METAL COMPLEXES AS MODELS FOR VITAMIN B_6 CATALYSIS

by

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Submitted in Partial Fulfillment
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Signature of Author Signature redacted
Department of Nutrition and Food Science
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Thesis Supervisor

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ABSTRACT

Chapter I. Historical introduction to Vitamin B_6 Complexes.

Chapter II. The aldimine complexes N(Salicylidene)glycinato and valinatozinc(II), N-(pyridoxylidene)valinatocopper(II) monohydrate and N-(3-Hydroxypyridyl-2-methylene)valinatocopper(II) hemihydrate have been prepared from L-valine. Synthetic methods and characterization data are given. Also prepared were the bis-chelate amino acid ester complexes, Bis[N-(2-ethoxycarbonyl-1-propyl)salicylaldiminato]copper(II) and Bis[N-(3-ethoxycarbonyl-2-propyl)salicylaldiminato]copper(II). The inertness of these two complexes to H-D exchange contrasts with the ready exchange in the absence of base of the complexes derived from α-amino acids. This result shows that facile exchange and racemization properties of Bis[N-(alkoxycarbonylalkyl)-salicylaldimino]metal(II) complexes derive principally from the direct attachment of the electron-withdrawing HC=NM and $COOC_2H_5$ groups to the asymmetric center. The base-catalyzed racemization rates of four copper(II)-aldimine complexes in 95% ethanol at 50° were found to increase in the order N-Salicylidene-L-valinatocopper(II), Cu(sal-L-val)<< N-Pyridoxylidene-L-valinatocopper(II)≤ N-3-Hydroxypyridyl-2-methylene-L-valinatocopper(II)<N-4-NO_2-Salicylidene-L-valinatocopper(II). This order is essentially the same as that of qualitative catalytic effectiveness of the constituent o-hydroxyarylcarbonyl compounds in nonenzymatic transamination and reinforces in semiquantitative fashion the prevailing model of ligand electronic features requisite to catalytic activity of these compounds.

Thesis Supervisor: Dr. Sanford Miller

Title: Professor of Nutritional Biochemistry and Metabolism

To my Father

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METAL COMPLEXES AS MODELS FOR

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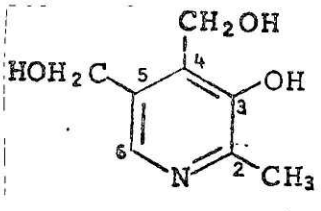
CHAPTER I

Introduction to Vitamin B_6 Metal Complexes

Vitamin B_6-containing enzymes catalyze a large number of transformations of α-amino and α-keto acids including transamination, racemization, decarboxylation and β-elimination. Many of these reactions are also catalyzed by metal ions in nonenzymatic systems.[1] The first part of this thesis is concerned with the nonenzymatic catalysis of racemization and transamination. Of particular interest are the role of the metal ion in nonenzymatic catalysis and the structural features of vitamin B_6 essential to catalysis; these are explored through quantitative racemization studies. An historical introduction is presented to acquaint the reader with concepts fundamental to understanding the research presented.

Vitamin B_6 is not the name for one specific molecule, but refers to a group of related compounds. Pyridoxine (1)



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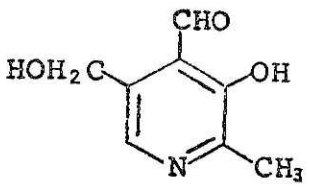

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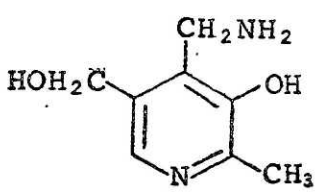

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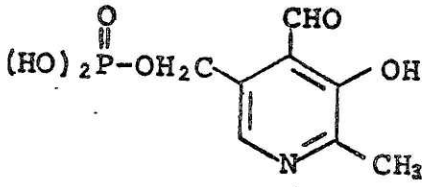

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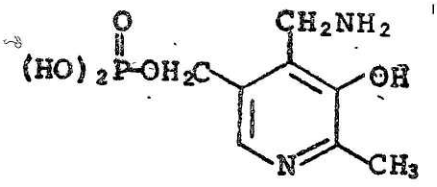
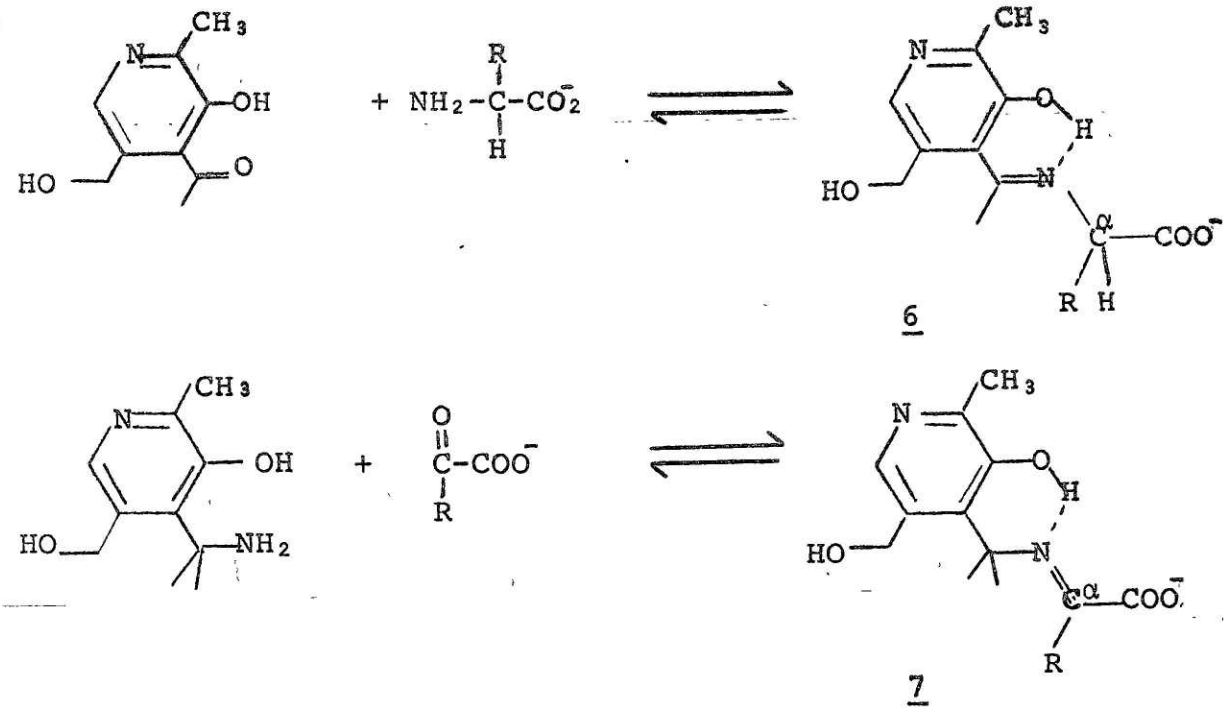


5

was the first to be isolated in 1938; it was fully characterized through structural determination and *in vitro* preparation by the end of 1939. However, Snell found in 1942 that there were other natural materials which also cured dermatitis in rats and promoted faster growth than pyridoxine in lactic acid bacteria. Through *in vitro* synthesis these were found to be pyridoxal (2) and pyridoxamine (3). The phosphate groups of 4 and 5 are essential for the coenzymatic activity of vitamin B_6; it is postulated that the phosphate group is necessary for attachment to the enzyme.[2-5] The most important function of vitamin B_6-dependent enzymes is to catalyze transformations of α-amino acids. Both pyridoxal and pyridoxamine reactoto form Schiff base compounds.



6

7

Aldimine type compounds (6) are formed from pyridoxal and an amino acid and ketimine type compounds (7) are formed from pyridoxamine and a keto acid. The Schiff bases 6 and 7 are tautomers differing only in the position of a double bond and a hydrogen atom.

The first observation that reactions catalyzed by vitamin B_6 enzymes could be reproduced nonenzymatically came in 1945 when Snell autoclaved pyridoxal with amino acids and noted the formation of pyridoxamine and a keto acid.[6] The coupling of the forward and reverse reactions (1) and (2) results in the overall transamination reaction:

(1) amino $acid_1$ + pyridoxal $\rightleftharpoons$ $ketoacid_1$ + pyridoxamine

(2) keto $acid_2$ + pyridoxamine $\rightleftharpoons$ amine $acid_2$ + pyridoxal

(3) amino $acid_1$ + keto $acid_2$ $\rightleftharpoons$ amino $acids_2$ + keto $acid_1$

In 1952 Snell found that the transamination reaction was

catalyzed by metal ions.[7] This finding prompted great interest in the reactions of metal ions with pyridoxal and amino acids, especially since it was thought at the time that a metal ion might be present at the active site of vitamin B_6 enzymes. There has been, however, no proof that metals are present at the active site of these enzymes, and in one enzyme, glutamic-aspartic transaminase, direct assay has shown that there is less than 0.4 mole of metal ion per mole of active site.[8] The reactions of Schiff base metal complexes, however, continue to be of interest as inorganic reactions of great diversity. Also, the metal ion may simulate some of the features of enzymatic active sites by promoting Schiff base formation and by increasing the lability of bonds formed by the α-carbon in metal complexes of compounds of type 6.

The question of whether the metal ion actually catalyzes the formation of the Schiff base, or simply acts in effect as a stabilizing trap for the intermediate carbinolamine and final product, but does not kinetically catalyze the reaction, has been studied most thoroughly for the system M(sal-gly).*[9,10] Salicylaldehyde has the same functional

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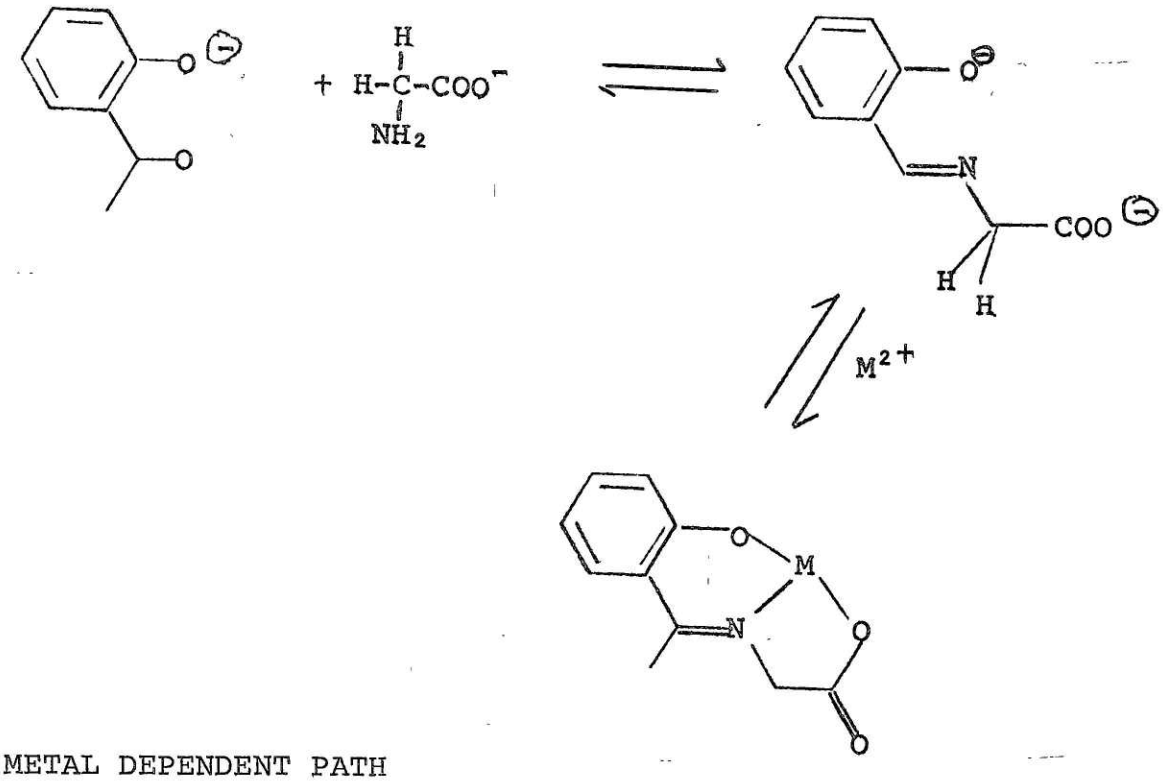
* Abbreviations are listed at the end of Part I.

groups for metal complexation as pyridoxal, o-OH and CHO, but has a simpler electronic spectrum and undergoes fewer side reactions because it lacks the ring constituents of pyridoxal. Using pH stat and spectrophotometric techniques in aqueous solution at pH 5-9, Leussing et.al.[9,10] have shown that the effect of metal complexation on Schiff base formation depends on the metal ion present. For some metals (Cu^{+2}, Ni^{+2}, Co^{+2}) there is a metal-independent path only, while for others there is both a metal-independent and metal-dependent path (Mg^{+2}, Mn^{+2}, Zn^{+2}, Cd^{+2}, Pb^{+2}). The two pathways are illustrated in Scheme 1. Those metals effective in catalyzing the metal-dependent pathway share the common feature of having no partially filled d-orbitals. Complexes of these metal ions are therefore likely to be labile in solution and to impose few geometrical constraints on the ligands. The salicylaldehyde and glycine can thus be bound to the metal in the proper spatial relationship for carbinolamine formation and subsequent rapid dehydration to occur. Although the reaction scheme described above may not be general for all o-hydroxyarylcarbonyl and amino acid reactants, the system PLP:glu:Cu^{+2} shows only metal-independent Schiff base formation at pH 4.[11]

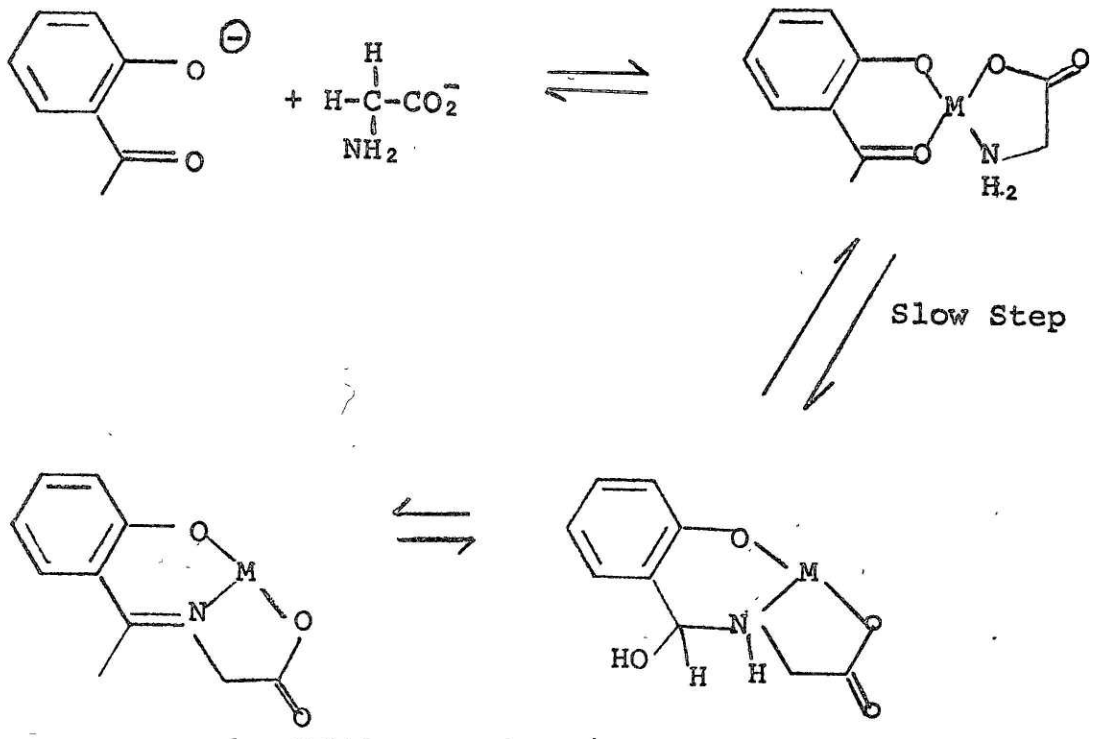
Metal complexes of the tridentate Schiff base ligand can have a 2:1 or a 1:1 ligand:metal ratio. This work is

SCHEME 1.

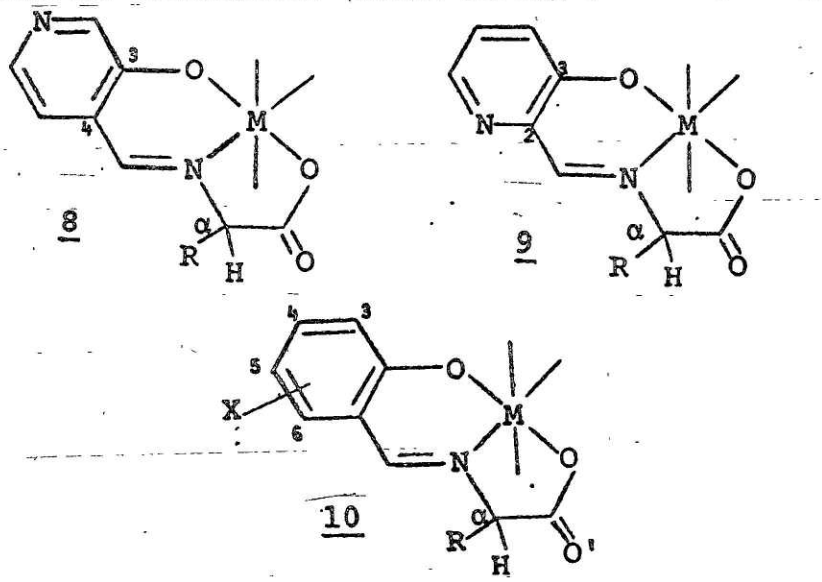
METAL INDEPENDENT PATH



METAL DEPENDENT PATH

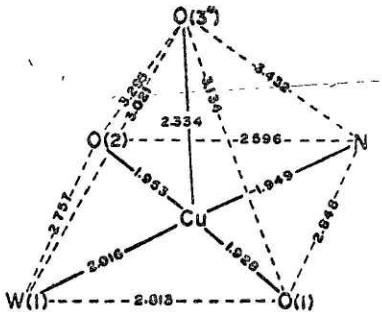


primarily concerned with the 1:1 complexes. Relevant examples are the complexes formed from pyridoxal, 3-hydroxy-2-pyridinealdehyde, and salicylaldehyde and amino acids, which have the general structures shown below:



8 9 10

A number of aldimine complexes of types 8,[12-16] 9,[16,17] and 10[16,18-22] and some with the pyridine ring protonated[15-17] have been isolated with metal ions such as Mn(II), Cu(II), and Zn(II). The crystal structures of [Cu(sal-gly)(H_2O)]·$\frac{1}{2}$ H_2O, 11, Cu(pyr-DL-val),[24] 12, and Cu(Ppyr-DL-Phala)(H_2O)],[25] 13 have been determined. In each case there is square pyramidal coordination around the metal with the Schiff base occupying three of the coordination positions in the basal plane. In 11 the fourth position in the plane is occupied by a water molecule and the apical oxygen is the free carboxyl oxygen from another molecule. The fourth basal position in the pyri-



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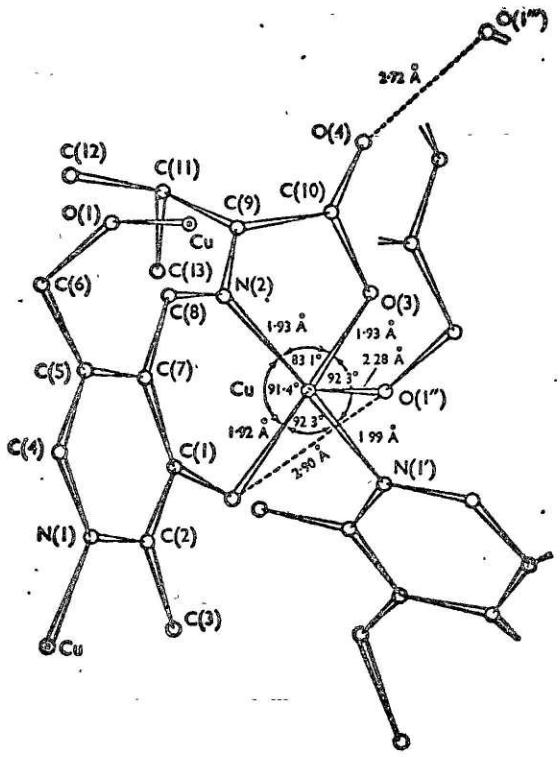
doxylidene complexes is occupied by a pyridine nitrogen, or a water molecule, and the apical position by a hydroxymethyl 12, or a phosphate, 13, oxygen molecule. Only a few attempts to isolate ketimine type complexes 14 and 15 have been reported[20,26] and no structural determinations have been done. It



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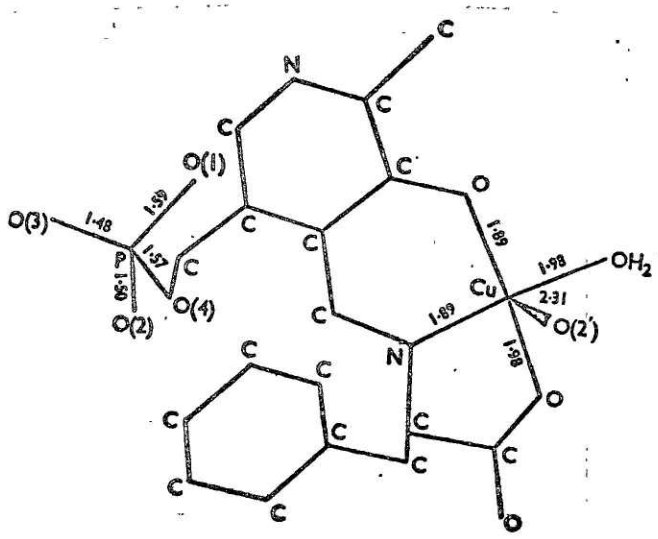

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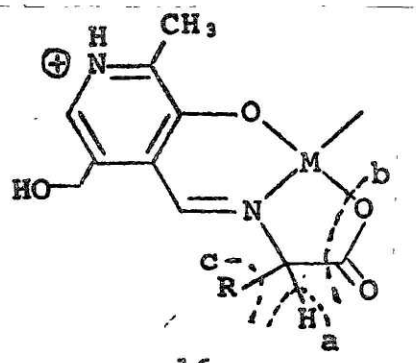
is assumed throughout that the structure is probably similar to that of the aldimine with the Schiff base acting as a planar tridentate ligand.

The reactions of the amino acids in complexes of type 8 and 16 have been grouped by Snell[4] according to the bond



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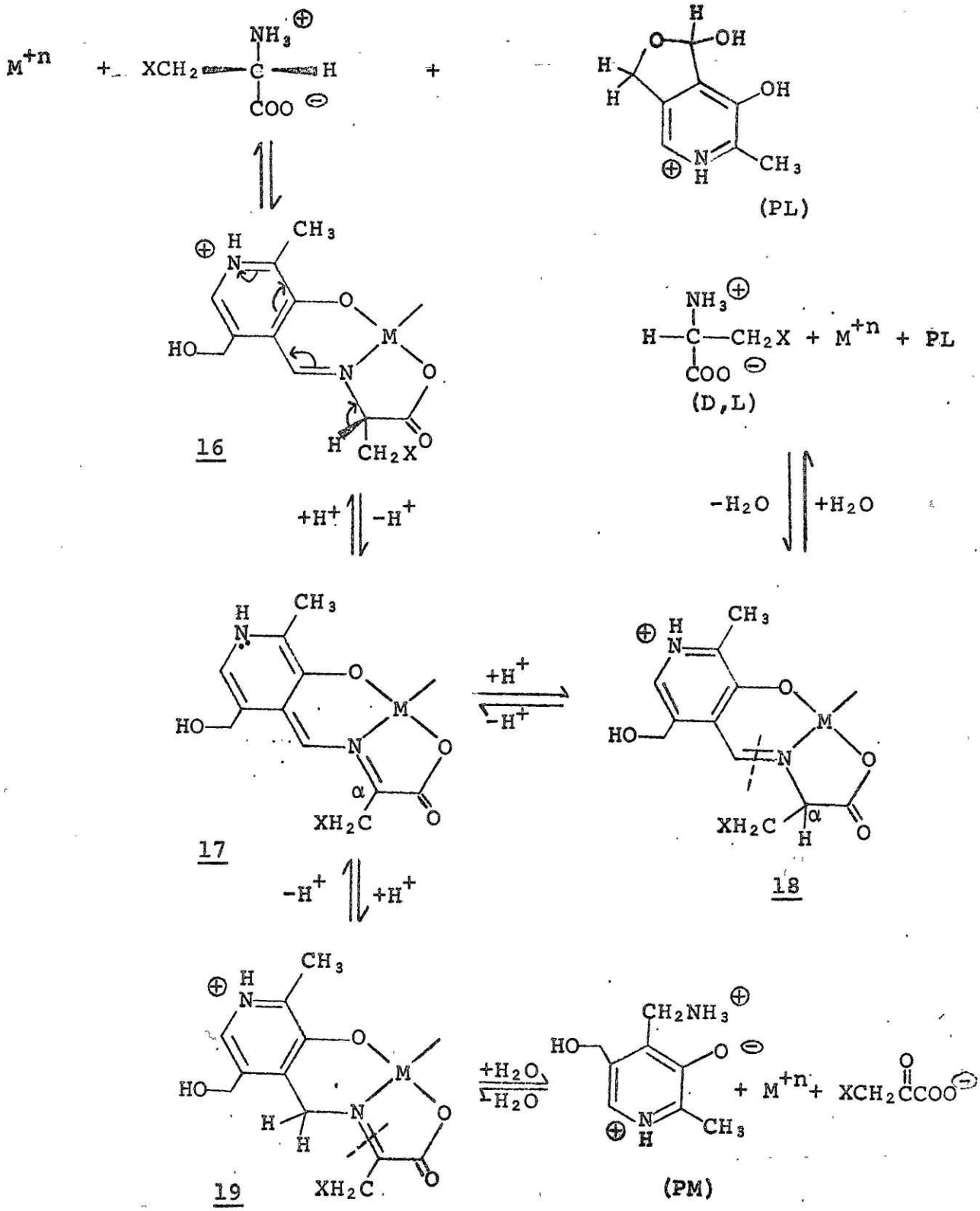
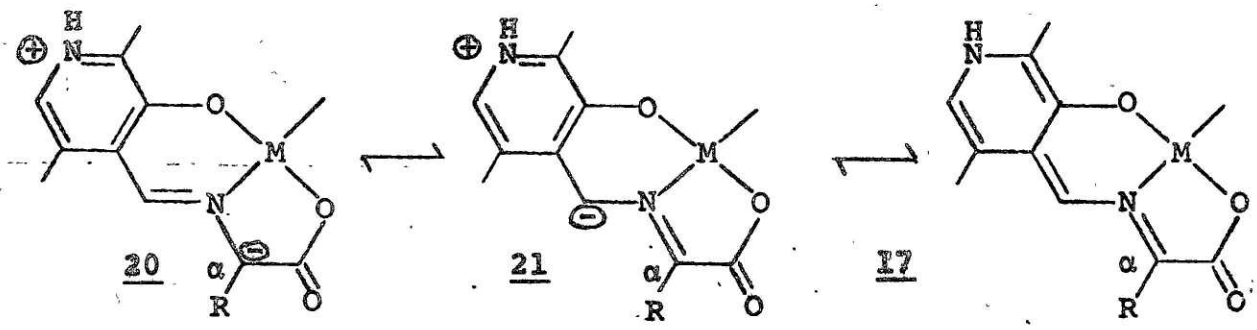
broken during the initiation of the reaction. A partial listing of the reactions of α-amino acids catalyzed by pyridoxal and metal ions in model systems is given in Table I, which is taken from reference 1. We will be concerned mainly with the transamination and racemization reactions. The mechanisms of these reactions was first proposed independently by Braunstein and Shemyakin[4] and Metzler, Ikawa and Snell[27] in 1953-1954 on the basis of model studies with pyridoxal and other o-hydroxyarylcarbonyl compounds, and metal ions as catalysts. The mechanisms proposed in Scheme 2 on the next page involve the formation of a 1:1 or 2:1 aldimine complex followed by the breaking of bond "a" (loss of the α-H) of the condensed amino



SCHEME 2. Mechanisms for Pyridoxal-Catalyzed Transamination and Racemization of L-Amino Acids.

acid in 8 or 16. If the proton recombines at the α-C in 17, the racemized complex 18 will be produced, if it recombines at the azomethine carbon the ketimine 19 will be formed. Subsequent hydrolysis of the imine bond will give pyridoxal plus racemized amino acid in the former case and pyridoxamine plus keto acid in the latter. The metal is postulated to increase the rate of the reaction in three ways:[1,4] (1) it promotes Schiff base formation in aqueous solution, (2) it increases thellability of the α-C-H bond through the inductive electron-withdrawing effect of the H-C=N-M grouping, (3) it serves to hold the ligand in a planar configuration. The importance of feature (3) is seen after the loss of the α-H when the carbanion formed can be stabilized by resonance 20 ↔ 21 ↔17, in which the conjugated system extends from the pyridinium nitrogen to the α-C. The electrophilic group on the ring ortho or para to the azomethine function is essential for this resonance stabilization. Thus, while 3-hydroxy-



pyridine-2- and -4-aldehyde catalyze the transamination reaction from glutamate to α-ketoglutarate,[4] salicylaldehyde, which has

the ortho hydroxyl and formyl groups essential for metal complexation but does not possess an electron-withdrawing group on the ring, is not catalytically active.[4] The series of compounds studied is given in Table II from Snell's review article.[4] It is interesting to note that some of the vitamin B_6 analogues which are unable to catalyze the nonenzymatic reaction of glutamate to α-keto glutarate are antagonists of vitamin B_6 in living systems (cf. Table III). One of the most effective and widely studied antagonists[28] 4-deoxypyridoxine has the groups necessary for attachment to the enzymes, but does not have the 4-hydroxymethyl group which can be converted to the aldehyde or amine necessary for catalysis. It has been shown to block in vivo some of the sites available for ^{3}H-pyridoxine, since tritium excretion was greater in rats fed a deficient diet plus deoxypyridoxine than in those fed the deficient diet without the antagonist.[28a]

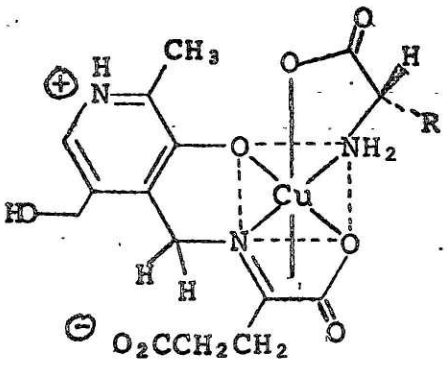
Racemization and transamination of species generated in solutionshave been studied both qualitatively and quantitatively by spectrophotometric and nuclear magnetic resonance techniques. Nonenzymatic racemization was first observed for L-alanine, L-phenylalanine, and L-valine in systems containing pyridoxal; the rate was increased by the addition of Al(III), Fe(III), or Cu(II).[29] For the system PL:L-ala:Cu(II) or Al(III), racemization is favored over transamination above pH ~9. The pyridine nitrogen is unprotonated at this pH. Racemization at low pH values where transamination is favored

probably occurs via formation of 19 and the reverse reaction to 18, followed by hydrolysis, whereas at pH <9 it is not necessary to postulate the formation of the ketimine 19 as an intermediate step. Racemization at high pH (pD ~10) has also been observed by pmr for $Al(pyr\text{-}\underline{L}\text{-}ala)_2^-$ in D_2O. As racemization occurs and the α-H is replaced by deuterium the alanine methyl doublet becomes a singlet.[30] Racemization rate studies at pH 10 in water have shown that the rate is dependent on the particular metal ion present and on the substituents on the benzene ring in pyridoxal analogues.[31] The fastest rates were observed for Cu^{2+} and for salicylaldehyde with an electron-withdrawing group in the 4-position. Substitution with an electron-releasing group produced rates slower than that of salicylaldehyde itself.[31]

The transamination reaction has been studied much more extensively than the racemization reaction. The transamination reaction for α-amino acids, outlined in equations (1)-(3), starts with the formation of an aldimine Schiff base complex, 16, breaking of bond "a" to form a resonance stabilized species such as 17, and reprotonation at the azomethine carbon to form a ketimine Schiff base complex, 19, followed by hydrolysis of the imine bond. Upon completion of the reverse reaction of pyridoxamine with a different keto acid to form 19 and a retracing of the steps above reaction (3) has been completed.

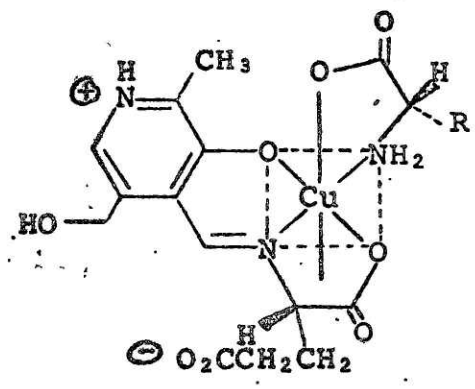
Usually the transamination reaction is only studied in one direction, viz., either (1) or (2) is followed since each of these is a transamination reaction in itself.

The metal ion not only increases the rate of transamination,[7] but in one report was found to produce optical selectivity in the transamination between D- or L-alanine or phenylalanine and α-ketoglutarate catalyzed by pyridoxal and Cu(II).[32a] The optically active amino acid was present in excess, and glutamic acid was formed with a 2-3% optical enrichment with the L- form predominant of L-alanine were present. The formation of the optically enriched amino acid must arise from stereoselectivity in the ketimine 19 ⟶ aldimine 17 conversion. Such stereoselectivity requires a dissymmetric intermediate which can be formulated as containing the optically active amino acid. This intermediate must be stable for longer than the time required for the tautomerization. The species 23 and 24 are possible structures



[Cu(Hpym-glt)(L-aa)]⁻

23



[Cu(Hpyr-L-glu)(L-aa)]⁻

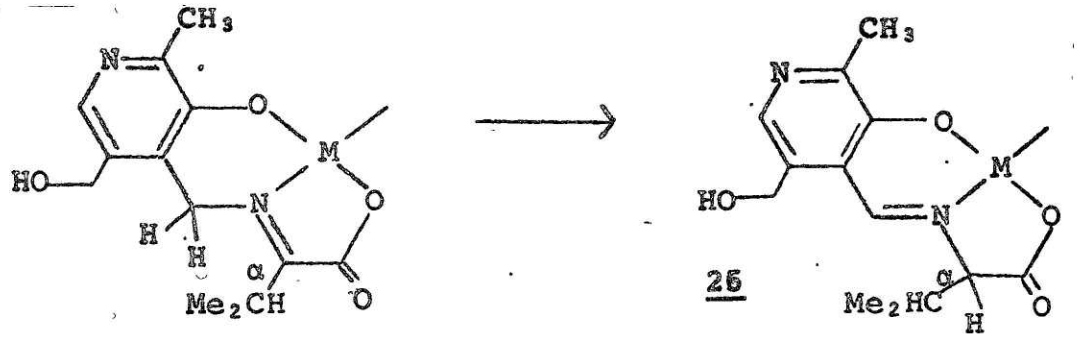
24

for the dissymmetric intermediates; hydrolysis of 24 yields L-glutamic acid. Although the optical selectivity is small, it is difficult to imagine there being any selectivity at all without formation of a metal complex.

The reaction of metal ions with pyridoxamine and α-ketoglutarate at 100° showed a rate enhancement of 7-35 over the metal-free systems.[32b] The efficiency of metal ions as catalysts was found to occur in the following order: Ga(III)>Cu(II)>>Al(III)>Fe(II)>Fe(III)≳Zn(II)>In(III)≳Ni(II)> Co(II)>Sc(III). Only slight catalytic activity was observed for the series Cd(II)>Cr(II)>Mn(II)>Mg(II). The order of catalytic activities for the divalent metal ions parallels the order of stability constants for the chelates formed by the reaction $M^{+2} + PL^{-} + val^{-} = M(pyr\text{-}val)$,[15] except for the inversion of Zn(II) and Ni(II). This finding supports the proposed mechanism in which metal chelation with the reactants is the first step in catalysis.

The aldimine→ketimine conversion 16→19 was originally proposed on the basis of the formation of uncomplexed transamination products. The first direct evidence of this reaction came from spectral studies of the system PM:pyruvate:Ni(II),[33] and the separation of ketimine and aldimine complexes from the system PL:glycine:Al(III) by paper chromatography.[34] Recent pmr work has clearly established the existence of both tautomers, and has been useful in measuring total ketimine and aldimine formation as a function of pH, establishing semi-

quantitative pK_a values for complexes, and providing structural information for those complexes for which ligand exchange is not fast on the pmr time scale.[30,35-40] The reaction ketimine ⟶ aldimine is usually followed due to the greater stability and more rapid rate of formation of the aldimine tautomers. The conversion of Al(III)-ketimine complexes[37] in the system PM:pyr:Al(III) to the corresponding aldimine is clearly shown in Fig. 1. The spectrum of the aldimine system PL:ala:Al(III) had been determined in a separate study. A recent spectrophotometric study has shown that in methanol unprotonated complexes from pyridoxamine



and α-ketoisovalerate convert to the aldimine 26 by a first order process.[41,42] The catalytic order of the metal ions when added to a preequilibrated mixture of PM and α-ketoisovalerate is Cu(II) > Zn(II) > Ni(II), which is the same as that found for the PM-glutarate transamination[32b] and is 1000-fold faster than the metal-free rate.[41,42] The reaction

rate is increased by the presence of base. An unprotonated form of 17 is suggested as the intermediate which is converted to the aldimine on reprotonation of the α-carbon .

No attempt has been made to be exhaustive in this introduction, as there are several recent reviews on the subject of vitamin B_6 metal complexes.[1,5,43] It should be noted that all previous racemization and transamination rate studies have been done on species generated in solution. In the kinetic studies reported in this thesis, however, preformed characterized metal complexes which maintain their integrity in solution have been studied, greatly reducing uncertainty as to the identity of the species undergoing reaction. The findings presented herein support the general mechanism proposed for racemization and transamination, while emphasizing both the independence of racemization from transamination at high pH and the stability of the aldimine vs. the ketimine complex.

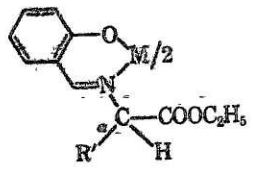
CHAPTER II

Racemization of Schiff Base Complexes

Introduction

The relation of the structural and electronic properties of pyridoxal and other o-hydroxyarylcarbonyl compounds to their ability to catalyze the transamination[4] reaction in model systems has been discussed in Chapter I. The minimal features of pyridoxal essential to the nonenzymatic catalysis of transamination are found in 3-hydroxypyridine-2- and 4-aldehydes. It is of interest to see if the same features are necessary for racemization.

The racemization reaction proceeds much faster for Schiff base complexes derived from amino acid esters (27)



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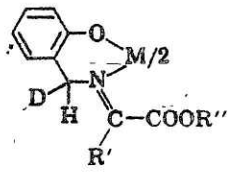
than for those from amino acids (10, X=H).[22,44] In fact the bis[N-(alkoxycarbonylalkyl)salicylaldimino]metal(II) complexes have not been isolated with a high degree of optical activity. If the optically active amino acid ester is reacted with

$M(sal)_2$ for as short a time as 30 seconds in ethanol (M=Cu) the metal complex isolated is optically inactive.[22] If the reaction is run in ethanol-1-d hydrogen-deuterium exchange is seen at the α-carbon only; exchange is about 65% complete at the end of the 30 second reaction time.[22] No deuterium substitution is seen at the azomethine carbon. In a related experimentain which the metal complex is made from bis(2-deuterioformylphenolato)copper(II), $Cu(d\text{-}sal)_2$, there is no loss of deuterium from the azomethine position and no deuterium incorporation at the α-carbon. These findings suggest that the ketimine (28) is not an intermediate in the



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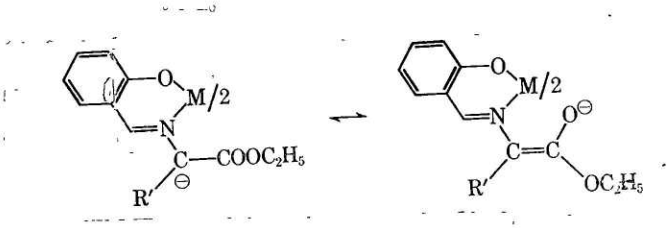
racemization reaction since an equilibrium between 27 and 28 would have to result in the exchange of a deuterium label between the azomethine and α-carbon atoms. The reaction scheme suggested involves loss of a proton from the α-carbon by dissociation or base (B = acetate or ethanol) assistance to form a carbanion which could be stabilized by enolate resonance.[22]



Experimental Section

Preparation of Complexes. The following groups of complexes were prepared by the indicated procedures. The degrees of hydration n indicated in the preparations were obtained from best fits of the analytical data; independent determinations by weight loss studies were not carried out. All quantities dependent upon formula weights were calculated using these n values. N-Salicylidene-L-valinatocopper(II)sesquihydrate and N-4-NO_2-Salicylidene-L-valinatocopper(II)monohydrate were prepared by Dr. M. J. O'Connor and reported previously.[16] N-(Salicylidene)glycinatozinc (II) anhydrous and monohydrate, Zn(sal-gly) and Zn(sal-gly)·H_2O (10, X = H, R = H). Salicylaldehyde (0.020 mol) was added to the amino acid (0.022 mol) in 30 ml of water and the mixture was warmed to 50°. Solid zinc acetate monohydrate (0.020 mol) was introduced and the mixture was stirred vigorously at 40-60° for ca. 90 min. After cooling to room temperature the crude complex was collected by filtration. The initially isolated species was boiled five times with 100 ml of 95% ethanol and 15 ml water and twice with 100 ml absolute ethanol. Drying at 80° (10^{-2}mm) gave the anhydrous complex. The monohydrate was obtained by by combining the preceding aqueous ethanol solutions and reducing the volume until white crystals separated. This solution

was allowed to stand for 2 weeks; the crystalline complex was collected by filtration and then dried at 80°; both complexes mp >360°.

Anal. Zn(sal-gly)·$0H_2O$, calcd. for $C_9H_7NO_3Zn$: C, 44.57; H, 2.89; N, 5.78. Found: C, 44.67; H, 2.84; N, 5.70.

Zn(sal-gly)·$1H_2O$, calcd. for $C_9H_9NO_4Zn$: C, 41.49; H, 3.46; N, 5.38. Found: C, 41.18; H, 3.44; N, 5.29.

N-(Salicylidene)valinatozinc(II)hemihydrate and monohydrate, Zn(sal-L-val)·$0.5H_2O$ and Zn(sal-L-val)·H_2O (10, X = H, R = $CH(CH_3)_2$). These complexes were obtained by the procedure used for the glycinato complexes. The initially isolated valinato complex was recrystallized three times from 5:1 v/v ethanol-H_2O and dried to constant weight at 80° (10^{-2}mm). The white product obtained was found to be the hemihydrate. The monohydrate was isolated as white crystals by volume reduction of the combined aqueous ethanol filtrates.

N-(Pyridoxylidene)valinatocopper(II)monohydrate, Cu(pyr-L-val)·H_2O (8, R = $CH(CH_3)_2$). Pyridoxal was obtained from its hydrochloride (Calbiochem) by treatment with aqueous potassium hydroxide. Hydrated lithium hydroxide (9.50 mmol) was added to a solution of 4.75 mmol of L-valine in 20 ml of degassed methanol at 5° under nitrogen and allowed to react for 1 hr. Pyridoxal (4.75 mmol) was then added and the mixture stirred for 15 min at 5°. An equivalent amount of aqueous cupric nitrate solution was

added dropwise, producing almost immediate crystallization of the product. Methanol (ca. 5 ml) was added and after additional stirring for 1 hr the complex was collected by filtration. It was obtained as a dark green solid after washing with ice-cold methanol (3x10 ml) and ether (5x10 ml) and drying at 25°/10^{-2} mm for 24 hrs; mp >360° with decomposition beginning at 235°.

Anal. calcd. for $C_{11}H_{18}N_2O_5Cu$: C, 45.15; H, 5.21; H, 8.10. Found: C, 45.16; H, 4.70; N, 7.90.

N-(3-Hydroxopyridyl-2-methylene)valinatocopper(II)hemihydrate, $Cu(3,2\text{-hpy-L-val})\cdot 0.5H_2O$ (9, R = $CH(CH_3)_2$). The ligand 3-hydroxypyridine-2-carboxaldehyde was synthesized by a published method[45,46] and purified by vacuum sublimation at 70-80° immediately before use. The complex was prepared by the procedure for the related pyridoxylidene complex. Analytically pure samples were obtained either by washing the precipitated product with methanol and ether or by recrystallizing it from a chloroform-methanol-isobutanol solvent mixture. Dark green crystals were obtained; mp 258-260°.

Anal. Calcd. for $C_{11}H_{13}N_2O_{3.5}Cu$: C, 45.13; H, 4.44; N, 9.57. Found: C, 45.13; H, 4.08; N, 9.52.

Bis[N-(2-ethoxycarbonyl-1-propyl)salicylaldiminato]Cu(II), $Cu(Etaibsal)_2$ (31). The ethyl ester hydrochloride of the amino acid was prepared by dissolving 0.010 mol of β-amino-

isobutyric acid in 30 ml of absolute ethanol and passing dry hydrogen chloride gas through the solution for 3 hrs. Removal of the solvent gave the crude hydrochloride as an oil. The free ester was obtained by dissolving the oil in 50 ml of dichloromethane and passing dry ammonia gas through the solution for 15 min. Ammonium chloride was filtered off, the dichloromethane evaporated, and 30 ml of absolute ethanol added to the residue. This solution was heated just to the boiling point and bis(salicylaldehydato)Cu(II) (0.0050 mol) added. The reaction was allowed to proceed for 30 sec, the solution filtered, and the brownish-green filtrate evaporated until crystallization began. The solution was maintained at 40° for 1 hr and the product filtered off. It was recrystallized twice from absolute ethanol and dried *in vacuo* for 3 hrs at room temperature. The pure product was obtained as greenish-brown crystals, mp 122-123°.

Anal. Calcd. for $C_{26}H_{32}N_2O_6Cu$: C, 58.70; H, 6.02; N, 5.27. Found: C, 58.38; H, 6.27; N, 5.10.

Bis[N-(3-ethoxycarbonyl-2-propyl)salicylaldiminato]Cu(II), Cu(Etab-sal)$_2$ (32). Crude 3-aminoethylbutyrate was obtained on a 0.030 mol scale from 3-aminobutyric acid using the method in the preceding preparation. It was reacted with 0.030 mol of salicylaldehyde in 15 ml of dichloromethane for 15 min at room temperature. Removal of solvent gave the Schiff base as a yellow oil which was distilled at 140°/10^{-2} mm. The

base (0.021 mol) was dissolved in 60 ml of dry t-butanol containing 0.025 mol of potassium t-butoxide under a nitrogen atmosphere. Tetraethylammonium tetrabromocuprate(II) (0.013 mol) was added and the reaction allowed to proceed for 3 hrs at 50°. Removal of the t-butanol yielded a brown tar which was extracted with warm, dry n-heptane. The solvent was removed in vacuo and the resultant brown oil was subjected to pumping at 30-50° for 2 hrs. The complex obtained in this way was found to be of adequate purity. Despite repeated attempts it could not be recrystallized to yield a solid. It could not be prepared in pure form by a method similar to that employed for complex 31.

Anal. calcd. for $C_{26}H_{32}N_2O_6Cu$: C, 58.70; H, 6.02; N, 5.27. Found: C, 58.65; H, 6.17; N, 5.16

N-(Salicylidene)-3-aminoisobutyrato-Cu(II), Cu(sal-aib) (33, R = H, R' = CH_3). 3-Aminoisobutyric acid (0.015 mol) was dissolved in 15 ml of water and 0.015 mol of salicylaldehyde added. The yellow solution was heated for 30 min at 60-70° and then 0.015 mol of cupric acetate monohydrate in 20 ml of hot water was added dropwise. The green complex precipitated as the addition was completed. Reaction was allowed to proceed for 1 hr at 60-70° and the solution filtered when hot to yield ca. 3.0 g of product. This material was recrystallized from 300 ml of absolute ethanol and dried to constant weight at 80°/10^{-2} mm. An anhydrous dark green solid was obtained; mp 281-283°.

Anal. calcd. for $C_{11}H_{11}NO_3Cu$: C, 49.12; H, 4.08; N, 5.21. Found: C, 49.28; H, 4.15; N, 5.08.

N-(Salicylidene)-3-aminobutyrato-Cu(II) hemihydrate, Cu(sal-ab)·0.5H_2O (33, R = CH_3, R' = H). This compound was obtained using the preceding method. It was recrystallized twice from 90% ethanol and obtained as a light green solid after drying at 80°/10^{-2} mm to constant weight; mp 258-260°.

Anal. calcd. for $C_{11}H_{12}NO_{3.5}Cu$: C, 47.42; H, 4.31; N, 5.03. Found: C, 47.68; H, 4.73; N, 5.26.

Magnetic Measurements. Magnetic moments of representative Cu(II) complexes at 25° were measured by the Faraday method using $HgCo(NCS)_4$ as a calibrant. Moments for four complexes are given in Table IV. Additional values are Cu(sal-ab)·$\frac{1}{2}H_2O$, 1.76 BM and Cu(sal-aib), 1.78 BM.

Acidity Measurements: Measurements of apparent hydrogen ion concentrations in 95% or other aqueous ethanol solutions were made with a Radiometer 26 pH meter equipped with a Radiometer combined glass-calomel electrode, which was equilibrated in the particular solvent medium prior to measurement.

Deuterium Exchange Studies of Bis(N-ethoxycarbonylpropylsalicylaldiminato)Cu(II) Complexes. The deuterium exchange properties of the proton attached to the asymmetric carbon in Cu(Etaibsal)$_2$ and Cu(Etabsal)$_2$ were investigated under neutral and basic conditions in ethanol-1-*d* solutions. After the treatments described below the complexes were reisolated,

dissolved in carbon tetrachloride, and the Schiff bases freed by passing hydrogen sulfide through the solutions. The precipitated sulfide was removed by filtration, the volume of the solution reduced to ca. 2-3 ml, and the pmr spectrum of the free ligand recorded. A 0.09 M solution of Cu(Etaib-sal)$_2$ was refluxed for 30 min and a 0.08 M solution containing equimolar ethanol-1-d was refluxed for 17 hrs. Solutions of Cu(Etab-sal)$_2$ 0.08 M in complex and sodium ethoxide were refluxed for periods up to 17 hrs and a similar solution containing a 1:5 mole ratio of complex to base was refluxed for 30 min. In all cases the pmr spectra revealed no deuterium exchange of the proton in question or of any other protons, and were the same as those of the separately prepared Schiff bases. The following chemical shift data (Hz, CCl_4 solution, TMS reference) were obtained: H(Etaib-sal), -73 (both CH_3's) triplet + doublet), -168 (β-H, quartet), -222 (N-CH_2, doublet), -247 (ester CH_2,,quartet), -420 (ring protons), -500 (HC=N); H(Etab-sal), -69 (ester CH_3, triplet), -74 (CH$\underline{CH_3}$, doublet), -148 (CH$\underline{CH_2}$, doublet), -240 (ester CH_2 + α-H), multiplet), -420 (ring protons), -499 (HC=N). All coupling constants are 6-7 Hz.

Measurement of Racemization Rates. Rates of racemization of Cu(sal-L-val)$\cdot\frac{3}{2}H_2O$, Cu(4-NO_2sal-L-val)$\cdot H_2O$, Cu(3,2-hpy-L-val)$\cdot\frac{1}{2}H_2O$, and Cu(pyr-L-val)$\cdot H_2O$ were measured polarimetrically

in basic 95% ethanol solutions at 50.0±0.1°. A Perkin-Elmer Model 141 Spectropolarimeter and a 10 cm jacketed cell attached to a circulating constant temperature bath were employed. Sample solutions (50 ml) were prepared by dissolving the complex in degassed 95% ethanol and adding a sufficient volume of standardized, degassed stock solution of sodium hydroxide in 95% ethanol to achieve an apparent base concentration equal to within ±2% of that of the complex (usually $1\text{-}1.5\times10^{-3}$ M). The ca. 0.05 M stock solution was standardized by pH titration with a 95% ethanol solution of benzoic acid. Measurements of optical rotations of the four complexes were made at 589 mμ and in several cases at 578 and 436 mμ also. At the concentrations employed (cf. Table V) initial rotations ranged from 0.150° to 0.350°. Kinetic runs were carried out for at least two half-lives and some were continued to zero rotation. The average number of measurements per run was 25, and as many as 50 measurements were made for the longer runs. The following control experiments were also performed. Solutions idential to those used in the kinetic runs, except for the presence of base, were maintained at 50° for times longer than two half-lives of the racemization reaction in basic solution. In all cases the values of $[\alpha]_{589}$ and the wave-

lengths and intensities of absorption band maxima in the 210-450 mμ region were unchanged. Spectral data are given in Table IV. As a check on possible decomposition of the complexes during the kinetic runs, spectra in the 210-800 mμ range were recorded at the beginning and end of these runs using solutions maintained at 50° and having the same concentrations as those employed in rate measurements. In the210-450 mμ range no new absorption features were observed, and band intensities changed by <6% with changes of <4% observed in most cases. In addition, the ORD, CD and absorption spectra of fresh solutions without base and in the presence of equimolar base were compared (cf. Figs. 2 and 3). Only slight changes were found in the ultraviolet absorption spectra and in the visible a weak shoulder was detectable on the trailing edge of the ultraviolet absorption at 490-525 mμ in basic solutions of the four complexes. Band maxima at 640-670 mμ in neutral solutions were shifted by 10-20 mμ to higher energies in the basic solutions. Intensities of these features changed by <10% during kinetic runs except for Cu(3,2-hpy-val)$\cdot\frac{1}{2}$ H_2O, whose bands at 650 mμ and 500 mμ (sh) increase considerably in intensity. Possible sources of spectral differences between neutral and basic solutions are mentioned in the text. To establish that the added base acted as a catalyst and was not consumed during loss of optical activity, glass electrode measurements were made on

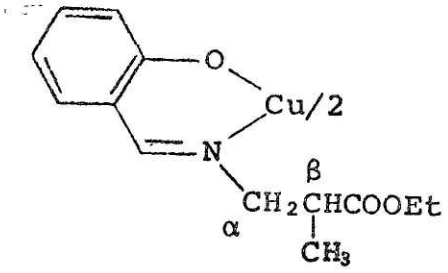
portions of solutions used in the kinetic runs. Prior to each run solutions were diluted under nitrogen to 50% aqueous ethanol composition using degassed distilled water and the apparent pH determined; the same procedure was followed at the end of each run. A 0.01 M succinic acid buffer in 50° ethanol was employed as a standard.[47] Changes of less than 10% in initial and final readings were found except for solutions of Cu(3,2-hpy-L-val)·$\frac{1}{2}H_2O$ where differences of 10-15% were observed. Plots of log α_{589} vs. time in all cases gave excellent straight lines for at least two half-lives from which pseudo first-order rate constants $k_{r(obsd)}$ (min^{-1}) were obtained by least squares fits of the data. Racemization rate constants were obtained from the relation $k_r(M^{-1}min^{-1}) = k_{r(obsd)}/(OH^-)$. Kinetic data are are set out in Table V.

Results and Discussion

Racemization of Bis[N-ethoxycarbonylalkyl)salicylaldiminato]-Metal(II) Complexes.

The rapid racemization of complexes of type 27 was proposed to result from the activation of the α-C-H bond by electron-withdrawing properties of the metal azomethine linkage and the carboethoxy group, and from resonance stabilization of the intermediate.[22] In order to discover which of

these effects was more important the metal complexes



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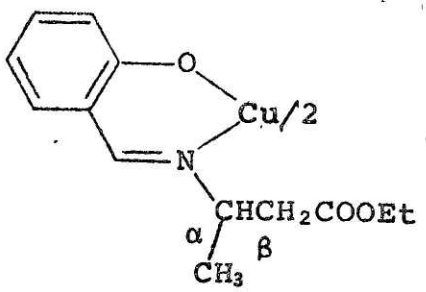


32

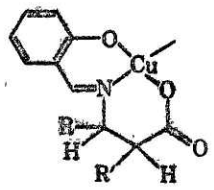
$Cu(Etaib\text{-}sal)_2$ (31) and $Cu(Etab\text{-}sal)_2$ (32) were prepared. In the former a methylene group has been inserted between the asymmetric carbon and the metal center and in the latter between the asymmetric carbon and the carboethoxy group. Because of the effort involved in making the optically active amino acids, the racemic amino acid esters were used to prepare the complexes, and possible hydrogen-deuterium exchange was monitored. The complexes were refluxed in ethanol-1-d in the presence and absence of base (sodium ethoxide) and then decomposed with hydrogen sulfide. PMR spectra of the free Schiff bases showed no H-D exchange at any position. This same procedure was used to show exchange at the α-carbon only in the type 27 complexes $Cu(Etgly\text{-}sal)_2$,[48] and $Cu(Etala\text{-}sal)_2$, $Cu(EtPhala\text{-}sal)_2$ and $Zn(Etala\text{-}sal)_2$.[22]

It had been hoped that the lability of the α-proton in 31 or 32 would be decreased just enough so that exchange could be studied on a convenient time scale; obviously the effect was

more extreme. Whereas 27 undergoes H-D exchange at the α-carbon in ethanol-1-d with no added base, 31 and 32 are perfectly stable in refluxing ethanol-1-d with added base. Direct attachment of the asymmetric carbon to one or the other of the two electron-withdrawing groups COOEt and HC=N-M is not enough to activate the C-H bonds to exchange. Thus all three features of the asymmetric center in 27, (1) electron withdrawal through the azomethine-metal linkage, (2) electron withdrawal by the carboethoxy group and (3) "enolate" resonance stabilization of the anionic intermediate by the carboethoxy group, appear to be necessary for rapid exchange and racemization to occur.

Schiff Base-Amino Acid-Metal(II) Complexes. The metal complexes of primary interest for the racemization studies are Cu(sal-L-val)·1.5H_2O, Cu(4-NO_4sal-L-val)·H_2O, Cu(3,2-hpy-L-val)·0.5H_2O and Cu(pyr-L-val)·H_2O, all of which were isolated as crystalline solids. The magnetic and spectral data for these complexes, which is presented in Table IV, indicate that they are simple spin-doublet species with electronic spectra similar to those for other Cu(II)-Schiff base complexes obtained from simple amino acids.[15,19-21,49-52] A common Cu(II) chromophore composed of the O_2N donor group from the Schiff base and two or three additional ligands,[23-25] which are probably ethanol or water molecules, is suggested

by the appearance of all ligand field absorptions in the visible region in the narrow range 14,900-15,600 cm^{-1}. These complexes all have a negative Cotton effect or negative CD band associated with strong absorption bands at 25,000-28,000 cm^{-1} which is typical for Cu(pyr-L-aa) and Cu(sal-L-aa) complexes.[50,53] The ORD and CD spectra of a typical complex are presented in Fig. 2. Two Schiff base amino acid complexes having two six-membered rings have also been synthesized. They have the general structure 33 and were prepared from



33

3-aminobutyric acid (R = CH_3, R' = H) and 3-aminoisobutyric acid (R = H, R' = CH_3). Preliminary studies indicated that racemization was slower in these complexes than in those prepared from valine, which is an α-amino acid.

Racemization Kinetics. The purpose of the kinetics measurements has been to obtain a quantitative indication of the activation of the α-C-H bond in a series of 1:1 Schiff base complexes derived from the same amino acid and metal ion, but differing in the o-hydroxyarylcarbonyl ligand component. The latter has been selected according to its reported catalytic activity (pyridoxal, 3-hydroxy-2-pyridinealdehyde, and 4-nitro-

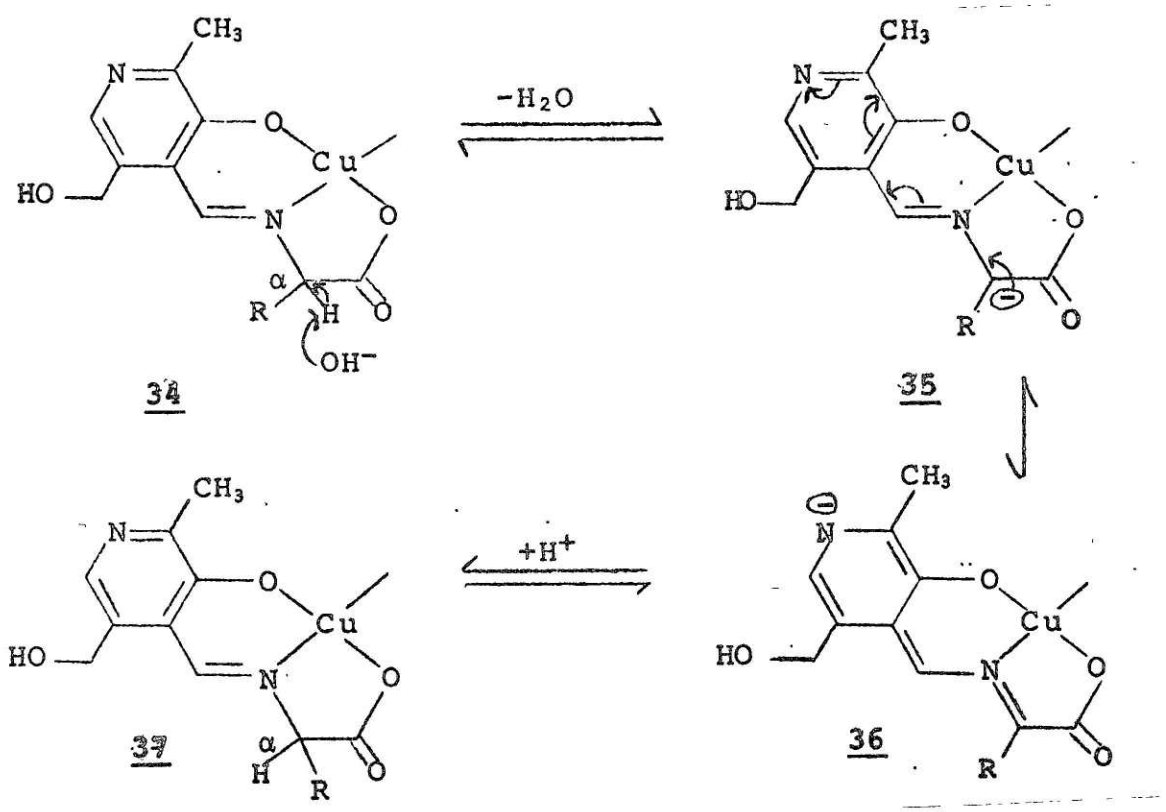
salicylaldehyde) or lack of same (salicylaldehyde) in glutamate → α-ketoglutarate transamination at pH ~5 in the presence of Al(III).[27,54] It should be noted here that during the transamination reaction 4-nitrosalicylaldehyde undergoes reduction of the nitro groups[54] which could result in oxidative deamination of theaamino acid.[43] This compound was chosen for study because it introduced an electron-withdrawing function para to the azomethine function in the Schiff base without alteringtthe basic benzene ring structure of salicylaldehyde. Cu(II) and L-valine were chosen for study because they form complexes whose reaction rates are conveniently studied by polarimetry. Finally, 95% ethanol was employed as a reaction medium in order to prevent the hydrolysis of the imine bond which occurs in water.

The control experiments described in the experimental section demonstrated that (i) racemization does not occur in solutions kept at 50° without added base, (ii) decomposition is slight during the course of the kinetic run, (iii) the apparent base concentration does not show significant change during a kinetic run. The only exception to these statements is for Cu(3,2-hpy-L-val) which does undergo some decomposition during the kinetic run, as evidenced by small changes in the visible region of the electronic spectrum. The rate constants for this complex are therefore considered to be the least reliable. The largest spectral changes were found for neutral solutions compared to solutions with equimolar base and com-

plex at the start of kinetic runs, and are illustrated by the ORD, CD, and absorption spectra of Cu(pyr-L-val)·H_2O in Figs. 2 and 3. The origin of these changes is obscure but may be due to the formation of a species such as, e.g., $[Cu(pyr\text{-}L\text{-}val)OH]^-$ in a labile preequilibrium step prior to onset of the racemization process. No new features or significant intensity alterations of ultraviolet bands which might arise from carbinolamine complexes or hydrolysis products were found in the presence of base.

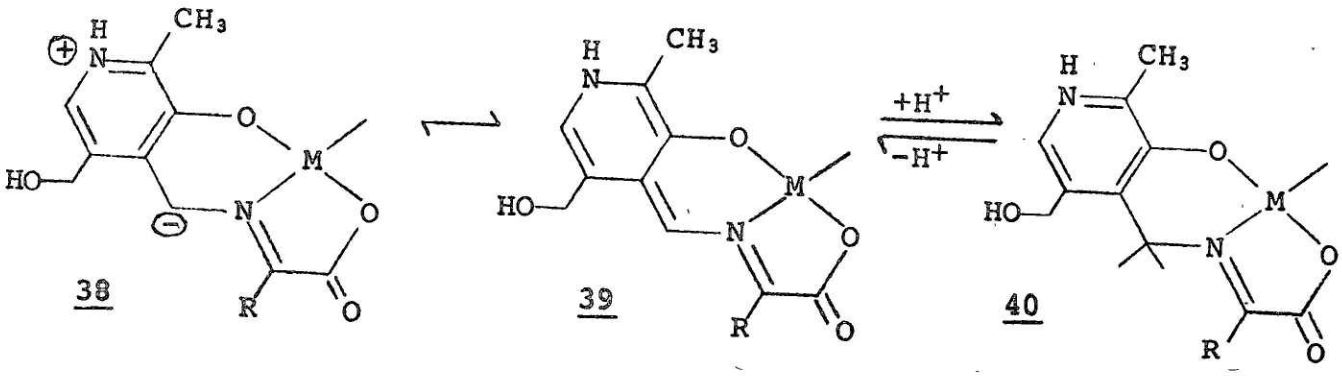
In order to obtain rate data suitable for comparison, racemization kinetics of the four complexes were determined over the same or nearly the same concentration range of complex and base; the two solutes were maintained at equimolar concentrations in all runs. Pseudo first-order rate constants $k_{r(obsd)}$ and racemization rate constants k_r corresponding to the relation rate= k_r(complex)(OH^-) are given in Table V. In the range of ca. $0.7\text{-}1.7\times10^{-3}$ M k_r values are reasonably independent of concentration. The apparent order of racemization rates under these experimental conditions is Cu(4-NO_2sal-L-val)>Cu(3,2-hpy-L-val)≥Cu(pyr-L-val)>> Cu(sal-L-val).

The kinetic data indicate that salicylaldehyde is the least effective racemization catalyst of the four o-hydroxyarylcarbonyl compounds studied. The proposed reaction mechanism provides some explanation of these results; the pyridoxal complex which is used to illustrate the mechanism is unprotonated



at the basic pH of the reaction mixture.[55] In the mechanism proposed the α-proton is lost by base attack in the slow step, and the carbanion formed 35 is stabilized by resonance with 36 in which the negative charge is delocalized onto the ring nitrogen. In 36 the conjugated system extends from the α-carbon to the ring nitrogen. The racemization reaction is completed by reprotonation at the α-carbon to form 37. Since salicylaldehyde lacks an electron-withdrawing group ortho or para to the azomethine function, it cannot be postulated to have a resonance form such as 36. Therefore, formation of the anionic intermediate 35 from the Cu(sal-L-val) complex requires a somewhat higher activation energy than for the other three complexes resulting in a slower rate, provided that preexponential factors are similar for all four complexes.

Loss of the α-H is postulated to be the first step in both the transamination and racemization reactions. Racemization and transamination are, however, competitive and distinct processes. The transamination reaction is favored over the racemization reaction at pH values below the pK_a values of the pyridinium nitrogen of the aldimine complexes.[55]



A reactive intermediate such as 38 is postulated to be important in the transamination reaction. This intermediate is stabilized by resonance with 39 in which the negative charge is delocalized onto the ring; the reaction is completed by the protonation of the azomethine carbon to form the ketimine tautomer 40. Although this same reaction sequence could occur for the unprotonated complex during racemization, it is thought

to be unlikely since the electronic spectra give no evidence of ketimine formation during the course of the reaction. The presence of an unprotonated ketimine similar to 40 and its rapid tautomerization to the racemized aldimine cannot, however, be completely ruled out by the present data.

Torchinski[50] observed that at pH 8.5 and 65-90° aqueous solutions of Cu(II), pyridoxal and several optically active amino acids showed a loss of CD intensity. This effect was ascribed without proof to an aldimine→ketimine conversion. Similarly Johns and Whelan[56] ascribed the α-deuteration of amino acids which they observed in aqueous solutions of Cu(II), salicylaldehyde and amino acids to an aldimine → ketimine →deuterated (racemized) aldimine sequence. There is no necessity to postulate ketimine formation to explain the results of either of these studies; on the basis of our data the conversion of optically active aldimine directly to racemized aldimine via the intermediate 35 ⇌ 36 is suggested. Further investigation of these systems, particularly electronic spectra and H-D exchange studies would be of interest to show if racemization without ketimine formation is a general phenomenon which would explain the results of these and other experiments.

The results of this study emphasize two aspects of the electronic factors important in vitamin B_6 catalysis.[4,27] First, the stability of aldimine structures of type 8 (and to a lesser extent 9) during the racemization reaction indicates that racemization is favored over transamination in the absence of the protonated pyridine unit. At low pH values racemization is thought to occur primarily by the reversal of transamination.[4] Second, the order of racemization rates of the four complexes studied in basic solution is the same as the qualitative order found for the o-hydroxyarylcarbonyl components as transamination catalysts in acid solution (salicylaldehyde<< 4-nitrosalicylaldehyde ~3-hydroxy-2-pyridinealdehyde~pyridoxal).[4] This relationship supports the contention that appreciable reaction rates for transamination in acid solution and racemization in basic solution derive from resonance stabilization of the appropriate intermediates (38 $\rightleftharpoons$ 39, 35 $\rightleftharpoons$ 36), for which an electron-withdrawing group ($-N=$, $\overset{+H}{-N}=$, $-NO_2$) ortho or para to the azomethine is required. Although the above findings are not directly related to the function of vitamin B_6 enzymes, one could certainly envisage the apoenzyme playing a similar, but even more effective and

and discriminating role than the metal ion in the enzymatic reaction. It could certainly replace the metal ion in the mechanistic picture presented (cf. Scheme 2); it could catalyze Schiff base formation, activate the α-CH bond and allow resonance stabilization of the intermediate, as well as altering the pK_a of the pyridoxal molecule in order to favor racemization or transamination in a particular enzyme system.

FIGURE LEGENDS

Figure 1. Pmr spectra (100 MHz) of D_2O solutions initially 0.1 M in both pyridoxamine and pyruvate, 0.05 M in Al(III):[37] (a) fresh solution showing the formation of free ketimine and the 1:1 Al complex; (b, c) spectra revealing transamination of Al-ketimine complexes to yield 1:1 and 2:1 Al-aldimine species. The three solutions are not at equilibrium. Unprefixed signals refer to free pyridoxamine: Al-A, aluminum aldimine, Al-K, aluminum ketimine complex; SB, side band; X, impurity.

Figure 2. ORD and CD spectra of Cu(pyr-L-val)·H_2O in 95% ethanol in presence of equimolar sodium hydroxide at the beginning of a kinetic run and in the absence of base. CD: -o-o-o-, no base; ~~o-o-o~~, with base; ORD: - Δ - Δ - Δ -, no base; ~~Δ-Δ-Δ~~, with base.

Figure 3. Absorption spectrum of Cu(pyr-L-val)·H_2O in 95% ethanol solution; -•-•-, no base; ——, in presence of equimolar sodium hydroxide at start of kinetic run; ----, in presence of equimolar sodium hydroxide at end of kinetic run.

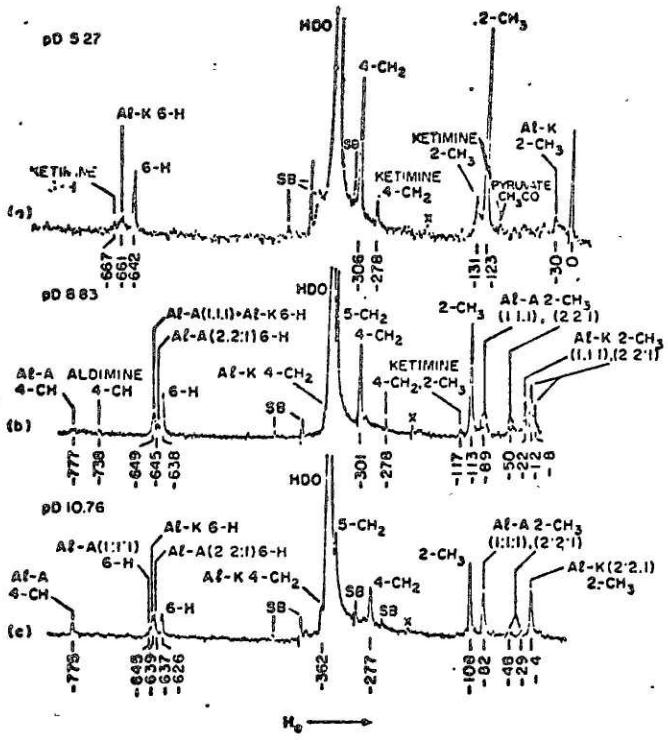

pD 5.27
HDO
4-CH2
Al-K 6-H
6-H
SB
KETIMINE 2-CH3
Al-K 2-CH3
KETIMINE 4-CH2
PYRUVATE CH3CO
(a)
-667
-661
-642
-306
-278
-131
-123
-30
0
pD 8.83
HDO
Al-A(1.1.1)+Al-K 6-H
Al-A(2.2.1)6-H
5-CH2
4-CH2
2-CH3
Al-A 2-CH3 (1.1.1), (2.2.1)
Al-K 2-CH3 (1.1.1),(2.2.1)
Al-A 4-CH
ALDIMINE 4-CH
6-H
Al-K 4-CH2
SB
KETIMINE 4-CH2, 2-CH3
(b)
-777
-738
-649
-645
-638
-301
-278
-117
-113
-89
-50
-22
-12.8
pD 10.76
HDO
Al-K 6-H
Al-A(1.1.1)
Al-A(2.2.1)6-H
6-H
5-CH2
2-CH3
Al-A 2-CH3 ((1.1.1),(2.2.1))
Al-K(2.2.1) 2-CH3
Al-A 4-CH
Al-K 4-CH2
SB
4-CH2
(c)
-775
-645
-639
-637
-626
-362
-277
-108
-82
-48
-29
-4
H0

Figure 1.

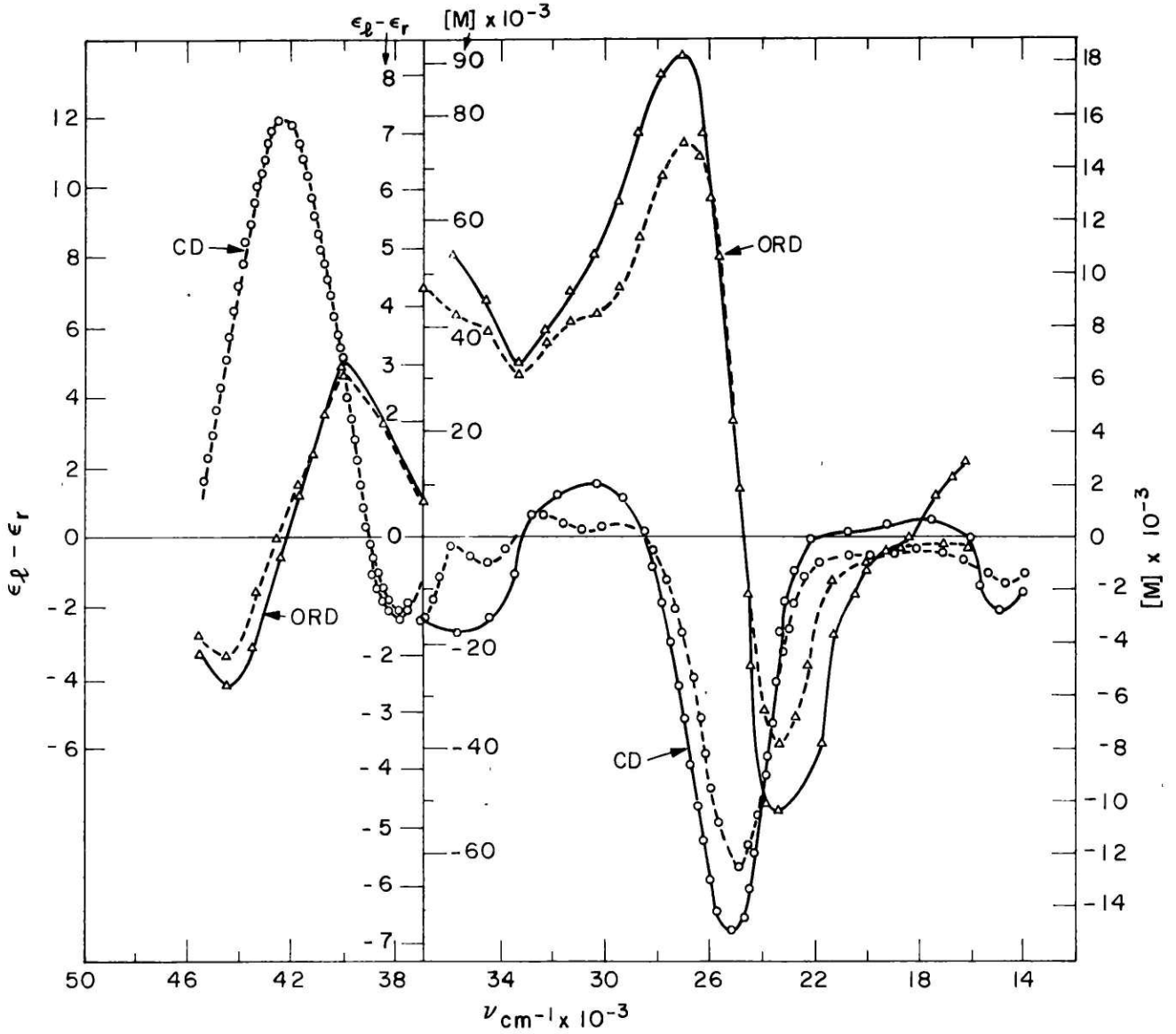

$\epsilon_l - \epsilon_r$
[M] x 10^{-3}
CD
ORD
ORD
CD
$\epsilon_l - \epsilon_r$
[M] x 10^{-3}
$\nu_{cm^{-1}}$ x 10^{-3}

Figure 2

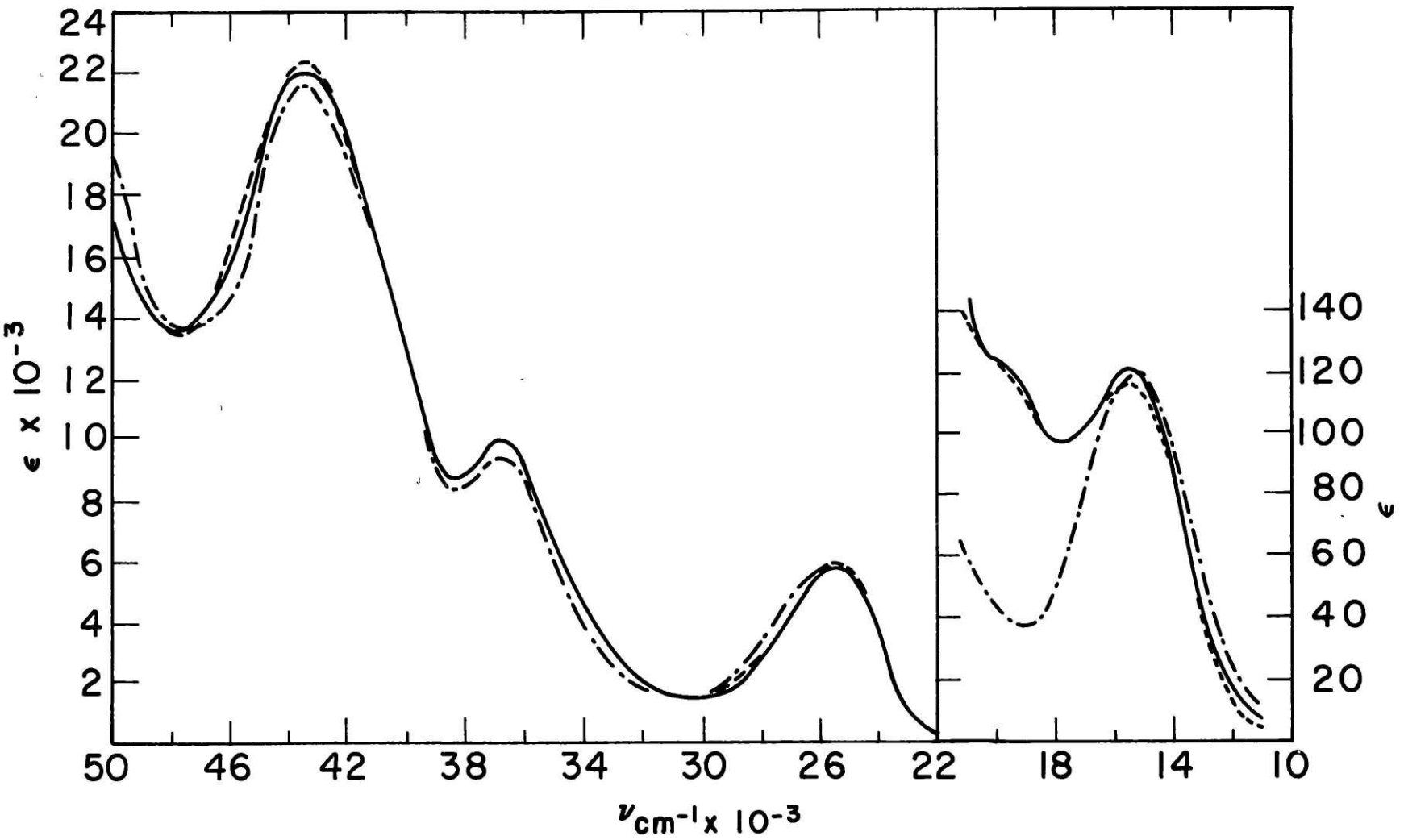

24
22
20
18
16
14
12
10
8
6
4
2
ε x 10⁻³
140
120
100
80
60
40
20
ε
50
46
42
38
34
30
26
22
18
14
10
ν cm⁻¹ x 10⁻³

Figure 3.

Table I Reactions of α-Amino Acids Catalyzed by Pyridoxal and Metal Ions in Model Systems

A. Reactions resulting from labilization of an α-hydrogen (18, bond a)

1. transamination: $RCH(NH_2)COOH + R'COCOOH \rightleftharpoons RCOCOOH + R'CH(NH_2)COOH$
2. racemization: $L\text{-}RCH(NH_2)COOH \rightleftharpoons D\text{-}RCH(NH_2)COOH$
3. β-elimination: dehydration of α-amino-β-hydroxyacids
   $HOCH_2CH(NH_2)COOH \rightarrow CH_3COCOOH + NH_3$
   desulfhydration of cysteine
   $HSCH_2CH(NH_2)COOH \rightarrow CH_3COCOOH + H_2S + NH_3$
4. tryptophan synthesis from serine and indole
5. β-proton exchange: $R_2CHCH(NH_2)COOH \rightleftharpoons R_2CDCD(NH_2)COOH$
6. γ-elimination: desulfhydration of homocysteine
   $HSCH_2CH_2CH(NH_2)COOH \rightarrow CH_3CH_2COCOOH + H_2S + NH_3$
7. Synthesis of α-amino-β-hydroxyacids from glycine and an aldehyde

B. Reactions resulting from labilization of the carboxyl group (18, bond b)

decarboxylation of amino acids

$RCH(NH_2)COOH \rightarrow RCH_2NH_2 + CO_2$

C. Reactions resulting from labilization of an R group (18, bond c)

degradation of α-amino-β-hydroxyacids to aldehydes and glycine

$RCH(OH)CH(NH_2)COOH \rightleftharpoons RCHO + H_2NCH_2COOH$

D. Oxidative Deamination

$RCH(NH_2)COOH + O_2 + H_2O \rightarrow RCOCOOH + NH_3 + H_2O_2$

TABLE II.

RELATION OF STRUCTURE TO ABILITY OF ALDEHYDES TO REACT WITH GLUTAMATE TO FORM α-KETOGLUTARATE[a,b]

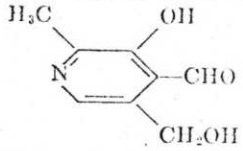
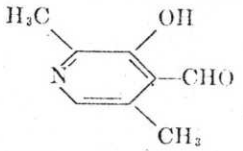
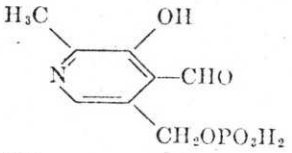
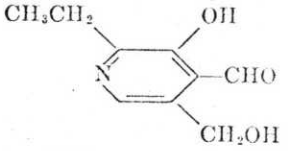
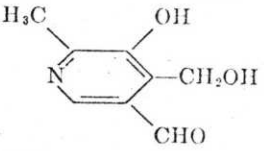
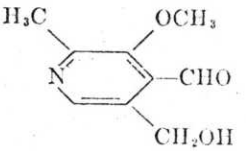
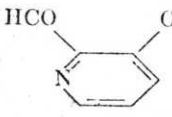
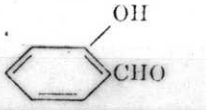
Aldehyde	Ketoglutarate formed[c] +Alum	Ketoglutarate formed[c] −Alum
1. Pyridoxal 	+++	+
2. 5-Deoxypyridoxal 	+++	+
3. Pyridoxal phosphate 	+++	+
4. ω-Methylpyridoxal 	+++	+
5. Isopyridoxal 	−	−
6. 3-*O*-Methylpyridoxal 	−	−
7. 2-Formyl-3-hydroxypyridine 	+++	−
8. Salicylaldehyde 	−	−

TABLE II. (continued)

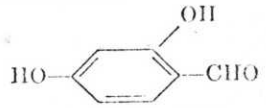
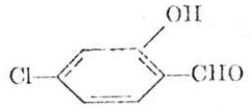
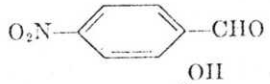
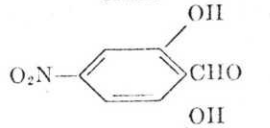
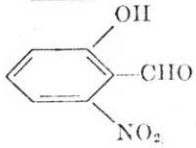
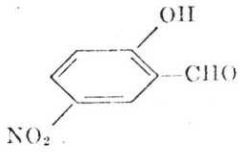
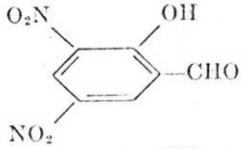
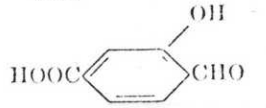
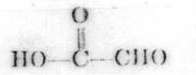
Aldehyde	Ketoglutarate formed[c] +Alum	−Alum
9. 2,4-Dihydroxysalicylaldehyde	 −	
10. 2-Hydroxy-4-chlorosalicylaldehyde	 −	
11. *p*-Nitrobenzaldehyde	 ±	±
12. 4-Nitrosalicylaldehyde	 +++	-
13. 6-Nitrosalicylaldehyde	 +++	+
14. 5-Nitrosalicylaldehyde	 −	
15. 3,5-Dinitrosalicylaldehyde	 −	
16. 4-Carboxysalicylaldehyde	 −	
17. Glyoxylic acid	 +++	

TABLE III.

EFFECT OF SOME VITAMIN B_6 ANALOGS ON GROWTH OF VARIOUS ORGANISMS

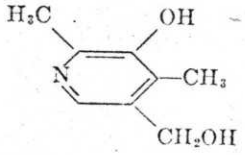
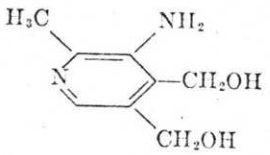
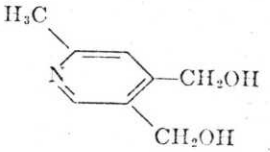
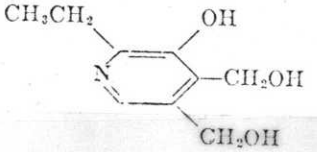
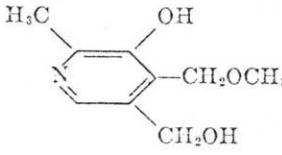
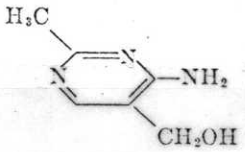
Compound	Structure	Effect on growth: Inhibits	Effect on growth: Replaces vitamin B_6
Class I. Compounds phosphorylated by pyridoxal kinase of yeast			
1. 4-Deoxypyridoxine		Rats, chicks, *Saccharomyces carlsbergensis*, *Neurospora sitophila*, *S. cerevisiae*	—
2. 2-Methyl-3-amino-4,5-bis(hydroxymethyl)pyridine		*N. sitophila*	—
3. 2-Methyl-4,5-bis(hydroxymethyl)pyridine		—	—
4. a. ω-Methylpyridoxine		*Ceratostomella ulmi*, *Saccharomyces carlsbergensis*, rats	Tomato roots, rats
b. ω-Methylpyridoxal	R—4—CHO	*Streptococcus faecalis*, rats	*Lactobacillus casei*, *S. faecalis*, rats
c. ω-Methylpyridoxamine	R—4—CH_2NH_2	Rats	*S. faecalis*, rats
5. 2-Methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine		Chicks	—
6. Toxopyrimidine		Rats and mice, *Saccharomyces carlsbergensis*	—

TABLE III. (continued)

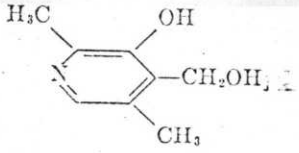
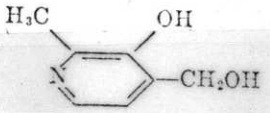
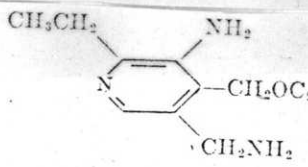
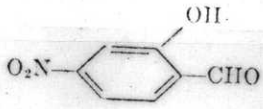
Compound	Structure	Effect on growth: Inhibits	Effect on growth: Replaces vitamin B_6
Class II. Compounds not phosphorylated by pyridoxal kinase			
7. a. 5-Deoxypyridoxine		*S. carlsbergensis*	
b. 5-Deoxypyridoxal	R—4—CHO	*S. carlsbergensis, Streptococcus faecalis, Lactobacillus helveticus*	
c. 5-Deoxypyridoxamine	R—4—CH_2NH_2	*Saccharomyces carlsbergensis, Streptococcus faecalis*	
8. 2-Methyl-3-hydroxy-4-hydroxymethylpyridine		*Saccharomyces cerevisiae*	
9. 2-Ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine		*Saccharomyces cerevisiae*	—
10. 4-Nitrosalicylaldehyde		*Streptococcus faecalis, Saccharomyces carlsbergensis*	—

TABLE IV

MAGNETIC MOMENTS AND ELECTRONIC SPECTRA OF Cu(II)–SCHIFF BASE–AMINO ACID COMPLEXES

Complex	μ_{eff},[a] BM	λ_{max}, cm^{-1} (ϵ)[b]
Cu(sal-L-val)·1.5H_2O	1.65	45,040 (20,500), 41,150 (21,900), 37,040 (13,800), 27,320 (5030), 15,600 (109)
Cu(4-NO_2sal-L-val)·H_2O	1.75	46,730 (17,700), 40,000 (19,900), 34,970 (14,000), 23,920 (4420), 14,900 (118)
Cu(3,2-hpy-L-val)·0.5H_2O	1.96	46,300 (26,300), 41,150 (13,900), 35,700 sh (2860), 27,470 (7960), 15,000 (104)
Cu(H-3,2-hpy-L-val)Br	1.73	46,300 (24,300), 41,150 (13,200), 35,700 sh (3100), 27,860 (7490), 14,800 (108)
Cu(pyr-val)·H_2O	1.72	43,480 (21,100), 36,760 (9300), 25,640 (5720), 15,200 (120)

[a] Solid. [b] 95% ethanol solution.

TABLE V

KINETIC DATA FOR THE BASE-CATALYZED RACEMIZATION OF Cu(II)–SCHIFF BASE–AMINO ACID COMPLEXES IN 95% ETHANOL SOLUTION AT 50°

Complex	Concn × 10^2, M	$k_{r(obsd)}$,[a] min^{-1}	$k_r = k_{r(obsd)}/(OH^-)$, M^{-1} min^{-1} × 10^4	$k_r(av)$, M^{-1} min^{-1}
Cu(sal-L-val)·1.5H_2O	1.67	0.394	0.0236	
	1.56	0.361	0.0231	0.0246
	1.06	0.278	0.0262	
	1.04	0.266	0.0256	
Cu(4-NO_2sal-L-val)·H_2O	1.58	9.25[b]	0.585	
	1.54	8.40	0.546	
	1.08	5.94	0.550	0.558
	0.87	4.90	0.563	
	0.74	4.04	0.547	
Cu(3,2-hpy-L-val)·0.5H_2O	1.54	5.17	0.336	
	1.53	4.95	0.324	0.309
	1.09	3.01	0.276	
	1.00	2.99	0.299	
Cu(pyr-L-val)·H_2O	1.57	3.53	0.225	
	1.56	3.56	0.228	
	1.06	2.53	0.238	0.236
	1.03	2.55	0.248	
	0.77	1.84	0.239	

[a] Determined from α_{589} data; concentrations of complex and sodium hydroxide are equal. [b] Determined from α_{578} data.

TABLE VI. Abbreviations

ala	free or condensed alanine
ab	free or condensed aminobutyric acid
aib	free or condensed β-aminoisobutyric acid
Etaa-sal	N-ethoxycarbonylalkylsalicylaldimine anion
glu	free or condensed glutamate
glt	free or condensed glutarate
hac-aa	N-o-hydroxyacetophenoneiminoaminoacidato dianion
hba-ka	2-o-hydroxybenzyliminoketoacidato dianion
3,2-hpy-aa	N-(3-hydroxypyridyl-2-methylene)aminoacidato dianion
ival	free or condensed α-ketoisovaleric acid
M	di- or trivalent metal ion
mhba-ka	α-Methyl-o-hydroxybenzyliminoketoacidato dianion
Phala	free or condensed phenylalanine
PL	pyridoxal
PLP	pyridoxal phosphate
pm	pyridoxamine
Ppyr	pyridoxylidene phosphate
prop	free or condensed pyruvate
pyr-aa	N-pyridoxylideneamino acidato dianion
X-sal-aa	ring substituted N-salicylideneamino acidato dianion (X = H not explicitly stated)
val	free or condensed valine

REFERENCES

1. R. H. Holm, "Vitamin B_6 Complexes," in "Inorganic Biochemistry," G. L. Eichhorn, Ed., Elsevier Publishing Company, Amsterdam, 1972, Chap. 32.

2. F. A. Robinson, "The Vitamin Cofactors of Enzyme Systems," Pergamon Press, Oxford, 1966, Chap. V.

3. A. F. Wagner and K. Folkers, "Vitamins and Coenzymes," Interscience Publishers, New York, N. Y., 1964, Chapt. IX.

4. E. E. Snell, Vitamins and Hormones, 16, 77 (1958).

5. cf. Collected papers in Vitamins and Hormones, 22, 361-884 (1968).

6. E. E. Snell, J. Am. Chem. Soc., 67, 194 (1945).

7. D. E. Metzler and E. E. Snell, J. Am. Chem. Soc., 74, 979 (1952).

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

9. K. S. Bai and D. L. Leussing, J. Am. Chem. Soc., 89, 6126 (1967).

10. D. Hopgood and D. L. Leussing, J. Am. Chem. Soc., 91, 3740 (1969).

11. M. E. Farago and T. Matthews, J. Chem. Soc., A, 609 (1970).

12. J. Baddiley, Nature (London), 170, 711 (1952).

13. D. E. Metzler, J. B. Longenecker, and E. E. Snell, J. Am. Chem. Soc., 76, 639 (1954).

14. H. N. Christensen, J. Am. Chem. Soc., 79, 4073 (1957).

15. L.F. Davis, F. Roddy, and D. E. Metzler, J. Am. Chem. Soc., 83, 127 (1961).

16. G. N. Weinstein, M. J. O'Connor and R. H. Holm, Inorg. Chem., 9, 2104 (1970).

17. D. Heinert and A. E.Martell, J. Am. Chem. Soc., 85, 1334 (1963).

18. R. P. Houghton and D. J. Pointer, J. Chem. Soc., 3306 (1964).

19. A. Nakahara, Bull.Chem. Soc. Japan, 32, 1195 (1959).

20. Y. Nakao, S. Sasaki, K. Sakurai, and A. Nakahara, Bull. Chem. Soc. Japan, 40, 241 (1967).

21. Y. Nakao, K. Sakurai, and A. Nakahara, Bull. Chem. Soc. Japan, 40, 1536 (1967).

22. M. J. O'Connor, R. E. Ernst, J. E. Schoenborn and R. H. Holm, J. Am. Chem. Soc., 90, 1744 (1968).

23. T. Ueki, T. Ashida, Y. Sasada, and M. Kakudo, Acta Cryst., 22, 870 (1967).

24. J. F. Cutfield, D. Hall and T. N. Waters, Chem. Commun., 785 (1967).

25. G. A. Bentley, J. M. Waters and T. N. Waters, Chem. Commun., 988 (1968).

26. B. E. C. Banks, A. A. Diamantis and C. A. Vernon, J. Chem. Soc., 4235 (1961).

27. D. E. Metzler, M. Ikawa, and E. E. Snell, J. Am. Chem. Soc., 76, 648 (1954).

28. (a) "Nutrition Reviews, Present Knowledge in Nutrition," The Nutrition Foundation, N. Y., N. Y., 1967, Chap. XVI. B. F. Chow, "The B Vitamins," in "Nutrition, A Comprehensive Treatise," G. F. Beaton and E. W. McHenry, Eds., Academic Press, 1964, Chap. 3.

29. J. Olivard, D. E. Metzler, and E. E. Snell, J. Biol. Chem., 199, 669 (1952).

30. E. H. Abbott and A. E. Martell, J. Am. Chem. Soc., 92, 5845 (1970).

31. M. Ando and S. Emoto, Bull. Chem. Soc. Japan, 42, 2624, 2628 (1969).

32. (a) J. B. Longenecker and E. E. Snell, Proc. Natl. Acad. Sci. U. S., 42, 221 (1956); (b) J. B. Longenecker and E. E. Snell, J. Am. Chem. Soc., 79, 142 (1957).

33. G. L. Eichhorn and J. W. Dawes, J. Am. Chem. Soc., 76, 5663 (1954).

34. P. Fasella, H. Lis, N. Siliprandi and C. Baglioni, Biochim. Biophys. Acta, 23, 417 (1957).

35. O. A. Gansow and R. H. Holm, J. Am. Chem. Soc., 90, 5735 (1968).

36. O. A. Gansow and R. H. Holm, J. Am. Chem. Soc., 91, 573 (1969).

37. O. A. Gansow and R. H. Holm, J. Am. Chem. Soc., 91, 5984 (1969).

38. E.H. Abbott and A. E. Martell, Chem. Commun., 1501 (1968).

39. E. H. Abbott and A. E. Martell, J. Am. Chem. Soc., 91, 6866 (1969).

40. E. H. Abbott and A. E. Martell, J. Am. Chem. Soc., 91, 6931 (1969).

41. Y. Matsushima and A. E. Martell, J. Am. Chem. Soc., 89, 1322, 1331 (1967).

42. A. E. Martell and Y. Matsushima, in ref. 5, pp. 33-52.

43. T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. II, W. A. Benjamin, Inc., New York (1966).

44. P. Pfeiffer, W. Offerman, and H. Werner, J. Prakt. Chem., 159, 313 (1942).

45. D. Heinert and A. E. Martell, Tetrahedron, 3, 49 (1958).

46. D. Heinert and A. E. Martell, J. Am. Chem. Soc., 81, 3933 (1959).

47. R. G. Bates, "Determination of pH, Theory and Practice," John Wiley and Sons, Inc., New York, N. Y., 1964, Chap. 8.

48. G. A. Auld and A. Davison, Inorg. Chem., 7, 306 (1968).

49. Y. Matsuo, J. Am. Chem. Soc., 79, 2011 (1957).

50. Yu. M. Torchinskii, Biochemistry (USSR), 31, 909 (1966).

51. Y. Matsushima and A. E, Martell, J. Am.Chem. Soc., 89, 1322 (1967).

52. Y. Matsushima, Chem. Pharm. Bull., 16, 2143 (1968).

53. Yu. M. Torchinskii and L. G. Koreneva, Dokl. Biochem., 155, 110 (1964); Biochem (USSR), 30, 31 (1965).

54. M. Ikawa and E. E. Snell, J. Am. Chem. Soc., 76, 653 (1954).

55. D. L. Leussing and N. Huq, Anal. Chem., 38, 1388 (1966).

56. R. B. Johns and D. Whelan, Australian J. Chem., 19, 2143 (1966).

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