Model Studies of Pyridoxal Schiff's Bases. Coplanarity and Intramolecular Hydrogen Bonding

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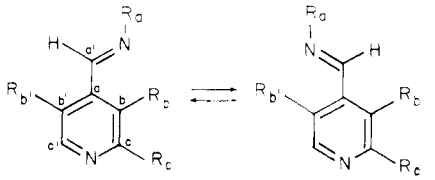
The interactions between the π cloud of the aromatic ring and the π-electron pair of the imine double bond of aromatic oximes as model compounds of pyridoxal Schiff's bases have been studied by high-resolution carbon-13 magnetic resonance spectroscopy. The coplanarity and intramolecular hydrogen bonding have been determined by $^{13}C-^{1}H$ long-range couplings. This detailed investigation of $^{13}C-^{1}H$ coupling also provides unambiguous proof of the existence of the "enol-imine" tautomers in chloroform and dimethyl sulfoxide solutions. The tautomerism between the "enol-imine" and "keto-enamine" is discussed.

Pyridoxal and pyridoxal phosphate Schiff's bases (1) are important intermediates in many enzymatic and nonenzymatic reactions of carbonyl compounds and amines. Such reactions, for example, the various transformations of amino acids,[1,2] involve a number of electron and hydrogen shifts and are presumably governed by subtle conformational[3] and tautomeric[1] changes.



1 (cis) 1 (trans)

Current views of the general mechanism of pyridoxal phosphate catalysis[1] invoke an interaction between the π cloud of the pyridine ring and the π-electron pair of the imine double bond, implying coplanarity of the two π planes. Recent NMR measurements have shown that the two-bond $^{13}C-^{1}H$ coupling constants [$^{2}J(^{13}C_a-^{1}H_{a'})$] in pyridoxal Schiff's bases are considerably smaller than in pyridoxal phosphate (pyridoxal phosphate, $^{2}J_{CH}$ = 21.4 Hz,[4] pyridoxal Schiff's bases, $^{2}J_{CH}$ < 11 Hz at pH ~8.5[5]), which could indicate a change in the degree of coplanarity in going from pyridoxal phosphate to the Schiff's bases. On the other hand, dependence of $^{1}J_{CH}$,[6] $^{2}J_{CH}$,[7] and $^{3}J_{CH}$[8-10] on substituent electronegativity has been established and hence this could provide an alternative explanation for the change in coupling constants.

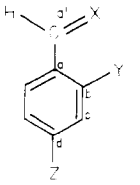
In order to distinguish between these two interpretations we conducted model studies with a series of simple benzaldehydes and pyridine-4-aldehyde and their respective oximes **2**-**6**.

Coplanarity can clearly be assumed for these sterically unhindered compounds and unequivocal proof for this assumption in the case of salicylaldehyde and its oxime (**4a** and **4b**) is the demonstration of intramolecular hydrogen bonding. The $^{2}J(C_a-H_{a'})$ values of these compounds as summarized in Table I clearly indicate that a large change of the coupling constants results from the variation in the substituent X. Therefore, the coupling constant changes between pyridoxal phosphate and the Schiff's bases are not necessarily indicative of a distortion of the coplanarity.

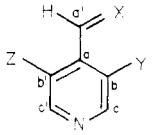
Considerable effort has been directed toward determining whether the conformation of the $C_a-C_{a'}$ bond in pyridoxal phosphate and model compounds (1) is cis or trans. Earlier ir[11] and uv[12] spectral analyses have indicated the cis conformation, in agreement with the more recent conclusions of Tumanyan's group[13] and our group,[14] which are based on nuclear Overhauser effect measurements and

Table I. Two-Bond $^{13}C-^{1}H$ Coupling Constants of Aromatic Aldehydes and Their Oximes

Compd	$^{2}J(C_a-H_{a'})$, Hz		
	a	b	c
2	23.8	8.2	
3	24.4	6.6	
4	21.4	8.2	
5	22.6		6.9
6	25.3	7.3	



2a, X = O; Y = H; Z = H
b, X = NOH (syn); Y = H; Z = H
c, X = $NOCH_3$ (syn); Y = H; Z = H
3a, X = O; Y = H; Z = Cl
b, X = NOH (syn); Y = H; Z = Cl
c, X = $NOCH_3$ (syn); Y = H; Z = Cl
4a, X = O; Y = OH; Z = H
b, X = NOH (syn); Y = OH; Z = H
c, X = $NOCH_3$ (syn); Y = OH; Z = H
5a, X = O; Y = OCH_3; Z = H
b, X = NOH (syn); Y = OCH_3; Z = H
c, X = $NOCH_3$ (syn); Y = OCH_3; Z = H



6a, X = O; Y = H; Z = H
b, X = NOH; Y = H; Z = H
7, X = NOH; Y = OH; Z = CH_2OH
8, X = O; Y = OH; Z = H

theoretical calculations, respectively. An x-ray structure analysis of pyridoxal phosphate oxime (1, R_c = CH_3; R_b = R_a = OH; $R_{b'}$ = $CH_2OPO_3^{2-}$) also shows the cis conformation.[15] On the other hand, a recent uv study by Metzler's group[16] indicates the presence of a mixture of the two conformers in solution and suggests that the "internal" Schiff's base in the enzyme aspartate aminotransferase may actually be in the trans conformation. Likewise, Turchin's ^{1}H NMR investigation[17] also indicates the trans conformation of pyridoxal and its derivatives based on the long-range $^{1}H-^{1}H$ coupling constants. Their argument is derived from model studies of 3-hydroxy-5-hydroxymethyl-4-pyridinealdoxime (**7**). The trans conformation was characterized by the coupling constants, $^{5}J(H_{a'}-H_{c'})$ = 0.55 Hz and $^{5}J(H_{a'}-H_c)$ = 0 Hz. It must be

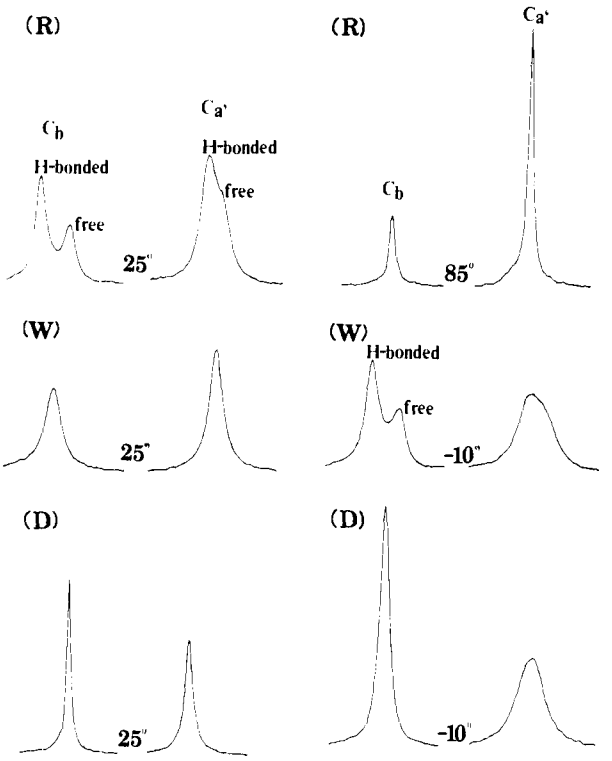


Figure 1. Carbon-13 proton-decoupled spectra of the C_b and $C_{a'}$ resonances of salicylaldoxime in deuteriodimethyl sulfoxide solution at different temperatures (°C): (R) in "regular" Me_2SO-d_6 [0.35% (w/w) of water]; (W) in "wet" Me_2SO-d_6 (1.8% of water); (D) in "dry" Me_2SO-d_6 (<0.001% of water).

noted, however, that this argument is not on firm grounds, because $H_{a'}$ couples with H_c and $H_{c'}$ via two completely different pathways which may theoretically result in different coupling constants.[18] Our three-bond ^{13}C–^1H coupling data[9,10] have shown unequivocally that the long-range coupling constants can be considerably reduced if coupling occurs through an oxygen-substituted carbon atom.

These discrepancies indicate that further investigations are necessary to clarify the conformation of the C_a–$C_{a'}$ bond. The cis conformation can be invoked if the existence of an intramolecular hydrogen bond between the nitrogen and the *o*-hydroxy group can be demonstrated. To probe for such hydrogen bonding by analysis of the long-range coupling patterns, the assignments of the C_c, C_b, and $C_{a'}$ resonances of **4b** are crucial. On the basis of ^{13}C chemical shift theory,[19] C_c can be assigned the most upfield and $C_{a'}$ the most downfield methine-carbon signals since C_c is ortho to the hydroxy group and $C_{a'}$ is α to the nitrogen atom. Similarly, the C_b resonance is the most downfield quaternary carbon signal. Based on our previous studies,[9,10] the ^{13}C–^1H long-range coupling pattern can be conveniently utilized to monitor the dynamic equilibrium of hydrogen bonding. The high-resolution ^{13}C spectrum of salicylaldoxime (**4b**) in deuteriochloroform solution at room temperature reveals that the coupling pattern of the C_c signal is comparable to that of *syn*-*p*-chlorobenzaldoxime (**3b**) in deuteriodimethyl sulfoxide solution [**4b** = 116.3 ppm, doublet of doublets, $^1J(C_c–H_c)$ = 160.9 Hz, $^3J(C_c–H_{c'})$ = 7.5 Hz; **3b** = 128.8 ppm, doublet of doublets, $^1J(C_c–H_c)$ = 167.1 Hz, $^3J(C_c–H_{c'})$ = 5.4 Hz], indicative of the lack of stable intramolecular hydrogen bonding. This might be the result of the catalytic effect of the oxime hydroxy group in enhancing the equilibration rate between the hydrogen bonded and free conformers, similar to the

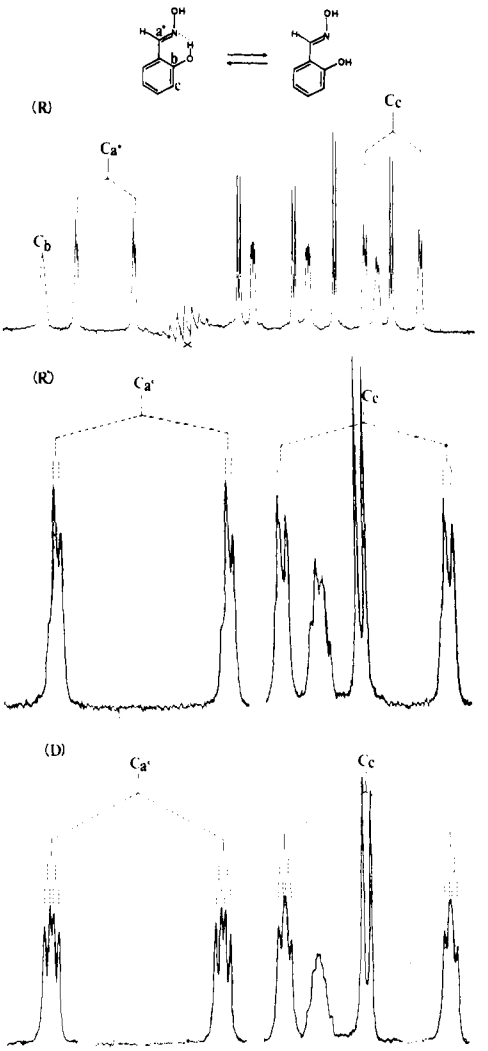
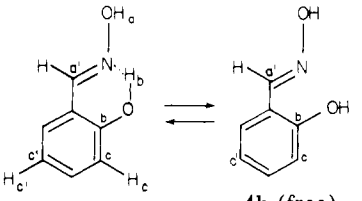


Figure 2. Carbon-13 proton-coupled spectra of salicylaldoxime: (R) in "regular" deuteriodimethyl sulfoxide [0.35% (w/w) of water]; (R′) expanded portion of the $C_{a'}$ and C_c signals of (R); (D) expanded portion of $C_{a'}$ and C_c in "dry" deuteriodimethyl sulfoxide.

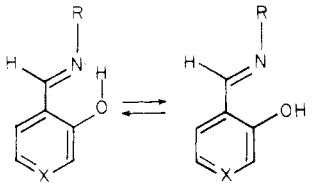
situation observed for salicylic acid in deuterioacetone solution.[10]



Dimethyl sulfoxide has been used as a solvent to inhibit proton exchange in ^1H NMR[20] and ^{13}C NMR.[10] In "regular" deuteriodimethyl sulfoxide solution of **4b** at 25 °C, the proton-decoupled ^{13}C spectrum of the C_b resonance appears as a doublet (Figure 1), and the proton-coupled spectrum of the C_c and $C_{a'}$ resonances displays extra shoulders (Figure 2). At 85 °C, however, the doublet becomes a singlet and the shoulders disappear (Figure 1), resembling the spectrum in deuteriochloroform solution. These spectral results imply the coexistence of two conformers or two tautomers.

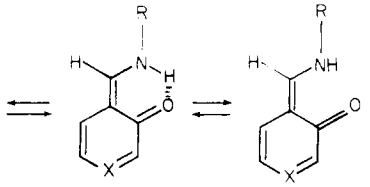
An investigation of the ir spectra of the amino acid Schiff's bases of 3-hydroxypyridine-4-aldehyde (**8**) and salicylaldehyde (**4a**) had shown the presence of the

"keto-enamine" tautomers (3′ and/or 4′),[11] while an analysis of the uv spectra indicates the presence of two tautomers, both the "enol-imine" (1′ and/or 2′) and the "keto-enamine" species.[12]



"enol-imine"

1′ 2′



"keto-enamine"

3′ 4′

X = N or CH

A spectral analysis of salicylaldoxime (**4b**) was therefore undertaken in "dry" deuteriodimethyl sulfoxide solution with a view toward reducing the equilibration rate and varying the isomeric ratio. However, only one sharp C_b resonance signal was observed in the proton-decoupled spectrum (Figure 1), suggesting the presence of a single isomer. In the proton-coupled spectrum, the C_c and $C_{a'}$ resonances both appear as doublet of double doublets (Figure 2) [C_c = 116.5 ppm, $^1J(C_c-H_c)$ = 160.3 Hz, $^3J(C_c-H_{c'})$ = 7.2 Hz, $^3J(C_c-OH_b)$ = 5.4 Hz; $C_{a'}$ = 149.2 ppm, $^1J(C_{a'}-H_{a'})$ = 166.1 ppm, $^3J(C_{a'}-H_{b'})$ = 8.7 Hz, $^3J(C_{a'}-OH_a)$ = 5.3 Hz]. These coupling patterns allow us to unequivocally assign the structure of the intramolecularly hydrogen-bonded "enol-imine" tautomers (1′, X = CH). The second isomer in "regular" deuteriodimethyl sulfoxide solution should be the free conformer (2′, X = CH). It is not likely to be either of the "keto-enamine" tautomers (3′ or 4′, X = CH) because there is no uv spectral change between the "dry" and the "wet" Me_2SO solutions.[21]

It is evident from these preliminary studies that the analysis of long-range coupling patterns can be extremely useful in the conformational analysis of biological molecules in solution. It is hoped that in conjunction with specific labeling by isotopic enrichment, ^{13}C or ^{15}N NMR may also become useful for the conformational analysis of substrates and cofactors bound to an enzyme or receptor.

Experimental Section

The NMR spectra were obtained in 10-mm (^{13}C NMR) and 5-mm (^1H NMR) spinning tubes. Tetramethylsilane (Me_4Si) was used as internal reference for ^1H NMR spectra. The ^{13}C resonances of deuteriodimethyl sulfoxide and deuteriochloroform serve as internal references for ^{13}C NMR spectra and converting the Me_4Si scale involved the following corrections.

$$\delta\ (Me_4Si) = \delta\ (Me_2SO\text{-}d_6) + 39.6 \text{ ppm}$$

$$\delta\ (Me_4Si) = \delta\ (\text{chloroform-}d_6) + 76.9 \text{ ppm}$$

The instruments employed were a Jeol PFT-100 spectrometer (^{13}C NMR) operating at 23 kG, interfaced with a Jeol EC-100 Fourier-transform computer with 20K memory, and a Varian EM-360 60-MHz spectrometer (^1H NMR). The normal spectra were recorded at ambient temperature using an internal deuterium lock; the coupling patterns were measured for 2500-Hz sweep width. The typical pulse width was 25.5 μs, and the repetition time between pulses were 8 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 gated decoupling technique was employed to measure proton-coupled spectra.

A "regular" deuteriodimethyl sulfoxide usually contained 0.2–0.5% (w/w) of water. A "dry" deuteriodimethyl sulfoxide contained less than 0.001% (w/w) of water and was prepared by distillation over calcium hydride prior to use. A "wet" deuteriodimethyl sulfoxide solution contained 1.5–2.5% of water.

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References and Notes

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(21) Uv λ_{max} 307 and 259 nm in "dry" Me_2SO; and 308 and 260 nm in "wet" Me_2SO-d_6 [molar ratio (water/oxime) = 15]. No significant absorption at ~420 nm due to the π_1 transition of the "keto-enamine" tautomer[1,12] can be observed. A detailed analysis of the uv and ir spectra will be published later.