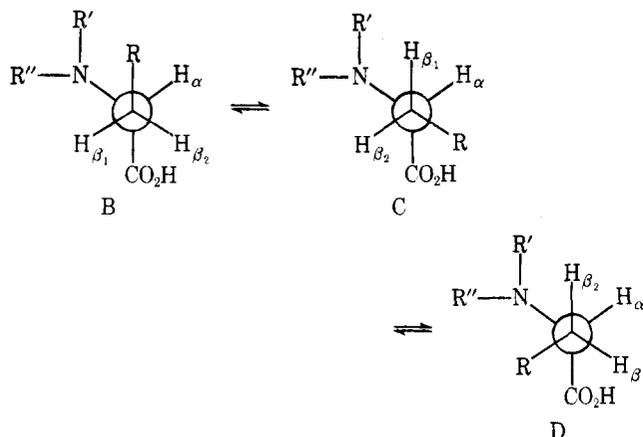
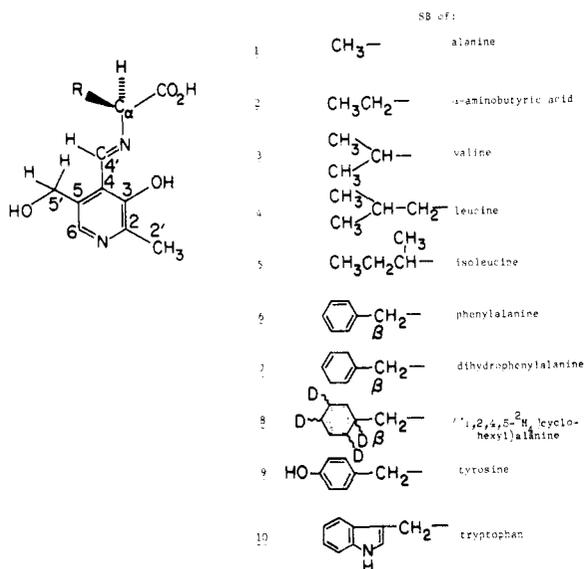


in aqueous solutions of various pH values, in dimethyl sulfoxide and in trifluoroacetic acid, have been reported (Wüthrich, 1976). The H_α and H_β portions of many amino acids containing two β hydrogens exhibit an ABX or ABC spin pattern. The time-averaged ^1H NMR spectra have been used to estimate the relative population of three classical staggered rotamers (B, C, and D). It is important to note that the designation of H_{β_1} and H_{β_2} involves an unexchangeable commitment concerning the assignment of these hydrogens to the *pro-R* and *pro-S* positions.



In this paper we report results of the conformational analysis of a series of pyridoxal-amino acid Schiff's bases (1-10) using ^1H and ^{13}C NMR techniques.



Materials and Methods

The amino acids were purchased from Sigma Chemical Co., except dihydrophenylalanine and cyclohexylalanine which were synthesized in our laboratory. The pyridoxal was obtained by neutralizing the pyridoxal hydrochloride purchased from Sigma with NaOH and filtering. The NMR solvents (CD_3OD , CD_3OH , dimethyl- d_6 sulfoxide) were purchased from Koch Isotopes Inc. and Merck Sharp & Dohme Ltd. NaOD (40% in D_2O) and DCl (38% in D_2O) were purchased from Norell Chemical Co., Inc. Other chemicals were of highest commercial grade available.

The NMR spectra were obtained in 10-mm (^{13}C NMR) and 5-mm (^1H NMR) spinning tubes. Tetramethylsilane (Me_4Si)¹

was used as internal reference for ^1H NMR spectra. The ^{13}C resonance of deuteriomethanol was used as internal reference for ^{13}C NMR spectra and the data were converted to the Me_4Si scale by the following correction: $\delta(\text{Me}_4\text{Si}) = \delta(\text{CD}_3\text{OD}) + 49.3$ ppm.

The instruments employed were a Jeol PFT-100 spectrometer operating at 23 kG, interfaced with a Jeol EC-100 Fourier transform computer with 20K memory. The normal ^{13}C NMR spectra were measured for 5 kHz sweep width and at ambient temperature using an internal deuterium lock. The typical pulse width was 25.5 μs , and the repetition time between pulses was 5 s. All proton resonances were decoupled by a broad band (2.5 kHz) irradiation from an incoherent 99.9-MHz source for proton noise-decoupled spectra. The aged decoupling technique was employed to measure proton coupled spectra. The instrumental resolution was normally ± 0.61 Hz for ^{13}C NMR and ± 0.24 Hz for ^1H NMR.

The normal Schiff's bases were prepared by mixing pyridoxal (1 mmol), amino acid (1 mmol), and 40% NaOD (1 mmol for monoanion, 2.5 mmol for dianion) in deuteriomethanol solution and stirring for 2 h at room temperature under a nitrogen atmosphere. Then the pD value was adjusted to 8.2 (monoanion) and 12.3 (dianion) using NaOD or DCl if necessary, and the total volume was adjusted to 2.0 mL. For those Schiff's bases which rapidly exchange H_α with solvent, e.g., the SB's of alanine, α -aminobutyric acid, phenylalanine, dihydrophenylalanine, and leucine, CD_3OH was used as solvent to observe the coupling constants $^1J(^{13}\text{C}-H_\alpha)$ and $^3J(^{13}\text{C}_\beta-H_\alpha)$. The ^{13}C chemical shifts at pH 7.9 and 12.0 in CD_3OH correspond to those at pD 8.2 and 12.3 in CD_3OD , respectively.

For the experiments in which we tried to detect the phenolic proton, the SB prepared as above was dried thoroughly under vacuum, dissolved in dry dimethyl- d_6 sulfoxide containing not more than 0.001% (w/w) of H_2O and then transferred to the NMR tube under dry argon.

The samples for the nuclear Overhauser effect study were exchanged once with CH_3OD , evaporated to dryness, and then taken up in CD_3OD to make a 0.4 M solution. The samples were then degassed by passing argon through them for 5 min and sealing the tubes.

The temperature variation study was carried out in 0.1 M CD_3OD solution. The spectrum width was narrowed to 1.0 kHz to improve the resolution to ± 0.12 Hz. Since the SB decomposes appreciably above 50 $^\circ\text{C}$, only the low temperature spectra were studied. Below -40 $^\circ\text{C}$ the resolution was very poor.

The (2S,3R)-[3- ^2H]phenylalanine was a gift of Professor G. W. Kirby. Ten milligrams of this sample was used to prepare the SB solution in CD_3OD and determine the first-order coupling constants between H_α and H_{β_2} . The observed $^3J(H_\alpha-H_{\beta_2})$ was very close to the smaller coupling constant of the two $^3J(H_\alpha-H_\beta)$ of phenylalanine SB (monoanion and dianion) obtained by analysis of the second-order spectra.

L-2,5-Dihydrophenylalanine (DHPA) was prepared using the method of Snow et al. (1968). Birch reduction was performed on 5 g of L-phenylalanine. The yield after subsequent purification was 3.2 g of DHPA. The melting point was identical with and the NMR spectrum was comparable with those published in the literature. L-([1,2,4,5- $^2\text{H}_4$]cyclohexyl)alanine was obtained by Pt-catalyzed hydrogenation of 450 mg of DHPA using deuterium gas. After removal of the catalyst by centrifugation, the crude mixture was passed onto the top of a 1×100 cm cation exchange column made of Bio-Rad AG50-X8 cation exchange resin (200-400 mesh, hydrogen form). The column was then eluted with 0.4 N sodium citrate

¹ Abbreviations used: SB, Schiff's base; Me_4Si , tetramethylsilane; ^{13}C NMR, carbon-13 nuclear magnetic resonance; ^1H NMR, proton magnetic resonance; DHPA, L-2,5-dihydrophenylalanine; NOE, nuclear Overhauser effect.

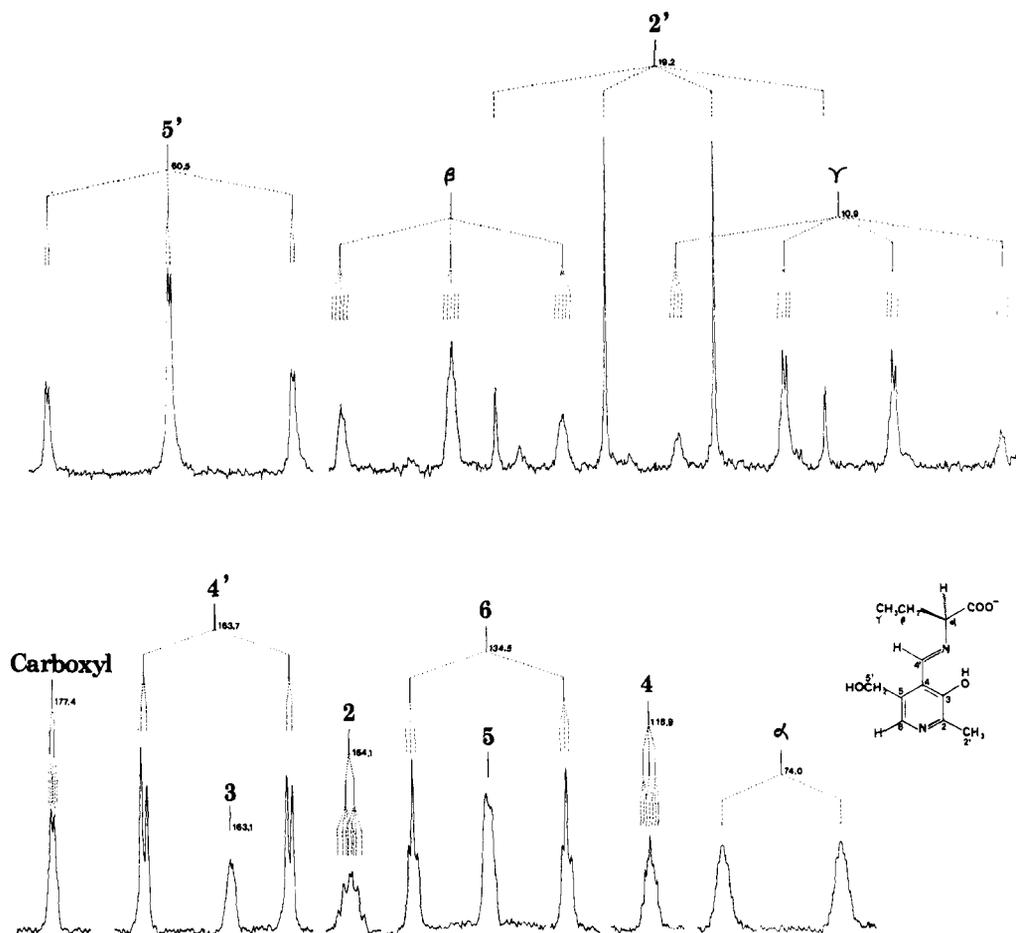


FIGURE 1: Proton-coupled ^{13}C NMR spectrum of the pyridoxal Schiff's base of α -aminobutyric acid.

buffer (pH 4.2) and 7-mL fractions were collected. The fraction from tubes 60 to 80 contained a compound which gave a blue spot of R_f 0.45 when spotted on silica gel TLC plates (5×20 cm, Brinkmann), developed with 1-butanol/glacial acetic acid/water (12:3:5) and sprayed with 1% ninhydrin in absolute ethanol (R_f of phenylalanine = 0.35). This fraction was desalted by passing onto the top of a Dowex 50W (Sigma) cation-exchange column (50–100 mesh, hydrogen form), washing thoroughly with water, followed by elution with 1 N ammonium hydroxide. The yield was 210 mg of L-([1,2,4,5- $^2\text{H}_4$]-cyclohexyl)alanine, identified by its NMR spectrum (D_2O): δ 3.4 (1 H, t, $J = 5$ Hz, H_α) and δ 1.2–1.7 (9 H, broad, methylene protons).

Results and Discussion

^{13}C Chemical Shifts and Couplings.² The chemical shifts of pyridoxal Schiff's bases (Table I; see paragraph at the end of this paper concerning supplementary material) are primarily assigned according to their multiplicity and model studies of pyridoxal derivatives (Witherup & Abbott, 1975; O'Leary & Payne, 1976; Harruff & Jenkins, 1976; Dalling et al., 1976). The ^{13}C chemical shift assignments of pyridoxal Schiff's bases

² All of our NMR studies were done in methanol solution since the Schiff's base formation is quantitatively complete in methanol but not in water. pD 8.2 and 12.3 were chosen for the monoanion and the dianion, respectively, based on our UV observations which indicated that, in the pH range 6.8 to 9.2, the SB solution in methanol contained mainly the two monoanionic species, the enolimine form and the ketoenamine form. In the pH range 8.0 to 9.2, the ratio of these two monoanionic species is not very sensitive to pH. At pH higher than 12.0 the dianion became the single species present.

of alanine and valine have recently been reported (Harruff & Jenkins, 1976; Dalling et al., 1976). The resonance signals of the side chains of other amino acids can be directly deduced from previous studies of amino acids (Wütherich, 1976). The ^{13}C - ^1H coupling constants of pyridoxal and simple related compounds have been partially interpreted (Witherup & Abbott, 1975; Harruff & Jenkins, 1976). We have undertaken an extensive study to unravel most of the ^{13}C - ^1H long-range couplings (Table I in supplementary material) on the basis of our previous work (Chang, 1976; Chang & Peck, 1976; Chang et al., 1977a,b). Some of the long-range coupling constants cannot be measured due to the second-order nature of the spectra or the line-broadening effect resulting from tautomeric isomerization and/or conformational fluctuation. The ^{13}C - ^1H coupling patterns are well illustrated in the proton-coupled ^{13}C spectrum of the pyridoxal Schiff's base of α -aminobutyric acid (Figure 1). The ^{13}C - ^1H couplings of all carbons are directly discernible except C_α , C_3 , and C_5 , which appear as unresolved peaks due to the long-range couplings with several protons. The larger coupling constant of C_4 (7.2 Hz) is assigned to $^2J(\text{C}_4-\text{H}_4)$ based on our previous model studies (Chang et al., 1977a). The long-range couplings $^2J(\text{COO}-\text{H}_\alpha)$ and $^3J(\text{COO}-\text{H}_\beta)$ are assigned by comparing the coupling patterns of the Schiff's bases of phenylalanine and [α - ^2H]phenylalanine.

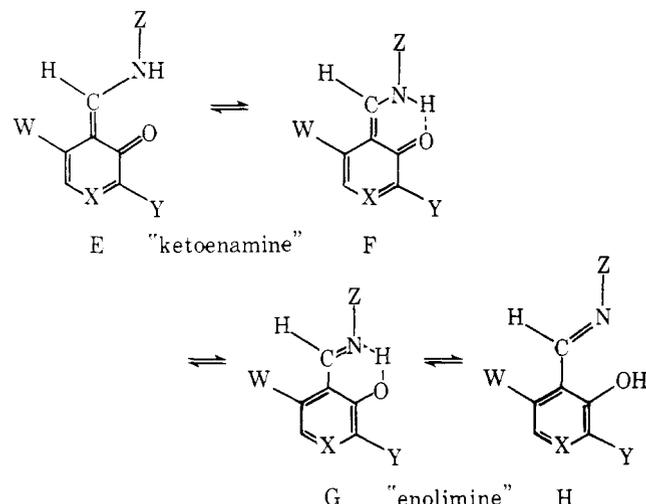
Coplanarity, Tautomerism, and Cis-Trans Conformation. The mechanism of pyridoxal phosphate catalysis involves an interaction between the π system of the pyridine ring and the π -electron pair of the imine double bond (cf. Dunathan, 1971), implying coplanarity of the two π planes which is influenced

TABLE II: Results of Nuclear Overhauser Effect Studies.^a

proton irradiated	proton measured	valine SB (%)		phenylalanine SB ^b (%)	
		monoanion	dianion	monoanion	dianion
H _{5'}	H _{4'}	21	11	22	12
H _{5'}	H ₆	49	34	45	35
H _{4'}	H _α	20	21		

^a The percentage enhancements are measured by integration with H₂ as the reference. The estimated maximum error is less than 10%.
^b Since the α proton of phenylalanine SB is exchanging with solvent deuterium, the α-deuterated sample was used for NOE studies.

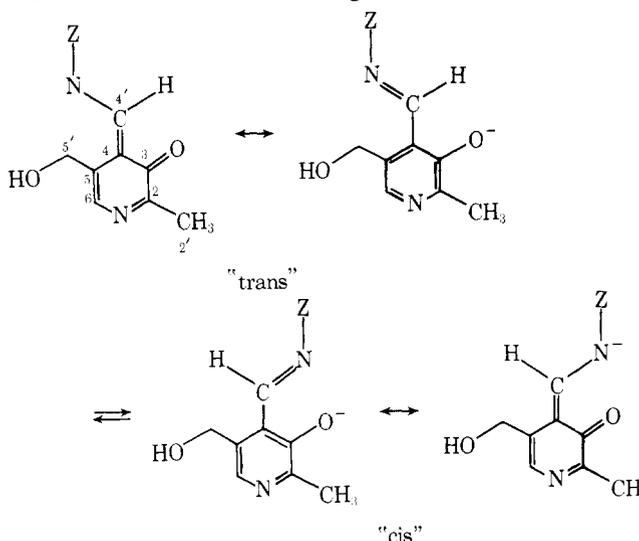
by the tautomeric isomerism (ketoenamine and enolimine isomers) and the cis-trans conformation of the C₄-C_{4'} bond.



Our recent ¹³C NMR analysis (Chang et al., 1977a) of a series of simple benzaldehydes, salicylaldehyde, pyridine-4-aldehyde, and their oximes and amino acid Schiff's bases, has shown that the reduction of the two-bond coupling constant (²J(¹³C₄-¹H_{4'})) in going from pyridoxal to its Schiff's bases is not necessarily indicative of a distortion of coplanarity. Meanwhile, using the ¹³C-¹H three-bond couplings (³J(C₂-OH₃) or ³J(C₄-OH₃)), we have been able to demonstrate this coplanarity, and to determine the tautomeric form and cis-trans conformation of oximes. In the case of salicylaldehyde-amino acid Schiff's bases,³ no coupling between OH and C₂ (or C₄), and between NH and C₄ can be measured in methanol or dimethyl sulfoxide solution. Neither the OH nor the NH signal of the pyridoxal-amino acid Schiff's bases in dry dimethyl sulfoxide solution may be clearly observed presumably due to the perturbation of the pyridine nitrogen atom and the tautomeric equilibration, revealed by the UV absorptions at 420 nm (ketoenamine) and 335 nm (enolimine) (Johnson & Metzler, 1970). In order to unequivocally demonstrate coplanarity of the two π systems and the cis conformation of the C₄-C_{4'} bond, nuclear Overhauser effect (NOE) measurements were carried out on the phenylalanine and valine Schiff's bases (Table II).

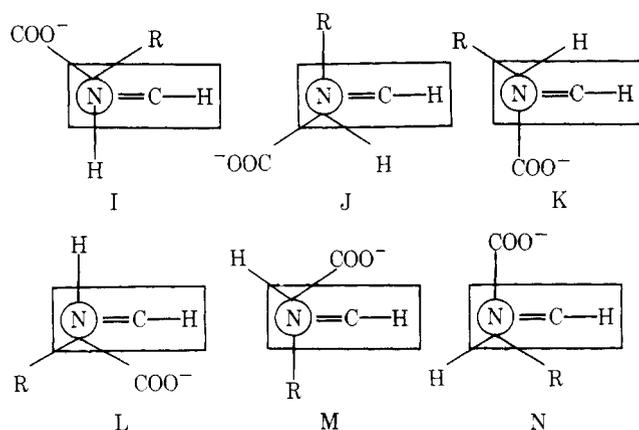
³ Methylamine, *n*-butylamine, glycine, valine, and phenylalanine Schiff's bases of salicylaldehyde have been prepared and their ¹H NMR and ¹³C NMR spectra have been thoroughly analyzed. In dry deuterio-dimethyl sulfoxide, the downfield signal (>12 ppm) is assigned to the proton attached to the heteroatom (N or O). The downfield chemical shift and the line width (*W*_{1/2} ~ 50-100 Hz) suggest the existence of intramolecular hydrogen bonding and dynamic equilibration of the two tautomeric isomers (F ⇌ G) (Chang, C.-j., Shieh, T.-L., & Floss, H. G., manuscript in preparation).

Irradiating at the resonance frequency of H_{5'}, a prominent NOE (21% on the H_{4'} and 49% on H₆ for valine; 22% on H_{4'} and 45% on H₆ for phenylalanine at pD = 8.2) was observed which is strongly indicative of the cis conformation. The reduction of the NOE on H_{4'} in going from the monoanion (pD = 8.2) to the dianion (pD = 12.3) may indicate an increasing contribution of the trans conformer because of the electrostatic repulsion between two electronegative atoms.



A similar NOE reduction on H₆ suggests a conformational change around the C₅-C_{5'} bond, which is also indicated by the spectral change of H_{5'}, from a singlet at pD = 8.2 to an AB quartet at pD = 12.3.

C_α-N Bond. The conformation of the C_α-N bond in solution can be viewed as a dynamic equilibrium of six staggered rotamers. The catalytic function of the pyridoxal Schiff's bases



may only rely on the population of these conformers. The coupling constants ³J(C₄-H_α) for all Schiff's bases are invariant within experimental error (Table I in supplementary material) and the average value is 6.3 ± 0.5 Hz (pD = 8.2) and 8.6 ± 0.5 Hz (pD = 12.3), suggesting that their conformations at the C_α-N bond are alike at the same pH. As shown in Table II, an approximately 20% NOE on the H_α is detected when irradiating at the resonance frequency of H_{4'} of pyridoxal-valine Schiff's base. Based on this NOE and the Lemieux graph (Lemieux et al., 1972) for correlating the dihedral angle and the ¹³C-¹H three-bond coupling constant, the predominance of conformers J and/or K is implied. In view of the relative constance of ³J(C₄-H_α), further elaboration of the detailed conformation of the pyridoxal phenylalanine Schiff's base was undertaken in order to provide an overall view of the rest of the compounds.

TABLE IV: Conformational Analysis of the C_α-C_β Bond of Aromatic Amino Acids and Their Schiff's Bases.

	amino acids			Schiff's bases (monoanions)		
	Phe ^a	Tyr ^a	Trp ^b	Phe	Tyr	Trp
$J_{\alpha\beta_1}$ (Hz)	7.9	7.6	7.3	10.3	9.8	10.7
$J_{\alpha\beta_2}$ (Hz)	5.2	5.0	4.1	4.2	3.8	3.3
P_B (%)	48	46	43	69	65	73
P_C (%)	24	22	23	15	10	7
P_D (%)	28	33	34	16	25	20

^a Kainosho & Ajisaka, 1975a. The conformational population of aromatic amino acids is insensitive to the pD value of the solution.

^b Cavanaugh, 1970.

C_α-C_β Bond. As uncertainty in the assignment of the two β protons of aromatic amino acids has been an obstacle to the conformational analysis in solution by ¹H NMR, proton spectral analysis of stereospecifically deuterium-labeled samples, (2*S*,3*R*)-[3-²H]phenylalanine and (2*S*,3*R*)-[3-²H]tyrosine has allowed an unambiguous assignment of H_{β1} and H_{β2} in the free amino acids (Sawada et al., 1975; Kainosho & Ajisaka, 1975b). Similarly, we have made the assignment for pyridoxal phenylalanine Schiff's base based on the stereospecifically deuterated sample. The ¹H NMR of phenylalanine SB shows a typical ABX pattern, from which three chemical shifts, δ_α, δ_{β1}, and δ_{β2}, and three coupling constants, $J_{\alpha\beta_1}$, $J_{\alpha\beta_2}$, and $J_{\beta_1\beta_2}$ can be determined (Table III in supplementary material). The upfield shift of H_{β1} relative to H_{β2} is probably due to the field shielding effect of the imino and the carbonyl groups. Furthermore, we can calculate the relative conformational population around the C_α-C_β bond of this Schiff's base (Table IV) by the method developed by Pachler (1964) (eq 1-3):

$$P_B = \frac{J_{\alpha\beta_1} - J_g}{J_t - J_g} \quad (1)$$

$$P_C = \frac{J_{\alpha\beta_2} - J_g}{J_t - J_g} \quad (2)$$

$$P_D = 1 - P_B - P_C \quad (3)$$

where $J_g = 2.60$ Hz and $J_t = 13.56$ Hz.⁴ This method has generally been used for the conformational analysis of amino acids (Kainosho & Ajisaka, 1975b). Alternatively, the conformational distribution can be calculated from the ¹³C-¹H three bond couplings $^3J(^{13}\text{CO}_2^- - \text{H}_{\beta_1})$ and $^3J(^{13}\text{CO}_2^- - \text{H}_{\beta_2})$ by the method developed by Espersen & Martin (1976) ($J_t = 9.8$ Hz; $J_g = 1.3$ Hz) and Batchelor & Feeney (1975) ($J_t = 11.9$ Hz; $J_g = 0.4$ Hz). The results obtained from these calculations using the coupling constants $^3J(^{13}\text{CO}_2^- - \text{H}_{\beta_1}) = ^3J(^{13}\text{CO}_2^- - \text{H}_{\beta_2}) = 2.4$ Hz are in good agreement with those calculated from ¹H-¹H coupling constants. The relative conformations of the C_α-C_β bond of tyrosine and tryptophan Schiff's bases can be established based on the results with phenylalanine Schiff's base (Table IV). It seems reasonable to assume that the population of C should increase and those of B and D should decrease upon formation of the Schiff's base because of the steric interference between the imine moiety and the aromatic side chain. Surprisingly, the relative population of conformer B increases significantly upon going from the free

⁴ Although different J_g and J_t values for ¹H-¹H three-bond coupling have been proposed by another group (Koppel et al., 1973), this does not alter the "relative" conformational distribution of free amino acid and the corresponding pyridoxal SB.

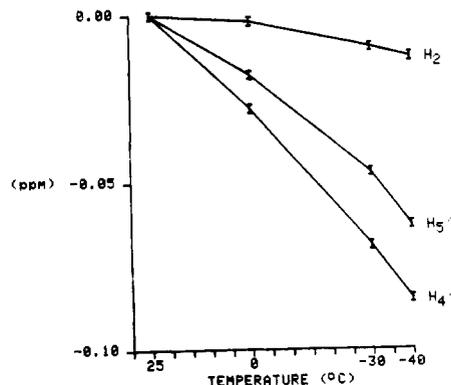
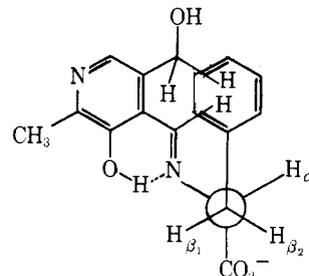


FIGURE 2: Variation of chemical shifts of H_{4'} and H_{5'} of phenylalanine SB with temperature at pD = 8.2. The chemical shifts at room temperature are set as zero for all the protons, and the upfield shift is designated as negative variation. The chemical shifts of other protons show little variation with temperature change.

aromatic amino acids to their Schiff's bases (Table IV), implying that other, nonsteric factors might be involved. One of the important stabilizing factors for conformer B in these Schiff's bases could be the π-π interaction between the aromatic nucleus and the pyridoxal π system.

The proton chemical shifts for the pyridoxal portion of the Schiff's bases of nonaromatic amino acids are fairly constant (Tables III (supplementary material) and V). On the other hand, both H_{4'} and H_{5'} of the Schiff's bases of aromatic amino acids undergo upfield shifts simultaneously (Table V). In accordance with the dominant population of conformer B, the observed upfield shift can be attributed to the diamagnetic anisotropic effect exerted by the aromatic ring which lies above H_{4'} and H_{5'}.



This provides support for the π-π interaction between the pyridoxal π system and the aromatic side chain of amino acids. At lower temperature, this anisotropic effect becomes even more pronounced (Figure 2). The reduction of the diamagnetic shift in dihydrophenylalanine and cyclohexylalanine⁵ pyridoxal Schiff's bases further substantiates the unique π-π interaction. The "simultaneous" upfield shift for H_{4'} and H_{5'} would also indicate a *cis* conformation for the C₄-C_{4'} bond, because such an upfield shift of both H_{4'} and H_{5'} is not possible for the *trans* conformation. Going from the monoanion (pD = 8.2) to the dianion (pD = 12.3), the diamagnetic effect on H_{4'} still remains, whereas the degree of upfield shift is reduced due to the change of electron density. On the other hand, the diamagnetic shift on H_{5'} is reduced to a much larger extent, reflecting the presence of more *trans* conformer in the dianion form. This observation is in agreement with the NOE studies.

⁵ ([1,2,4,5-²H₄]cyclohexyl)alanine SB (8) was used in order to avoid complication of the H_α-H_β couplings by the γ proton. Due to the peak overlaps in the ¹H NMR of this SB, the populations P_B, P_C, and P_D cannot be calculated. However, the coupling pattern of H_α of this SB is very close to a triplet, implying that predominance of conformer B over C and D no longer exists.

TABLE V: Chemical Shifts of H₄' and H₅' (δ Values in ppm) of Schiff's Bases.

	average of 1 to 5, δ	6		7		8		9		10	
		δ	$\Delta\delta$								
monoanion H ₄ '	8.90 \pm 0.04	8.28	-0.62	8.64	-0.26	8.93	0	8.21	-0.69	7.86	-1.04
H ₅ '	4.77 \pm 0.02	4.45	-0.32	4.69	-0.08	4.77	0	4.46	-0.31	4.06 ^a	-0.71
dianion H ₄ '	8.95 \pm 0.05	8.62	-0.33	8.81	-0.14	8.95	0	8.63	-0.32	8.72	-0.23
H ₅ '	4.50 \pm 0.03 ^a	4.44 ^a	-0.06	4.45 ^a	-0.05	4.51 ^a	0	4.43 ^a	-0.07	4.44 ^a	-0.06

^a Taken as the center of AB quartet.

In conclusion, we have been able to determine the conformations of the C₄-C₄', C _{α} -C _{β} , and N-C _{α} bonds of pyridoxal-aromatic amino acid Schiff's bases by application of the vicinal spin-spin couplings, the diamagnetic anisotropic shift, and the nuclear Overhauser effect in conjunction with stereospecific deuterium labeling. These results can be useful in understanding the mechanism of reactions of pyridoxal Schiff's bases. In particular, the results provide an explanation for the "abnormal" reactivity of phenylalanine SB, compared with Schiff's bases of nonaromatic amino acids with pyridoxal, in reactions involving C _{α} -H _{α} bond cleavage (Tsai et al., 1978).

Acknowledgments

We are indebted to Professor G. W. Kirby, University of Glasgow, for providing the stereospecifically deuterated phenylalanines.

Supplementary Material Available

¹³C chemical shifts and ¹³C-¹H coupling constants (Table I) and ¹H chemical shifts and ¹H-¹H coupling constants (Table III) of Schiff's bases (4 pages). Ordering information is given on any current masthead page.

References

- Barrett, A. N., & Palmer, R. A. (1969) *Acta Crystallogr., Sect. B* 25, 688.
- Batchelor, J. G., & Feeney, J. (1975) *J. Chem. Soc., Chem. Commun.*, 503.
- Cavanaugh, J. R. (1970) *J. Am. Chem. Soc.* 92, 1488.
- Chang, C.-j. (1976) *J. Org. Chem.* 41, 1881.
- Chang, C.-j., & Peck, G. E. (1976) *J. Pharm. Sci.* 65, 1019.
- Chang, C.-j., Shieh, T.-L., & Floss, H. G. (1977a) *J. Med. Chem.* 20, 176.
- Chang, C.-j., Floss, H. G., & Steck, W. (1977b) *J. Org. Chem.* 42, 1337.
- Dalling, D. K., Grant, D. M., Horton, W. J., & Sagers, R. D. (1976) *J. Biol. Chem.* 251, 7661.
- Dunathan, H. C. (1966) *Proc. Natl. Acad. Sci. U.S.A.* 55, 712.
- Dunathan, H. C. (1971) *Adv. Enzymol.* 35, 79.
- Espersen, W. G., & Martin, R. B. (1976) *J. Phys. Chem.* 80, 741.
- Fisher, T. C., & Metzler, D. E. (1969) *J. Am. Chem. Soc.* 91, 5323.
- Gutowky, H. S., Belford, G. G., & McMahon, P. E. (1962) *J. Chem. Phys.* 36, 3353.
- Harris, C. M., Bennett, C., & Metzler, D. E. (1975), paper presented at the Conference on Vitamin B₆ and Its Function in Enzymes, Ames, Iowa, October 10-11.
- Harruff, R. C., & Jenkins, W. J. (1976) *Org. Magn. Reson.* 8, 548.
- Heinert, D., & Martell, A. E. (1962) *J. Am. Chem. Soc.* 84, 3257.
- Johnson, R. J., & Metzler, D. E. (1970) *Methods Enzymol.* 18, 433.
- Kainosho, M., & Ajisaka, K. (1975a) *J. Am. Chem. Soc.* 97, 1488.
- Kainosho, M., & Ajisaka, K. (1975b) *J. Am. Chem. Soc.* 97, 5630.
- Kopple, K. D., Wiley, G. R., & Tanke, R. (1973) *Biopolymers* 12, 627.
- Lemieux, R. U., Nagabhushan, T. L., & Paul, B. (1972) *Can. J. Chem.* 50, 773.
- Mizushima, S. (1954) *Structure of Molecules and Internal Rotation*, Academic Press, New York, N.Y.
- O'Leary, M. H., & Payne, J. R. (1976) *J. Biol. Chem.* 251, 2248.
- Pachler, K. G. R. (1964) *Spectrochim. Acta* 20, 581.
- Sawada, S., Kumagai, H., Yamada, H., & Hill, R. K. (1975) *J. Am. Chem. Soc.* 97, 4334.
- Snell, E. E., & Dimari, S. J. (1970) *Enzymes, 3rd Ed.* 2, 355-370.
- Snow, M. L., Lauinger, C., & Ressler, C. (1968) *J. Org. Chem.* 33, 1774.
- Tsai, M.-D., Weintraub, H. J. R., Byrn, S. R., Chang, C.-j., & Floss, H. G. (1978) *Biochemistry* 17 (following paper in this issue).
- Tumanyan, V. G., Mamaeva, O. K., Bockarov, A. L., Ivanov, U. I., Karpeisky, M. Ya., & Yakovlev, G. I. (1974) *Dokl. Akad. Nauk SSSR* 214, 1452.
- Turchin, K. F., Bystrev, V. F., Karpeisky, M. Ya., Olkhovoy, A. S., Florentiev, V. L., & Sheinker, Yu. N. (1968) in *Pyridoxal Catalysis: Enzymes and Model Systems* (Snell, E. E., Braunstein, H. E., Severin, E. S., & Tarchinsky, Yu. M., Eds.) pp 67-79, Interscience, New York, N.Y.
- Witherup, T. H., & Abbott, E. H. (1975) *J. Org. Chem.* 40, 2229.
- Wütherich, K. (1976) *NMR in Biological Research: Peptides and Proteins*, North-Holland Publishing Co., Amsterdam.