Mechanism of Formation, Structure, Stereochemistry, and Racemization of Bis[pyridoxylidene(amino acidato)]aluminum(III) Complexes[1]

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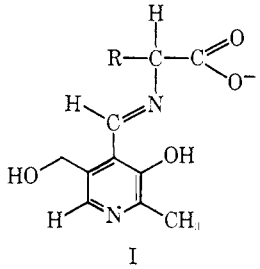
Abstract: The nuclear magnetic resonance spectra of D_2O solutions containing the heterocyclic aldehyde pyridoxal, an α-amino acid, and aluminum(III) ion are described. Using glycine, alanine, α-aminobutyric acid, norvaline, valine, leucine, serine, and threonine, it is shown that the amino acids condense with pyridoxal to form Schiff bases which coordinate the aluminum(III) ion as planar, terdentate ligands. The bis aluminum(III) complexes of these Schiff bases are novel and represent an advance in the field of nuclear magnetic resonance of diamagnetic complexes, in that they are demonstrated to exist as three diastereoisomers which are readily distinguishable and whose structures are directly deduced from the 2-CH_3 resonances of their pyridine aldehyde moieties. The detection of isomerism is possible because the 2-CH_3 of one ligand is in the shielding region of the azomethine π system of the other ligand, and so its chemical shift is hypersensitive to the small steric interactions with the asymmetric center of the amino acid portion of the other ligand. This phenomenon is so sensitive to slight changes in structure that in complexes of two Schiff bases differing only in the amino acid moiety, the mixed-ligand complexes which are formed can readily be detected by their 2-CH_3 resonances. The shape of the shielding region of this π system is discussed. Changes in the relative populations of the diastereoisomers during the formation process are interpreted as showing that the rate-determining step in the formation of the bis complex is the addition of a second Schiff base to the 1:1 complex. Exchange of the α proton of the amino acid part of the complex occurs in the region pD (pH) 10. That this reaction results in racemization of the amino acid asymmetric center is demonstrated by the appearance of the diastereoisomer of the racemic complex in solutions initially containing but one of the amino acid enantiomers.

Vitamin B_6 is an essential cofactor for many enzymic reactions of amino acids. It has long been established that most of these reactions also proceed in the absence of enzyme in amino acid–pyridoxal systems, and that in many cases addition of metal ions to these binary systems results in enhanced reaction rates. The mechanisms proposed for these nonenzymic reactions[3-5] involve formation of a Schiff base (I) as a reactive intermediate, and further catalysis by coordination of the terdentate Schiff base to the metal ion. Thus it seems



I

that the metal ion serves as a model for the enzyme, and the enzyme-catalyzed pathway may be taken to be analogous in some respects to the metal-ion-catalyzed pathway, although there are also many ways in which they differ. Aluminum(III) has been found to be one of the most effective metal ions in enhancing the reaction rate in these systems. Therefore, as part of a continuing investigation into structure and mechanism in these diverse reactions, we have investigated the structures of species in the aluminum pyridoxal–amino acid systems and have studied several reactions which are readily detected in these systems.

Nuclear magnetic resonance (nmr) has proved to be an effective method for investigation of both metal-free[6-8] and metal-ion-containing[9-13] pyridoxal systems. Structures have been confirmed and deprotonation schemes have been verified for the forms of vitamin B_6[7] and for its Schiff bases.[11] The aluminum(III) complexes of pyridoxylidenealanine have also been studied, and it has been reported that the 1:1 complex and 2:1 complex can be observed in equilibrium with complexes in which the carboxyl group of a Schiff base moiety was uncoordinated.[11] Recently we have suggested that this "dangling carboxyl" species is improbable and have explained the results by pointing out that the aluminum(III) complexes of the tridentate pyridoxal Schiff bases can exist in three diastereoisomeric forms and showing that these isomers are readily distinguishable by nmr, owing to a fortunate accident of their structure.[13] In this paper additional evidence is presented supporting this point of view, the mechanism of formation of these complexes is discussed, and the nmr detection of a subsequent reaction is reported.

(1) This work was supported by Research Grant No. AM11694 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

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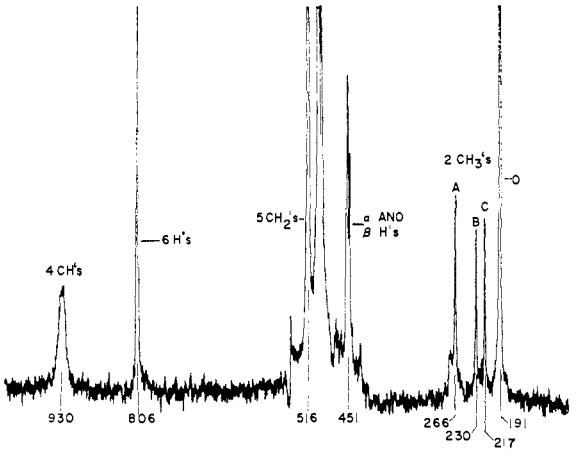


Figure 1. The 100-MHz nmr spectrum of a solution of 0.10 *M* DL-serine, 0.10 *M* pyridoxal, and 0.05 *M* aluminum(III) in D_2O at pD 9.50, μ = 1.0 (NaCl), temperature = 30 ± 2°. Chemical shifts are measured with respect to HMDS in an internal capillary.

Experimental Section

Pyridoxal hydrochloride and the amino acids were obtained from Mann Laboratories. NaOD was prepared as previously described.[12] Aluminum(III) solutions were prepared from the hydrated sulfate by adding D_2O and evaporating to dryness several times. The solutions were filtered and then were standardized by Schwarzenbach's method of back-titration with zinc(II).[14]

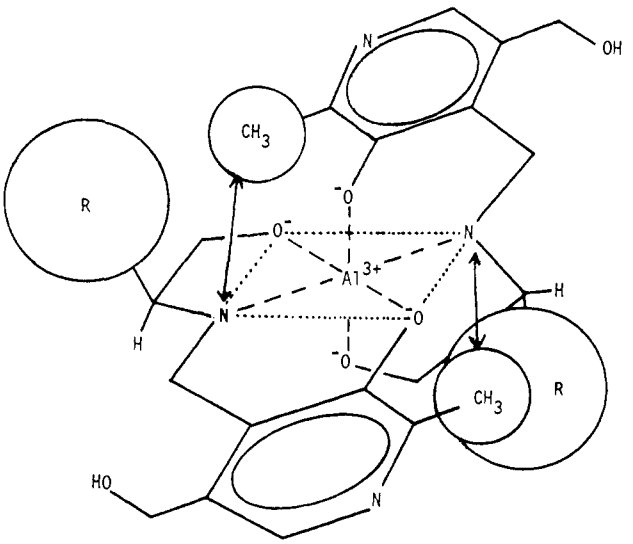
Spectra were taken with a Varian HA-100 nuclear magnetic resonance spectrometer. The probe temperature was 30 ± 2° and the ionic strength was maintained at unity with NaCl. Chemical shifts are reported with respect to an internal capillary of hexamethyldisiloxane (HMDS), whose proton resonance was also used as an internal locking signal. In this paper, pD is the negative logarithm of the deuterium ion concentration. The deuterium ion activity was measured by Covington's method[15] and was converted to concentration by means of tabulated activity coefficients.[16]

Precipitation was often observed in the neutral region; however, measurements in the presence of precipitates are justified since the solid phase does not contribute to sharp-line nmr spectra, and since the purpose of this paper is to identify resonances and to suggest structures rather than to determine equilibrium constants.

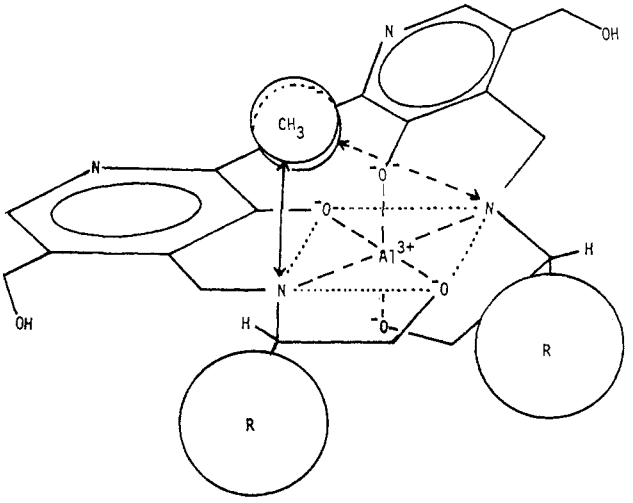
Results and Discussion

Assignment of Nmr Resonances. Figure 1 shows the 100-MHz nmr spectrum of the bis(DL-pyridoxylidene-serinato)aluminum(III) complex and is typical of the spectra of all the racemic bis complexes investigated. As the ratio of ligand to metal is lowered toward 2:1, resonances assignable to the free Schiff base and its components decrease in relative intensity with respect to those of the bis complex. Precipitation occurs below the stoichiometric (2:1) ratio in the pD region from 7 to 10.5. Below pD 5.5, complexes are formed which have stoichiometry of one Schiff base to one aluminum(III) ion. As the pD is raised toward 5.5 the resonances attributable to the 1:1 complexes disappear while those of the 2:1 complexes become more intense. In the region of pD 5, one of the water molecules coordinated in the 1:1 complex should hydrolyze to a hydroxide ion. As will be shown below, the Schiff base ligand undergoes deprotonation in this pD region and becomes formally dinegative. Thus, the 1:1 complex should be neutral and relatively insoluble in this pD region. Above a pD of 5.5, no 1:1 complexes are observed under the conditions described in this paper either because they are insoluble or because they are thermodynamically unstable to disproportionation. The 1:1 complexes are the subject of an investigation at lower pD's which will be reported shortly.

The resonances outside of the methyl region are easily assigned.[11] The resonance at 930 Hz is typical of azomethine protons and is of unit intensity; therefore it is the 4-CH resonance. The 6-H resonance is of equal area and is located at 806 Hz, in the immediate region of vitamin B_6 group protons in general.[7] The resonance at 516 Hz is of double intensity and is assigned to the 5-CH_2 resonance of the bis complex. Having established the sources of these three resonances, the resonances A, B, C, and D can be assigned to the 2-CH_3 group of the complex, and integration shows that their sum is of triple intensity. The remarkable feature of Figure 1 is that despite the appearance of single



II MAXIMUM STERIC REPULSION: MINIMUM SHIELDING



III MINIMUM STERIC REPULSION; MAXIMUM SHIELDING

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resonances for the 4-CH, 5-CH_2, and 6-H protons, the 2-CH_3 resonances are spread over 75 Hz of the spectrum. These unusual resonances in the methyl region can be explained in the following way.

If it is hypothesized that the bis complex is formed from two approximately planar pyridoxylideneserinato ligands, the one bound to the two polar and one of the equatorial sites, and the other bound to the remaining three equatorial sites, then the 2-CH_3 group of one ligand is directly above the π cloud of the azomethine nitrogen of the other ligand. The methyl resonance is then expected to be strongly shifted to higher field by the anisotropy of the azomethine nitrogen, and this is the reason for the wide range over which the 2-CH_3 resonances of the bis complex are found. In addition, if, as in the serine case, there is optical activity at the α carbon atom of the amino acid, the 2-CH_3 of the one ligand should be influenced by the steric requirements of the groups on the α carbon atom of the amino acid moiety of the other ligand. This is shown in II and III. In the case of the Schiff base formed from a single enantiomorph of an optically active amino acid, two configurations are possible: that in which the 2-CH_3's of both ligands are crowded, as in II, and that where neither is crowded, as in III. If, however, a D and an L Schiff base are each coordinated to the same metal ion, one methyl group is crowded and the other is not. Thus, in the case of the D,L complex, the two methyl groups are nonequivalent.

Figure 2 contrasts the 2-CH_3 resonances of the bis(pyridoxylidenevalinato)aluminum(III) complex when it is prepared from the racemic amino acid and from the pure L isomer. The racemic complex has two resonances, B and C, which are absent from the spectrum of the pure L complex. These are assigned to the crowded and uncrowded 2-CH_3 resonances of the identical D,L and L,D complexes. Therefore, the doubly crowded D,D and L,L 2-CH_3's give rise to the resonance at A in Figure 2. As expected, the most abundant species are the double-uncrowded D,D and L,L forms. Their identical methyl groups give rise to resonance D.

Interestingly, in the D,L and L,D cases, crowding, and subsequent relative deshielding, at one methyl results in deshielding at the other methyl group as well. The effect is as expected because the aromatic portions of these ligands are quite rigid and thus steric interference which forces one 2-CH_3 group from its favored position above an azomethine nitrogen is transmitted as a shearing force to the other azomethine nitrogen, also forcing it away from its 2-methyl group, but probably by a smaller distance than at the crowded position. Accordingly, in Figures 1 and 2 the resonance labeled B is assigned to the crowded methyl group and the one labeled C is assigned to the uncrowded methyl group of the D,L and L,D complexes.

The nmr spectrum of the bis(pyridoxylideneglycinato)aluminum(III) complex has but a single methyl resonance, which appears in the region of resonance D. This is as expected, since glycine does not have an asymmetric α carbon and so the corresponding Schiff base complex can exist only in a single form. The bis(pyridoxylideneglycinato)aluminum(III) also has a relatively broad 4-CH resonance, as in the serinato complex in Figure 1. This broadening was not temperature dependent and therefore appears to be a result of the

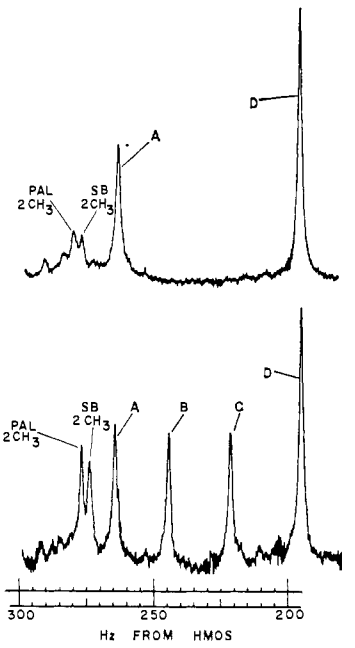

Figure 2. The 100-MHz nmr spectra of: top, 0.102 *M* L-valine, 0.104 *M* pyridoxal, and 0.050 *M* aluminum(III) at pD 8.9; bottom, 0.102 *M* DL-valine, 0.102 *M* pyridoxal, and 0.050 *M* aluminum(III) at pD 9.7; temperature = 30 ± 2°; μ = 1.0 (NaCl). Chemical shifts are with respect to HMDS; Pal = pyridoxal; SB = Schiff base.

interaction of the aluminum-27 nucleus with the 4-CH protons of the bis complexes.

In addition to serine, valine, and glycine, several other amino acids were investigated. The 2-CH_3 resonances of their bis complexes are summarized in Table I. It

Table I. 2-CH_3 Resonances of Selected Bis[pyridoxylidene(amino acidato)]aluminum(III) Complexes between pD 9 and 10

Amino acid	Resonance,[a] Hz from HMDS			
	A	B	C	D
Glycine				204
DL-Alanine	244	210	203	189
DL-α-Aminobutyric	239	215	204	191
DL-Norvaline	243	216	203	190
DL-Homoserine	258	223	208	191
DL-Valine	261	241	215	191
DL-Serine	266	239	216	192
DL-Threonine	270	250	225	190

[a] Letters A, B, C, and D refer to the notation used in the narrative for the four observed 2-methyl resonances.

was found that the chemical shift of resonance D is nearly unaffected by the size of the α substituent. However, the shifts of A, B, and C with respect to D, and to each other, are strongly affected. A good correlation was noted between the size of the α substituent and the amount of deshielding with respect to the double uncrowded resonance. This correlation is depicted in Figure 3.

Deprotonation. Above pD 7.5, the resonances of the various 2-CH_3 groups are nearly invariant with pD. Below this point, they shift to lower field as the pD is lowered. This is demonstrated in Figure 4 for the valine case. The sharp break near pD 5.8 is associated

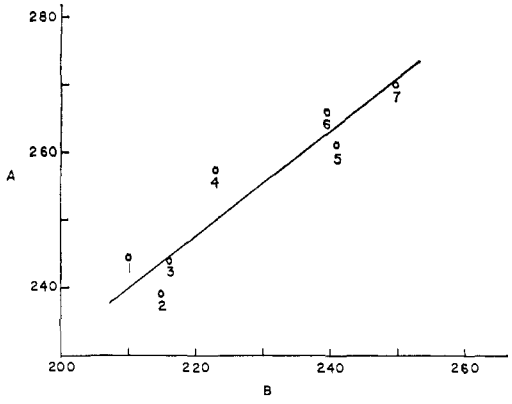

Figure 3. Correlation of the chemical shift of the 2-CH_3 resonance of the doubly hindered bis complex (A) with one of the 2-CH_3 resonances of the singly hindered bis complex (B). Chemical shifts are with respect to HMDS. 1 = alanine, 2 = α-aminobutyric acid, 3 = norvaline, 4 = homoserine, 5 = valine, 6 = serine, 7 = threonine.

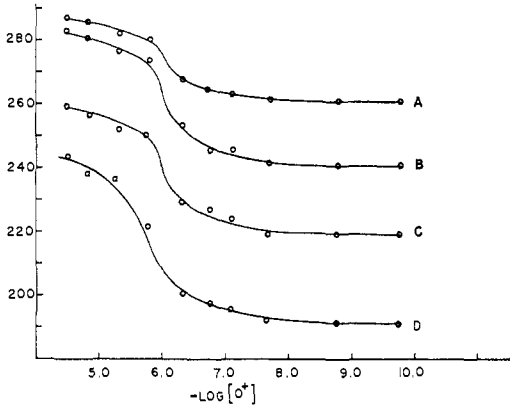

Figure 4. The chemical shifts of the 2-CH_3 resonances of bis(pyridoxylidenevalinato)aluminum(III) with respect to pD.

with the deprotonation of the heterocyclic nitrogen and is analogous to the metal-free results.[7] Evidently, the pK's of the diastereoisomers are identical within the power of the nmr approach to distinguish.

In general, upfield shifts of neighboring-group protons accompany the deprotonation of species in aqueous media, and this has been verified for vitamin B_6 and its Schiff bases.[6-8] This would, presumably, predict that the shifts of A, B, C, and D would vary with pD by the same amount in a given bis complex; however, the ordering D > C $\doteq$ B > A was observed, as shown in Figure 4. This effect can be ascribed to one of two factors. Either protonation of the heterocyclic nitrogen affects the π system in the region of the azomethine nitrogen to restrict its ability to shield the 2-CH_3 groups, or charge repulsion of the protonated heterocyclic nitrogen forces these different 2-CH_3 groups away from the shielding region by different amounts. The latter reason seems to be the most probable interpretation, although there are no means to test it at the present time.

Nature of Shielding of Azomethine Nitrogen. The isoshielding contours of benzene and related aromatic rings have been dealt with successfully by means of theoretical approaches,[17-19] and these results are well

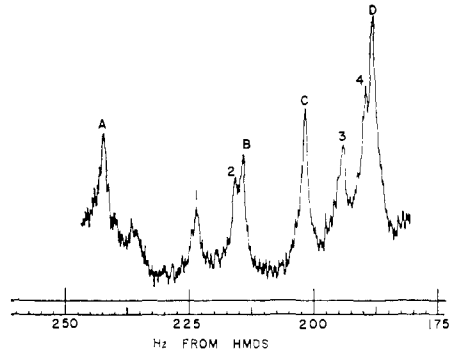

Figure 5. The 100-MHz nmr spectrum of a solution 0.10 M in pyridoxal, 0.060 M in DL-norvaline, 0.040 M in glycine, and 0.050 M in aluminum(III) at pD 9.4, μ = 1 (NaCl). Resonances A, B, C, and D arise from bis(pyridoxylidenenorvalinato)aluminum(III) (Table I). There is also a small contribution of the 2-CH_3 resonance of bis(pyridoxylideneglycinato)aluminum(III) to C. Resonances 1, 2, 3, and 4 arise from the mixed-ligand complex pyridoxylideneglycinatopyridoxylidenenorvalinatoaluminum(III) and are described in the text.

supported by experiment. The same cannot be said for other unsaturated systems,[20] although the regions of shielding and deshielding are generally well known. It was hoped when the significance of the large shift of the 2-CH_3 resonances was first discovered,[13] that it would be possible to arrive at some quantitative estimate for the shape of the shielding region above the azomethine nitrogen. Although this did not prove possible, some conclusions can be drawn. It seems likely, for example, that as the methyl group spins on its axis, its protons pass through extreme gradients in shielding and possibly are in deshielding regions part of the time. Models indicate that the steric forces should be slight, even in the crowded case, and that the methyl groups are pushed only a small distance from their favored position above the azomethine nitrogen. Thus, it would seem that if the shielding region were a cone, as is drawn for the carbonyl group, small shifts in position would not have such a profound effect on the shielding. The shielding contours are probably quite steep above the azomethine nitrogen and the pattern is probably relatively complex.

Mixed-Ligand Complexes. Further support for the structures suggested above comes from an examination of spectra taken from solutions in which two different Schiff bases are coordinated to the aluminum(III) ion. A simple case occurs when one of the amino acids is glycine, since it has a symmetrical substituted α carbon atom. In such systems, there are two possible diastereoisomers of the mixed-ligand complex, the one with a crowded 2-CH_3 and the other with an uncrowded 2-CH_3. The 2-CH_3's of each complex are nonequivalent. As shown in Figure 5, the mixed-ligand complex pyridoxylideneglycinatopyridoxylidenenorvalinatoaluminum(III) has four clearly distinguishable resonances (numbered 1-4), the theoretical maximum. Using the principle expounded above that the more strained the 2-CH_3 group is, the lower the field at which it is found, resonances 1 and 3 are assigned to the 2-CH_3 of the pyridoxylideneglycinato ligands which are, respectively, crowded and uncrowded, while 2 and 4 are

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assigned to the crowded and uncrowded 2-CH_3 groups of the pyridoxylidenenorvalinato portions.

The extreme sensitivity of the 2-CH_3 chemical shift to environment is underscored in Figure 5 by noting that the 2-CH_3 resonance of the uncrowded pyridoxylideneglycinato ligand is shifted from 204 Hz in the bis complex (Table I) to 197 Hz in this mixed-ligand complex, even though models show that there is little steric interference from the other ligand in either the bis or the mixed ligand complexes.

Several investigations were also made on the most complicated case, the mixed-ligand complexes formed from two different racemic Schiff bases. In such systems there are 16 different 2-CH_3 resonances; however, they cannot always be resolved from one another. This is a universal problem in the region of 189 Hz where the 2-CH_3's of the doubly uncrowded diastereoisomers ("D" resonances) of the bis complexes and the two nonequivalent 2-CH_3's of the doubly crowded mixed-ligand complex all are found. As an example, the 2-CH_3 chemical shifts in the relatively favorable pyridoxal, DL-valine, DL-norvaline, aluminum(III) system are presented in Table II together with assignments of their sources.

Table II. Resonances in the 100-MHz Nmr Spectrum of the System 0.05 *M* DL-Valine, 0.050 *M* DL-Norvaline, 0.10 *M* Pyridoxal, and 0.050 *M* Aluminum(III) at pD 8.9

Shift with respect to HMDS	Assignment
261	Bisvalinato "A" resonance
255	Two mixed-ligand resonances
244	Bisnorvalinato "A" re4onance
241	Bisvalinato "B" resonance
233	Mixed-ligand resonance
222	Two mixed-ligand resonances
216	Bisnorvalinato "B" resonance
215	Bisvalinato "C" resonance
206	Mixed-ligand resonance
203	Bisnorvalinato "C" resonance
191	Bisvalinato "C" resonance + mixed-ligand resonance
189	Bisnorvalinato "C" resonance + mixed-ligand resonance

Mechanism of Formation of 2:1 Complexes. The kinetics of formation of the transition metal complexes of Schiff bases have been the subject of many recent publications[21-25] and a variety of mechanisms have been proposed to explain the data. These involve "template" and "promnastic" effects, rate-determining dehydration of an intermediate carbinolamine complex, and inhibition by amine complexation. In this section evidence is presented showing that bis(pyridoxylidenevalinato)aluminum(III) is formed by the attack by the carboxyl group of the Schiff base pyridoxylidenevaline upon an axial site of a 1:1 Schiff base–aluminum complex.

In Figure 6 part of the methyl region of an nmr spectrum of a solution initially 0.10 *M* in DL-valine, 0.10 *M* in pyridoxal, and 0.050 *M* in aluminum(III) ion is shown at various times at pD 10.2. It has been shown above that the relative energies of the isomers are A greater than that giving rise to B and C greater than D and that these differences are probably due to the small steric repulsions between the alkyl group of the amino acid of one ligand and the 2-CH_3 of the complementary ligand. Figure 6, however, shows that the two higher energy species are initially formed in approximately the statistical ratio of 1:2, and that the lower energy form is initially formed to a much smaller extent. As equilibrium is approached, the concentration of the lower energy form, D, increases as expected. Thus, though the formation of the bis complexes occurs before the first spectrum can be obtained, the low contribution of the most stable diastereoisomer to the initial spectra must mean that steric forces operate to interfere with its formation relative to the other diastereoisomers. Therefore, the mechanism of formation of the bis complexes must have an activated complex of higher energy for the doubly unhindered complex than for the other diastereoisomers.

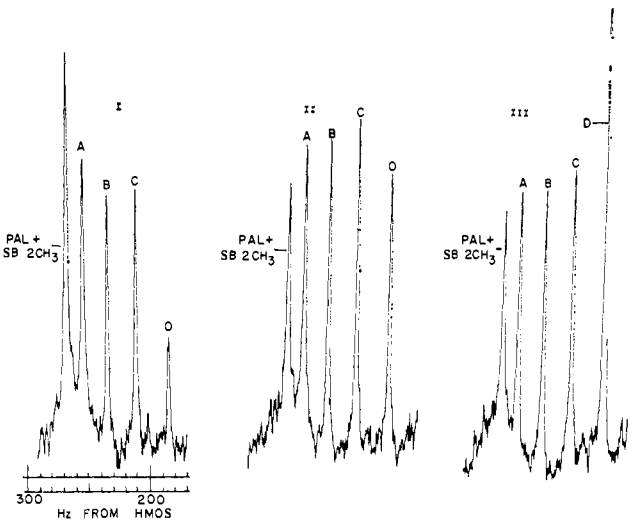

Figure 6. The 100-MHz nmr spectrum of 0.10 *M* DL-valine, 0.10 *M* pyridoxal, and 0.050 *M* aluminum(III) at pD 10.2, ionic strength 1.0 (with NaCl), and temperature 30 ± 2°: I, initially; II, at 20 min; III, at 1 hr.

This result may be interpreted in the following way: consider a 1:1 Schiff base–aluminum(III) complex in which the Schiff base is coordinated to three equatorial sites of an octahedral aluminum(III) ion. One equatorial site and two axial sites remain unbound. If the complexed Schiff base is derived from an optically active amino acid such as DL-valine, then the alkyl group of the amino acid can be either above or below the equatorial plane of the 1:1 complex. Attack by the carboxyl group of a free Schiff base on one of the two axial sites available can then lead to three situations.

(1) The attacking Schiff base is of the same absolute configuration as the coordinated Schiff base and the axial site attacked is on the same side of the equatorial plane as the alkyl group of the coordinated Schiff base. If this is the case, severe steric interaction will occur between the alkyl groups of the attacking Schiff base and the coordinated Schiff base.

(2) The attacking Schiff base is of different absolute configuration from the coordinated Schiff base. If

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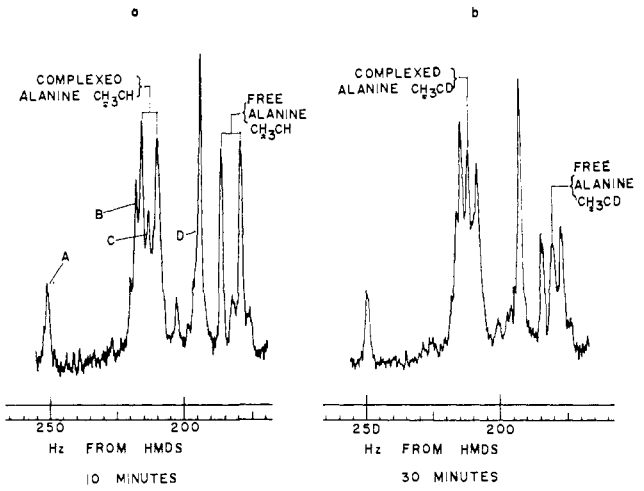


Figure 7. The 100-MHz spectrum of the methyl region of a solution of 0.10 *M* DL-analine, 0.10 *M* pyridoxal, and 0.025 *M* aluminum(III) at pD 10.2, ionic strength 1.0 (with NaCl), and temperature 30 ± 2°. The reference is hexamethyldisiloxane (HMDS); (a) after 10 min and (b) after 30 min.

this is the case, there is no steric interaction of alkyl groups whether the alkyl group of the coordinated Schiff base is on the same or opposite side of the equatorial plane as the axial site being attacked.

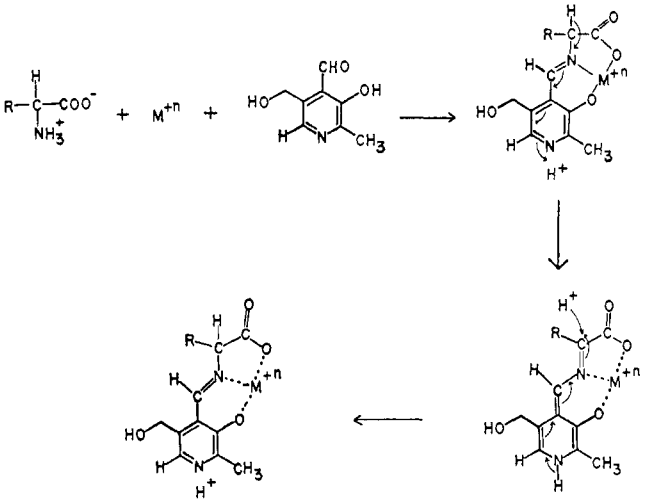
(3) The attacking Schiff base is of the same absolute configuration as the coordinated Schiff base, but attack occurs at the axial site on the other side of the equatorial plane of the 1:1 complex from its alkyl group. Here again, no steric effects are observed.

If the coordination of the azomethine nitrogen and phenolic oxygen follows carboxyl coordination directly, situation 1 will lead to the complexes giving resonance D (whose 2-CH_3's are unhindered), situation 2 will lead to the complex giving B and C (with one 2-CH_3 hindered and one 2-CH_3 unhindered), and situation 3 will lead to A (with both 2-CH_3's hindered). Thus, such a mechanism explains that the most stable complex, that giving rise to D, is initially formed in low quantity. In addition, the initial 1:2 ratio of A to B + C is readily understood by observing that in situation 2, either axial site may be attacked, while in 3, the only site that may be attacked is the one on the opposite side of the equatorial plane from the alkyl group of the previously coordinated Schiff base. Thus, there are two ways to make the complex giving B and C and only one way to make the complex giving A.

The bulky amino acids threonine and leucine behave similarly. A mechanism involving a rate-determining "template" or "promnastic" effect (in which the second Schiff base is formed in the coordination sphere from previously coordinated components) does not give rise to steric effects which explain the results, nor does attack of the azomethine nitrogen at the one remaining equatorial site. Rate-determining attack of the phenolic oxygen entails considerable steric interference of the 2-CH_3 against the previously coordinated ligand. Therefore, the results are best explained by rate-determining attack by the carboxyl group of a free Schiff base on an axial site of the 1:1 complex, followed by rapid coordination of the azomethine nitrogen and phenolic oxygen. This means that the activation energy for the reaction forming the bis complexes is greater for the most stable diastereoisomer. Thus the formation of the bis[pyridoxylidene(amino acidato)aluminum(III) complexes is a clear example of the crossing of reaction profiles.

Racemization and α Proton Exchange. The racemization of optically active amino acids is a reaction well known to be catalyzed by enzymes requiring vitamin B_6 for activity.[26] A nonenzymic model system is known for these reactions insofar as amino acids have been shown to racemize when heated at 100° with pyridoxal and transition metal ions.[27] The mechanism shown in Scheme I has been proposed to explain this

Scheme I. Metal-Ion-Catalyzed Racemization of α-Amino Acids



metal ion and pyridoxal catalysis of racemization of α amino acids.

Recently, evidence has been presented which indicates that this reaction is very slow at room temperature and that if it proceeds *via* Schiff base formation, the rate must be negligible.[11] The results of our investigations show that racemization proceeds rapidly in the region of pD 10 at room temperature and that the progress of this reaction is conveniently followed by nmr for certain amino acids, giving evidence of the structures of intermediates and the mechanism of the reaction.

Figure 7 shows the changes which occur in the methyl region of a solution initially 0.1 *M* in DL-alanine, 0.10 *M* in pyridoxal, and 0.025 *M* in aluminum(III) ion at pD 10.2. Resonances are labeled A, B, C, and D, according to the conventions established above. The methyl resonance of the alanine of the Schiff base ligand has nearly the same chemical shift as B and C in this case, and so in solutions in which the stoichiometric ratio of metal to ligand is examined, as in an earlier work,[11] this unfortunate overlap renders unequivocal detection of α deuteration very difficult. By investigating conditions under which free amino acid is in equilibrium with the complex, and by investigating a series of amino acids in which superposition is not a problem, it becomes clear that rapid α exchange is occurring.

Comparison of Figures 7a and 7b shows that the doublets of the alanine methyl portion of the ligand and of the free alanine are gradually replaced by broadened singlets shifted to slightly higher field. The shift[28] to

(26) W. A. Wood and I. C. Gunsolus, *J. Biol. Chem.*, **190**, 403 (1951).
(27) J. Olivard, D. E. Metzler, and E. E. Snell, *ibid.*, **199**, 669 (1952).
(28) H. S. Gutowsky, *J. Chem. Phys.*, **31**, 1683 (1959).

higher field and loss of multiplicity is precisely as expected for exchange of the α proton with a deuteron of the solvent. After the reaction has proceeded to completion and the pD lowered to destroy the complex, only a singlet is found for the methyl of the alanine spectrum. As expected, the quartet of the α proton has vanished.

Apparently, the half-life for ligand exchange is considerably less than the half-life for reaction because the resonances from the α deuterated species appear equally rapidly in both free and coordinated alanine. Support for the mechanism in Scheme I, however, is found in the observation that increasing the aluminum(III) concentration increases the reaction rate. As previously observed[27] the reaction rate is base catalyzed. The fact that it slows down above pH 11 is undoubtedly due to hydrolysis of the aluminum complex to free ligand (and its components) and to the very stable aluminate ion.

Other amino acids also have been found to undergo racemization under these conditions. L-Serine was investigated and found to react more rapidly than alanine. In this case, the resonances arising from the D,L complex (corresponding to B and C in Figure 7) are absent initially; however, they rapidly appear in the spectrum and attain their equilibrium intensity within half an hour, providing conclusive evidence that racemization accompanies α proton exchange. Serine is known to undergo an aldolase model reaction under these conditions[3] reversibly forming glycine and formaldehyde, and so in this case racemization could proceed by a different mechanism. L-Valine was studied by the same technique and DL-valine was investigated by following the disappearance of its α proton doublet in solutions quenched by addition of D_2SO_4. The valine

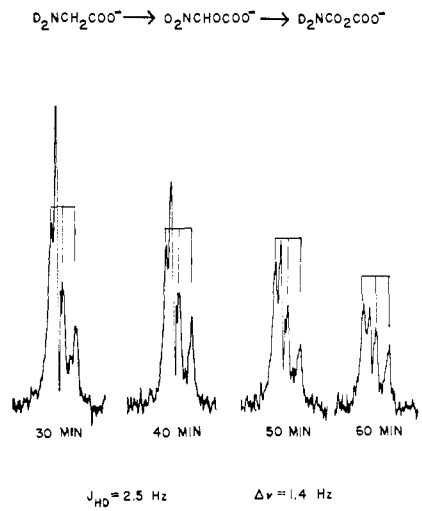


Figure 8. The 100-MHz nmr spectrum of the changes in the region of the glycine methylene resonance of a solution 0.20 *M* in glycine, 0.10 *M* in pyridoxal, and 0.050 *M* in aluminum(III) at pD 9.8, after 30, 40, 50, and 60 min, all at 30 ± 2°.

racemization is found to be much slower than the alanine reaction at a given pD. The methylene protons of glycine exchange under these conditions at a rate comparable to that of alanine. The glycine singlet is gradually replaced by a 1 : 1 : 1 triplet (J = 2.5 Hz) shifted about 1.4 Hz to higher field, as is illustrated by Figure 8. Within 2 hr, the geminal HD glycine resonances disappear entirely. The racemization reaction is under spectropolarimetric investigation at the present time in these laboratories and will be reported on in greater detail at a later date.