Coordination Modes of Histidine. 2.[1] Stereochemistry of the Reaction between Histidine Derivatives and Pyridoxal Analogues. Conformational Properties of Zinc(II) Complexes of Histidine Schiff Bases

Luigi Casella* and Michele Gullotti

Contribution from the Istituto di Chimica Generale ed Inorganica dell'Università, Centro CNR, 20133 Milano, Italy. *Received February 24, 1981. Revised Manuscript Received May 18, 1981*

Abstract: Pyridoxal reacts with L-histidine, L-histidine methyl ester, histamine and N^{τ}-methylhistamine to form 4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole compounds through the formation of Schiff base intermediates. The 4,6-trans isomers of the products are formed with a high degree of stereoselectivity in the case of L-histidine and L-histidine methyl ester. The stereochemistry of these products has been assigned on the basis of their ^{1}H NMR and CD spectra. Salicylaldehyde reacts similarly, albeit slower, and with a much lower degree of stereoselectivity than pyridoxal. The biochemical significance of these reactions has also been discussed. Zinc(II) ions can function as a trap for Schiff base intermediates, and a number of zinc(II) complexes of *N*-pyridoxylidene-L-amino acids and *N*-salicylidene-L-amino acids have been obtained. The circular dichroism spectra of these complexes correlate with the mode of binding of the amino acid residues. Zinc(II) complexes derived from L-histidine behave like those of L-amino acids with nonpolar side chains, indicating that the histidine residues chelate glycine-like. By contrast, the circular dichroism spectra of zinc(II) complexes derived from L-histidine methyl ester bear a mirror-image relationship to those of corresponding complexes containing L-amino acidato residues and suggest the adoption of a histamine-like coordination mode by these histidine residues. The conformational properties of zinc(II) complexes of amino acid Schiff bases are discussed in relation to vitamin B_6 model reactions.

Vitamin B_6 is an essential cofactor for many enzymic reactions of amino acids. Most of these reactions also proceed in nonenzymatic pyridoxal–amino acid systems, and in many cases addition of metal ions to these binary systems has been found to enhance their reaction rates. The role of metal ions and the Vitamin B_6 group of compounds, together with their analogues and homologues, in the catalysis of these reactions have been extensively reviewed.[2,3] Metal ions may simulate some of the features of enzymic active sites by acting as a trap for the Schiff base formed between pyridoxal and the amino acid and, more importantly, by labilizing the bonds adjacent to the coordinating groups of the amino acid residue. Despite the large interest in metal ion containing model systems which reproduce some of the transformations of amino acids effected by pyridoxal-dependent enzymes, most of the studies have been carried out in solution, and only a limited number of Schiff base chelates related to pyridoxal catalysis have been isolated. These include 1:1 and 2:1 complexes of *N*-pyridoxylideneamino acids,[4-6] *N*-salicylideneamino acids,[7-13] *N*-(+)-(hydroxymethylidenecamphorato)amino acids,[1,12,13] and *N*-(3-hydroxypyridyl-2-methylidene)amino acids,[14] but in a number of instances characterization has been incomplete. The chelate ring structure of these compounds has been established by several X-ray structural studies[11,15] and involves coordination of the tridentate Schiff base ligand through the phenolic oxygen, the imine nitrogen, and carboxylate oxygen donors. However, to the best of our knowledge reports of metal complexes of imines derived from histidine are completely absent, and only recently some papers describing properties of histamine Schiff base chelates have appeared.[16]

This paper describes the synthesis and characterization of a series of zinc(II) complexes with the Schiff bases of histidine derivatives and pyridoxal, salicylaldehyde, and pyruvic acid. The zinc ions have been selected because of their relatively high ef-

(1) Part 1: Casella, L.; Gullotti, M. *Inorg. Chem.* **1981**, *20*, 1306-1308.

(2) (a) Snell, E. E.; Braunstein, A. E.; Severin, E. S.; Torchinsky, Yu. M., Eds. "Pyridoxal Catalysis: Enzymes and Model Systems"; Interscience: New York; 1968. (b) Mahler, H. R.; Cordes, E. H. "Biological Chemistry"; Harper and Row: New York, 1971; Chapter 8. (c) Martell, A. E. In "Metal Ions in Biological Systems"; Sigel, H., Ed.; Marcel Dekker: New York, 1973; Vol. 2, pp 208-263. (d) Holm, R. H. In "Inorganic Biochemistry"; Eichhorn, G. L., Ed.; Elsevier: New York, 1973; pp 1137-1167. (e) Braunstein, A. E. *Enzymes, 3rd Ed.* **1973**, *9*, 379-481.

(3) (a) Pasini, A.; Casella, L. *J. Inorg. Nucl. Chem.* **1974**, *36*, 2133-2144. (b) Phipps, D. A. *J. Mol. Catal.* **1979**, *5*, 81-107. (c) Metzler, D. E. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1979**, *50*, 1-40.

(4) (a) Christensen, H. N. *J. Am. Chem. Soc.* **1957**, *79*, 4073-4078; *Ibid.* **1958**, *80*, 2305-2308. (b) Abbott, E. H.; Martell, A. E. *J. Inorg. Nucl. Chem.* **1971**, *33*, 567-569. (c) Wrobleski, J. T.; Long, G. J. *Inorg. Chim. Acta* **1979**, *36*, 155-160. (d) Long, G. J.; Wrobleski, J. T.; Thundathil, R. V.; Sparlin, D. M.; Schlemper, E. O. *J. Am. Chem. Soc.* **1980**, *102*, 6040-6046.

(5) Davis, L.; Roddy, F.; Metzler, D. E. *J. Am. Chem. Soc.* **1961**, *83*, 127-134.

(6) Wrobleski, J. T.; Long, G. J. *Inorg. Chem.* **1977**, *16*, 2752-2762.

(7) Burrows, R. C.; Bailar, J. C. *J. Am. Chem. Soc.* **1966**, *88*, 4150-4156.

(8) Weinstein, G. N.; O'Connor, M. J.; Holm, R. H. *Inorg. Chem.* **1970**, *9*, 2104-2112.

(9) (a) Nakahara, A. *Bull. Chem. Soc. Jpn.* **1959**, *32*, 1195-1199. (b) Nakao, Y.; Sakurai, K.; Nakahara, A. *Ibid.* **1967**, *40*, 1536-1538. (c) Theriot, L. J.; Carlisle, G. O.; Hu, H. J. *J. Inorg. Nucl. Chem.* **1969**, *31*, 2841-2844, 2891-2894, 3303-3308. (d) Carlisle, G. O.; Theriot, L. J. *Ibid.* **1973**, *35*, 2093-2096. (e) Zyzyck, L. A.; Frummer, H.; Villa, J. F. *Ibid.* **1975**, *37*, 1653-1657. (f) Chow, S.-T.; Johns, D. M.; McAuliffe, C. A.; Machin, D. J. *Inorg. Chim. Acta* **1977**, *22*, 1-5. (g) Bowden, F. L.; Carpenter, R. P.; Parish, R. V.; Pollock, R. D. *Ibid.* **1977**, *23*, 35-36. (h) Casella, L.; Pasini, A.; Ugo, R.; Visca, M. *J. Chem. Soc., Dalton Trans.* **1980**, 1655-1663.

(10) Percy, G. C. *J. Inorg. Nucl. Chem.* **1975**, *37*, 2071-2073.

(11) Belokon', Yu. N.; Belikov, V. M.; Vitt, S. V.; Savel'eva, T. F.; Burbelo, V. M.; Bakhmutov, V. I.; Aleksandrov, G. G.; Struchkov, Yu. T. *Tetrahedron* **1977**, *33*, 2551-2564.

(12) Casella, L.; Gullotti, M.; Pasini, A.; Rockenbauer, A. *Inorg. Chem.* **1979**, *18*, 2825-2835.

(13) Casella, L.; Gullotti, M.; Pasini, A.; Visca, M. *Inorg. Chim. Acta* **1976**, *19*, L9-L13.

(14) Heinert, D.; Martell, A. E. *J. Am. Chem. Soc.* **1963**, *85*, 1334-1337.

(15) (a) Willstadter, E.; Hamor, T. A.; Hoard, J. L. *J. Am. Chem. Soc.* **1963**, *85*, 1205-1206. (b) Cutfield, J. F.; Hall, D.; Waters, T. N. *Chem. Commun.* **1967**, 785-786. (c) Bentley, G. A.; Waters, J. M.; Waters, T. N. *Ibid.* **1968**, 988-989. (d) Capasso, S.; Giordano, F.; Mattia, C.; Mazzarella, L.; Ripamonti, A. *J. Chem. Soc., Dalton Trans.* **1974**, 2228-2233. (e) Casella, L.; Gullotti, M.; Pasini, A.; Ciani, G.; Manassero, M.; Sironi, A. *Inorg. Chim. Acta* **1978**, *26*, L1-L2. (f) Hämäläinen, R.; Ahlgrén, M.; Turpeinen, U.; Rantala, M. *Acta Chem. Scand., Ser. A* **1978**, *A32*, 235-240, 549-553. (g) Aoki, K. Yamazaki, H. *J. Chem. Soc., Chem. Commun.* **1980**, 363-365.

(16) (a) Simmons, M. G.; Wilson, L. J. *J. Chem. Soc., Chem. Commun.* **1978**, 634-636. (b) Grzybowski, J. J.; Merrell, P. H.; Urbach, F. L. *Inorg. Chem.* **1978**, *17*, 3078-3082. (c) Nakao, Y.; Mori, W.; Okuda, N.; Nakahara, A. *Inorg. Chim. Acta* **1979**, *35*, 1-4. (d) Kovacs, D.; Shepherd, R. E. *J. Inorg. Biochem.* **1979**, *10*, 67-88.

0002-7863/81/1503-6338$01.25/0

ficiency among diamagnetic ions in catalyzing Vitamin B_6 model reactions, and because a large number of comparative data on equilibria, reactivity and structure in solution are available for zinc systems.[2,3] In view of the importance of the histidyl residue as metal-binding site in biological systems,[17] complexes of this type may well simulate structural features of enzymic active sites. This work is part of a systematic study of metal complexes of histidine Schiff bases which has been undertaken to the end of establishing firm correlations between spectral properties of the complexes and mode of binding of the histidine residue.[1,18] The condensation reaction between pyridoxal, or salicylaldehyde, and histidine derivatives in the absence of metal ions, its possible biological significance, and the stereochemistry of the cyclic products formed will also be discussed here.

Experimental Section

All reagents were reagent grade and used as received; N^{τ}-methylhistamine was prepared according to a literature method.[19] Elemental analyses were from the microanalytical laboratory, the University of Milan. The NMR spectra were obtained with a Bruker WP-80 spectrometer operating at 80 MHz and using a pulsed Fourier transform technique. The internal reference standard used in D_2O solutions was sodium 3-(trimethylsilyl)propionate-d_4. Electronic and circular dichroism spectra were recorded on Beckman DK-2A and on Jobin-Yvonne Mark III instruments, respectively. Infrared spectra were recorded on a Beckman Acculab 1 spectrophotometer and MS spectra (70 eV) recorded on a Varian MAT 112 spectrometer.

Preparation of Tetrahydropyrido[3,4-*d*]imidazole Compounds III–VI and IX–XI.[20] Tetrahydropyrido[3,4-*d*]imidazole compounds derived from pyridoxal (III–VI) were prepared by reacting equimolar amounts of pyridoxal hydrochloride and of the histidine derivative (2 mmol) in water (20 mL) at pH ~9. The derivative of histidine (III) was isolated as described in an earlier report.[23a] The derivatives of histamine (IV) and L-histidine methyl ester (VI) precipitated within a few hours and were collected by filtration, washed with water, and dried under vacuum. The rection between N^{τ}-methylhistamine and pyridoxal was much slower, and precipitation of the tetrahydropyridine derivative V occurred only after approximately 20 h. The product was collected after ~40 h and was largely contaminated by the isomeric Schiff base Va.

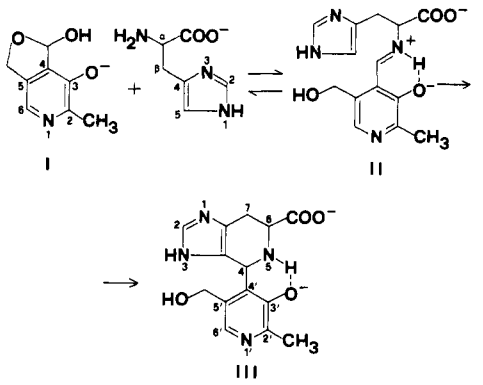
Tetrahydropyrido[3,4-*d*]imidazole compounds derived from salicylaldehyde IX and XI were prepared by reaction of equimolar amounts of salicylaldehyde and the histidine derivative (2 mmol) in 1:1 water–methanol (20 mL) at pH ~9. The derivative of histamine X was prepared similarly but at pH ~10. After about 50 h the solution was concentrated under vacuum until precipitation of the product occurred and cooled. The precipitate was collected by filtration, washed with water–methanol, and dried under vacuum. Elemental analyses of the tetrahydropyrido[3,4-*d*]imidazole compounds IV–VI and IX–XI are collected in Table I.[21]

Preparation of Zinc(II) Complexes of Schiff Bases.[22] The complexes Zn(sal-him)Cl, Zn(sal-L-hisOCH_3)Cl, Zn(pdx-him)Cl, and Zn(pdx-L-hisOCH_3)Cl were prepared according to the following procedure. Equimolar amounts of salicylaldehyde, or free pyridoxal, and histamine dihydrochloride, or L-histidine methyl ester dihydrochloride (2 mmol), were dissolved in 1:1 water–methanol (40 mL). Methanolic 1 M sodium hydroxide solution (4 mmol) was added to neutralize the acidity. Then zinc(II) nitrate hexahydrate (2 mmol) and methanolic sodium hydroxide (2 mmol) were added to the solution under stirring. After several hours the zinc complex of the Schiff base precipitated as a light yellow powder, was collected by filtration, was washed with water–methanol, and was dried under vacuum.

The compound Zn(sal-L-his) was obtained by treating equimolar amounts of salicylaldehyde, L-histidine, and zinc(II) acetate dihydrate (2 mmol) in 1:1 water–methanol (40 mL) under stirring. The precipitate thus formed was filtered, washed with water–methanol, and dried under vacuum. This same procedure was followed to obtain the complexes Zn(sal-L-ala), Zn(sal-L-val), and Zn(sal-L-phe). In several instances the use of zinc(II) nitrate instead of the acetate salt gave the same result.

The complexes Zn(pdx-L-his), Zn(pyv-L-his), Zn(pdx-L-phe), Zn(pyv-L-phe), and Zn(pyv-L-ala) were obtained by reaction of free pyridoxal, or pyruvic acid (2 mmol), the amino acid (2 mmol), and zinc(II) oxide (~1.8 mmol) in 1:1 water–methanol (40 mL) under stirring. After several hours the precipitate was collected by filtration, washed with water–methanol, and dried under vacuum. Elemental analyses of the zinc complexes are collected in Table I.[21]

Scheme I



I II III

Results and Discussion

The Reaction between Histidine Derivatives and Pyridoxal Analogues. The reaction between histidine and pyridoxal in weakly basic medium has been reported to occur with formation of the tetrahydropyrido[3,4-*d*]imidazole derivative III (Scheme I).[23] Similar products have been obtained from pyridoxal 5-phosphate and histidine or histamine.[24] Some evidence that the reaction proceeds through the formation of a Schiff base intermediate (II) was obtained by the changes observed in the electronic spectra throughout the course of the reaction.[24] More convincing evidence for this reaction path can be obtained following the reaction by ^{1}H NMR and CD spectroscopy. When equimolar solutions of pyridoxal and L-histidine in D_2O at pD ~9 are mixed, the proton NMR spectrum of Figure 1a is obtained. The aldimine proton resonance of II can be observed at δ 8.33, though the tetrahydropyridine ring is already forming, as indicated by the presence of the 4-CH signal of III at δ 5.81. Free pyridoxal and histidine are also present, as inferred from the 4-CH signal of pyridoxal hemiacetal form I at δ 6.56[25] and from the 5-H resonance of the histidine imidazole ring at δ 7.05. Formation of III is complete in approximately 3 h, and after this time the signals of II and of the starting reagents have disappeared (Figure 1b). The intermediate II can be detected also in the electronic and CD spectra. The spectra recorded after dissolution of pyridoxal and L-histidine (1:1) in water at pH ~9 are shown in Figure 2a. Free pyridoxal contributes to the absorption bands at ~250, 300, and 400 nm.[26,27] The CD bands at 290 and 420 nm (of negative sign) and that at

(17) (a) Sundberg, R. J.; Martin, R. B. *Chem. Rev.* **1974**, *74*, 471–517. (b) Schneider, F. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 583–592.

(18) Part 3: Casella, L.; Gullotti, M.; Pacchioni, G., submitted for publication.

(19) Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Roe, A. M.; Slater, R. A. *J. Med. Chem.* **1976**, *19*, 923–928.

(20) The systematic Chemical Abstracts names of these compounds are as follows: III, 4-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole-6-carboxylic acid; IV, 4-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole; V, 4-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-3-methyl-4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole; VI, methyl 4-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole-6-carboxylate; IX, 4-(1-hydroxy-2-phenyl)-4,5,6,7-tetrahydropyrido[3,4-*d*]-imidazole-6-carboxylic acid; X, 4-(1-hydroxy-2-phenyl)-4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole; XI, methyl 4-(1-hydroxy-2-phenyl)-4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole-6-carboxylate.

(21) Supplementary material.

(22) Abbreviations employed for the ligands: *N*-pyridoxylideneamino acidato anion = pdx-aa; *N*-salicylideneamino acidato anion = sal-aa; *N*-pyruvylideneamino acidato anion = pyv-aa; condensed amino acid anion = aa; histidinate anion = his; alaninate anion = ala; valinate anion = val; phenylalaninate anion = phe; histidine methyl ester = hisOCH_3; histamine = him.

(23) (a) Heyl, D.; Harris, S. A.; Folkers, K. *J. Am. Chem. Soc.* **1948**, *70*, 3429–3431. (b) Matsushima, Y. *Chem. Pharm. Bull.* **1968**, *16*, 2046–2055. (c) Abbott, E. H.; Martell, A. E. *J. Am. Chem. Soc.* **1970**, *92*, 1754–1759.

(24) (a) Kierska, D.; Maslinski, Cz. *Biochem. Pharmacol.* **1971**, *20*, 1951–1959. (b) Kierska, D.; Sasiak, K.; Boguslawski, M.; Maslinski, Cz. *Agents Actions* **1975**, *5*, 15–19.

(25) (a) Koritnyk, W.; Singh, R. P. *J. Am. Chem. Soc.* **1963**, *85*, 2813–2817. (b) Gansow, O. A.; Holm, R. H. *Ibid.* **1969**, *91*, 573–581; *Tetrahedron* **1968**, *24*, 4477–4487.

(26) (a) Metzler, D. E.; Snell, E. E. *J. Am. Chem. Soc.* **1955**, *77*, 2431–2437. (b) Nakamoto, K.; Martell, A. E. *Ibid.* **1959**, *81*, 5857–5863, 5863–5869.

(27) Matsushima, Y.; Martell, A. E. *J. Am. Chem. Soc.* **1967**, *89*, 1322–1330.

Table III. Proton NMR Data of Tetrahydropyrido[3,4-*d*]imidazole Derivatives in D_2O

	δ^a							
compd	2-H	6'-H	4-H	6-H	7-H	2'-CH_3	N-CH_3	$COOCH_3$
III[b]	7.78 (s)	7.64 (s)	5.90 (s, br)	4.15 (dd, $J_1 + J_2 = 16.8$ Hz)	2.9–3.5 (m)	2.40 (s)		
IV[b]	7.72 (s)	7.60 (s)	5.86 (t, $J = 1.8$ Hz)	3.0–4.1 (m)		2.36 (s)		
V[b,c]	7.61 (s)	7.51 (s)	5.90 (s, br)	2.7–3.8 (m)		2.37 (s)	3.06 (s)	
VI[b]	7.91 (s)	7.60 (s)	5.74 (t, $J = 1.9$ Hz)	4.12 (dd, $J_1 + J_2 = 15.2$ Hz)	3.0–3.3 (m)	2.38 (s)		3.87 (s)

	δ^a					
compd	2-H	phenyl ring	4-H	6-H	7-H	$COOCH_3$
IX[d]	7.79 (s)	6.9–7.5 (m)	5.84 (t, $J = 1.7$ Hz)	4.22 (dd, $J_1 + J_2 = 16.8$ Hz)	2.9–3.4 (m)	
X	7.70 (s)	6.8–7.4 (m)	5.54 (s)	3.30 (t, $J = 5.5$ Hz)	2.89 (t)	
XI[d,e]	7.76 (s)	6.9–7.4 (m)	5.98 (s)	3.6–4.0 (m)[f]	2.9–3.3 (m)	3.75 (s)

[a] Signal multiplicity is given in parentheses: s = singlet, t = triplet, dd = doublet of doublets, m = multiplet, br = broad. [b] The signals of pyridoxyl 5'-CH_2 groups are obscured by or close to solvent absorption (δ 4.6–4.9). [c] This product contains approximately 40% of isomeric Schiff base Va. The azomethine signal occurs at δ 8.53. [d] Major isomer. [e] Recorded in CD_3OD-Me_4Si. [f] The signals of the two isomers overlap considerably.

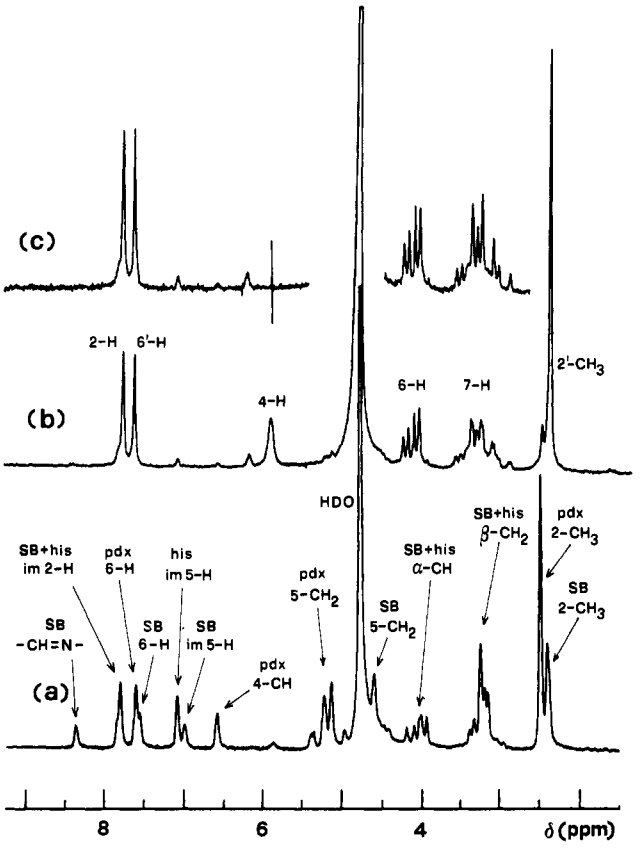


Figure 1. Proton NMR spectra of an equimolar solution of pyridoxal and L-histidine in D_2O at pD ~9: (a) immediately after mixing of the reagents (pdx = pyridoxal, SB = Schiff base, his = histidine, im = imidazole; numbering corresponds to position on pyridine or imidazole rings; Greek letters correspond to position on amino acid residue); (b) after approximately 3 h (numbering refers to III); (c) upon irradiation of the 5.81-ppm signal of b.

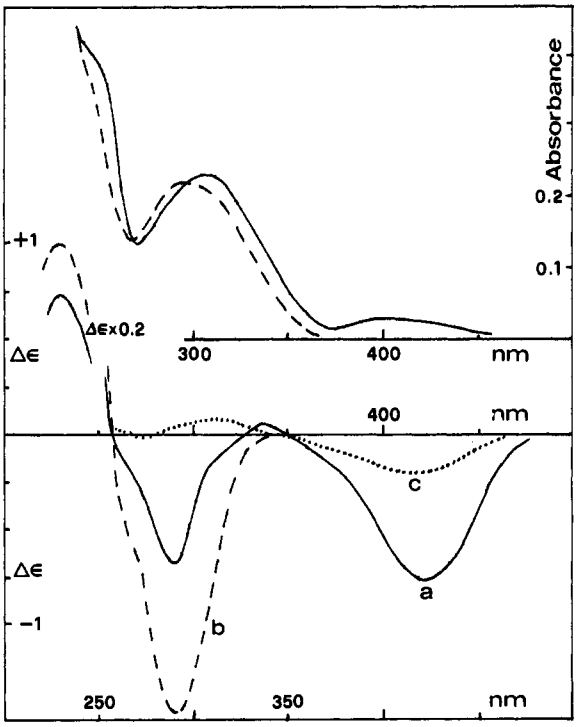
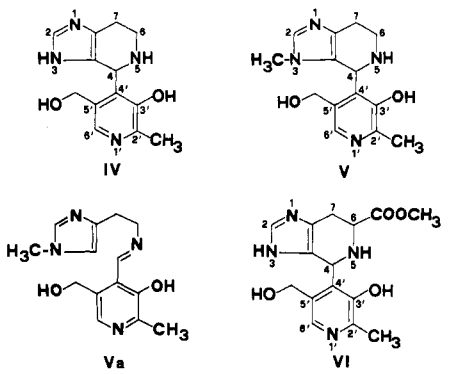


Figure 2. Electronic and CD spectra of an equimolar aqueous solution of pyridoxal and L-histidine at pH ~9 (concentration 5×10^{-2} M; cell path 0.01 cm): (a) —, after mixing of the reagents; (b) ---, after approximately 3 h. The spectrum c (···) is in the same conditions as a but at pH ~7.

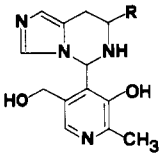
230 nm (positive) are related to the Schiff base II and tetrahydropyridine III. The 420-nm CD band can be assigned to the Schiff base II[27,28] and that at 290 nm to the tetrahydropyridine III chromophores, respectively. Both these compounds are contributing to the 230-nm CD band. Formation of III is accompanied by an increase in intensity of the 290-nm CD band and extinction of I and II by a corresponding decrease in the electronic and CD bands in the 400-nm region. After approximately 3 h, when only III is present, the electronic and CD spectra shown in Figure 2b are obtained. By lowering the pH of the solution to ca. 7 the rate of formation of II and III is also lowered, and only the Schiff base can be detected in an appreciable amount immediately after the reagents are mixed (Figure 2c).

Pyridoxal reacts similarly with histamine, N^{τ}-methylhistamine, and L-histidine methyl ester to form the tetrahydropyrido[3,4-*d*]imidazole derivatives IV–VI. Formation of V rules out the

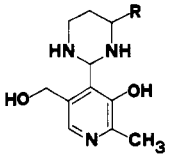


(28) (a) Metzler, D. E. *J. Am. Chem. Soc.* **1957**, *79*, 485–490. (b) Heinert, D.; Martell, A. E. *Ibid.* **1962**, *85*, 183–188, 188–193. (c) Metzler, C. M.; Cahill, A.; Metzler, D. E. *Ibid.* **1980**, *102*, 6075–6082.

possibility that the condensation products had structure VII, which



VII

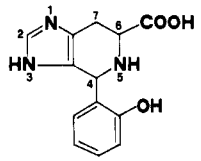


VIII

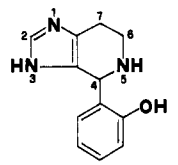
is analogous to the geminal-diamine-type structure VIII obtained by condensation of pyridoxal with 1,3- or 2,4-diamines.[28c,29] The rate of formation of V from the intermediate pyridoxylidene-N^{τ}-methylhistamine Schiff base, however, is slower than that of the other histidine derivatives due to the increased basicity of the N^{τ}-methylimidazole nucleus. The product V isolated after reaction of pyridoxal and N^{τ}-methylhistamine at pH ~9 for 40 h contains approximately 40% of the isomeric Schiff base Va (Table III).

The importance of these cyclization reactions undergone by Schiff bases derived from pyridoxal and histidine or histamine is related to their possible occurrence in vivo, where they may lead to inhibition of pyridoxal-dependent enzymes such as diamine oxidase, thereby providing a regulatory mechanism of biogenic amine metabolism.[30] It can be noted that tetrahydroisoquinoline derivatives structurally corresponding to III–VI are formed by similar cyclization reactions between pyridoxal, or pyridoxal 5-phosphate, and ring-substituted aromatic amino acids,[31] or arylalkylamines.[32] These processes appear to be related to the inhibitory activity of a variety of enzymes such as amino-transferase,[33] amino acid decarboxylase,[31,34] and pyridoxal kinase.[35]

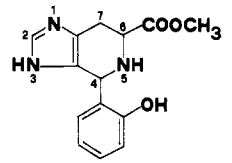
Tetrahydropyrido[3,4-*d*]imidazole compounds IX–XI, the



IX



X



XI

salicyl analogues of III–VI, can be obtained by reaction of the histidine derivative with salicylaldehyde in weakly basic aqueous alcoholic solution. These reactions proceed at a rate slower than in the case of pyridoxal, as expected from the reduced reactivity of the salicylaldehyde carbonyl group, compared with that of pyridoxal. For instance, the reaction between L-histidine and salicylaldehyde in 1:1 water–methanol at pH ~9.5 needs over 20 h to reach completion. There is some evidence that also pyruvic acid reacts with derivatives of histidine to form corresponding tetrahydropyridine compounds. The reactions are extremely slow in aqueous medium but occur at a reasonable rate in hot alcohol. The ^{1}H NMR spectra of the crude reaction mixtures show a marked decrease in intensity of the 5-H signal of the histidine imidazole ring, which seems an indication that cyclization at this ring position has occurred. Competing processes such as pyruvate dimerization[36] are apparently favored in the conditions required by the condensation reaction and we have been unable so far to characterize the condensation products. However, it is noticeable that if also pyruvate can undergo such cyclization reactions, these may provide a route for inactivation of pyruvate-containing enzymes, e.g., bacterial histidine decarboxylase,[37] similar to that of pyridoxal phosphate dependent enzymes.[38]

Table IV. Electronic and CD Spectra of Tetrahydropyrido[3,4-*d*]imidazole Derivatives in Methanol

compd	UV λ_{max}, nm (ϵ)	CD λ_{max}, nm ($\epsilon_l - \epsilon_r$)
III	289 (5150), 221 (13 000)	290 (−2.02), 225 (+8.72)
IV	289 (5420), 224 (14 700)	
V	420,[a] 335,[a] 285, 250,[a] 218	
VI	289 (5150), 221 (11 700)	290 (−2.80), 225 (+9.54)
IX	278 (2960), 220 (10 300)	278 (−1.00), 222 (−7.83)
X	276 (3400), 221 (14 900)	
XI	276 (2500), 221 (10 000)	275 (−0.07), 256 (+0.03)

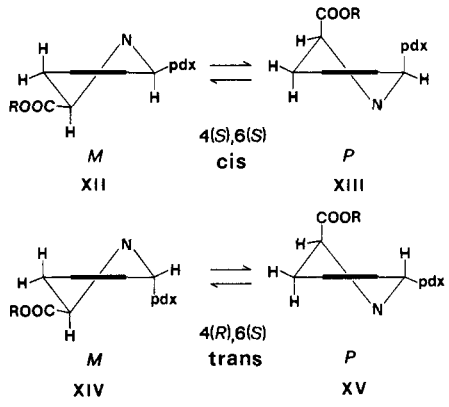
[a] Schiff base absorption.

Characterization of Tetrahydropyrido[3,4-*d*]imidazole Compounds. Compounds III–VI and IX–XI have been isolated, and their spectral properties confirm their tetrahydropyrido[3,4-*d*]-imidazole structure (Tables II,[21] III, and IV). The absence of isomeric Schiff base impurities in all the products isolated, except V, is confirmed by lack of azomethine absorption bands in either the IR spectra (near 1640 cm^{-1})[39] or the UV spectra (at 350–400 nm). Similarly, the proton NMR spectra lack resonances to lower field than the aromatic signals. The presence of a proton signal near δ 6, which suggests a saturated 4-CH group, and of a single imidazole ring proton indicates the cyclic structure of the compounds (Table III). The electronic and CD spectra of III–VI and IX–XI display two intense bands at 280–290 and 220–225 nm. These can be assigned to the $\pi \rightarrow \pi^*_1$ and $\pi \rightarrow \pi^*_2$ transitions, respectively, which characterize the Vitamin B_6 group of compounds (Table IV).[27]

The tetrahydropyrido[3,4-*d*]imidazole compounds derived from L-histidine or L-histidine methyl ester should consist of 4-C epimeric mixtures. It is expected, though, that cyclization of the Schiff base intermediates occurs with some degree of stereoselectivity, and, in each case, one of the two 4-C epimers should predominate. The ^{1}H NMR spectra of III and VI show, in fact, a single 4-CH resonance near δ 6. However, the spectra of the corresponding tetrahydropyridines derived from salicylaldehyde, IX and XI, show two resonances referable to 4-CH protons. In the ^{1}H NMR spectrum of IX these two signals occur at δ 5.84 and 6.07 (ratio 75:25), and two sets of resonances are also clearly exhibited by the 6-CH proton and by the 2-H proton of the fused imidazole residue. The spectrum remains unchanged when the sample is heated up to 90 °C in D_2O solution. Such behavior can be interpreted in terms of the presence of either two very stable conformers, the interconversion of which is slow on the NMR time scale, or two 4-C epimers. The former possibility, however, is

(29) Abbott, E. H.; Martell, A. E. *J. Am. Chem. Soc.* **1971**, *93*, 5852–5856.
(30) (a) Mondovi, B.; Scioscia-Santoro, A.; Rotilio, G.; Costa, M. T. *Enzymologia* **1965**, *4*, 228–234. (b) Sjaastad, O. V. *Acta Physiol. Scand.* **1967**, *71*, 6–15. (c) Krajewska, J.; Maslinski, Cz. *Eur. J. Pharmacol.* **1969**, *7*, 300–306. (d) Roscoe, H. G.; Kupfer, D. *Anal. Biochem.* **1972**, *47*, 418–425. (e) Sasiak, K.; Kierska, D.; Boguslawski, M.; Maslinski, Cz. *Agents Actions* **1975**, *5*, 25–30.
(31) Schott, H. F.; Clark, W. G. *J. Biol. Chem.* **1952**, *196*, 449–462.
(32) (a) Heyl, D.; Luz, E.; Harris, S. A.; Folkers, K. *J. Am. Chem. Soc.* **1952**, *74*, 414–416. (b) Raso, V.; Stollar, B. D. *Ibid.* **1973**, *95*, 1621–1628.
(33) (a) Fellman, J. H.; Roth, E. S. *Biochemistry* **1971**, *10*, 408–414. (b) Trewyn, R. W.; Nakamura, K. D.; O'Connor, M. L.; Parks, L. W. *Biochim. Biophys. Acta* **1973**, *327*, 336–344. (c) Borri-Voltattorni, C.; Orlacchio, A.; Giartosio, A.; Conti, F.; Turano, C. *Eur. J. Biochem.* **1975**, *53*, 151–160.
(34) (a) Kierska, D.; Maslinski, Cz. *Agents Actions* **1975**, *5*, 471–475. (b) Orlacchio, A.; Borri-Voltattorni, C.; Turano, C. *Biochem. J.* **1980**, *185*, 41–46.
(35) (a) Neary, J. T.; Menecly, R. L.; Grever, M. R.; Diven, W. F. *Arch. Biochem. Biophys.* **1972**, *151*, 42–47. (b) Ebadi, M. S.; Russell, R. L.; McCoy, E. E. *J. Neurochem.* **1968**, *15*, 659–665. (c) Ebadi, M.; Govitrapong, P. *Ibid.* **1979**, *32*, 845–853. (d) Albercrombie, D. M.; Martin, D. L. *J. Biol. Chem.* **1980**, *255*, 79–84.
(36) Leussing, D.; Stanfield, C. K. *J. Am. Chem. Soc.* **1964**, *86*, 2805–2810.
(37) Snell, E. E. *Trends Biochem. Sci. (Pers. Ed.)* **1977**, *2*, 131–135.
(38) In this regard, of special interest is the observation that mammalian histidine decarboxylase, unlike that from bacterial sources, is a pyridoxal phosphate dependent enzyme: (a) Hakanson, R. *Eur. J. Pharmacol.* **1967**, *1*, 383–390. (b) Battersby, A. R.; Joyeau, R.; Staunton, J. *FEBS Lett.* **1979**, *107*, 231–232.
(39) Heinert, D.; Martell, A. E. *J. Am. Chem. Soc.* **1962**, *84*, 3257–3263.

Scheme II



incompatible with the observation of a single species, i.e., a single set of resonances, in the ^{1}H NMR spectra of the pyridoxyl tetrahydropyridines and appears to require an exceedingly high activation free enthalpy for the assumed conformational interconversion process. The NMR behavior of XI is similar (4-CH signals at δ 5.32 and 5.98, ratio 40:60), but in this case the spectrum was recorded in CD_3OD, for the low solubility of the compound in water, and heating of the sample was therefore limited to 50 °C. The low optical activity displayed by XI (Table IV), however, indicates that the product isolated is probably a mixture of diastereomers at both 4-C and 6-C.[40]

The tetrahydropyrido[3,4-*d*]imidazole compounds derived from pyridoxal, III and VI, therefore, consist of a single isomer, and ^{1}H NMR and CD spectroscopy can be used to provisionally assign their stereochemistry. Regular half-chair conformations, viewed from the imidazole ring toward the tetrahydropyridine ring, for the 4(*S*),6(*S*) (4,6-cis) and 4(*R*),6(*S*) (4,6-trans) isomers are represented by XII and XIII, and XIV and XV, respectively (Scheme II). In the ^{1}H NMR spectra, the splitting pattern of 6-CH proton should account for axial–axial and axial–equatorial couplings with the 7-CH_2 protons in XII and XIV and for axial–equatorial and equatorial–equatorial couplings in XIII and XV. The experimental values of $J_{AX} + J_{BX}$ (A = H(7), B = H(7′), X = H(6)) for III and VI are 16.8 and 15.2 Hz, respectively. These data agree with the conformations containing the axial–axial coupling (XII and XIV), since the axial–equatorial and especially the equatorial–equatorial couplings usually show rather small splittings. The CD spectra of III and VI are apparently consistent with this result. In XII–XV the left- or right-handed sense of ring chirality is described by its *M* or *P* helicity, respectively. According to the helicity rule of the cyclohexane (piperideine, dihydropyrane, ...) ring, which applies also to tetralines, tetraisoquinolines, etc.,[41] *P* helicity leads to positive Cotton effects within the bands at the longest wavelengths and *M* helicity to negative ones. Therefore, the negative CD band at 290 nm displayed by III and VI (Table IV) reflects *M* helicity of the tetrahydropyridine ring (XII or XIV). To decide whether III and VI are 4,6-cis (XII) or 4,6-trans (XIV) isomers, we note that the 4-CH signal in their proton NMR spectra is long-range coupled (J = 1–2 Hz) with the 7-CH_2 protons (Table III). This was confirmed by appropriate spin-decoupling experiments, as shown in Figure 1c for III. Such a coupling can be accounted for if the proton at 4-C is in an equatorial position, as in XIV. Therefore, we assign the 4,6-trans stereochemistry to III and VI. The presence of an axial pyridoxyl group in XIV may make uncertain the assumption of this as the more stable trans conformer. The axial pyridoxyl group, however, is devoid of the interaction due to $A^{(1,2)}$ strain[42] between position 4 and the imidazole N–H. Recent studies in the field of 1,2,3,4-tetrahydro-β-carbolines have shown that this type of interaction is significant.[43] This interpretation seems supported by the finding that of the two 4-CH signals observed in the NMR spectra of IX and XI, that lying at lower field is a singlet, while that at higher field is a triplet (J = 1.7–2.0 Hz). These should correspond to protons in axial and equatorial 4-CH positions, respectively, and, at least in the case of IX, to the epimeric 4,6-cis and 4,6-trans salicyl tetrahydropyrido[3,4-*d*]imidazole derivatives. Moreover, the 6-CH signals of these 4,6-trans and 4,6-cis epimers of IX, centered at δ 4.22 and 4.02, respectively, show $J_{AX} + J_{BX}$ values of 16.8 (trans) and 15.0 Hz (cis) and indicate structures corresponding to XIV (trans) and XII (cis) as the most stable conformers for these isomers. Although only the 4,6-trans isomers of III and VI were isolated, even in the case of pyridoxal the tetrahydropyridine ring forming reaction does not occur with complete stereoselectivity. For instance, the small signals at δ 6.15 and 2.50 in Figure 1b must be attributed to the 4-CH and 2′-CH_3 groups of the minor isomer of III.

Zinc(II) Complexes of Schiff Bases. IR and NMR Spectra. The easy cyclization to pyridoxyl tetrahydropyrido[3,4-*d*]imidazole compounds prevents the isolation of Schiff bases from pyridoxal and histidine derivatives in reasonable purity and yield. The corresponding imines derived from salicylaldehyde can possibly be prepared, but the synthesis of zinc(II) complexes of Schiff bases is more easily carried out by mixing the reagents in 1:1:1 ratios in neutral aqueous methanol solution. The complexes precipitate as light yellow solids. In these conditions cyclization to tetrahydropyridines does not occur, and also zinc(II) complexes of Schiff bases derived from pyruvic acid can be obtained, despite the rather low reactivity of this carbonyl compound toward amino acids in metal-free systems.[44] It is possible, therefore, that besides functioning as a trap for the Schiff base, the metal ion is kinetically active in promoting Schiff base formation.[44] A number of zinc(II) complexes of Schiff bases derived from amino acids with nonpolar side chains were also prepared to gain a full insight into the conformational properties of this class of compounds. The complexes isolated from the reaction mixtures were in a sufficiently pure form. Recrystallization was not effected for the known thermal lability of these systems, especially when containing amino ester groups.[8,45] It is worth mentioning that if the synthesis of zinc complexes of imines derived from pyridoxal is carried out in slightly acidic solution, the resulting products are contaminated by large amounts of the corresponding complexes containing the pyridine ring protonated form of pyridoxal and one additional counterion (usually chloride, when this ion is present).[4a,5] We have also found contamination by the hydrochloride salt in complexes of the type Zn(pdx-aa) prepared in neutral medium when pyridoxal was not freed from its hydrochloride salt.[46]

The Schiff base structure of the ligand in the zinc(II) complexes is readily assigned on the basis of their spectral properties. The IR spectra of complexes derived from pyridoxal or salicylaldehyde show a strong band at 1630–1640 cm^{-1} (Table V,[21] VII) which is related to the stretching mode of the imine C=N bond[6,10] and is absent in the IR spectra of the tetrahydropyrido[3,4-*d*]imidazole compounds III–VI and IX–XI (this ν(C=N) band occurs at 1660

(40) In a separate preparation of XI carried out in the same conditions as described in the Experimental Section but at pH ~10, an analitically pure but optically inactive material was obtained. It is likely that 6-C diastereomers are formed by racemization at α-carbon atom of the Schiff base intermediate before cyclization to the tetrahydropyridine takes place, since in the case of VI a single isomer is obtained.

(41) (a) Snatzke, G.; Kajtâr, M.; Snatzke, F. In "Fundamental Aspects and Recent Developments in ORD and CD"; Ciardelli, F.; Salvadori, P., Eds.; Heyden: London, 1973; Chapter 3.4. (b) Snatzke, G. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 363–377. (c) Craig, J. C.; Lee, S.-Y. C.; Chan, P. K.; Wang, I. Y.-F. *J. Am. Chem. Soc.* **1977**, *99*, 7996–8002. (d) Sagiv, J. *Tetrahedron* **1977**, *33*, 2315–2320.

(42) Johnson, F. *Chem. Rev.* **1968**, *68*, 375–413.

(43) Ungemach, F., Soerens, D.; Weber, R.; DiPierro, M.; Campos, O.; Mokry, P.; Cook. J. M.; Silverton, J. V. *J. Am. Chem. Soc.* **1980**, *102*, 6976–6984.

(44) Leussing, D. L. In "Metal Ions in Biological Systems"; Sigel, H., Ed.; Marcel Dekker: New York, 1976; Vol. 5, Chapter 1.

(45) (a) O'Connor, M. J.; Ernst, R. E.; Schoenborn, J. E.; Holm, R. H. *J. Am. Chem. Soc.* **1968**, *90*, 1744–1752. (b) Gillard, R. D.; Wootton, R. *J. Chem. Soc. B* **1970**, 364–371.

(46) Contamination by salts at the pyridine nitrogen may be the origin of the exceedingly low values found for hydrogen in the elemental analyses of similar (*N*-pyridoxylideneamino acidato)copper(II) complexes which were instead supposed to contain large amounts of lattice water.[6]

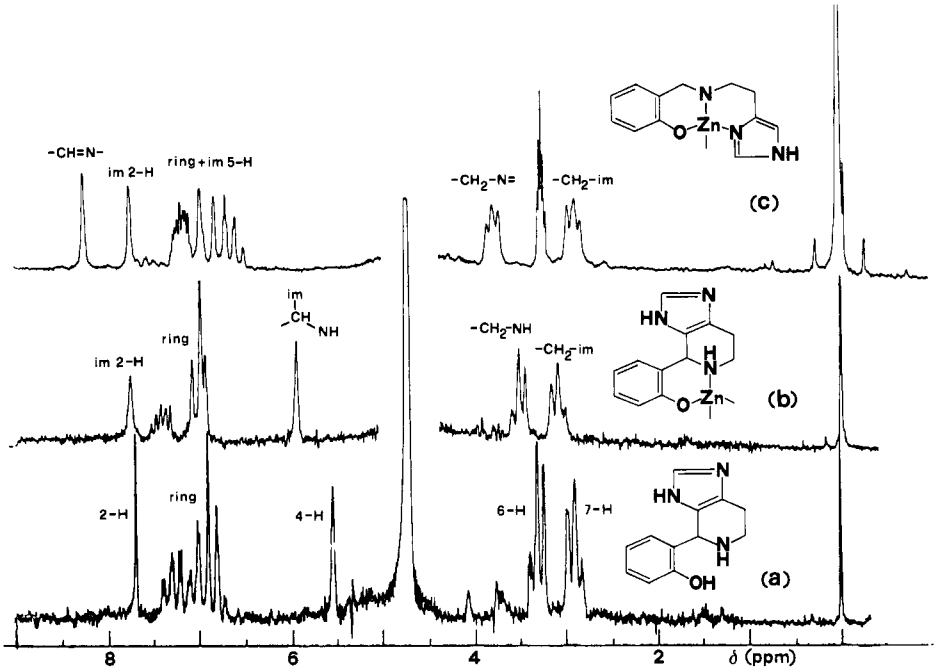


Figure 3. Proton NMR spectra of (a) the 4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole compound X in D_2O, (b) the zinc(II) complex of X[51] in D_2O, and (c) Zn(sal-him)Cl, recorded in CD_3OD–Me_4Si (im = imidazole).

−1670 cm^{-1} in the spectra of pyruvic acid derivatives). In the IR spectra of complexes containing an amino acid carboxylate group the azomethine band is flanked by the asymmetric carboxylate stretch, ν_{as}(COO), near 1600 cm^{-1}.[47] This is clearly broadened on its low-energy side by overlap with aromatic ring–carbon stretching when phenyl or pyridine nuclei are present. The position of the symmetric carboxylate stretch, ν_s(COO), is uncertain and is probably obscured by other absorptions. It may occur in the region 1370–1400 cm^{-1}, where the derivatives of histamine or histidine methyl ester exhibit sharper absorptions. In the spectra of Zn(sal-L-hisOCH_3)Cl and Zn(pdx-L-hisOCH_3)Cl the ester carbonyl stretching frequencies do not indicate significantly strong interaction of the C=O group with the metal and are in the range of other noncoordinated amino ester groups.[1,45a,48] The complexes derived from salicylaldehyde possess a medium band at 1545 cm^{-1} which is lacking in the spectra of the other zinc complexes. Previous assignment of this band to carboxyl stretch, ν(C=O), lowered in position by coordination to the metal ion of an adjacent molecule,[10] is clearly incorrect since the band is present unshifted in the spectra of Zn(sal-him)Cl and Zn(sal-L-hisOCH_3)Cl. This band may originate from the coupling between the C=N bond and the phenyl ring.[49] Corresponding bands in the spectra of complexes derived from pyridoxal may be shifted to higher or lower energy. Most of the absorption bands related to the imidazole group of histidine or histamine residues are hardly discernible within other absorptions. However, typical for the imidazole nucleus can be considered a weak band near 3150 cm^{-1}, assigned to ν(CH), and a weak to medium but clearly recognizable band at 1510 cm^{-1}, assigned to ν(C=C).[50] These bands are absent, for instance, in the IR spectra of Zn(sal-L-ala) and Zn(sal-L-phe), and only Zn(pdx-L-phe) exhibits very weak absorption in the 1510–1520-cm^{-1} region. Broad bands roughly centered at 3400 cm^{-1} in the spectra of all zinc complexes are related to imidazole ν(N-H) and to water or hydroxy group absorptions.

Figure 3 compares the ^1H NMR spectrum of Zn(sal-him)Cl with that of the tetrahydropyrido[3,4-*d*]imidazole compound X and its Zn(II) complex.[51] The azomethine proton of Zn(sal-him)Cl is clearly observed at δ 8.25, while the 4-CH signal of X and of its zinc complex, at δ 5.5–6.0, is absent in the spectrum of Zn(sal-him)Cl. Integration of the multiplet comprised between δ 6.5 and 7.5 accounts for four protons in the spectrum of either X or its zinc complex and for five protons in that of Zn(sal-him)Cl, indicating that the imidazole 5-H signal of this latter compound is included in the multiplet (probably near δ 7). This imidazole 5-H signal occurs, for instance, at δ 7.05 in the ^1H NMR spectrum of Zn(pdx-him)Cl (Table VI). Unfortunately, the zinc(II) complexes derived from histidine are too little soluble for investigating their solution properties by NMR. Also, the ^1H NMR

Table VI. Proton NMR Data of the Zn(II) Complexes of Schiff Bases in CD_3OD–Me_4Si

compd	δ[a] (group)
Zn(sal-him)Cl	8.24 (CH=N, s), 7.73 (im 2-CH, s), 6.98 (im 5-CH, s), 6.5–7.3 (ring, m), 3.78 (CH_2N=, t, J = 5.5 Hz), 2.87 (CH_2-im, t)
Zn(pdx-him)Cl	8.84 (CH=N, s), 8.00 (im 2-CH, s), 7.56 (pdx 6-H, s, br), 7.05 (im 5-CH, s), 4.85[c] (pdx 5-CH_2, s), 3.8–4.1 (CH_2N=, m), 2.8–3.3 (CH_2-im, m), 2.47 (pdx 2-CH_3, s)
Zn(sal-L-hisOCH_3)-CH_3COO[b]	8.35 (CH=N, s), 7.86 (im 2-CH, s), 6.5–7.4 (ring, im 5-CH, m), ~4.5[c] (CHN=, m), 3.70 (OCH_3, s), ~3.3[c] (CH_2-im, m), 2.03 (CH_3COO, s)
Zn(pdx-L-hisOCH_3)-CH_3COO[b,d]	8.83 (CH=N, s), 7.96 (im 2-CH, s), 7.52 (pdx 6-H, s), 7.11 (im 5-CH, s), 4.70[c] (pdx 5-CH_2, s), 3.66 (OCH_3, s), ~3.4[c] (CH_2-im, m), 2.47 (pdx 2-CH_3, s), 2.00 (CH_3COO, s)

[a] Signal multiplicity is given in parentheses: s = singlet, t = triplet, m = multiplet, br = broad. Imidazole = im; pyridoxal = pdx. [b] Obtained from the chloride by exchange with silver acetate in methanol. [c] Obscured by solvent absorptions. [d] The α-CH resonance seems completely buried under HDO.

(47) Nakamoto, K. "Infrared and Raman Spectra of Inorganic and Coordination Compounds", 3rd ed.; Wiley: New York, 1978; Chapter III-6.
(48) (a) Springer, M. P.; Curran, C. *Inorg. Chem.* **1963**, *2*, 1270–1275. (b) Alexander, M. D.; Busch, D. H. *Ibid.* **1966**, *5*, 602–606.
(49) Ledbetter, J. W., Jr. *J. Phys. Chem.* **1977**, *81*, 54–59.
(50) Hodgson, J. B.; Percy, G. C.; Thornton, D. A. *J. Mol. Struct.* **1980**, *66*, 81–92.

(51) The zinc(II) complex of X was prepared by reacting equimolar amounts of histamine dihydrochloride and salicylaldehyde (2 mmol) in 1:1 water–methanol (20 mL) at pH ~9 for about 40 h. Then, zinc(II) nitrate hexahydrate (2 mmol) was added to the solution, and the precipitate formed was collected by filtration, washed (water–methanol), and dried under vacuum. Anal. Calcd for $ZnC_{12}H_{12}N_3ClO \cdot H_2O$: C, 43.27; H, 4.24; N, 12.62. Found: C, 43.33; H, 4.17; N, 12.11.

Table VII. Electronic and CD Spectra in Methanol Solution and Selected Infrared Data of Zn(II) Complexes of Schiff Bases

compd	Vis-UV λ_{max},[a] nm (ϵ)	CD λ_{max}, nm ($\epsilon_l - \epsilon_r$)	ν,[c] cm^{-1} [mode]
Zn(sal-him)Cl	357 (4570) 270 sh (7650), 265 (7950) 236 sh (18 800), 225 (21 700)		1640 s [ν(C=N)];[e] 1610 sh m, 1550 m, 1510 w [ν(C=C)]
Zn(sal-L-his)·3H$_2$O	366 (5300) 272 (9400) 237 sh (18 000), 223 (21 700)	363 (−6.00) 292 (+0.49), 280 (−1.81) 268 (+0.98) 228 (+10.46)	1640 s [ν(C=N)]; 1610 s [ν_{as}(COO)]; 1600 sh m, 1545 m, 1510 w [ν(C=C)]
Zn(sal-L-hisOCH$_3$)Cl·0.5H$_2$O	364 (5100) 271 (9450) 268 sh (9100), 264 sh (6000) 236 sh (18 400), 226 (19 500)	358 (+1.14) 280 (−0.77) 264 (+1.48) 232 (−1.30), <220 (+3.00)[b]	1740 s [ν(C=O)]; 1630 s [ν(C=N)]; 1600 sh m, 1545 m, 1510 w [ν(C=C)]
Zn(sal-L-ala)·1.5H$_2$O	360 (5200) 270 (9450) 235 (23 000), 223 (23 800)	360 (−1.20) 283 (+0.45), 268 (−1.70) 235 (+1.87)	1640 s [ν(C=N)]; 1610 s [ν_{as}(COO)]; 1600 sh m, 1550 m [ν(C=C)]
Zn(sal-L-phe)·1.5H$_2$O	361 (5800) 272 (9800) 269 sh (9500) 235 (19 000), 226 (18 500)	359 (−7.60) 286 (+0.39) 270 (−7.60, 265 (−7.60) 230 (+6.32)	1640 s [ν(C=N)]; 1610 s [ν_{as}(COO)]; 1590 sh m, 1545 m [ν(C=C)]
Zn(sal-L-val)·1.5H$_2$O	359 (5500) 272 sh (11300) 269 (11900), 262 sh (9500) 235 (26600), 226 (28400)	358 (−3.44) 272 (−4.45) 256 (+0.37) 235 (+3.85)	1640 s [ν(C=N)]; 1610 s [ν_{as}(COO)]; 1590 sh w, 1545 m [ν(C=C)]
Zn(pdx-him)Cl·2H$_2$O	381 (4500) 273 sh (3950), 267 (4350) 227 (14700)		1640 s [ν(C=N)]; 1590 w, 1510 w [ν(C=C)]
Zn(pdx-L-his)·2H$_2$O	395 (4350) 275 (3460), 270 (3500) 225 (16600)	395 (−3.53) 278 (+1.59) 233 (+7.21)	1630–1580 s, br [ν(C=N), ν_{as}(COO), ν(C=C)]; 1510 m [ν(C=C)]
Zn(pdx-L-hisOCH$_3$)Cl·2H$_2$O	386 (5000) 278 sh (4200), 269 (5050) 227 (18000)	385 (+0.88) 275 (−0.10), 252 (+0.15) 230 (−1.13)	1730 s [ν(C=O)]; 1630 s [ν(C=N)]; 1600 sh m, 1510 m [ν(C=C)]
Zn(pdx-L-phe)·2.5H$_2$O	387 (6900) 280 sh (4300), 273 (5250) 230 (16200)	386 (−6.52) 275 (+1.93) 232 (+5.24)	1640–1580 s, br [ν(C=N), ν_{as}(COO), ν(C=C)]; 1520–1510 vw [ν(C=C)]
Zn(pyv-L-his)·2H$_2$O	260 sh (1100) 235 sh (2000), 222 (3200)	270 (+1.00) 225 (+1.51)	1660 s, 1640 s, 1620 s, 1600 s [ν(C=N), ν_{as}(COO)], 1510 w [ν(C=C)]
Zn(pyv-L-ala)	270 sh (660) 230 sh (2800), 210 (4370)	280 sh (+0.82) 242 (+3.13), <220 (−2.06)[b]	1670 s [ν(C=N)]; 1600 vs [ν_{as}(COO)]
Zn(pyv-L-phe)·0.3H$_2$O	262[d] (3100) 219 (5270)	280 (+0.90), 250 (+1.41) <220 (−5.18)[b]	1670 s [ν(C=N)]; 1600 vs, br [ν_{as}(COO), ν(C=C)]

[a] Shoulder = sh. [b] Maximum below solvent cutoff, $\Delta\epsilon$ in parentheses is at 220 nm. [c] Recorded as Nujol mulls. [d] Resolved shoulders on this band appear at 269, 256, and 250 nm. [e] s = strong; m = medium; w = weak; br = broad; sh = shoulder.

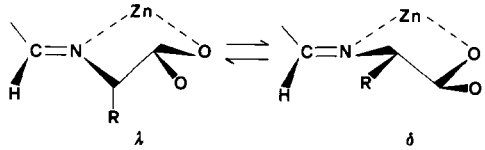
spectra of Zn(sal-L-hisOCH$_3$)$^+$ and Zn(pdx-L-hisOCH$_3$)$^+$ (Table VI) could be recorded only on their acetate salts, after exchange of the chloride salts with silver acetate in methanol. From the data in Table VI it can be seen that the position of the azomethine proton signal occurs near δ 8.3 in complexes derived from salicylaldehyde and near δ 8.8 in those derived from pyridoxal, in agreement with previous assignments of similar zinc(II) complexes of amino acid Schiff bases.[8,45a,52]

Electronic and Circular Dichroism Spectra. The electronic and CD spectra for the present series of zinc(II) chelates of amino acid Schiff bases are summarized in Table VII, and representative spectra are given in Figures 4 and 5. In general, the zinc complexes derived from salicylaldehyde and pyridoxal possess a low-energy absorption band near 360 and 390 nm, respectively, which can be attributed to a $\pi \rightarrow \pi^*$ transition originating mainly in the azomethine chromophore. The bathochromic shift undergone by this band, compared to its position in the free amino acid Schiff bases,[27,28,53] is related to an increase of conjugation occurring in

(52) Gansow, O. A.; Holm, R. H. *J. Am. Chem. Soc.*, **1969**, *91*, 573–581.

(53) The dianion form of the amino acid Schiff base should be considered.

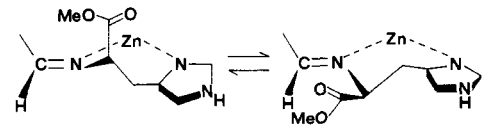
the molecules upon coordination.[12,54] Comparable shifts can be observed in the spectra of corresponding complexes containing other metal ions.[5-8,12,18,27] At higher energies, the zinc(II) complexes of salicylidene- and pyridoxylideneamino acid Schiff bases exhibit intense absorption bands, with maxima near 270 and 225 nm, associated with benzene or pyridine ring $\pi \rightarrow \pi^*$ transitions. Badly resolved shoulders on these bands appear in the spectra of most of the chelates. The CD spectra are more informative than the corresponding absorption spectra. Careful comparison of the CD spectra of Zn(sal-L-aa) complexes shows that the sign pattern of the Cotton effects associated with the main bands, near 360, 270, and 230 nm, corresponding to the maxima of electronic absorption, is constant within the entire series (Figure 4). This behavior must reflect the adoption of a common conformation by the coordinated ligands. It is rather well established on the basis of X-ray structural analysis of a number of (salicylidene)-, (pyridoxylidene)- and related (amino acidato)metal complexes[11,15] that the amino acid chelate ring of the coordinated Schiff base ligand is in a puckered λ conformation when the amino acid has L absolute configuration. In this conformation of the ligand, the amino acid side chain is in a pseudoaxial position and shows the least amount of steric interaction with the azomethine hydrogen atom. In solution, the complexes can undergo a conformational inversion such that the amino acid side chain will occupy either a pseudoxial or pseudoequatorial position, depending on whether the chelate ring conformation is λ (XVI) or δ (XVII). However,



XVI XVII

the similarity of the CD spectra within the entire Zn(sal-L-aa) series, and especially the increasing magnitude of the Cotton effects with increasing the bulkiness of the amino acid side chain (Table VII), indicates that these complexes prefer the λ conformation (XVI). The complex Zn(sal-L-his) behaves like the other Zn(sal-L-aa) complexes; therefore the histidine residue chelates through the imine nitrogen and carboxylate oxygen donors (glycine-like). Imidazole coordination can occur in roughly an apical position, but this is difficult to ascertain, since there are no prominent features in the electronic or CD spectra which can be directly related to imidazole coordination. Thus, for instance, the electronic spectrum of Zn(sal-him)Cl is very similar to those of Zn(sal-L-aa) complexes. This result is not unexpected, given the spectral transparency of bis(L-histidinato)zinc(II) and of zinc(II)–imidazole complexes at wavelengths above ca. 225 nm.[55]

Consistent with this conformational model is the observation that the CD spectrum of Zn(sal-L-hisOCH$_3$)Cl displays a sign pattern of the bands near 360, 270, and 230 nm opposite to that of the Zn(sal-L-aa) complexes and appears almost enantiomeric to the CD spectrum of Zn(sal-L-ala) (Figure 4). In Zn(sal-L-hisOCH$_3$)Cl the ester carbonyl group is noncoordinated, and the histidine residue can chelate only through the imine and imidazole nitrogen donors (histamine-like). In this conformation the disposition of the L-histidine side chain is the same as for D-amino acids; i.e., if the ester group prefers to be pseudoaxial, the six-membered histidine chelate ring bound through two nitrogen



XVIII XIX

donors will adopt a δ conformation (XVIII). The chirality associated with this conformation is opposite to that of the five-membered chelate ring of amino acids with the same absolute configuration bound through one amino nitrogen and one carboxyl oxygen donors, and this leads to the observed inversion of the Cotton effects.

The zinc complexes derived from pyridoxal display the same behavior as their salicyl analogues. Thus, the signs of the CD bands of Zn(pdx-L-aa) complexes near 390, 270, and 230 nm, corresponding to the maxima of electronic absorption, bear a mirror-image relationship to those of Zn(pdx-L-hisOCH$_3$)Cl (Figure 5). The amino acid chelate ring, therefore, prefers a λ conformation in Zn(pdx-L-aa) (XVI) and a δ conformation in Zn(pdx-L-hisOCH$_3$)Cl (XVIII). It can be noted that for zinc(II) complexes derived from either pyridoxal or salicylaldehyde the Cotton effects associated with the azomethine and the higher energy benzenoid band are dissignate, but their signs hold constant for a given conformation of the ligand. Although the preferred conformation of the coordinated amino acid residue is not simply related to the absolute configuration at the α-carbon atom, we have the important result that the preferred conformation is dictated by the mode of binding of the amino acid residue. Therefore, ambiguities can arise only in the case of potentially tridentate amino acids such as histidine. For amino acids containing nonpolar side chains, the sign of the Cotton effect at 360–390 nm or at 230 nm in the CD spectra of their Zn(sal-aa) and Zn(pdx-aa) derivatives correlates with the absolute configuration of the amino acid. Thus, for instance, a negative azomethine CD band corresponds to negative (λ) conformation chirality (left-handed helicity)[56] and L absolute configuration of the amino acid residue. A similar unambiguous correlation between the CD spectrum and the absolute configuration of the amino acid is not found in the metal-free systems. For instance, in the case of *N*-salicylidene-L-amino acids the Cotton effect associated with the azomethine band is positive for the derivatives of aliphatic L-amino acids, and negative for those of β-aryl-L-α-amino acids (including histidine).[57,58] The interpretation of the CD spectra of zinc(II) complexes derived from pyruvic acid is less straightforward. The CD spectrum of Zn(pyv-L-his) seems too dissimilar to those of Zn(pyv-L-ala) and Zn(pyv-L-phe) to involve a common ligand conformation (Table VII). On the basis of the order of stability of fused chelate ring systems,[44,59,60] the five-membered chelate ring of the pyruvic residue in Zn(pyv-L-his) should favor the adoption of the six-membered (histamine-like) over the five-membered (glycine-like) chelate ring structure by the histidine residue. This has been actually found in the case of the corresponding copper(II) complexes.[18] However the CD features of Zn(pyv-L-his) are too little informative to infer the histidine coordination mode. It is possible that these complexes involving pyruvate give rise to mixed species in solution and that a consistent contribution to the equilibrium species is given by carbinolamine complexes.[60,61] An indirect evidence for the

(54) (a) Bosnich, B. *J. Am. Chem. Soc.* **1968**, *90*, 627–632. (b) Downing, R. S.; Urbach, F. L. *Ibid.* **1969**, *91*, 5977–5983. (c) Perry, S. J.; Quinn, R. S.; Dudek, E. P. *Inorg. Chem.* **1968**, *7*, 814–818.

(55) Fawcett, T. G.; Bernaducci, E. E.; Krogh-Jespersen, K.; Schugar, H. J. *J. Am. Chem. Soc.* **1980**, *102*, 2598–2604.

(56) (a) IUPAC *Inorg. Chem.* **1970**, *9*, 1–5. (b) Hawkins, C. J. "Absolute Configuration of Metal Complexes"; Wiley-Interscience: New York, 1971; Chapter 1.

(57) (a) Smith, H. E.; Burrows, E. P.; Marks, M. J.; Lynch, R. D.; Chen, F.-M. *J. Am. Chem. Soc.* **1977**, *99*, 707–713. (b) Smith, H. E.; Neergaard, J. R.; Burrows, E. P.; Chen, F.-M. *Ibid.* **1974**, *96*, 2908–2916. (c) Smith, H. E.; Padilla, B. G.; Neergaard, J. R.; Chen. F.-M. *J. Org. Chem.* **1979**, *44*, 1690–1695.

(58) Although specific rules have been derived to correlate the CD spectra of the *N*-salicylidene derivatives of a number of amino acids with their absolute configuration,[57a] the interpretation of the CD spectra is complicated by the presence in solution of enamine and Schiff base tautomers. These species are present in comparable amounts at the equilibrium,[28b] and each contributes to the CD spectrum with a number of low-energy conformers. Only the Schiff base tautomer, however, has been considered for spectral correlation.

(59) (a) Nakahara, A.; Yamamoto, H.; Matsumoto, H. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 1137–1142. (b) Nakao, Y.; Ishibashi, H.; Nakahara, A. *Ibid.* **1970**, *43*, 3457–3461.

(60) Leussing, D. L.; Hanna, E. M. *J. Am. Chem. Soc.* **1966** *88*, 693–696, 696–699.

(61) (a) Leussing, D. L.; Stanfield, C. K. *J. Am. Chem. Soc.* **1964**, *86*, 2805–2810. (b) Leussing, D. L.; Schultz, D. C. *Ibid.* **1964**, *86*, 4846–4850.

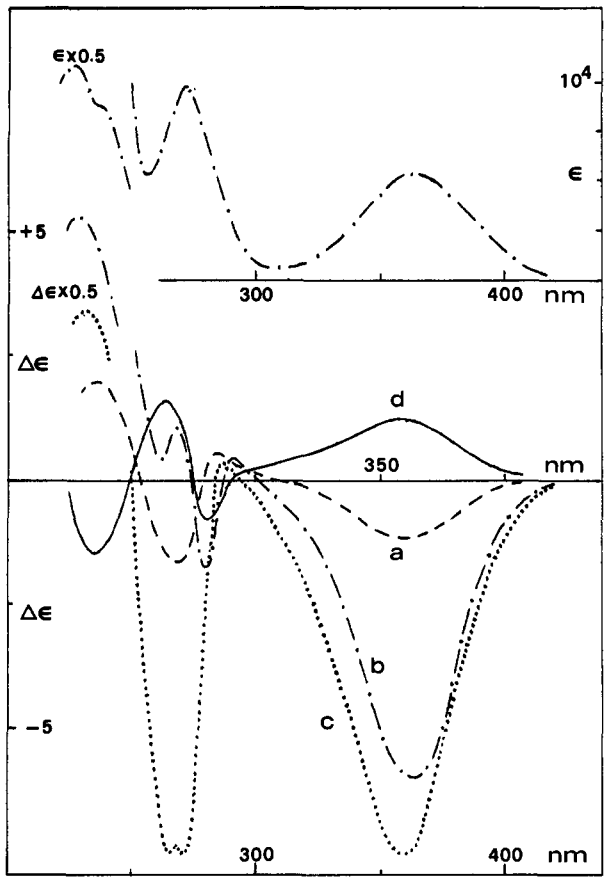


Figure 4. Circular dichroism spectra in methanol solution of (a) ---, Zn(sal-L-ala), (b) ····, Zn (sal-L-his), (c) ···, Zn(sal-L-phe), and (d) —, Zn(sal-L-hisOCH$_3$)Cl. The electronic spectrum of Zn(sal-L-his) is also reported.

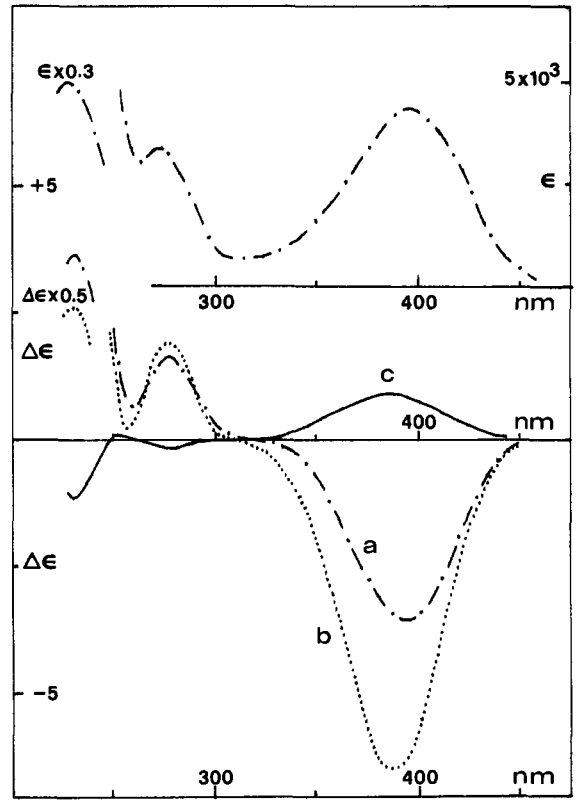


Figure 5. Circular dichroism spectra in methanol solution of (a) ····, Zn(pdx-L-his), (b) ···, Zn(pdx-L-phe), and (c) —, Zn(pdx-L-hisOCH$_3$)Cl. The electronic spectrum of Zn(pdx-L-his) is also reported.

presence of some strain in these systems is our inability to obtain complexes of the type Zn(pyv-L-hisOCH$_3$)X. Repeated preparations of this complex led to the isolation of products in which complete hydrolysis of the amino ester group had occurred.

Conclusions

The condensation between pyridoxal, or salicylaldehyde, and derivatives of histidine, or histamine, in weakly basic medium proceeds through the formation of a Schiff base intermediate and leads to 4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole compounds. The formation of these products seems to be linked to the inhibitory activity of a number of pyridoxal-dependent enzymes. The reaction of pyridoxal with L-histidine or L-histidine methyl ester occurs with a rather high degree of stereoselectivity to provide the 4,6-trans tetrahydropyrido[3,4-*d*]imidazole derivative as the major isomer. The stereochemistry of these products has been assigned on the basis of their ^{1}H NMR and CD spectra.

Zinc(II) ions can function as a trap for the Schiff base intermediates, and a number of zinc(II) complexes of amino acid–pyridoxal and –salicylaldehyde Schiff bases have been prepared by template synthesis in neutral aqueous methanol. These zinc(II) chelates exhibit interesting conformational properties and provide a basis for the qualitative understanding of stereochemical aspects of Vitamin B_6 model reactions. Dunathan has suggested that cleavage of a bond to the amino acid α-carbon atom can be accomplished by orienting that bond perpendicular to the plane of the extended conjugated system in order to optimize orbital interactions.[62] This stereoelectronic requirement seems completely fulfilled in pyridoxal-dependent enzymic reactions[63] and has been recently recognized for the cleavage of the C_α–H bond in model reactions involving free[64] or metal complexed[65] amino acid–pyridoxal Schiff bases. When the cleavage of this C_α–H bond is taken into account, the first step in a number of pyridoxal-mediated enzymic reactions reproduced by model systems,[2,3] it can be noted that the preferred conformation of Zn(pdx-L-aa) and Zn(sal-L-aa) complexes (λ, XVI) carries the C_α–H bond in an unfavorable (equatorial) position for an easy breaking process. Only in the minor conformer (δ, XVII) of these complexes the C_α–H bond is perpendicular to the coordination plane containing the conjugated system. A similar situation is present in the corrsponding Cu(pdx-L-aa)[18] and Cu(sal-L-aa)[12] complexes, and this qualitatively accounts for the low reactivity attainable by model systems. The magnitude of the Cotton effects (Table VII) indicates that the amount of δ conformer to the conformational $\lambda \rightleftharpoons \delta$ equilibrium decreases with increasing the bulkiness of the amino acid side chain. On steric grounds, therefore, it is expected that cleavage of the C_α–H bond will be hindered by the presence of bulky side chains on the amino acid residues. This effect is apparently operating in the observed trend of transamination rates of zinc(II)–amino acid–pyridoxal 5-phosphate systems.[66]

A special interest is deserved by the complexes containing a histidine residue. Simple metal complexes with this potentially tridentate amino acid undergo competing equilibria in solution between its' various chelating forms,[67] and it is usually difficult to infer structural–spectral correlations of the complexes. The relatively rigid structure of the Schiff base ligand in zinc(II) complexes derived from pyridoxal or salicylaldehyde, and their copper(II) analogues,[18] provides an useful frame for correlating the glycine-like or histamine-like coordination mode of histidine residues and the chiroptical properties of the complexes.

Acknowledgment. This work was supported by the Italian

(62) (a) Dunathan, H. C. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 712–716. (b) Dunathan, H. C. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1971**, *35*, 79–134.
(63) Vederas, J. C.; Floss, H. G. *Acc. Chem. Res.* **1980**, *13*, 455–463.
(64) Tsai, M. D.; Weintraub, H. J. R.; Byrn, S. R.; Chang, C.; Floss, H. G. *Biochemistry* **1978**, *17*, 3183–3188.
(65) Fischer, J. R.; Abbott, E. H. *J. Am. Chem. Soc.* **1979**, *101*, 2781–2782.
(66) Hopgood, D. *J. Chem. Soc., Dalton Trans.* **1972**, 482–487.
(67) Chow, S. T.; McAuliffe, C. A. *Prog. Inorg. Chem.* **1975**, *19*, 51–103.

C.N.R. We thank Professor Renato Ugo for helpful discussions, Mrs. M. Bonfà for recording the NMR spectra, and Mrs. P. Russo for recording the MS spectra.

Supplementary Material Available: Listings of elemental analysis (Table I), mass spectral data of 4,5,6,7-tetrahydropyrido[3,4-*d*]imidazole derivatives (Table II), and infrared data of zinc(II) complexes of amino acid Schiff bases (Table V) (4 pages). Ordering information is given on any current masthead page.

Angular Distortions at the Carbon Bound to Cobalt in Coenzyme B_{12} Models. Implications with Regard to Co–C Bond Cleavage in Coenzyme B_{12} and Other Alkylcobalamins

Lucio Randaccio,* Nevenka Bresciani-Pahor, Paul J. Toscano, and Luigi G. Marzilli*

Contribution from the Istituto di Chimica, Universita di Trieste, 34127 Trieste, Italy, and the Department of Chemistry, Emory University, Atlanta, Georgia 30322. *Received March 30, 1981*

Abstract: The crystal and molecular structures of the complexes *trans*-bis(dimethylglyoximato)neopentyl(pyridine)cobalt(III) (I) and *trans*-bis(dimethylglyoximato)((trimethylsilyl)methyl)(pyridine)cobalt(III) (II) are reported. Both compounds crystallize in space group $P3_1$ with a = 8.814(8) Å, c = 23.57 (1) Å, and D_{measd} and D_{calcd} = 1.37 and 1.38 g cm^{-3}, respectively, for I and a = 8.870 (5) Å, c = 24.72 (1) Å, and D_{measd} and D_{calcd} = 1.36 and 1.35 g cm^{-3} respectively, for II. For both, Z = 3 and 2780 reflections were measured. The structure of I was solved by conventional Patterson and Fourier methods. Block-diagonal least-squares refinement led to a final R value of 0.037. The structure of II was refined similarly to a final R value of 0.038 by starting with the coordinates of I and substituting Si. The primary coordination sphere about the Co is pseudooctahedral with the usual arrangement of the dimethylglyoximato (DH) ligands occupying the four equatorial positions and the pyridine and alkyl group trans to each other. The $Co(DH)_2$ unit is relatively planar. Of particular note, the Co–C–Y angle (Y = C or Si) is 130.3 (4)° in I and 127.7 (3)° in II. These unusual values permit the $Y(CH_3)_2$ fragment to lie in a plane nearly parallel to the $Co(DH)_2$ plane. Compound I is the first neopentyl transition-metal compound which has been structurally characterized. Angular distortions are believed to be pronounced in unstable alkylcobalamins, and in the B_{12} coenzyme this angle is 125°. The implications of the present results are discussed in terms of the factors which affect the trans influence and trans effect of the alkyl group. With the addition of the results on I and II, sufficient data are now available on complexes of the type *trans*-alkylbis(dimethylglyoximato)(pyridine)cobalt(III) to discern a clear distortion in the C–Co–N (equatorial) bond angle. Also the Co–N(pyridine) bond length variation in these compounds can now be compared to spectroscopic trends such as ^{13}C NMR shifts. Brief comparisons are made to ligand-exchange reactions.

Wider acceptance has recently been accorded to the possibility that a conformational (mechanochemical) change triggers the important Co–C cleavage step in catalytic reactions involving coenzyme B_{12}.[1-3] Soluton studies on alkylcobalamins (species in which another alkyl group is substituted for 5′-deoxyadenosine in the coenzyme) have led some investigators to suggest that the Co–C bond length is responsive to steric effects in these organocobalt(III) compounds.[4-6] Steric factors are known to be important in destabilizing the Co–C bond in such compounds[7,8] but the exact nature of the distortions, if any, is uncertain. In model compounds, we have found that the Co–C bond length does indeed respond to steric rather than electronic effects.[9,10] The bond lengths in structurally characterized complexes vary over a remarkably broad range of ~0.2 Å.[10] Spectroscopic evidence was presented that even longer bonds occur in more sterically hindered systems which have thus far proved to be too unstable for X-ray structural characterization.

An additional factor, which may be an important controller of Co–C bond lability, is a distortion of the Co–C–C bond angle away from the normal tetrahedral value.[5] Indeed, this angle is 125° in the coenzyme.[11] Such a distortion could be viewed as altering the degree of carbon s and p character used in the bond to Co. Of the sterically hindered alkyl groups employed in studies of alkylcobalamins,[4-6] the neopentyl group frequently exhibited some of the most pronounced effects. However, to our knowledge, no neopentyl organometallic complex has ever been structurally characterized.

We have prepared neopentyl complexes of one class of vitamin B_{12} coenzyme model compounds which are known as cobaloximes (the trivial name for complexes such as those containing the $Co(DH)_2$ unit with DH equal to the monoanion of dimethylglyoxime).[12] Cobaloximes have been shown to respond structurally to changes in the electronic and steric properties of axial ligands in pseudooctahedral complexes of the type $LCo(DH)_2X$, where L equals neutral and X equals negative monodentate ligands, respectively.[9,10,13-16] (See structure **1**).

* Address correspondence as follows: L.R., Universita di Trieste; L.G.M., Emory University.

(1) Halpern, J. *Ann. N. Y. Acad. Sci.* **1974**, *239*, 2.
(2) Pratt, J. M. In "Techniques and Topics in Bioinorganic Chemistry"; McAuliffe, C. A., Ed.; Macmillan: London, 1975; pp 109–204.
(3) Toraya, T.; Krodel, E.; Mildvan, A. S.; Abeles, R. H. *Biochemistry* **1979**, *18*, 417 and references therein.
(4) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2259.
(5) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2267.
(6) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2274.
(7) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601.
(8) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541.
(9) Marzilli, L. G.; Toscano, P. J.; Randaccio, L.; Bresciani-Pahor, N.; Calligaris, M. *J. Am. Chem. Soc.* **1979**, *101*, 6754.
(10) Randaccio, L.; Bresciani-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1980**, *102*, 7372.
(11) Lenhert, P. G. *Proc. R. Soc. London, Ser. A* 1968, *303*, 45.
(12) Schrauzer, G. N. *Acc. Chem. Res.* 1968 *1*, 97.
(13) Bresciani-Pahor, N.; Calligaris, M.; Randaccio, L.; Marzilli, L. G. *Inorg. Chim. Acta* **1979**, *32*, 181.
(14) Randaccio, L.; Bresciani-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *Inorg. Chem.* **1981**, *20*, 2722.
(15) Marzilli, L. G.; Toscano, P. J.; Ramsden, J. H.; Randaccio, L.; Bresciani-Pahor, N. *Adv. Chem. Ser.* **1981**, *No. 196*.
(16) Bresciani-Pahor, N.; Calligaris, M.; Randaccio, L. *Inorg. Chim. Acta* **1980**, *39*, 173.

0002-7863/81/1503-6347$01.25/0