Conformations of Pyridoxal Schiff Bases of Amino Acids. A Circular Dichroism Study

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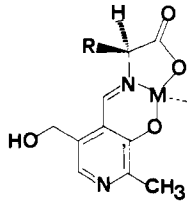
Abstract: The conformations of a series of pyridoxal–L-amino acid Schiff bases in methanol solution were deduced from their circular dichroism spectra. The *N*-salicylidene analogues of the pyridoxal Schiff bases were used as appropriate reference compounds. The predominant conformation of the C_α–N bond was found to be approximately the same for all the Schiff bases and involves a pseudoequatorial disposition of the C_α–H bond with respect to the plane of the extended π system. The sign of the Cotton effects near 420 and 340 nm varies with the nature (polar, nonpolar, aromatic) of the amino acid side chain, according to the chirality of the dominant interaction of the amino acid residue with the pyridoxylideneamino chromophore. The circular dichroism spectra of zinc(II) complexes of *N*-pyridoxylidene-L-amino acids were also discussed in relation to the metal-free systems.

Pyridoxal phosphate is the essential cofactor for most of the enzymic reactions undergone by amino acids during metabolism.[1] Pyridoxal catalysis usually involves formation of a Schiff base with the amino acid and labilization of one of the three bonds to the amino acid α-carbon atom. According to Dunathan[2] the bond to be labilized must be oriented perpendicular to the π system of the Schiff base; thus, a key function of the enzyme is to achieve reaction specificity by controlling the conformation of the C_α–N bond. This stereoelectronic requirement seems fulfilled in pyridoxal-dependent enzymic reactions[3,4] and has been recognized for the C_α–H bond cleavage in model systems involving free[5] and metal-complexed[6] pyridoxal–amino acid Schiff bases.

We have recently investigated the stereochemistry of zinc(II)[7] and copper(II)[8] complexes of *N*-pyridoxylideneamino acids (I)



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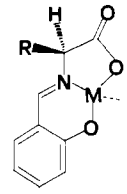
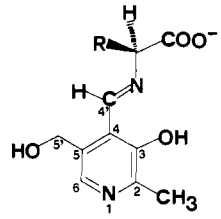


II

and *N*-salicylideneamino acids (II) and found that the preferred conformation of the amino acid residue can be easily inferred from the circular dichroism spectra of the complexes. This is possible because the conformational mobility about the C_α–N bond in those systems is restricted within the amino acid chelate ring. In free pyridoxal–amino acid Schiff bases (III), a much wider range of



III

conformations about the C_α–N bond is theoretically possible, though recent nuclear magnetic resonance studies[9] and semiempirical conformational calculations[5] indicate that the number of preferred conformers is rather limited. In this paper we wish to relate the features of the CD spectra of pyridoxal–amino acid Schiff bases to the preferred conformations of the C_α–N bond. Despite the importance of recognizing the stereochemical factors that rule the conformational preferences in model systems, detailed CD studies of *N*-pyridoxylideneamino acids are completely lacking, and correlations of the CD spectra with the conformations of amino acid Schiff bases are currently limited to *N*-salicylideneamino acids.[10]

Experimental Section

All reagents were of the highest commercial grade available. The compounds L-N^τ-methylhistidine,[11] (*R*)-*trans*-1,2-cyclohexanediamine, and (*R*)-1,2-propanediamine[12] were prepared according to literature procedures. The normal Schiff bases were prepared in situ by mixing equimolar amounts of free pyridoxal (0.1 mmol), the amino acid, and methanolic 0.1 M sodium hydroxide in degassed absolute methanol (final volume 10 mL) and stirring for 2 h at room temperature under a stream of dry nitrogen. Aliquots of the solutions were diluted 1:25 immediately before the spectral measurements. In the case of L-glutamic acid and L-aspartic acid, 2 molar equiv of methanolic sodium hydroxide was used, while for L-cysteine the final volume was 100 mL and the reaction was followed spectrally without further dilution. The spectra of pyridoxal–L-histidine and –L-N^τ-methylhistidine Schiff bases were recorded immediately after mixing of the reagents and 1:25 dilution. The pyridoxal–L-3,4-dihydroxyphenylalanine Schiff base was prepared by mixing solutions of sodium L-3,4-dihydroxyphenylalaninate (0.1 mmol) and free pyridoxal (0.1 mmol) in degassed absolute methanol (final volume 100 mL); the spectra were recorded immediately after 2:5 dilution. The pH of all the reaction solutions ranged between 8.5 and 9.4. The Schiff bases between pyridoxal or salicylaldehyde and (*R*)-*trans*-1,2-cyclohexanediamine or (*R*)-1,2-propanediamine were obtained by warming under nitrogen in a water bath for 2 h equimolar amounts of the reagents (1 mmol) in absolute methanol (final volume 100 mL). Solutions of the zinc(II) complexes of *N*-pyridoxylidene-L-alanine, -L-valine, and -L-leucine were obtained by stirring for 2 h zinc(II) nitrate hexahydrate (0.1 mmol), pyridoxal hydrochloride (0.1 mmol), the amino acid (0.1 mmol), and methanolic 0.1 M sodium hydroxide (0.3 mmol) in absolute methanol (final volume 100 mL). Quartz cells of paths ranging from 0.01 to 0.5 cm were used as needed for observation of the reported electronic absorption and CD maxima. Results are reported in terms of ϵ (molar absorption coefficient) and $\Delta\epsilon = \epsilon_l - \epsilon_r$ (molar CD coefficient), in L mol^{-1} cm^{-1}. Electronic spectra were recorded on a Beckman DK-2A spectrophotometer. Circular dichroism spectra were obtained with a Jobin-Yvonne Mark III dichrograph, calibrated with a solution of isoandrosterone in dioxane ($\Delta\epsilon$ = +3.31 at 304 nm).

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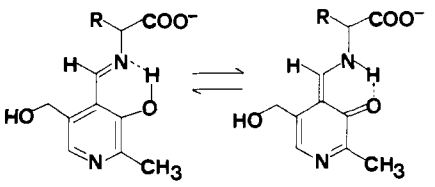
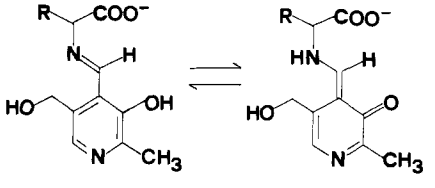
Results

The present spectral investigation was carried out with methanol solutions since in this solvent,[9] but not in water,[13] Schiff base formation between pyridoxal and an amino acid is quantitatively complete. The electronic spectra of pyridoxal–amino acid Schiff bases display four bands near 420, 340, 290, and 250 nm (Table I). The 290-nm band appears as a shoulder on the 250-nm band. Additional poorly resolved shoulders near 270 nm can often be detected, especially in the spectra of the derivatives of aromatic amino acids. Higher energy absorptions occur below 230 nm, the maximum being too close or below the solvent cutoff (220–225 nm) to be clearly detected. In the pH range studied here, the methanol solution of the Schiff base contains mainly the two monoanionic species, the enol imine form IV and the keto enamine



IV V

form V.[9] Intramolecular hydrogen bonding stabilizes the cis conformation of the C_4–$C_{4'}$ bond in both tautomers. The enol imine and keto enamine species can be differentiated by their electronic absorption bands. The bands near 420 and 290 nm characterize the keto enamine form V and are assigned to the $\pi \rightarrow \pi_1^*$ transition (mainly localized in the azomethine chromophore) and to the $\pi \rightarrow \pi_2^*$ transition (more typically a pyridine ring transition), respectively, while the bands near 340 and 250 nm are ascribed to the corresponding $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ transitions of the enol imine form IV.[14] The tautomers IV and V are present in comparable amounts at equilibrium in solution, though the keto enamine form is predominant in aqueous solution while the enol imine form predominates in methanol or nonpolar solvents.[15] Minor species containing ring-protonated forms of IV and V are also contributing to the complex solution equilibria of pyridoxal–amino acid Schiff bases,[15b] while expectation of any significant contributions by trans conformers such as VI and VII



VI VII

have recently been discarded.[9] Dianionic species become increasingly important at higher pH and are predominant at pH ~12. The spectra in such basic solutions were not investigated since the dianions of the Schiff bases contain significant amounts of species with trans conformation of the C_4–$C_{4'}$ bond,[9] giving rise to exceedingly complicated systems for conformational studies.

The circular dichroism spectra of pyridoxal–amino acid Schiff bases are summarized in Table I and representative spectra are given in Figures 1 and 2. In general, all the compounds exhibit CD bands close to the positions of the $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ transitions of tautomers V (~420 and ~290 nm) and IV (~340 and ~260 nm). Although the ~260-nm CD band overlaps considerably with that near 290 nm, its position appears somewhat red shifted compared with the corresponding electronic absorption maximum, and it is possible that transitions other than $\pi \rightarrow \pi^*$ are contributing to the CD activity in this region, for instance the $n \rightarrow \pi^*$ transitions of the azomethine group,[16,17] which are obscured by the more intense $\pi \rightarrow \pi^*$ transitions in the electronic spectra. In addition, the derivatives of aromatic amino acids may also exhibit CD bands between 250 and 300 nm originating from $\pi \rightarrow \pi^*$ ring transitions of the amino acid residue. The CD activity displayed by *N*-pyridoxylideneamino acids below ~230 nm is not considered here because the CD extrema lie too close or below the solvent cutoff to be determined with accuracy. Inspection of Table I shows that for the derivatives of L-amino acids the higher

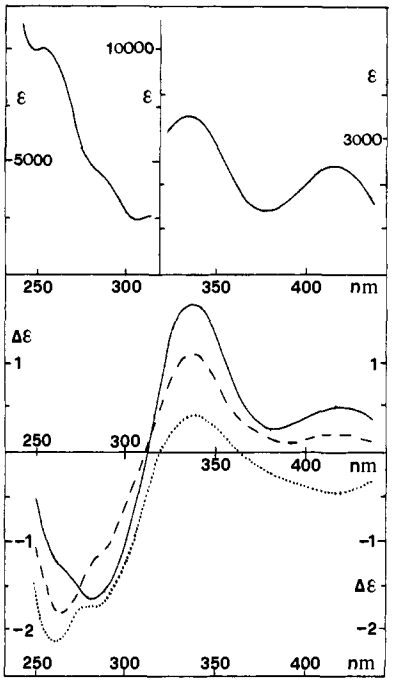


Figure 1. Circular dichroism spectra in slightly basic methanol solution of (a) (—) *N*-pyridoxylidene-L-alanine, (b) (--) *N*-pyridoxylidene-L-serine, and (c) (···) *N*-pyridoxylidene-L-aspartic acid. The electronic spectrum of *N*-pyridoxylidene-L-alanine is also reported.

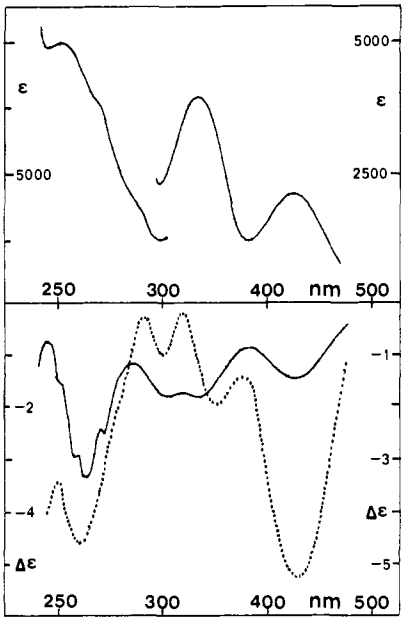


Figure 2. Circular dichroism spectra in slightly basic methanol solution of (a) (—) *N*-pyridoxylidene-L-phenylalanine, and (b) (···) *N*-pyridoxylidene-L-tryptophan. The electronic spectrum of *N*-pyridoxylidene-L-phenylalanine is also reported.

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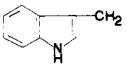
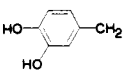
Table I. Electronic and CD Spectra of *N*-Pyridoxylidene Derivatives of α-Amino Acids ($NH_2CH(R)COOH$) Formed in Situ in Methanol

N-pyridoxylidene deriv	R	UV, λ_{max}, nm[a] (ϵ)	CD, λ_{max}, nm ($\epsilon_l - \epsilon_r$)
L-alanine	CH_3	421 (2350) 336 (3550) 285 sh (4300) 251 (10200)	420 (+0.50) 338 (+1.65) 285 (−1.70) 263 sh (−1.25)
L-valine	$(CH_3)_2CH$	420 (2750) 337 (3550) 290 sh (4500) 255 (10000)	420 (+0.43) 340 (+1.38) 293 (−2.95) 268 sh (−1.98)
L-leucine	$(CH_3)_2CHCH_2$	422 (2400) 388 (3900) 290 sh (4300) 255 (10300)	420 (+0.23) 340 (+1.05) 292 (−3.00) 265 sh (−1.88)
L-serine	$HOCH_2$	420 (2250) 336 (3700) 285 sh (3500) 253 (9600)	420 (+0.16) 338 (+1.10) 285 sh (−1.20) 262 (−1.80)
L-threonine	$CH_3C(OH)H$	420 (2150) 336 (3800) 288 sh (4250) 255 (9900)	420 (+0.40) 340 (+1.45) 285 (−1.95) 265 sh (−1.85)
L-methionine	$CH_3SCH_2CH_2$	423 (1650) 336 (3850) 290 sh[b] 253 (9750)	423 (−0.18) 342 (+0.45) 297 (−2.58) 260 sh (−1.00)
L-aspartic acid	$HOOCCH_2$	422 (2750) 335 (3250) 290 sh (4000) 250 sh (9800)	422 (−0.43) 338 (+0.38) 285 sh (−1.75) 262 (−2.15)
L-glutamic acid	$HOOCCH_2CH_2$	420 (2350) 336 (3500) 290 sh (4500) 254 (10000)	422 (−0.17) 340 (+0.38) 290 sh (−1.13) 265 (−1.38)
L-cysteine[c]	$HSCH_2$	405 (715) 335 (1000) 294 (5550) 272 sh (3300), 250 sh (3400)	422 (−0.10) 336 (−0.29) 294 (+2.25) 252 (−2.10)
L-phenylalanine	$C_6H_5CH_2$	425 (2100) 335 (3950) 290 sh (3500) 272 sh (7500) 255 (10000)	423 (−1.48) 334 (−1.83) 302 (−1.80) 270 sh (−2.50) 262 (−3.38)
L-histidine[d]		412 (1400) 335 (2400) 285 sh (5000) 253 (9750)	423 (−0.58) 335 (−0.38) 282 (−1.87) 262 (−2.38)
L-N^{τ}-methylhistidine[d]		423 (2000) 336 (3800) 290 sh[b] 252 sh (11000)	422 (−1.08) 337 (−0.40) 285 sh (−1.50) 265 (−3.02)
L-tyrosine	p-$HOC_6H_4CH_2$	425 (2600) 335 (3750) 285 sh (6000) 270 sh (8000), 250 sh (10000)	424 (−1.88) 340 (−1.93) 285 sh (−1.70) 270 (−3.12)
L-tryptophan		426 (3650) 340 (3750) 290 (8500) 278 sh (10000), 255 (11500)	430 (−5.25) 352 (−1.90) 300 (−1.15) 260 (−4.63)
L-3,4-dihydroxyphenylalanine[e]		406 (1780) 330 sh (1650) 283 (8750) 255 sh (8500)	420 (−0.70) 340 (−0.58) 292 (−2.13) 278 (−1.88)

[a] Shoulder = sh. [b] Poorly resolved. [c] Concentration of the reagents was 10^{-3} M; spectra recorded after 2 h. Some free pyridoxal is present. [d] Spectra recorded within a few minutes after mixing of the reagents. Some free pyridoxal is present. [e] Concentration of the reagents was 10^{-3} M; spectra recorded within a few minutes after mixing of the reagents. Some free pyridoxal is present.

energy CD bands (near 290 and 260 nm) are always of negative sign, while those at lower energy (near 420 and 340 nm) exhibit a sign pattern that depends upon the nature of the amino acid side chain. Thus, for amino acids with nonpolar side chain and for β-hydroxy-α-amino acids the latter two bands are both of positive sign, while for dicarboxylic amino acids and methionine

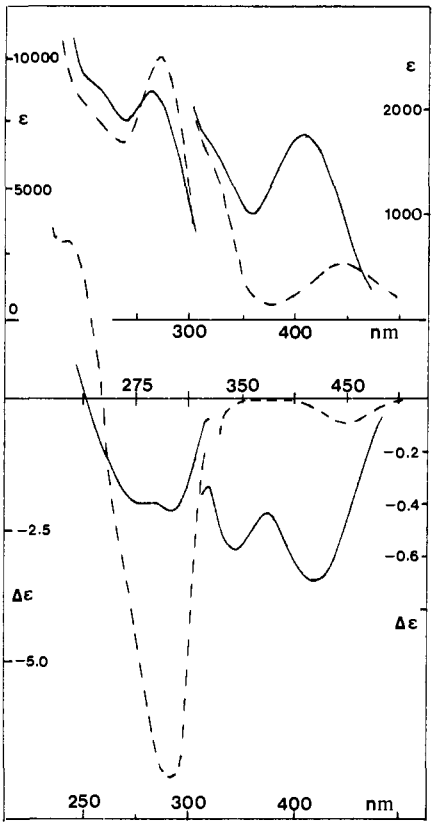


Figure 3. Electronic and CD spectra of an equimolar solution of pyridoxal and L-3,4-dihydroxyphenylalanine in slightly basic methanol: (a) (—) after mixing of the reagents; (b) (- -) after 6 h.

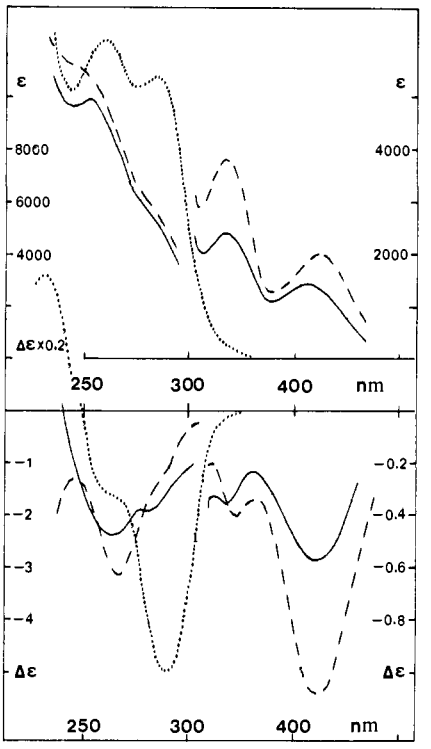


Figure 4. Electronic and CD spectra in slightly basic methanol solution of (a) (—) equimolar amounts of pyridoxal and L-histidine, recorded after mixing of the reagents; (b) (···) same as (a), recorded after approximately 3 days; (c) (- -) equimolar amounts of pyridoxal and L-N^{τ}-methylhistidine, recorded after mixing of the reagents.

the pattern is negative (420 nm) and positive (340 nm), and for aromatic amino acids two negative bands are observed.

The Schiff bases derived from pyridoxal, or other aldehydes, and histidine, tyrosine, 3,4-dihydroxyphenylalanine, or tryptophan can undergo cyclization reactions to give tetrahydropyrido[3,4-*d*]imidazole,[7,8,19] tetrahydroisoquinoline,[20] and tetrahydro-β-carboline[21] derivatives, respectively. The reactions of tyrosine– and tryptophan–pyridoxal Schiff bases are apparently very slow in the conditions employed here, since the electronic and CD spectra of these systems do not undergo any appreciable change within 2 days. On the other hand cyclization of the 3,4-dihydroxyphenylalanine Schiff base is complete in a few hours and that of the histidine Schiff base is significant within times of the order of a day. In order to observe the features of the Schiff bases, we have therefore recorded the spectra of these systems immediately after mixing of the reagents and, in the case of 3,4-dihydroxyphenylalanine, using a 10-fold dilution of the reaction solution. Figure 3a shows the electronic and CD spectra recorded after dissolution of pyridoxal and 3,4-dihydroxyphenylalanine in slightly basic methanol solution. Free pyridoxal contributes to the absorption bands near 400, 330, and 260 nm. The CD bands at 420 and 340 nm are quite certainly due to the tautomeric forms of the intermediate Schiff base, while those below 300 nm are probably contributed by both the Schiff base and the tetrahydroisoquinoline derivative. Progress of the cyclization reaction is accompanied by a decrease in intensity of the 420- and 340-nm CD bands and by an increase of CD activity below 300 nm. Figure 3b shows the spectra obtained after 6 h, when only the tetrahydroisoquinoline is presumably present. Similar arguments account for the spectral changes undergone by the pyridoxal–L-histidine system (Figure 4). The system derived from L-N^{τ}-methylhistidine was also studied, since the presence of a N^{τ}-methyl group on the imidazole nucleus is known to reduce the rate of cyclization of the Schiff base.[7] Therefore, the negative CD bands at 420, 335, 285, and 260 nm in the spectra recorded after mixing of the reagents can be assumed to represent features of the pyridoxal–L-histidine and –L-N^{τ}-methylhistidine Schiff bases. It can be noted that cyclization of the pyridoxal–L-histidine Schiff base appears to be slower in methanol than in water; this may possibly be related to different tautomeric equilibria in the two solvents.

Cysteine reacts with pyridoxal to give a 4-thiazolidinecarboxylic acid derivative.[18,22] The reaction in water is assumed to proceed through the formation of a Schiff base intermediate, though evidence for this reaction path is lacking since no Schiff base apparently accumulates in the reaction mixture. We have followed the course of the reaction by recording electronic and CD spectra at different times of a rather dilute (10^{-3} M) slightly basic methanol solution of pyridoxal and L-cysteine (Figure 5). It can be seen from Figure 5 that the CD bands related to the Schiff base (near 420 and 335 nm) develop rather slowly, while the CD bands at 292 and 250 nm increase at a much faster rate and reach a maximum of intensity in approximately 1 h. Since the latter CD bands are likely to be related to the thiazolidine derivative, we are apparently led to the somewhat surprising conclusion that in methanol solution, the *N*-pyridoxylidenecysteine Schiff base

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Table II. Electronic and CD Spectra of Zinc(II) Complexes of *N*-Pyridoxylidene Derivatives of Aliphatic L-Amino Acids in Methanol Solution[a]

complex[b]	UV, λ_{max} nm (ϵ)	CD, λ_{max} nm ($\Delta\epsilon$)
Zn(pdx-L-ala)	387 (7700)	390 (−1.70)
	280 sh (5000), 271 (6700)	320 br[c] (+0.25)
	268 sh (6400), 265 sh (6000)	262 (−1.80)
	226 (35 000)	232 (+3.00)
Zn(pdx-L-val)	386 (8100)	390 (−3.50)
	280 sh (5700), 272 (7500)	315 br (+0.60)
	268 sh (7000), 265 sh (6500)	263 (−2.95)
	227 (35 000)	232 (+7.80)
Zn(pdx-L-leu)	386 (8200)	390 (−3.90)
	280 sh (5800), 271 (7500)	315 br (+0.35)
	268 sh (7100), 265 sh (6600)	262 (−3.10)
	227 (36 000)	232 (+7.10)

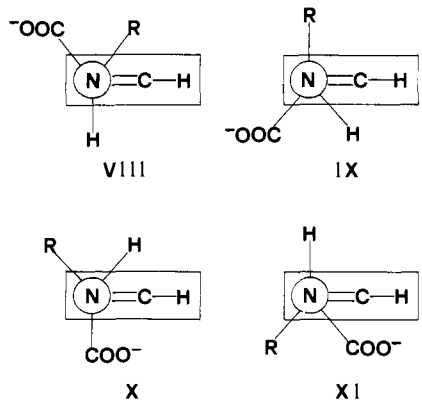
[a] Recorded at 10^{-3} M concentration. [b] Abbreviations: *N*-pyridoxylidene-L-alaninate dianion = pdx-L-ala; *N*-pyridoxylidene-L-valinate dianion = pdx-L-val; *N*-pyridoxylidene-L-leucinate dianion = pdx-L-leu. [c] Broad = br.

is formed through a path independent from that leading to the thiazolidine. On longer reaction times the electronic and CD bands near 420 and 335 nm decrease markedly, while those at 292 and 250 nm remain approximately constant. This seems to exclude that a simple Schiff base–thiazolidine equilibrium is established in solution, and other species are likely to be involved. A more detailed investigation is needed to assess these points.

For comparison purposes we have recorded the electronic and CD spectra of zinc(II) complexes of *N*-pyridoxylidene derivatives of aliphatic L-amino acids (I) (alanine, valine, and leucine) in neutral methanol solution, since only the derivatives of aromatic amino acids were reported previously.[7] In these complexes the ligand is in the enol imine form, the cis conformation of the C_4–$C_{4'}$ bond being imposed by metal chelation. The electronic and CD spectra display bands near 390 and 270 nm, corresponding to the $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ transitions, respectively. The former is bathochromically shifted compared to its position in the free ligands due to an increase of conjugation occurring upon coordination. The sign of these CD bands is negative. Additional positive CD activity is observed near 300 nm, with a weak and broad maximum, and below 240 nm. The spectral data are summarized in Table II.

Discussion

The conformation of the C_α–N bond of pyridoxal–amino acid Schiff bases in solution can be considered as a dynamic equilibrium of various rotamers. The most important conformers are VIII–XI,



while the other staggered rotamers with both the carboxyl and R substituents gauche to the azomethine group are expected to give contributions of only minor entity. Since intramolecular

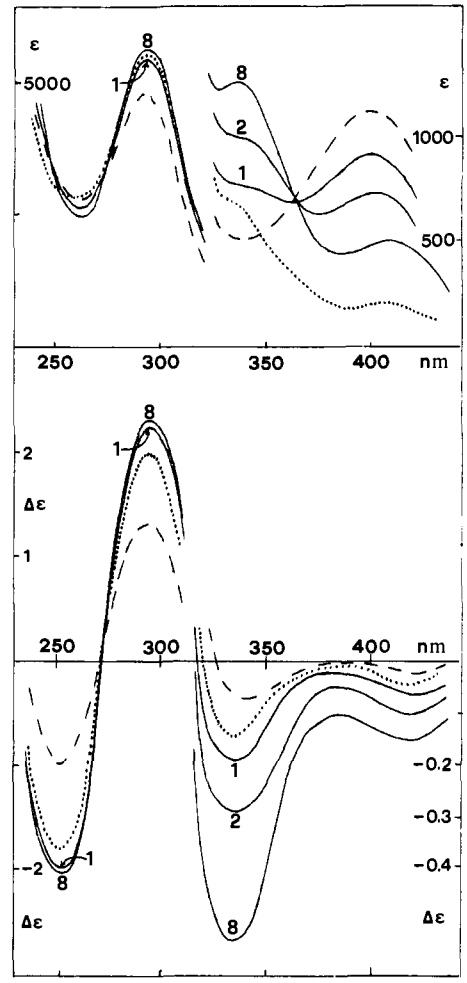


Figure 5. Electronic and CD spectra of an equimolar solution of pyridoxal and L-cysteine in slightly basic methanol solution (concentration 10^{-3} M): (a) (- -), recorded after 0.25 h; (b) (—), recorded after 1, 2, and 8 h, respectively; (c) (···) recorded after approximately 5 days.

hydrogen bonding stabilizes the cis enol imine (IV) and keto enamine (V) forms of the Schiff base, it can be assumed that the conformation of the C_α–N bond is ruled by the same stereochemical preferences in both tautomers and that, therefore, VIII–XI represent the preferred conformers for both tautomers. This seems confirmed by the observation that for the *N*-pyridoxylidene derivatives of L-amino acids with nonpolar side chains the sign pattern of the CD bands associated with the $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ transitions is the same for IV and V, i.e., positive ($\pi \rightarrow \pi_1^*$) and negative ($\pi \rightarrow \pi_2^*$). A similar conclusion can be drawn for the derivatives of aromatic L-amino acids, where all the CD bands are of negative sign, while for derivatives of L-amino acids with polar side chains the observed discrepancies may arise from additional interactions between the polar groups and the pyridoxylidene chromophore.

According to recent NMR studies, conformers IX and X are predominant in solution.[9] In order to assess which of the conformers VIII–XI determines the observed CD spectra, we note that the magnitude of the Cotton effects and the general features of the CD spectra of *N*-pyridoxylidene-L-amino acids indicate that the dominant mechanism operative in generation of the Cotton effects is coupling of the electronic transition moments of the pyridoxylideneamino chromophore with electric or magnetic transition moments of the amino acid residue.[23] For the *N*-pyridoxylidene chromophore the directions of the transition moments are not known. However, we can utilize the results obtained

(23) (a) Schellman, J. A. *Acc. Chem. Res.* **1968**, *1*, 144–151. (b) Buckingham, A. D.; Stiles, P. J. *Ibid.* **1974**, *7*, 258–264. (c) Snatzke, G. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 363–377.

from the analysis of optical and CD spectra of the parent *N*-salicylideneamino acids, even though only the enol imine tautomers of the Schiff bases were considered there.[10] The dominant conformer for generation of Cotton effects in an aliphatic amino acid *N*-salicylidene derivative is XII, and application of the salicylideneamino chirality rule[24] indicates that the sign of the CD bands at longer wavelengths (corresponding to the $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ transitions in the pyridoxylidene series) can be predicted from the chirality of the carboxylate group attachment bond and the phenyl group–methine carbon bond in the salicylideneamino chromophore.[10] In XII this chirality is positive (right-handed screw) and accounts for the observed positive Cotton effects within the CD bands at longer wavelengths. The carboxylate transition that most likely couples with the salicylideneamino chromophore is one of the doubly degenerate $\pi \rightarrow \pi^*$ transitions,[25] namely, that with component along the symmetry axis of the carboxylate group, while the perpendicular component is ineffective because of rotational averaging about the axis.[10] The pyridoxylidene analogue of XII is XIII, and if the transition moment directions for $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ were parallel to those of the corresponding transitions of the salicylideneamino chromophore, positive Cotton effects should be expected to arise within these bands by interaction with the carboxylate group transitions. The CD bands associated with the $\pi \rightarrow \pi_1^*$ transitions of both tautomers of the *N*-pyridoxylidene derivatives of aliphatic L-amino acids actually display positive Cotton effects, though the CD bands associated with the $\pi \rightarrow \pi_2^*$ transitions are of negative sign (Table I). The $\pi \rightarrow \pi_1^*$ transition is mainly localized within the azomethine chromophore, and therefore its polarization assignment is expected essentially coincident with that of the salicylideneamino chromophore. A slight difference in transition moment direction for $\pi \rightarrow \pi_2^*$ in the pyridoxylideneamino and salicylideneamino chromophores or the presence of other optically active transitions in the same spectral region may give account of the opposite sign of the corresponding CD bands observed in the two systems. This interpretation seems confirmed by the observation that the *N*,*N*′-dipyridoxylidene and *N*,*N*′-disalicylidene derivatives of (*R*)-*trans*-1,2-cyclohexanediamine, for which the preferred conformer is dictated by the rigidity of the diamine,[12,24] display Cotton effects of opposite sign within the CD band near 260 nm (Figure 6). The corresponding derivatives of (*R*)-1,2-propanediamine behave similarly (Figure 6). Therefore, XIII appears the conformer determining the features of the CD spectra of aliphatic L-amino acid *N*-pyridoxylidene derivatives. We note that XIII is the eclipsed conformer intermediate between the staggered rotamers IX and X which are the predominant conformers according to NMR spectroscopy.[9]

The presence of a β-hydroxyl substituent on an aliphatic amino acid *N*-pyridoxylidene derivative has little effect on its CD spectrum, while either sulfur-containing or carboxyl substituents affect the sign of the CD bands at longest wavelengths (Figure 1). We believe these changes result from strong interaction between the polar group on the side chain and the pyridoxylideneamino chromophore in XIII, rather than from the involvement of a different conformation of the C_α–N bond. These additional interactions apparently make a negative contribution to the CD of the bands associated with the $\pi \rightarrow \pi_1^*$ transitions of both the keto enamine and enol imine forms of the Schiff base. Thus, for the *N*-pyridoxylidene derivatives of methionine, aspartic acid, and glutamic acid the CD band at 420 nm is negative while that at

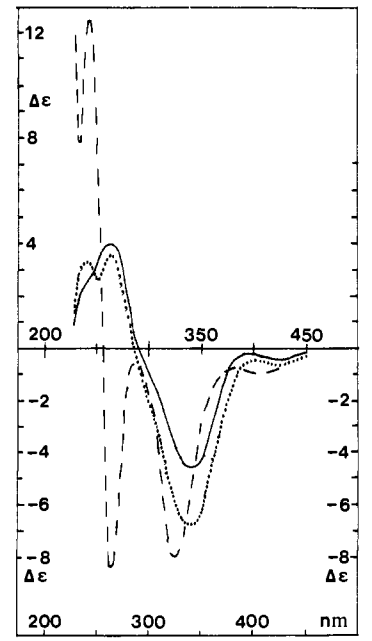


Figure 6. Circular dichroism spectra in methanol solution of (a) (—) (*R*)-*trans*-*N*,*N*′-dipyridoxylidene-1,2-cyclohexanediamine; (b) (- -) (*R*)-*trans*-*N*,*N*′-disalicylidene-1,2-cyclohexanediamine; and (c) (···) (*R*)-*N*,*N*′-dipyridoxylidene-1,2-propanediamine.

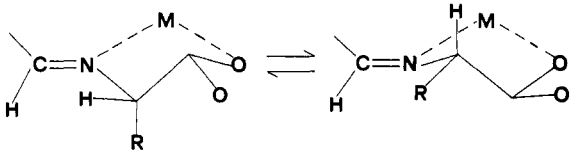
340 nm is positive but substantially reduced in rotational strength. Similar behavior is exhibited by the *N*-salicylidene derivatives of the same amino acids.[10]

The rotatory contribution of the carboxylate group in the *N*-salicylidene derivatives of aromatic amino acids is overshadowed by the interaction of the β-aryl group with the salicylideneamino chromophore.[10] Since negative CD bands are associated with the $\pi \rightarrow \pi_1^*$ and $\pi \rightarrow \pi_2^*$ transitions of *N*-pyridoxylidene derivatives of aromatic L-amino acids (phenylalanine, tyrosine, tryptophan, 3,4-dihydroxyphenylalanine, histidine, N^τ-methylhistidine), these behave exactly as their salicylidene analogues. Application of the salicylideneamino chirality rule to the latter systems indicates that negative CD bands are associated with negative chirality (left-handed screw) of the attachment bonds to the aryl and salicylideneamino groups.[24] Thus, XIV and XV are the most important conformers for dichroic absorption of *N*-salicylidene and *N*-pyridoxylidene derivatives of aromatic amino acids, respectively. We note that XV is also the predominant conformer according to NMR spectroscopy, where an upfield shift of $H_{4'}$ and $H_{5'}$ has been found to result from a π–π interaction between the aromatic side chain of the amino acid and the pyridoxal π system.[9] Since the conformation of the C_α–N bond in XV is the same as that of the eclipsed rotamer XIII, we are led to the conclusion that the conformations determining the features of the CD spectra are approximately the same for all the *N*-pyridoxylideneamino acid Schiff bases and are restricted within the narrow range depicted by IX and X. The CD results are, therefore, in substantial agreement with the previous NMR studies.[9] On steric grounds, the presence of minor amounts of the conformers containing an axial C_α–H bond (VIII and XI) may qualitatively account for the low reactivity attainable by model systems in reproducing the

(24) Smith, H. E.; Neergard, J. R.; Burrows, E. P.; Chen, F.-M. *J. Am. Chem. Soc.* **1974**, *96*, 2908–2916.
(25) Snyder, P. A.; Vipond, P. M.; Johnson, W. C., Jr. *Biopolymers* **1973**, *12*, 975–992.

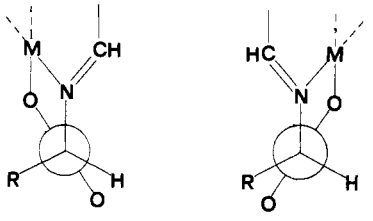
enzymic processes involving cleavage of that bond as a preliminary step (racemization, transamination, β elimination, γ elimination, retroaldolization). However, it can also be noted that while the conformation with an axial carboxyl group (X) may give non-negligible contribution to the conformational equilibrium, decarboxylation of *N*-pyridoxylideneamino acids is not usually observed in model systems and cleavage of that bond is apparently ruled by other factors. Although the conformational data are of limited value in explaining the reactivity of model systems, correlations between CD spectral data and the conformation of the C_α–N bond of *N*-pyridoxylidene-L-amino acids may be important to infer the structure of the coenzyme–substrate complex in the enzymic reactions, where the conformational mobility of the system is blocked by a rigid protein matrix.

The conformation of the ligand containing a pseudoaxial side chain (XVI) is preferred also in metal complexes of *N*-pyridoxylideneamino acids (I) and *N*-salicylideneamino acids (II), where a dynamic conformational XVI ⇌ XVII equilibrium can



XVI XVII

be present.[7,8] Therefore, the differences observed in the CD spectra of the free ligands and their metal complexes deserve some comment, at least in the case of zinc(II) complexes, where contributions to the CD from charge transfer or d–d transitions are absent. Metal coordination prevents the free rotation of the carboxylate group about its symmetry axis. As a result, the interaction between the carboxylate $\pi \rightarrow \pi^*$ transition with component perpendicular to the group's symmetry axis and the pyridoxylideneamino or salicylideneamino chromophore becomes effective in the complexes. Since the transition moment for the azomethine band for *N*-salicylidene- and *N*-pyridoxylideneamino acids can be regarded as essentially parallel to its attachment bond, the rotatory contribution by the additional interaction of the carboxylate group with the chromophore can be predicted by the chirality of the attachment bond to the azomethine group and the carboxylate plane. Newman projections of XVI and XVII show that for the conformation with pseudoaxial side chain (XVIII) this chirality



XVIII XIX

is negative (left-handed screw) while it is positive (right-handed screw) for the conformation with pseudoequatorial side chain (XIX). This interaction apparently overshadows that contributed by the carboxylate transition with component parallel to the group's axis since the CD spectra of zinc(II) complexes of aliphatic *N*-salicylidene-[7] and *N*-pyridoxylidene-L-amino acids display a negative (i.e., opposite to the free ligands) azomethine band (Table II). Similar arguments should apply to the interactions of the carboxylate transitions with the benzenoid or pyridine ring transitions, though the uncertainty in transition moment directions for the latter bands and the possible occurrence at similar energy of electronic transitions of different nature (e.g., $n \rightarrow \pi^*$) that can also couple with the carboxylate transitions make reliable predictions more difficult. We note that for zinc(II) complexes of aromatic *N*-salicylidene- and *N*-pyridoxylidene-L-amino acids the sign of the azomethine CD band is the same (negative) as that in the free ligands.[7] The origin of the Cotton effects within this CD band, though, is different in the metal-free systems and in the zinc complexes. In the free Schiff bases of aromatic L-amino acids the sign of the azomethine CD band is determined by its interaction with the gauche aryl group (XIV, XV). The X-ray structural determination of metal complexes of aromatic *N*-salicylideneamino acids show that the conformation of the C_α–C_β bond contains the aryl group roughly antiperiplanar to the azomethine group.[26] This conformation involves rather large separation and near anticollinearity between the interacting transition moments and is expected to lead to a negligible rotational strength contribution. It is very likely that a similar preferred conformation of the ligand exists in solution, where steric interaction with solvent molecules apically coordinated to the metal center destabilizes the conformers with gauche aryl groups. Therefore, the negative azomethine CD band observed for zinc complexes of aromatic L-amino acid Schiff bases is determined, as for their aliphatic analogues, by the interaction between the azomethine chromophore and the carboxylate group transitions.

Acknowledgment. This work was supported by a grant from the Italian CNR.

Registry No. I (M = Zn, R = CH_3), 83984-08-1; I (M = Zn, R = $CH(CH_3)_2$), 83984-09-2; I (M = Zn, R = $CH_2CH(CH_3)_2$), 83984-10-5; III (R = CH_3), 13933-84-1; III (R = $CH(CH_3)_2$), 13933-94-3; III (R = $CH_2CH(CH_3)_2$), 13933-97-6; III (R = CH_2OH), 13933-86-3; III (R = $CH(OH)CH_3$), 13933-92-1; III (R = $CH_2CH_2SCH_3$), 13933-91-0; III (R = CH_2COOH), 13934-01-5; III (R = CH_2CH_2COOH), 13934-03-7; III (R = CH_2SH), 13933-88-5; III (R = $CH_2C_6H_5$), 13934-07-1; III (R = 4-imidazolylmethyl), 14029-59-5; III (R = 1-methyl-4-imidazolylmethyl), 83984-06-9; III (R = *p*-$HOC_6H_4CH_2$), 13934-09-3; III (R = 3-indolylmethyl), 75281-26-4; III (R = 3,4-dihydroxyphenylmethyl), 83984-07-0; pyridoxal, 66-72-8.

(26) (a) Hämäläinen, R.; Ahlgrén, M.; Turpeinen, U.; Rantala, M. *Acta Chem. Scand., Ser. A* **1978**, *A32*, 235–240. (b) Hämäläinen, R.; Turpeinen, U.; Ahlgrén, M.; Rantala, M. *Ibid.* **1978**, *A32*, 549–553.