transamination (amino acid to keto acid)

Arthur E. Martell, a native of Massachusetts, received the B.S. degree in chemistry at Worcester Polytechnic Institute in 1938 and the Ph.D. degree in organic chemistry at New York University in 1941. After a year of teaching at WPI, he moved to Clark University, rising through the ranks to Professor in 1951 and Chairman in 1959. In 1961 he moved to Illinois Institute of Technology as Chairman of the Department of Chemistry. In 1966 he was appointed Head of the Chemistry Department at Texas A&M University, a position he held unit 1980. He became Distinguished Professor of Chemistry in 1976. He has authored, coauthored, and coedited 20 books and has published over 400 research papers on metal complexes in solution, including vitamin B_6 catalysis, dioxygen complexes, and the thermodynamics and kinetics of chelate, macrocyclic, and cryptate complexes.

(1) *Current Topics in Illness and Disease. Vol. 13. Vitamin B6: Its Role in Health and Disease*; Reynolds, R. D., Leklem, J. E., Eds.; A. R. Liss: New York, 1985.
(2) (a) Folkers, K. Priestly Award Lecture, 191st Meeting of the American Chemical Society, New York, April 1986. (b) Folkers, K.; Wolanuik, A.; Vodhanavikit, S. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7076.
(3) See, for example, papers by: Miles, E.; Jansonius, J.; and Metzler, D. In *Biochemistry of Vitamin B6. Proceedings 7th International Congress on Chemical and Biological Aspects of Vitamin B6 Catalysis*; Korpela, T., Christen, P., Eds.; Birkhauser Verlag: Basel, 1987.
(4) *Pyridoxal Catalysis*; Snell, E. E., Braunstein, A. E., Severin, E. S., Torchinsky, T. M., Eds.; Interscience: New York, 1968.
(5) *Chemical and Biological Aspects of Pyridoxal Catalysis*; Snell, E. E., Fasella, P. M., Braunstein, A. E., Ross, A., Eds.; MacMillan: New York, 1963.
(6) Holm, R. H. In *Inorganic Biochemistry*; Eichhorn, G., Ed.; Elsevier: New York, 1975; Vol. 2, Chapter 31.
(7) Snell, E. E.; DiMari, S. J. In *The Enzymes*, 3rd ed.; Academic Press: New York, 1970; Vol. 7.
(8) Kallen, R. G.; Korpela, T.; Martell, A. E.; Matsushima, Y.; Metzler, C. M.; Metzler, D. E.; Morozov, Y. V.; Ralston, I. M.; Savin, F. A.; Torchinsky, Y. M.; Ueno, H. In *Transaminases*; Christen, P.; Metzler, D. E., Eds.; John Wiley: New York, 1984; Chapter 2.
(9) Martell, A. E. *Adv. Enzymol.* **1982**, *53*, 163.
(10) Martell, A. E. In *Chemical and Biological Aspects of Vitamin B-6 Catalysis; Part A*; Evangelopoulos, A. E., Ed.; A. R. Liss: New York, 1984; pp 63–78.
(11) Leussing, D. L. In *Pyridoxal Phosphate: Chemical, Biochemical, and Medical Aspects, Part A*; Dolphin, D., Ed.; John Wiley: New York, 1968; pp 69–115.

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2. α-proton exchange
3. racemization of a chiral center at the α-carbon
4. C–C bond scission at the α,β-position of the amino acid (dealdolation)
5. α,β-elimination when the β substituent is electronegative
6. decarboxylation at the α-position (carboxyl bound to the α-carbon)
7. decarboxylation at the γ-position (carboxyl bound to the γ-carbon)

B. Ketimines (from pyridoxamine or pyridoxamine analogues with α-keto acids)

8. transamination (keto acid to amino acid)
9. decarboxylation at the β-position
10. dephosphonylation at the β-position
11. β-proton exchange
12. elimination of electronegative groups in the γ-position

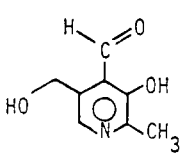
The discovery of the nonenzymatic and enzymatic reactions involving vitamin B_6 that are listed above occurred more or less simultaneously, and in many cases, model reactions have led the way. Transamination, for example, was discovered first in model systems,[12] allowing prediction of the nature of the enzymatic reaction. Similar suggestions were made by Braunstein and Shemyakin,[13] although they did not actually work with model compounds. In addition, α-racemization, dealdolation, α,β-elimination, and α-decarboxylation were first reported by Snell and coworkers (see ref 7 and references therein). Two model reactions, γ-decarboxylation and β-dephosphonylation, first reported by the present author, have yet to be discovered in enzymatic systems.

This paper is mainly concerned with model vitamin B_6 reaction pathways and is based on the results of detailed kinetics and mechanistic studies, carried out mainly by NMR measurements with support from spectrophotometric studies. A number of new reaction types discovered in the course of these studies are also described.

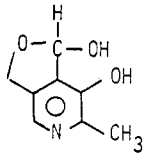
Relevance of Model Reactions to Biological Systems. The advantage of research on model compounds relative to enzyme systems is the ability to observe reactions over wide variations of pH and concentrations of reacting species, thus making it possible to determine dependence of the rate on each reactant, as well as on $[H^+]$, on $[OH^-]$, and on the nature of the particular form or modification of the coenzyme being employed. The relevance of such model studies to enzymatic reactions would also seem reasonably clear in the case of the vitamin B_6 coenzyme, because the reactions catalyzed in the model systems closely parallel those catalyzed by the corresponding enzymes.

It has been pointed out by Metzler et al.,[12] this investigator,[9,10] and Leussing[11] that the use of metal ions in the study of the catalytic effects of the coenzyme in model systems increases the degree of formation of the Schiff base (both the aldimine and the ketimine) and thus is advantageous in studying these reactions since all involve Schiff bases as intermediates. It has also been pointed out[14] that while the metal ions are not catalysts in enzymic reactions, the Schiff base metal chelates are closely analogous to the metal-free systems because the metal ions replace the proton covalently bound to the azomethine nitrogen in the Schiff base. The proton and metal ion are both also coordinated to the adjacent phenolate and carboxylate donor groups.[14]

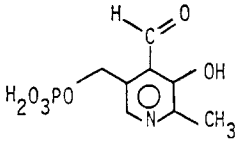
Forms of the Coenzyme and Their Analogues. The forms of the coenzyme employed in studies of vitamin B_6 catalysis are indicated by **1–5**. Modifications of these coenzymes that may be employed to achieve electronic and other constitutional effects on reaction intermediates, and hence on reaction kinetics, are indicated by formulas **6–8**. The structural changes



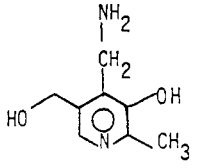
1 Pyridoxal



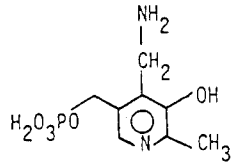
2 Pyridoxal hemiacetal



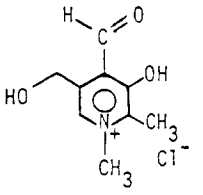
3 Pyridoxal phosphate



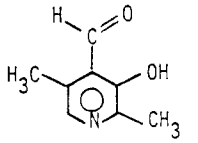
4 Pyridoxamine



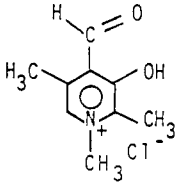
5 Pyridoxamine Phosphate



6 Pyridoxal-N-methochloride



7 5'-Deoxypyridoxal



8 5'-Deoxypyridoxal-N-methochloride

(relative to pyridoxal and pyridoxamine) represented by formulas **6–8** are designed to influence the properties of the Schiff base intermediates formed by the coenzyme with amino acids and keto acids. Methylation of the pyridine nitrogen in **6** and **8** greatly increases the electron-withdrawing effect of the pyridine ring on the azomethine group of the Schiff base, thus strongly influencing the reactions of amino and keto acids occurring through Schiff base formation. Removal of the hydroxyl group in **7** and **8** prevents the formation of the hemiacetal (as in the case of pyridoxal) and thus greatly increases the degree of formation of Schiff bases with amino acids, thereby increasing the rates of pyridoxal-catalyzed reactions of amino acids. These 5′-deoxy analogues thus have an effect on catalysis that parallels pyridoxal phosphate used in place of pyridoxal. The pyridoxal analogues **7** and **8** have the advantage of

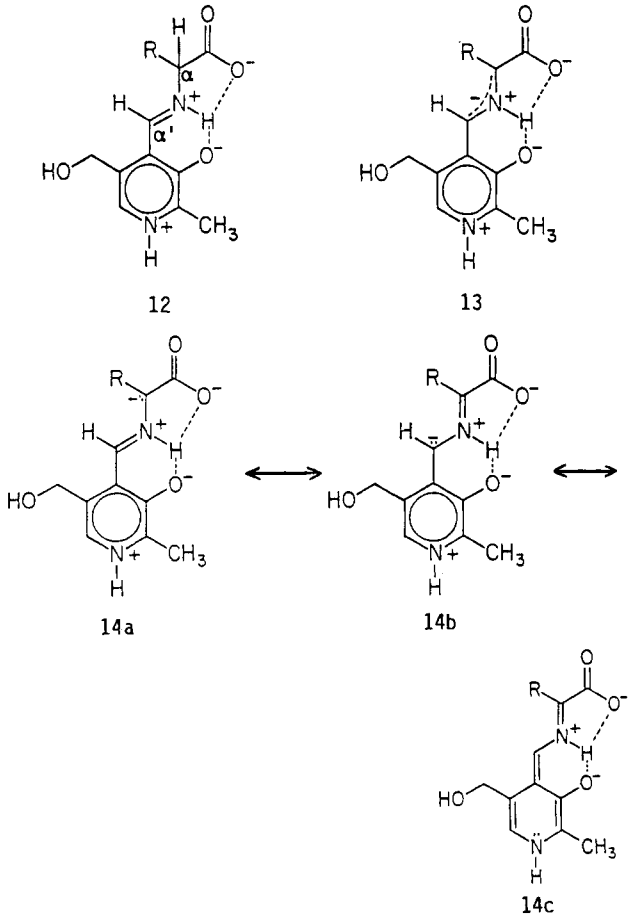
(12) Metzler, D. E.; Ikawa, M.; Snell, E. E. *J. Am. Chem. Soc.* **1954**, *76*, 648. Compare ref 19.

(13) Braunstein, A. E.; Shemyakin, M. M. *Biokhimiya (Moscow)* **1953**, *18*, 393.

(14) Martell, A. E. *Proceedings of Symposium on Chemical and Biological Aspects of Pyridoxal Catalysis*, Rome, 1962; Pergamon Press: London, 1963; pp 13–28.

avoiding the complications caused by the phosphate group in following the reaction pathways by NMR, and by the participation of the phosphate group in the Schiff base protonation equilibria.

Transamination and Related Reactions. The reaction mechanism for the pyridoxal-catalyzed conversion of an α-amino acid to an α-keto acid and the reverse reaction catalyzed by pyridoxamine is illustrated in Scheme I. The role of the aldimine Schiff base in catalysis of transamination and racemization was first demonstrated experimentally by Metzler, Ikawa, and Snell.[12] The importance of the protonated azomethine nitrogen in activating the α-hydrogen of the amino acid and the analogous effect of a coordinated metal ion were pointed out by Martell,[14] and a common α,α'-deprotonated carbanionic intermediate **10** was suggested for both the forward and reverse reactions. It was subsequently pointed out[8,9] that the diprotonated Schiff base **12** would further stabilize the delocalized carbanion



through its electron-withdrawing effect as in **13**. The principal resonance forms of the carbanion intermediate are represented by formulas **14a–c**. The greater catalytic effect of the diprotonated Schiff base species is counteracted by the lower degree of Schiff base formation at low pH. This is a good example of the helpful effects of metal ions, which tend to increase the degree of Schiff base formation in solution.

Of the three resonance forms of the carbanionic intermediate of the diprotonated Schiff base, **14c** corresponds to the "Metzler–Ikawa–Snell intermediate" indicated as being formed by tautomerization of **9**. It was suggested by Martell[9] that, other factors aside, the tendency to reprotonate at the α-position (at the amino acid residue) would be expected to predominate because the aldimine thus formed would be stabilized by conjugation with the aromatic ring. However, electron-releasing groups attached to the α-position would favor distortion of the delocalized electron cloud to increase the relative contribution to **14b** over **14a**, thus increasing reprotonation at the α'-position and leading to a higher yield of ketimine. Such an effect would also be promoted by electron-attracting substituents in the pyridine ring and by alkyl and branched alkyl substituents in the amino acid side chain.

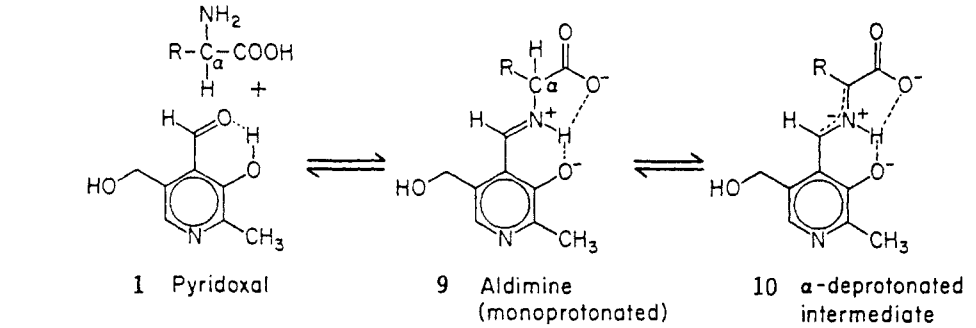
A paper by Abbott and Martell[15] on the Al(III) chelates of aldimines and a more recent report[16] present NMR evidence for the α-deprotonated intermediate (**14a–c**) of the Al(III) chelate of the Schiff base formed from pyridoxal and alanine. In still more recent studies,[17,18] this work was extended to other amino acids and to *N*-methylpyridoxal **6** as catalyst. The existence of this intermediate in measurable concentrations as the Al(III) chelate is considered due to the additional stabilization of **14a** and **14b** by metal ion coordination; i.e., coordination of the Al(III) ion at the azomethine nitrogen tends to localize negative charge in the vicinity of the metal ion and thus increase the population of **14a** and **14b** relative to **14c**. Further studies of this type of intermediate to determine its usefulness in predicting the rates of transamination and of α,α'-proton exchange are recommended.[62]

Decarboxylation. Many enzyme systems containing pyridoxal phosphate as the coenzyme catalyze the decarboxylation of α-amino acids,[19] a fact that gave rise in the past to the use of "codecarboxylase" as an alternate term for vitamin B_6. The decarboxylation of α-alkyl-substituted α-amino acids in model systems has been reported by Kalyankar and Snell.[20] Generally, however, it has been found that α-decarboxylation of mono-α-amino monocarboxylic acids does not readily occur in model systems and that other reactions initiated by labilization of the α-proton take place more rapidly.[19] Perhaps the major property of pyridoxal–amino acid Schiff bases that contributes to lack of α-carboxyl labilization is the coordination through hydrogen bonding of the carboxylate group to the protonated azomethine nitrogen of the Schiff base, and the coordination of the carboxylate group to the metal ion in metal complexes of Schiff bases. This results in the carboxylate group being drawn toward the plane of the aromatic ring. The most favorable orientation for release of the bonding electron pair for overlap with the π-system of the Schiff base would be a position perpendicular or nearly perpendicular to the plane, a consideration first pointed out by Dunathan.[21] The fact that the carboxylate group is coordinated to the protonated azomethine nitrogen, or to the metal ion in the Schiff base chelate, is in itself seemingly sufficient to prevent α-decarboxylation, because the coordinate bonding would shift the electron pair involved in carboxylate C–C bonding in the direction opposite to that required for decarboxylation.

Ketimine Schiff bases provide low-energy electron pathways for the decarboxylation of α-amino acids

(15) Abbott, E. H.; Martell, A. E. *J. Am. Chem. Soc.* **1973**, *95*, 5014.
(16) Martell, A. E.; Taylor, P. *Inorg. Chem.* **1984**, *23*, 2734.
(17) Taylor, P. A.; Martell, A. E. *Inorg. Chim. Acta* **1988**, *152*, 181.
(18) Tatsumoto, K.; Martell, A. E., to be submitted.
(19) Snell, E. E. *Vitam. Horm. (N.Y.)* **1958**, *16*, 77.
(20) Kalyankar, G. D.; Snell, E. E. *Biochemistry* **1962**, *1*, 594.
(21) Dunathan, H. C. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 712.

Scheme I
Vitamin B_6 Catalyzed Transamination, Racemization, and α-Proton Exchange



Transamination

1 → 9 → 10 → 11 → 4

4 → 11 → 10 → 9 → 1

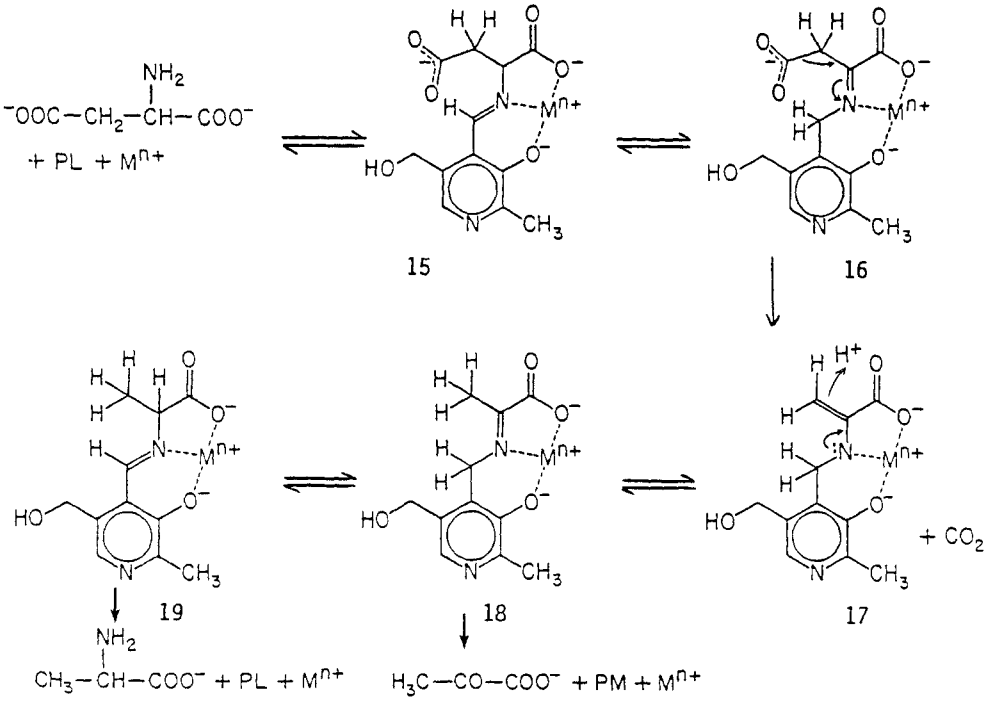
Racemization and α-Proton Exchange

1 → 9 → 10 → 9 → 1
↓
11 → 10 → 9 → 1

α'-Proton Exchange

4 → 11 → 10 → 11 → 4
↓
9 → 10 → 11 → 4

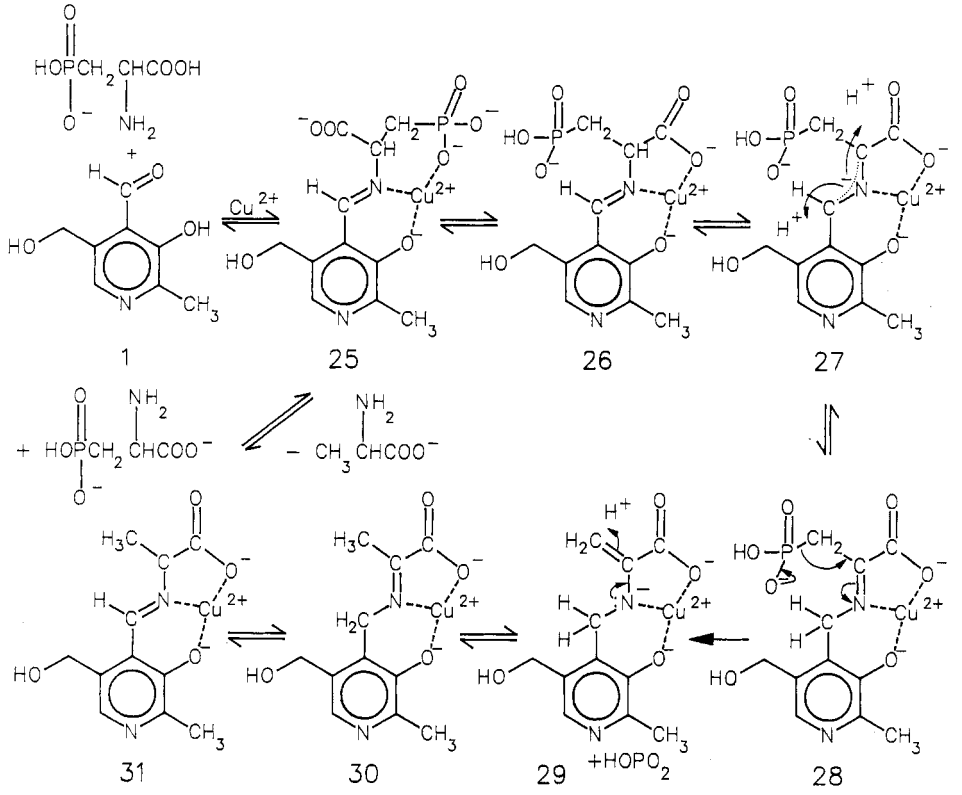
Scheme II
Mechanism of Metal Ion and Pyridoxal Catalyzed Decarboxylation of Aspartic Acid



having carboxyl groups bound to the β-position. Sakkab and Martell[22] studied pyridoxal catalysis of the decarboxylation of aspartic acid and suggested the mechanism shown in Scheme II, involving conversion of the aldimine **15** to ketimine **16** before the bond-breaking step involved in decarboxylation can take place. When the ketimine (**16**) is formed directly from pyridoxamine and oxaloacetic acid, the decarboxylation reaction is nearly instantaneous, with complete conversion to reaction product before the first NMR measurements could be carried out. This result is in accord with the reaction mechanism shown in Scheme II, since the conversion of aldimine to ketimine is a slow process; apparently in this system the equilibrium favors the aldimine so strongly that conversion to ketimine turns out to be relatively very slow. Reprotonation of the intermediate **17** would give first the ketimine **18**, which under most reaction conditions would probably be converted completely or nearly completely to the aldimine **19**. The reaction that was carried out in acid solution by Sakkab and Martell[22] yielded pyruvic acid, probably because of rapid hy-

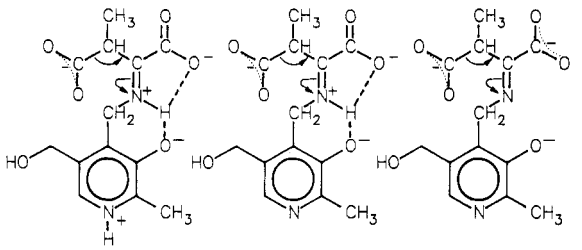
(22) Sakkab, N.; Martell, A. E. *Bioinorg. Chem.* **1975**, *5*, 67.

Scheme III
Metal Ion and Pyridoxal Catalyzed Dephosphonylation of 2-Amino-3-phosphonopropionic Acid



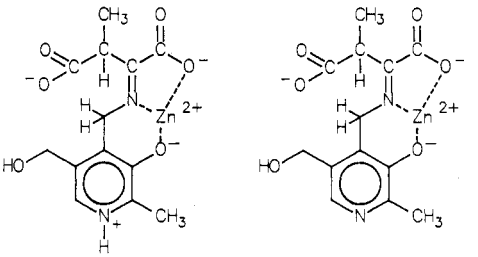
drolysis of the imine group of the ketimine under the reaction conditions employed. In most cases, however, one would expect conversion of the intermediate Schiff bases to the aldimine form and the production of the decarboxylated α-amino acid as the main reaction product; this is apparently the product usually formed in biological systems.

Detailed mechanistic studies have been carried out by Kubala and Martell on 2-oxalopropionic acid,[23] its metal chelates,[24] and its pyridoxamine Schiff base.[25] The results demonstrate that only protonated forms of the Schiff base are reactive intermediates, that the diprotonated species **20** is over 2 times as reactive as the monoprotonated form, **21**, and that the deprotonated Schiff base **22** shows very little activity (rate constants are 4.2 s^{-1}, 1.9 s^{-1}, and 0.3 s^{-1}, respectively).



protonated species of the pyridoxamine–oxalopropionic acid Schiff base

Additional work on the zinc(II)-catalyzed reactions of the Schiff base, which has recently been completed,[26] also shows increased reactivity for the protonated metal chelate **23**, over the deprotonated metal chelate, **24**, with rate constants 89 s^{-1} and 24 s^{-1}, respectively.

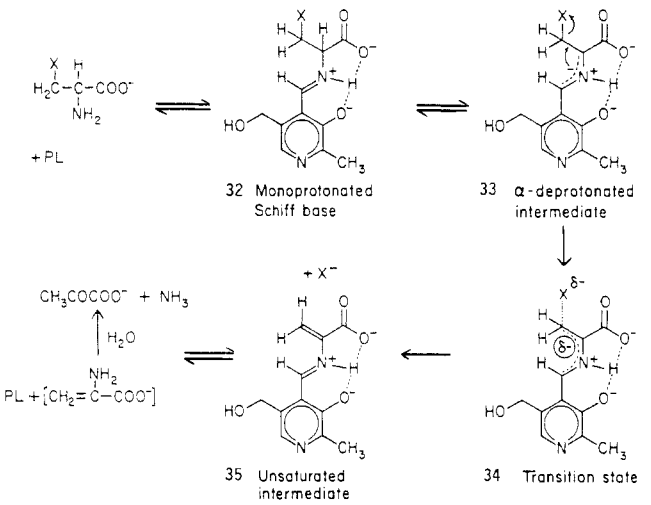


The first example of pyridoxal-catalyzed decarboxylation of a carboxyl group bound to the γ-carbon atom of an α-amino acid (i.e., in β-hydroxyglutamic acid) has been reported.[27] The fact that α,β-elimination may produce a double bond that transmits the activation by the azomethine group beyond the α-position of the amino acid side chain suggests many possible new vitamin B_6 catalyzed reactions.[62]

Dephosphonylation. The pyridoxal and metal ion catalyzed dephosphonylation of a natural amino phosphonic acid, 2-amino-3-phosphonopropionic acid, has been reported by Langohr et al.[28-30] The mechanism suggested is analogous to that proposed for the decarboxylation of aspartic acid and requires prior transamination to the ketimine before dephosphonylation can take place. A detailed equilibrium study and kinetic analysis of the pyridoxal phosphate–2-amino-3-phosphonopropionic acid system, in the presence and

(23) Kubala, G.; Martell, A. E. *J. Am. Chem. Soc.* **1981**, *103*, 7609.
(24) Kubala, G.; Martell, A. E. *J. Am. Chem. Soc.* **1982**, *104*, 6602.
(25) Kubala, G.; Martell, A. E. *J. Am. Chem. Soc.* **1983**, *105*, 449.
(26) Basak, A.; Martell, A. E. *Inorg. Chem.*, submitted.
(27) Tatsumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1981**, *103*, 6203.
(28) Langohr, M. F.; Martell, A. E. *J. Chem. Soc., Chem. Commun.* **1977**, 342.
(29) Langohr, M. F.; Martell, A. E. *J. Inorg. Nucl. Chem.* **1978**, *40*, 149.
(30) Martell, A. E.; Langohr, M. F.; Tatsumoto, K. *Inorg. Chim. Acta* **1985**, *108*, 105.

Scheme IV
Proposed Mechanism for Pyridoxal-Catalyzed β-Elimination



absence of Zn(II), Al(III), and Ga(III), have been carried out by Szpoganicz and Martell.[31-34] The results indicate that the major Schiff base species formed in solution must rearrange through preequilibrium to a minor species before transamination and dephosphonylation can take place, as indicated in Scheme III.

The scheme described above for pyridoxal and pyridoxal phosphate catalyzed dephosphonylation provides the first description of a reaction pathway in which a carbon–phosphorus bond is broken, and thus this reaction may have important implications for metabolism of phosphonic acids in biological systems. More research on this type of reaction is therefore strongly indicated.[62]

α,β-Elimination. Tatsumoto and Martell[35,36] studied the kinetics of elimination of electronegative groups substituted in the β-position of amino acids, such as serine, phosphoserine, and β-chloroalanine. Catalysis by pyridoxal in the presence and absence of metal ions was found to be dependent on factors controlling two rate-determining steps, dissociation of the α-proton and removal of the electronegative substituent at the β-position. The proposed mechanism in Scheme IV shows the probable reaction sequence for the monoprotonated form of the Schiff base, which was selected as the most reactive species (see below). The first step in the reaction sequence involves dissociation of the α-proton to give the carbanionic intermediate indicated by formula **33**. This reaction is rate determining inasmuch as the preequilibrium must take place before elimination can occur. The negative charge in the reaction intermediate **33** can be relieved by the loss of the electronegative substituent in the β-position with its bonding pair of electrons. This step is illustrated schematically with a proposed transition state **34**, which indicates the charge of the delocalized electron pair at the α- and α′-positions to be partially transferred to form an incipient double bond between the α- and β-carbon atoms and weakening of the single bond between the β-carbon atom and the electronegative substituent X. Completion of this process is illustrated by the formation of an aldimine **35**, which hydrolyzes to pyridoxal and an unsaturated amino acid. Since such amino acids are unstable in aqueous solution, the final products are seen to be pyridoxal, an α-keto acid (in this case pyruvic acid), and ammonia.

The variation of rates of β-elimination as a function of pH is due to the differences in reactivities of protonated Schiff base species in solution. In the pH range from about 6.5 to 10, the rate of β-elimination at first increases rapidly with pH, reaches a maximum between 8 and 9, and decreases somewhat at high pH values. Determination of the acid dissociation constants of the diprotonated and monoprotonated Schiff bases together with the rate law indicated by eq 1 made possible the calculation of the specific rate constants of each species, H_2SB, HSB^-, and SB^{2-}.[36] The rate constants assigned to the monoprotonated species are 10–30 times those of the diprotonated forms. Moreover, even the completely deprotonated Schiff bases, which may be assumed to have very weak catalytic properties, have rate constants that are comparable to those of the diprotonated forms.

$$k_{obsd}[SB_T] = k'[H_2SB] + k''[HSB^-] + k'''[SB^{2-}] \quad (1)$$

$$k_{obsd}[SB_T] = k'[H_2SB] + k''[HSB^-] + k_{OH}''[HSB^-][OH^-] \quad (2)$$

$$k''' = k_{OH}''K_{HSB}^{H}K_w$$

K_{HSB}^{H} = first protonation constant of SB^{2-}

K_w = ion-product constant of water

The fact that the monoprotonated Schiff bases are more reactive than the diprotonated species is due to their relative effects on the two rate-determining steps illustrated in Scheme IV. The diprotonated Schiff base would increase the concentration of the α,α′-deprotonated intermediate (analogous to **33**) to a greater extent than would the monoprotonated form. On the other hand, its higher positive charge would slow the departure of the electronegative substituent. Of these opposing tendencies, the latter effect seems to be more important.

The α,β-elimination reaction had long been recognized as being dependent on α-proton labilization.[12] Also, the influence of the leaving group on the rate of reaction was pointed out by Longenecker and Snell.[37] Thus chloride ion of β-chloroalanine[38] and the ester groups of *O*-carbamoylserine[37] and phosphoserine[37] are eliminated more rapidly than is the hydroxide ion from serine. The kinetic studies described above[35,36] added quantitative rate data to articulate further the proposed mechanism. These studies also provided new information on the influence of the protonation of the Schiff base intermediate on the relative populations of the reactive molecular species in solution, the effect of the metal ion on catalysis, and clarification of the relationship between the two rate-determining steps in the proposed reaction mechanism.

β,γ-Elimination. Early work by Abbott and Martell[39,40] on the kinetics of β-proton exchange in Schiff

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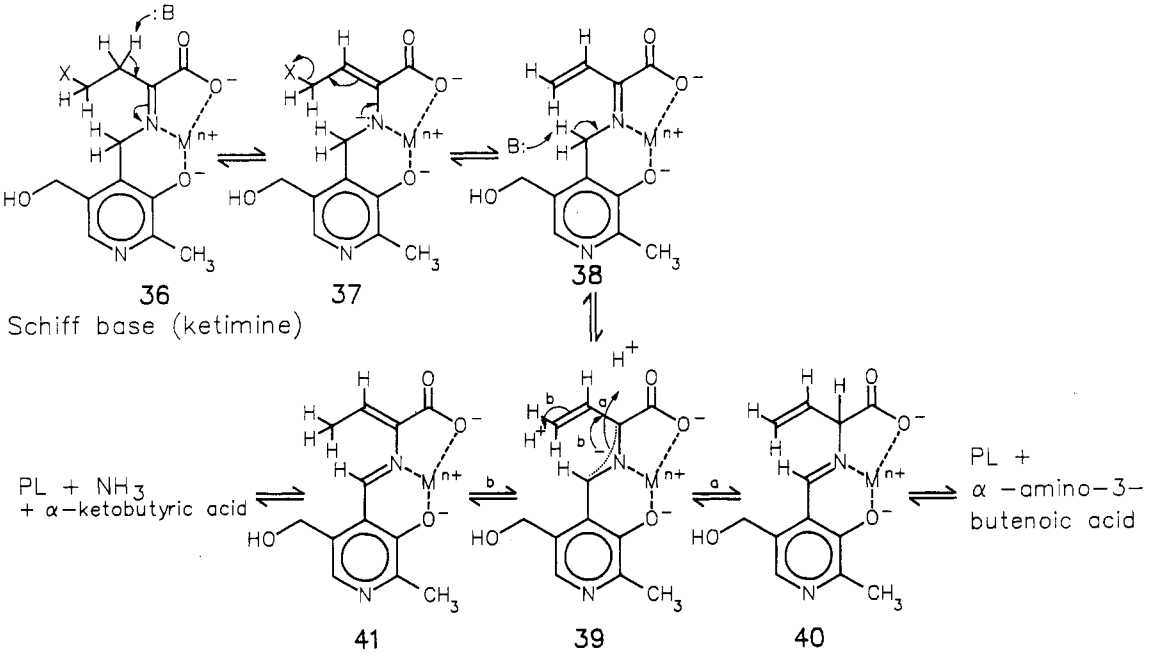
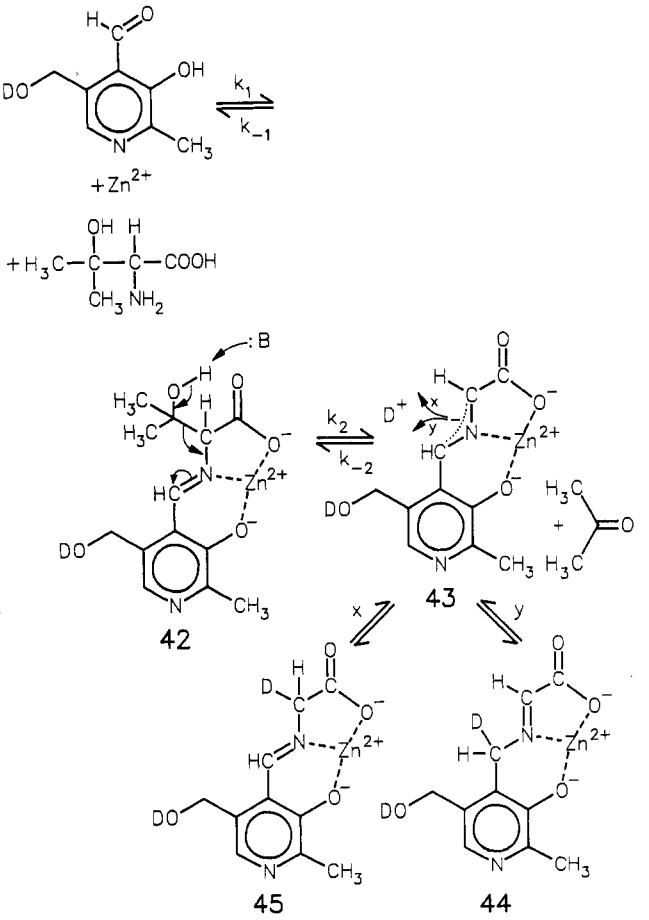
Scheme V
Mechanism Proposed for β-Proton Exchange and Elimination of γ Electronegative Substituents



bases of pyridoxal and α-amino acids, illustrated by Scheme V, provided a suggestion for the first reasonable mechanism of β,γ-elimination of amino acids in biological systems.[41-43] This work has been extended to a kinetic study of vitamin B_6 catalyzed β-proton exchange of α-ketobutyric acid in D_2O solution.[44] With quantitative information on the rates of deuteration, it became possible to undertake kinetic studies of the final step in β,γ-elimination, the rates of removal of the electronegative substituent in the γ-position of α-amino acids. The fact that only one example of β,γ-elimination (the elimination of methanethiol from methionine) has been reported[45] for model systems indicates that much more work should be done in this area.[62]

Pyridoxal-Catalyzed Dealdolation. The dealdolation of β-hydroxy-α-amino acids, first reported by Snell and co-workers,[12,37,46] occurs readily at high pH in aqueous solution at room temperature in the presence of pyridoxal and metal ions. The reaction rates generally increase rapidly with pH in alkaline solution to about pH 10 and then level off, indicating the presence of more than one catalytic species and probable variation of the concentrations of these species with pH. Kinetic measurements of dealdolation of threonine[47,48] in the presence of pyridoxal and aluminum ion gave only qualitative evidence, since the acetaldehyde produced rapidly undergoes aldol condensation with itself under the reaction conditions employed. With β-hydroxyvaline, however, the acetone produced on dealdolation is stable in alkaline solution and good kinetic data were obtained.

Scheme VI
Mechanism for Pyridoxal and Metal Catalyzed C–C Fission of β-Hydroxy-α-amino Acids



The mechanism proposed for the dealdolation of β-hydroxyvaline is given in Scheme VI. Although the bond-breaking process is not illustrated, it is apparent that fission of the α,β carbon–carbon bond cannot occur without a concomitant shift of electrons resulting in

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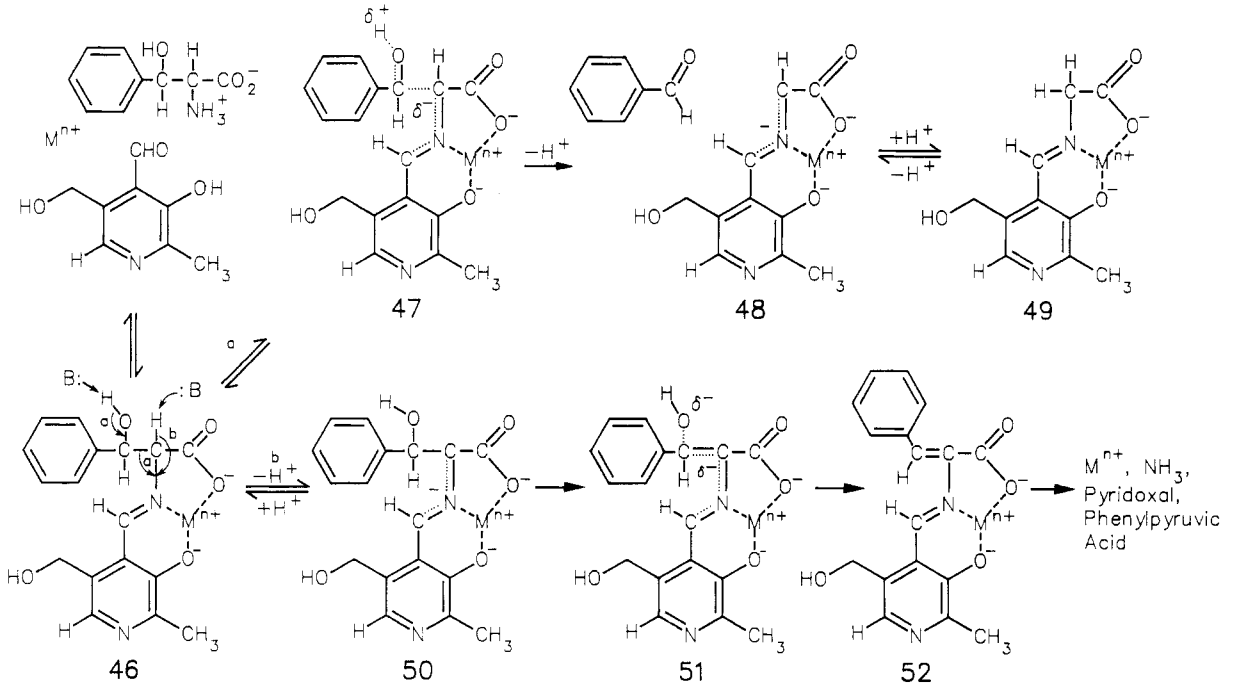
Scheme VII
Mechanism Proposed for Pyridoxal-Catalyzed Dealdolation and β-Elimination of β-Phenylserine



formation of a carbonyl group at the β-carbon and transfer of the hydroxyl proton to an appropriate base. The carbanion intermediate **43** thus formed is then deuterated in the α-position to give a partially deuterated glycinaldimine. Deuteration may also occur in the α′-position to give the ketimine of glyoxylic acid. Deuteration was observed to increase in the reaction mixture, indicating the reversibility of the protonation reactions x and y of the intermediate to give **44** and **45**. Experimental evidence for this mechanism is the observed catalysis by hydroxide ion, as well as the detection of H,D-glycine in the reaction mixture. The latter is eventually converted to D_2-glycine as the result of subsequent deuteration processes in solution under the reaction conditions employed.

An interesting system described by Tatsumoto and Martell[49] involves the pyridoxal and metal catalyzed reactions of β-phenylserine. In this system, dealdolation takes place readily to form benzaldehyde, as expected, but elimination of hydroxide occurs as a parallel reaction to form phenylpyruvic acid, with yield ratios of benzaldehyde to phenylpyruvic acid of approximately 2:1. This result is very interesting because hydroxide ion is a poor leaving group and is not eliminated from serine in the presence of pyridoxal and metal ions under conditions similar to those employed for phenylserine. A possible rationale for this difference in behavior may be seen in the suggested mechanisms for these reactions illustrated in Scheme VII. The initial Schiff base complex **46** undergoes two simultaneous reactions, involving the dealdolation sequence **47** → **48** → **49** and a parallel β-elimination sequence **50** → **51** → **52**. A clue to the reason for the promotion of the competing β-elimination reaction by the phenyl ring is seen in transition-state **51** and the final β-eliminated product **52**. The bond-forming and bond-breaking processes, which are initiated by the formation of the carbanion intermediate **51**, are seen to result in the incipient formation of a carbon-carbon double bond conjugated with the benzene ring as the hydroxide group is eliminated with its bonding electron pair. Simultaneously the aldimine carbon–nitrogen double bond becomes localized so that the result is a completely conjugated system (**52**) containing eight double bonds. This reaction sequence, therefore, is promoted by the tendency of the phenyl substituent on the amino acid moiety to favor the formation of more highly conjugated systems. The dealdolation sequence, on the other hand, produces a much lower degree of conjugation, the phenyl ring ending up with only one external conjugated double bond in benzaldehyde, and with the pyridine ring acquiring a single external conjugated double bond in the aldimine–glycine chelate. The reaction shown in Scheme VII provides a good example of how substitution on the amino acid moiety can profoundly influence the course of metal ion and pyridoxal catalyzed reactions.

The kinetics of dealdolation of a series of para-substituted phenylserines has been described by Marcello and Martell.[50] The results of these studies indicate that para-electronegative substituents, such as nitro, chloro, and amino groups, have relatively little effect (i.e., show only a slight increase) on the dealdolation rate relative to that of the parent compound, phenylserine itself. Inspection of the reaction mechanism indicated in Scheme VII suggests a number of factors that may tend to decrease or increase the observed rate of dealdolation. In general, electron-attracting substituents would be expected to oppose the electron-withdrawing effect of the coordinated azomethine nitrogen. Electronegative substituents would increase the acidity of the α-hydrogen and stabilize somewhat the delocalized carbanion intermediate, **50**, thus favoring elimination. On the other hand, an increase in electron attraction by the phenyl substituent would tend to favor the dissociation of a proton from the hydroxyl group adjacent to the

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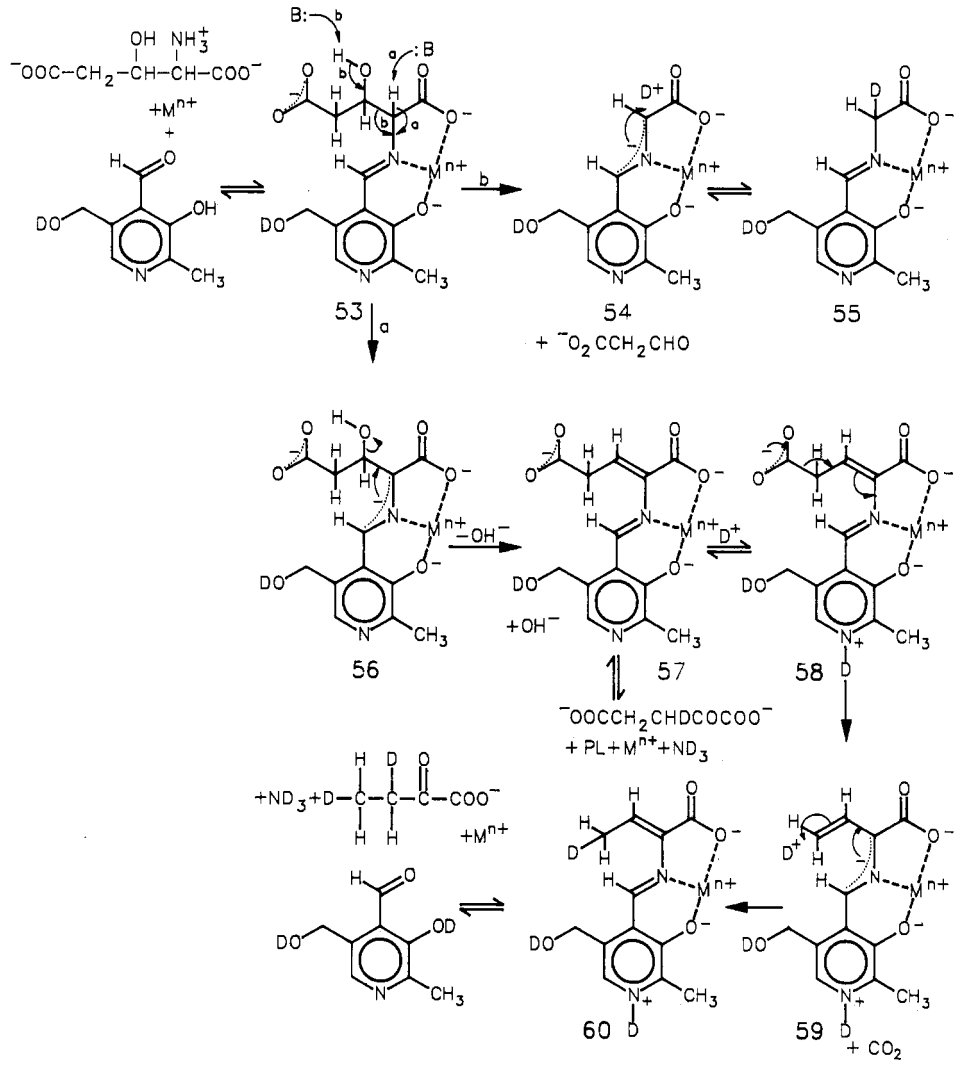
Scheme VIII
Suggested Mechanism for Pyridoxal and Metal Ion Catalyzed Dealdolation, β-Elimination, and -Decarboxylation of β-Hydroxyglutamic Acid



ring, and the shift of an electron pair toward the ring to form the carbon–oxygen double bond indicated by formula **47**, thus favoring dealdolation relative to elimination. Another effect favoring dealdolation would be the influence of electronegative substituents on the phenyl rings in hindering the removal of the negative leaving group attached to the adjacent carbon atom, thus slowing down the second of the two rate-determining steps in the competing β-elimination reaction. The small effects of the electronegative substituents may therefore be considered the result of opposing tendencies that nearly cancel each other.

Parallel Elimination, Dealdolation, and Decarboxylation. An interesting example of a pyridoxal amino acid Schiff base system that undergoes three pyridoxal-promoted reactions is the report by Tatsumoto and Martell[49] of the dealdolation, β-elimination, and γ-decarboxylation of β-hydroxyglutamic acid. In accordance with the reaction mechanism shown in Scheme VIII, dealdolation and β-elimination are parallel reactions of the type illustrated above for phenylserine. In this case, however, the unsaturated aldimine intermediate **57** produced in the course of β-elimination results in the transmission of the electronic effect of the azomethine nitrogen to the γ- and δ-carbon atoms. This intermediate provides the electronic pathway necessary for the electron shift that results in decarboxylation of the δ-carboxyl group and fission of the γ,δ carbon–carbon bond. The negative charge thus generated in the metal chelate is neutralized by protonation to give the aldimine of deuterated 2-amino-2-butenoic acid **60**, which hydrolyzes to the corresponding keto acid. The sequential elimination and decarboxylation reactions occurs only over a narrow pH range, since relatively low pH values favor the formation of the diprotonated Schiff base described above in the discussion of Scheme IV. In this species the removal of the electronegative leaving group is retarded, and the formation of the unsaturated intermediate necessary for conjugation between the γ-carbon atom of the amino acid and the azomethine group is thus inhibited. At higher pH, the monoprotonated Schiff base is the predominant reactive species in solution. These reaction conditions, however, favor the competing dealdolation reaction, which is further catalyzed in alkaline solution by hydroxide ion. Thus the conditions of decarboxylation in this system are met by a balance of favorable and unfavorable tendencies with maximum probability of reaction at around neutral pH.

An obvious extension of the reaction system shown in Scheme VIII for β-hydroxyglutamic acid would be catalysis by pyridoxal and metal ions of reactions at the

γ- and δ-carbon atoms of the amino acid.[62] The electronic effects that result in decarboxylation of β-hydroxyglutamic acid would also be expected to promote proton exchange and dissociation at the γ-position, perhaps followed by the elimination of an electronegative group from the δ-carbon of the amino acid side chain. While these suggestions seem speculative, the reaction pathways suggested are reasonable, and the investigation of appropriately designed amino acids might readily demonstrate the validity of this kind of extension of the catalytic effects of pyridoxal to remote positions of the amino acid side chains.[62]

Oxidation of Amino Acids to Keto Acids by Dioxygen. The amino acid oxidase enzymes[51-53] require copper(II) for catalysis in the oxidation of α-amino acids to α-keto acids. For a time, these enzymes had been considered to require pyridoxal phosphate as a coenzyme. For these enzyme systems, it has been observed that copper(II) does not change its valence in the course of the reaction. The initial work on pyridoxal-type models for this type of enzymic oxidase reaction by Ikawa and Snell[54] has been followed up by reports from Hamilton and Revesz[55] and Hill and Mann[56] and some recent work from the author's laboratory.[57] In pyridoxal-catalyzed model reactions, it has been found that a Schiff base is formed as an intermediate and that pyridoxamine is not formed, indicating that transamination has no role in the process. It was recently found that, in addition to formation of the keto acid, the azomethine nitrogen is hydroxylated to form hydroxylamine.[57] For these model systems, only metal ions capable of undergoing facile redox reactions, such as Mn^{2+}, Fe^{2+}, Co^{2+}, and Cu^{2+}, were found to be active.

Recent investigations have shown that the organic cofactor in copper-containing amine oxidases is not pyridoxal phosphate, but pyrroloquinoline quinone, covalently bound to the protein.[58-61] Therefore, this vitamin B_6 catalyzed reaction type probably should no longer be considered a model for amine oxidase enzymes.

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(62) **Note Added in Proof.** Further studies of several vitamin B_6 catalyzed reactions strongly recommended in this paper probably will not be carried out by the author, in view of the recent termination of financial support.