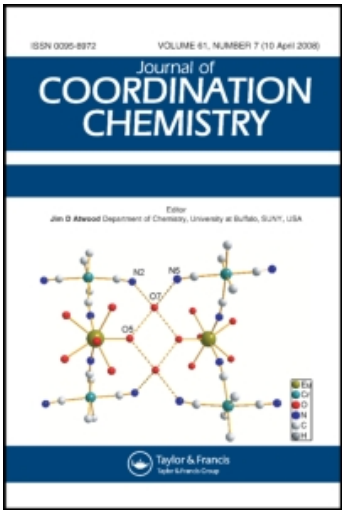

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Vitamin B-6 Model Reactions (V): Synthesis, Conformation and Differential Reactivity of the A-(S,S) and A-(S,S) Diastereoisomers of Bis(Pyridoxylidene-L-Valinato)Cobalt(III) Complexes

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VITAMIN B-6 MODEL REACTIONS (V): SYNTHESIS, CONFORMATION AND DIFFERENTIAL REACTIVITY OF THE Λ-(S,S) AND Δ-(S,S) DIASTEREOISOMERS OF BIS(PYRIDOXYLIDENE-L-VALINATO)COBALT(III) COMPLEXES

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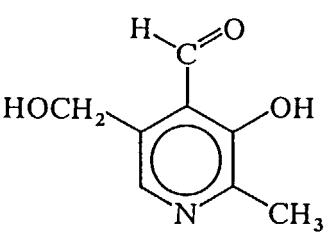
The Λ(S,S) and Δ(S,S) diastereoisomers of the bis(pyridoxylidene-*L*-valinato)cobalt(III) complex have been synthesized and separated from one another. Nuclear magnetic resonance investigations show that the Schiff base chelate ring conformations are different for the two isomers. These measurements also determine the absolute configuration. The rate of carbon–hydrogen bond breaking has been studied by monitoring the intial rate of loss of the amino acid α-proton resonance for each complex. In acidic solution, the Δ isomer reacts about five times more rapidly than the Λ isomer. Four-bond proton–proton spin coupling constants show that the most rapidly reacting complex is the one where the bond to be broken is most nearly dihedrally perpendicular to the plane of the aromatic ring. However, conductivity studies show substantial differences in solvation which could also explain differences in reactivity.

Keywords: Vitamin B-6, Schiff base, pyridoxal, aminoacids, diastereoisomers

INTRODUCTION

Transition metal complex chemistry has provided many useful models for biological reactions. Among the bioinorganic systems investigated by Martell and co-workers, models for the vitamin B-6 enzymes have yielded many important insights.[1] Vitamin B-6 is, in one of its forms, the heterocyclic aldehyde pyridoxal, I. In nature, it is a



I

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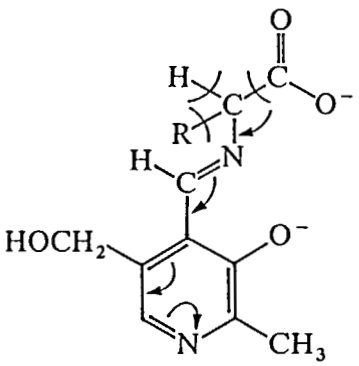
cofactor for a large number of enzymes catalyzing diverse transformations of amino acids. Model system work showed that these reactions often proceed by formation of a Schiff base between the pyridoxal and the amino acid followed by the electron shift indicated in **II**.[2-4]



II

Schiff bases like **II** are terdentate ligands and form coordination compounds with many metal ions. These complexes often react to form amino acid derivatives like those formed in vitamin B-6 enzyme-catalyzed reactions. Such reactions have been studied in detail by Martell and others and have helped to develop much of our knowledge of how vitamin B-6 enzyme reactions proceed.

We have been interested in analyzing the effects of stereochemistry on reactivity in vitamin B-6 model systems. The electron shift in **II** can result in breaking any of three bonds to the amino acid α-carbon atom, as indicated. Some years ago Dunathan hypothesized that the enzyme selects the bond to be broken by forcing it into the conformation whereby its electrons can flow most easily into the π orbitals of the azomethine group.[5] This can be accomplished by a rotation about the carbon–nitrogen single bond so that the group to be cleaved is dihedrally perpendicular (orthogonal) to the plane of the aromatic–azomethine system.

Cobalt(III) complexes of amino acid Schiff bases are useful as models for the study of the effect of Schiff base conformation on reactivity in these systems. Cobalt(III) complexes can be readily synthesized.[6,7] The bonds to the metal ion are inert on the time scale of vitamin B-6 model reactions. This means that the Schiff base is held fairly rigidly during the reaction. As demonstrated in this paper and in others, it is possible to create conditions wherein different Schiff base conformations are adopted so that reaction rates of different conformations can be compared to one another.[8-11]

EXPERIMENTAL

Pyridoxal hydrochloride and *L*-valine were obtained from Sigma Chemicals. All other reagents used were analytical grade.

Electronic spectra of the samples were recorded on a Cary Model 14 spectrophotometer and infrared spectra on a Perkin-Elmer 521 spectrometer. NMR spectra were obtained using JEOL PFT-100 or Bruker WM 250 spectrometers. Chemical

shifts are referenced externally to TMS. Coupling constants of less than 2.0 Hz were measured from spectra which had been resolution-enhanced through Gaussian multiplication. Conductivity measurements were carried out on a LKB conductivity bridge.

Preparation of the Complexes

Sodium Bis(pyridoxylidine-*L*-valinato)colbaltate(II), $Na_2[Co(PLV)_2]$

Pyridoxal hydrochloride (4.06 g) and *L*-valine (2.34 g) were suspended in water (20 cm^3) and 2 M sodium hydroxide (20 cm^3) was added. The solution was shaken for about 20 minutes and $CoCl_2.6H_2O$ (2.37 g) in water (10 cm^3) was added. The precipitate obtained was quickly filtered and washed several times with water, then methanol, and dried. Yield: 75% sodium bis(pyridoxylidene-*L*-valinato)cobaltate(II), $Na_2Co(PLV)_2.4\frac{1}{2}H_2O$.

Sodium Bis(pyridoxylidene-*L*-valinatocobaltate(III), $Na[Co(PLV_2]$

$Na_2[Co(PLV)_2]$ (1 g) was suspended in 98% methanol (60 cm^3) and 2 M sodium hydroxide ($\sim$1 cm^3) was added slowly with shaking until all but a small amount of solid remained undissolved. The solution was filtered and an additional amount of methanol (40 cm^3) was added. The cobalt(II) was then oxidized in the presence of charcoal (0.5 g) by passing a vigorous stream of air for one hour. The solution was then filtered and the solvent was removed under vacuum keeping the temperature below 25°C.

The product, $NaCo(PLV)_2$, obtained in quantitative yield, is a mixture of Λ- and Δ-$Na[Co(PLV)_2]$ in comparable amounts. *Anal.*: calcd. for $NaCoC_{26}H_{32}N_4O_8 \cdot 4.5 H_2O$: C, 45.1; H, 6.00; N, 8.10; Co, 8.52%. Found: C, 44.9; H, 6.09; N, 7.84; Co, 8.49%.

Separation of Isomers

Method (1)

One gram of the isomeric mixture, $Na[Co(PLV)_2]$, was extracted with 120 cm^3 of 40:1 acetone (AR): methanol (AR). After filtering, the solid was washed several times with 25:1 acetone:methanol and dried. The isomer obtained is Λ(*S,S*)-$Na[Co(PLV)_2]$. Its specific rotation at 25°C and 546 nm is −589°, in methanol. The filtrate on evaporation under vacuum below room temperature gives Δ-(*S,S*)-$Na[Co(PLV)_2]$.

Method (2)

Tetramethylammonium chloride (TMACl, 1.7 g) and 1 gm of the isomeric mixture of $Na[Co(PLV)_2]$ was shaken with water (3 cm^3) for about 5–10 minutes, when most of the Λ isomer precipitated as the TMA salt. To this suspension, AR acetone (120 cm^3) was added and the mixture was kept shaking for about 30 minutes in ice water. The solution was then filtered and yellowish green crystalline Λ-(*S,S*)-$TMA[Co(PLV)_2]$ was washed several times with a few drops of ice-cold water, followed by acetone and water (50/50), then acetone, and dried. *Anal*: calcd. for $CoC_{31}H_{44}N_4O_8 \cdot 3H_2O$: C, 49.9; H, 9.72; N, 8.16; Co, 8.52%. Found: C, 50.33; H, 10.04; N, 8.16; Co, 7.19%.

The filtrate on evaporation under vacuum below room temperature gives Δ-(*S*,*S*)-TMA[Co(PLV)$_2$] which was further purified by extraction with dry acetone and drying as before. The solid was dried under vacuum for about 10 hours to remove traces of tenaciously held acetone. Its specification rotation at 25°C and 546 nm is −121° in methanol.

Notes on Preparation

The main problem encountered in the preparation of the complexes is racemization of the valine in the Schiff base. Formation of the *R*,*S*-isomer of the Co(PLV)$_2$ complex by racemization makes separation of the isomers difficult. Under the conditions reported, racemization occurs only to a small extent. To prevent racemization, all operations must be carried out without delay and the temperature must be kept below 25°C. It is found that, although the separation of the isomers gives very pure Λ-isomer, the Δ-isomer often contained a small amount (2–5%) of the *R*,*S* diastereoisomer. It may be noted that the Δ-isomer racemizes much more rapidly than the Λ-isomer (*vide infra*).

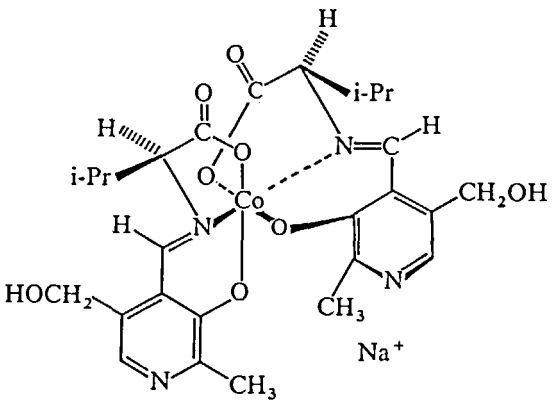
Properties of the Isomers

The sodium salts of the isomers are extremely soluble in water, methanol and other polar solvents. Λ-(*S*,*S*)-Na[Co(PLV)$_2$] is insoluble in dry acetone while the Δ-isomer has good solubility. The solubility of Λ-(*S*,*S*)-TMA[Co(PLV)$_2$] is limited in water at neutral pH, but it is very soluble below pH 4. However, Δ-(*S*,*S*)-TMA[Co(PLV)$_2$] is quite soluble in water (all pH's), methanol, acetone, *etc.* Both isomers are insoluble in ether, benzene, dioxane, chloroform, etc.

The solid complexes are stable at room temperature for indefinite periods. Methanolic solutions are stable for days at room temperature. At 0°C, no racemization could be observed even after three weeks. In water, at lower pH (2–3), the compounds are quite stable, although very slow formation of a new unidentified species was observed. All complexes undergo racemization (isomerization) in the pH range 5–10. At pH 11 and higher, slow formation of cobalt(II) could be observed as indicated by broadening and shift in pmr signals. Treatment with Chelex-100 removes cobalt(II) and narrows the pmr signals.

RESULTS

The synthesis of bis(pyridoxylidenevalinato)cobalt(III) complexes results in Λ-(*S*,*S*) and Δ-(*S*,*S*) diastereomers when *L*-valine is the starting material. If *D*,*L*-valine is the starting material, an additional diastereomer, Λ-(*S*,*R*) is formed as well as its enantiomer, Δ-(*R*,*S*). The presence of these complexes is most simply revealed in the proton spectrum of the 2-CH_3 region of the pyridine ring. When the starting material is optically pure, at pH 8.0, resonances are observed at 0.880 ppm and 0.930 ppm corresponding to Λ(*S*,*S*) and Δ(*S*,*S*) diastereomers. When racemic valine is the starting material, these resonances are present along with resonances at 0.899 ppm and 0.928 ppm. The latter resonances, therefore, must arise from the Λ(*S*,*R*) diastereomer and its Δ(*R*,*S*) enantiomer. The 2-methyl groups of this diasteromer are non-equivalent. This is illustrated in **III**.



III

Most conspicuously, in one case the amino acid side chain is on the same side of the amino acid α-carbon atom as is the 2-CH_3 group of the other ligand, whereas for the other 2-CH_3 group the amino acid side chain is on the opposite side of the α-carbon atom and therefore farther from the 2-CH_3 group of the other ligand. The isomerism and spectral analysis are entirely analogous to what is observed for the corresponding Al(III) complexes.[12]

The resonances at 0.938 ppm and 0.880 ppm are assigned to the $2CH_3$ resonances of the Δ-(*S*,*S*) and Λ-(*S*,*S*) isomers, respectively. In the Δ-(*S*,*S*) isomer the valine isopropyl group approaches the 2-CH_3 of the other ligand more closely. Models indicate a substantial steric interaction between these groups in the Δ-(*S*,*S*) isomer. This force destabilizes that isomer and also affects the dihedral angles of the chelated ring, including the amino acid α-carbon atom (*vide infra*). Table I lists proton chemical shifts and assignments for the Λ-(*S*,*S*)- and Δ-(*S*,*S*)-diastereoisomers.

TABLE I

Proton chemical shifts and assignments for the Λ- and Δ-diastereoisomers of bis(pyridoxylidenevalinato)-cobalt(III).

	Λ-(*S*,*S*)		Δ-(*S*,*S*)	
pD	7.79	3.46	8.44	3.24
2-CH_3	0.880(s)	1.428(s)	0.939(s)	1.503(s)
4'-CH	8.123(d)	8.391(d)	8.216(d)	8.508(d)
5'-CH_2	4.138(m)	4.280(m)	4.068(m)	4.188(m)
6-H	6.863(s)	7.006(s)	6.776(s)	7.029(s)
α-H	4.216(m)	4.216(m)	3.886(m)	4.188
β-H	1.752(m)	1.936(m)	2.230(m)	2.184
γ-CH_3	0.510(d)	0.457(d)	0.574(d)	0.606(d)
	0.438(d)	0.430(d)	0.364(d)	0.438(d)

Conformation of the Chelate Ring Containing the α-Carbon Atom

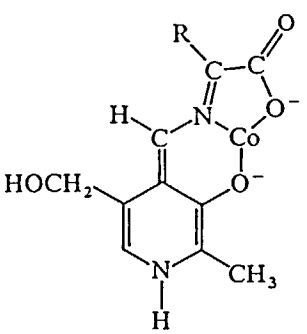
Allylic and pseudoallylic $^4J_{HH}$ couplings have been extensively studied and are a useful measure of the dihedral angle between the respective C–H bonds.[13] At pD 3.3

where the pyridine nitrogens are protonated, the coupling constants are 1.75 Hz for the Δ-complex and 1.42 Hz for the Λ-complex. When the pyridine nitrogens are deprotonated, the coupling constants are 1.84 Hz and 1.11 Hz for Δ and Λ respectively. In both cases, the dihedral angle is larger between the α-CH and the plane of the π system for Δ as compared to Λ. These angles support the preceding assignment of the chemical shifts for the diastereoisomers. More importantly, from the Dunathan hypothesis, the C–H bond in the Δ-complex is predicted to be more reactive than the C–H bond of the Λ-complex since it better overlaps the azomethine π orbital.

It should also be noted that the coupling constants indicate small differences in the average dihedral angle between C–H(α) and C–H(β) in the valine moieties of each complex. Angles are 126° and 131° for the Λ- and Δ-complexes, respectively. While it is not germane to the present work, conformation differences of this nature should influence the rates of β-elimination of amino acids which have electronegative substituents in the β-position.[10,14,15]

Reactions of the Diastereomers

Two reactions were observed for these diastereoisomers. New resonances are observed at 7.46, 5.98, 4.2, and 1.91 ppm. The integrals of these resonances are in the ratio of 1:1:2:3 and they are best assigned to **IV** where the other Co(III) binding sites are coordinated by a Schiff base ligand. A similar situation exists with aluminium(III) complexes.[16,17] This complex is an intermediate in both transamination and racemization. We are attempting to crystallize this compound so that we can determine its structure in detail.



IV

The loss of an α-proton resonance is the first step in both racemization and transamination. We are interested in this reaction because the dihedral angle between the α-hydrogen and the π-system of the pyridine ring should be an important factor in determining the rate of reaction because the bonding electron pair must migrate into the aromatic ring. Overlap between the π-system and this pair will influence the reaction rate. Table II lists rate data for this reaction. Significantly different rates are observed under weakly acidic conditions where the pyridine nitrogen atoms are protonated but the difference in rate is smaller at higher pH's. Since the Δ-complex

has the large pseudoallylic coupling constant, the dihedral angle between the valine α-C–H bond and the plane of azomethine π system is larger than for the Δ-complex. The Dunathan hypothesis, therefore, predicts that the Δ-complex should react more rapidly than the Λ-complex. This is what is observed.

TABLE II

Rate constants for the loss of α-proton signal in bis(pyridoxylidine-*L*-valinato)cobalt(III) complexes, $Co(PLV)_2$, at 24°C.

Complex	pD	Rate Constant (sec^{-1})[a]
Λ-$Co(PLV)_2$	4.7[b]	$7.7 \cdot 10^{-5}$
Δ-$Co(PLV)_2$	4.7	$3.2 \cdot 10^{-5}$
Λ-$Co(PLV)_2$	5.5[b]	$3 \cdot 10^{-5}$
Δ-$Co(PLV)_2$	5.5	$6.3 \cdot 10^{-5}$
Λ-$Co(PLV)_2$	10.0[c]	$8.7 \cdot 10^{-6}$
Δ-$Co(PLV)_2$	10.0	$2.6 \cdot 10^{-5}$

[a] Pseudo-first-order rate constants for total complex concentration of 5.7×10^{-2} M. [b] Self-buffered. [c] Carbonate/bicarbonate buffer.

Conductivity Studies

The Λ-(*S,S*) and Δ-(*S,S*) diastereomers have strikingly different molar conductances, as shown in Table III. For the sodium salts, the Λ-diastereomer has the higher conductivity while, for the tetramethylammonium salt, the Δ-isomer has higher conductivity. The factors responsible for these differences in conductivity are also very likely responsible for the considerable differences in solubility used to separate the diastereomers.

TABLE III

Molar conductances of bis(pyridoxylidene-*L*-valinato)cobalt(III) complexes in water and methanol. TMA is the tetramethylammonium cation. Units are $\Omega^{-1}\,cm^2/mol$.

	Solvent	
Complex	Methanol	Water
Λ-Na[$Co(PLV)_2$]	78	97
Δ-Na[$Co(PLV)_2$]	60	64
Λ-TMA[$Co(PLV)_2$]	76	51
Δ-TMA[$Co(PLV)_2$]	108	102
t-butylammonium iodide	81	95

DISCUSSION

The Λ- and Δ-diastereomers participate in Vitamin B-6 model reactions at different

rates. The relative rates are qualitatively interpretable by the Dunathan hypothesis which states that the bond most nearly dihedrally perpendicular to the plane of the π system should be the one most readily broken. The present system differs, interestingly, from a closely related case to be reported separately.[18] In the present work, the largest difference in reactivity between isomers is observed when the pyridine nitrogens are protonated. However, if the amino acid is glycine it is observed that when the pyridine nitrogen is protonated there is a two-fold difference between the reactivity of the two methylene protons; when the pyridine nitrogen is deprotonated, the methylene protons become strongly non-equivalent and the one whose C–H bond best overlaps the π-system reacts the fastest by eight-fold.

A note of caution is indicated by the conductivity data. Substantial differences in conductivity clearly indicate substantial differences in the solvation of the different diastereomers. The reaction we are studying is a proton transfer reaction which entails the approach of a base towards the amino acid α-carbon-hydrogen bond. With such major differences in solvation, it is easy to envisage that solvent shell structure could play a major role in determining the magnitude the reaction rates we have observed. Moreover, Belokon has suggested that steric factors can determine the stereoselectivity of reactions of salicylaldehydeamino acid Schiff bases.[9] With the large isopropyl side chains of valine moieties, steric effects could be very important.

ACKNOWLEDGEMENTS

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