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Chirality in pyridoxal

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Abstract. Pyridoxal and its iminium derivatives form cyclic compounds via an intramolecular nucleophilic attack. This results in a configurational chirality on C4' which can be related to out-of-plane orientations around the C4-C4' bond in the open forms. ¹H- and ¹³C-NMR structural studies of the cyclic compounds were performed in solution and in the solid state (¹³C NMR only). It was found that the presence of an additional chiral centre in the iminium moiety gives rise to an excess of one of the diastereomers for the cyclic aminal. This indicates a preference for one of the axially chiral C4-C4' conformers prior to ring closure. The present results may be of relevance with respect to the function of the coenzyme pyridoxal phosphate, which forms iminium compounds with L or D amino acids. Axial chirality in these systems has been proposed by us as a mechanism for the stereospecificity of enzymatic amino acid transformations.

Introduction

In various enzymatic transformations of amino acids (*e.g.*, racemization and transamination), the coenzyme pyridoxal phosphate plays an important role by forming a Schiff base with the amino acid¹. Recently, we have carried out quantum-chemical calculations on these compounds which indicate the possibility of an out-of-plane orientation around the C4-C4' bond, leading to a location of the iminium side-chain on either the A or B side of the asymmetric pyridinium ring².

Fig. 1. Schiff base formed from pyridoxal phosphate and an amino acid showing the numbering and A/B side definition.

The resulting axial chirality was found to be coupled to the amino acid configuration and it was therefore suggested that the presence of out-of-plane conformations allows the enzyme to position the amino acid for the initial process step, *i.e.*, deprotonation of C α . This mechanism may be of importance for the stereochemistry of the enzymatic reactions.

In model systems derived from pyridoxal, the axial chirality can be transformed into a configurational chirality via an intramolecular cyclization reaction. Therefore, studying the cyclic systems could provide insight into the initial conformational preferences around the C4-C4' bond in the open systems.

In this paper, we will describe the presence of open or cyclic forms for pyridoxal, pyridoximines and pyridoximinium systems. The preference for the different stereoisomers which arise from the chiral centre on C4' in the cyclic forms is investigated and explored with additional chirality.

Results and discussion

1. Pyridoxal

It is known from UV spectroscopy³ and 25-MHz ¹³C-NMR studies⁴ that pyridoxal predominantly adopts the cyclic hemi-acetal form **1b** in aqueous solution. We have studied pyridoxal in DMSO-*d*₆ solution using 200-MHz ¹H- and 50.3-MHz ¹³C-NMR spectroscopy and established the presence of the hemi-acetal exclusively.

The H4' resonance at 6.4 ppm (estimated for C(O)H: 9–10 ppm) and the C4' signal at 99.5 ppm (estimated for C(O)H: 190–200 ppm) clearly demonstrate this structural

31.25 assignment. Additional evidence was provided by the presence of a long-range coupling constant (*ca.* 2 Hz) between H4' and the 5'-methylene protons, which can only occur in the cyclic form.

Using cross-polarization magic-angle-spinning (CP-MAS) ¹³C NMR spectroscopy, we were able to study the structure of pyridoxal in the solid state. The presence of the hemi-acetal could also be established in the solid compound from a clear C4' resonance at 100.0 ppm. This is in agreement with earlier indications obtained from the solid-state IR spectrum of pyridoxal⁵.

The conformation of the five-membered hemi-acetal ring in **1b** in solution (both DMSO and water) can be inferred from the spectral parameters of the 4'- and 5'-protons (see Table I).

Table I Spectral parameters of the 4'- and 5'-protons in the pyridoxal hemiacetal **1b** in D₂O and DMSO-*d*₆.

	D ₂ O		DMSO- <i>d</i> ₆	
	δ (ppm)	⁴ J _{4'5'} (Hz)	δ (ppm)	⁴ J _{4'5'} (Hz)
H4'	6.40	1.4	6.38	1.5
H5'a	4.95	0	4.90	0
H5'b	5.10	1.4	5.05	1.5

A marked difference in chemical shift is seen between the two 5'-protons (*ca.* 0.15 ppm), while a long-range coupling constant to H4' (1.4 Hz) is only observed for the downfield H5'b resonance, as was ascertained via selective irradiation experiments. The difference between the 5'-protons can be rationalized by the well-known anomeric effect⁶, which favours a pseudo-axial position for the 4'-hydroxyl group. The preferred location can be obtained by a puckering of the five-membered hemi-acetal ring via a displacement of O5' away from the A side of the pyridinium ring for the 4'-*S* enantiomer (see Fig. 2). The situation is exactly reversed for the 4'-*R* isomer, where the hydroxyl is on the other (B) side of the ring, and the hemi-acetal ring pucker will locate O5' away from the B side.

Fig. 2. Conformation of the five-membered hemi-acetal ring in the 4'-*S* enantiomer of **1b** as derived from the ¹H-NMR data.

As a result of the puckering in the 4'-*S* enantiomer, the H5'a proton will also be located pseudo-axially and will be shielded by the nearby 4'-hydroxyl. The pseudo-equatorial H5'b proton, on the other hand, is found further downfield and is oriented in a perfect way (all-*trans* geometry) for long-range coupling with H4'. A similar situation arises in the 4'-*R* isomer, where H5'b is pseudo-axial (upfield) and only the pseudo-equatorial H5'a (downfield) couples with H4'.

2. Pyridoximines

Reaction of the pyridoxal hemi-acetal with a primary amine gives rise to Schiff base formation, *i.e.* an imine compound. For the methyl imine of pyridoxal, it was found from ¹H- and ¹³C-NMR spectroscopy in DMSO-*d*₆ that

only the open form **2a** is present. This is in good agreement with earlier NMR studies on pyridoximines^{4c}.

The H4' resonance is found at 8.9 ppm, while the location of the *N*-methyl signal (3.5 ppm) is incompatible with an amine. The C4' resonance at 164.0 ppm also indicates an imine instead of an aminal form (estimated: 80 ppm). The formation of **2a** most likely occurs via ring opening of pyridoxal, which may be base-catalyzed by deprotonation of the 4'-hydroxyl in the hemi-acetal form. The Schiff base formation then occurs with the aldehyde **1a**. The absence of any cyclization of **2a** into **2b** can be explained by the lower electrophilicity of the imine, as compared to the carbonyl moiety.

However, in most cases, it is found that the imine nitrogen is protonated, indicating the presence of an iminium system. As will be shown, this iminium readily forms a cyclic compound. Indeed, protonation of **2a** with hydrochloride gas in chloroform resulted in the formation of the pyridinium hydrochloride salt of the cyclic aminal **2b**. This followed clearly from the ¹H NMR spectrum in DMSO-*d*₆, in which the H4' resonance shifted upfield to 6.1 ppm, and the *N*-methyl signal was found at 2.0 ppm, as expected for an amine.

For **2b**, the presence of a puckered five-membered aminal ring was also observed. The H5' protons differed markedly in chemical shift (*ca.* 0.3 ppm) and only the downfield resonance displayed a long-range coupling to H4'. Evidently, the anomeric effect also favours a pseudo-axial amine group in the aminal **2b**.

3. Pyridoximinium systems

After reaction of the pyridoxal hemi-acetal **1b** with a secondary amine, the resulting iminium compound can form a cyclic aminal, since the electrophilic character of the C=N⁺ bond is strongly enhanced by the presence of a positive charge. It has been reported by Nakagawa *et al.*⁷, on the basis of 60-MHz ¹H NMR and IR spectroscopic studies, that the cyclic form is present for various amine substituents. We found a similar behaviour after reaction of pyridoxal with dimethylamine to give compound **3**, which to date has not been studied.

The ¹H- and ¹³C-NMR spectra in DMSO-*d*₆ clearly showed the presence of the cyclic form **3b** (H4' δ 6.0 ppm; C4', δ 97.9 ppm) instead of the iminium **3a** (estimated: H4', 9–10 ppm; C4', 150–160 ppm). Also in the solid state, the aminal form was found to be present, as based on the CP-MAS ¹³C NMR spectrum (C4', δ 98.9 ppm). The rigid conformation in the solid state gives rise to two separate *N*-methyl ¹³C-NMR resonances, whereas in solution they coalesce due to rapid rotation around the C4'–N bond.

This clearly shows that the C4'-N linkage is a single bond and also proves that the cyclic aminal form is predominant. Analysis of the ^1H NMR spectrum showed that both H5' protons are almost identical in **3b** and both have a long-range coupling to H4'. It must be concluded that the conformation of the five-membered aminal ring is now close to planar. Most probably this is due to the presence of two bulky groups on the amine, which lead to unfavourable steric interactions with a H5' proton when the amino group is in a pseudo-axial position.

In order to study the preference for the different configurations at C4' in the presence of another chiral centre, we synthesized aminal compounds with chiral secondary amines, viz. methyl[(*S*)- and (*R*)- α -methylbenzyl]amine.

The presence of the two diastereoisomeric forms for both (α -*S*)- and (α -*R*)-**4b** could be clearly seen in the ^1H - and ^{13}C -NMR spectra, which contain two separate sets of resonances. For both compounds, it was found that one of the diastereoisomers is consistently present in a slight excess (*ca.* 55:45). The dominant isomers have exactly the same chemical shifts in the NMR spectra for *R* and *S* chirality on C α , and must therefore be enantiomers. This implies that the configuration on C4' is reversed in the dominant isomer of each compound together with the C α chirality, i.e. the chiral centre on C α determines the configurational preference on C4' after ring closure.

Based on the theoretical studies on the Schiff bases of pyridoxal phosphate and amino acids², we propose that the iminium side-chain in the open form **4a** has a preference for one of the out-of-plane locations, which is related to the chirality in the amine. Cyclization into **4b** then leads to a predominance of one of the configurations on C4'. Further studies are now in progress to afford more direct support for the mechanistic proposal that the initial axial preference around the C4-C4' bond can be related to the mode of ring closure, leading to a fixed chirality.

Experimental

^1H NMR spectra were recorded at 200 MHz and ^{13}C NMR spectra at 50.3 MHz, both on a Bruker AC 200 spectrometer. Proton chemical shifts were related to the residual signal of the deuterated solvent (D_2O , 4.68 ppm; $\text{DMSO-}d_6$, 2.50 ppm) or TMS (CDCl_3). ^{13}C chemical shifts were referenced to the CDCl_3 triplet (77 ppm). The solid-state CP-MAS ^{13}C NMR spectra were recorded at 75.5 MHz on a Bruker CXP 300 spectrometer equipped with a double-air bearing CP-MAS probe. Chemical shifts were related to adamantane. The cross-polarization measurements were carried out with flipback of the proton magnetization and spin-temperature inversion. For the CP-MAS experiments, we used a time domain of 1 K data points and a spectral width of 17.2 kHz. Pyridoxal and pyridoxal hydrochloride were used as received.

5-Hydroxy-6-methyl-4-(methyliminomethyl)-3-pyridinemethanol (**2a**)

A solution of 2 g (0.01 mol) of pyridoxal hydrochloride in 80 ml of methanol p.a. was stirred with 2 g (0.024 mol) of sodium bicar-

bonate at 50°C for 1 h. The inorganic salt was removed by filtration and excess of methylamine was added to the filtrate. After standing overnight at room temperature, the solution was evaporated to dryness under reduced pressure. The residue was extracted with 100 ml of dichloromethane, giving a bright yellow solid in a yield of 1.5 g (83%). M.p. 146–147°C. A sample was recrystallized from dioxane/hexane (1/4); m.p. 149–150°C⁸. $\text{C}_8\text{H}_{12}\text{N}_2\text{O}_2$ (168.19) calcd.: C 59.98, H 6.71, N 15.55; found: C 60.47, H 6.74, N 15.82%. ^1H NMR ($\text{DMSO-}d_6$): δ 2.35 (3H, s, 2- CH_3), 3.50 (3H, s, N- CH_3), 4.62 (2H, s, 5'- CH_2), 7.85 (1H, s, H6), 8.89 (1H, s, H4'). ^{13}C NMR (CDCl_3): δ 18.4 (2- CH_3), 45.9 (N- CH_3), 59.9 (C5'), 119.8 (C4), 131.8 and 136.8 (C5, C6), 150.2 (C3), 155.4 (C2), 163.95 (C4').

1,3-Dihydro-6-methyl-1-(methylamino)furo[3,4-c]pyridin-7-ol (**2b**)

Hydrochloride gas was bubbled through a solution of **2a** in chloroform until a white precipitate formed. Filtration and drying yielded the hydrochloride of **2b**. ^1H NMR ($\text{DMSO-}d_6$): δ 1.95 (3H, s, N- CH_3), 2.05 (3H, s, 2- CH_3), 4.62 (1H, d, H5'b), 4.95 (1H, dd, H5'a), 6.10 (1H, d, H4'), 7.75 (1H, s, H6).

1-(Dimethylamino)-1,3-dihydro-6-methylfuro[3,4-c]pyridin-7-ol (**3b**)

This compound was prepared according to Nakagawa et al.⁷ To a suspension of pyridoxal (2.5 g, 0.012 mol) in 40 ml benzene was added dropwise a solution of dimethylamine (2.0 g, 0.044 mol) in 20 ml benzene. The resulting solution was heated to reflux and the water was azeotropically removed using a Dean-Stark apparatus. The precipitated dimethylamine hydrochloride was removed by filtration and the filtrate was evaporated to dryness. The residue was triturated with dry diethyl ether to give 1.7 g (71%) of the desired product. M.p. 161–162°C. Recrystallization from hexane afforded a sample with m.p. 169–170°C. $\text{C}_9\text{H}_{14}\text{N}_2\text{O}_2$ (182.22) calcd.: C 61.83, H 7.26, N 14.43; found: C 62.07, H 7.31, N 14.85%. ^1H NMR ($\text{DMSO-}d_6$): δ 2.38 (6H, s, N- CH_3), 2.45 (3H, s, 2- CH_3), 5.05 (2H, s, 5'- CH_2), 5.95 (1H, s, H4'), 8.00 (1H, s, H6). ^{13}C NMR (CDCl_3): δ 17.70 (2- CH_3), 38.96 (N- CH_3), 70.43 (C5'), 97.91 (C4'), 130.16 (C4), 133.08 and 134.32 (C5, C6), 144.78 and 146.37 (C2, C3).

1,3-Dihydro-6-methyl-1-[methyl[(*S*)/(*R*)- α -methylbenzyl]amino]furo[3,4-c]pyridin-7-ol (**4b**)

This compound was prepared according to the procedure described for **2b** from pyridoxal and methyl[(*S*)/(*R*)- α -methylbenzyl]amine. The dominant diastereoisomer is referred to as isomer **a**. ^1H NMR (CDCl_3): δ 1.50 (3H, d, CH_3 benzyl, isomer **a**), 1.55 (3H, d, CH_3 benzyl, isomer **b**), 2.12 (3H, s, N- CH_3 , **a**), 2.15 (3H, s, N- CH_3 , **b**), 2.45 (6H, 2- CH_3 , **a** + **b**), 4.00 (1H, m, H-benzyl, **b**), 4.15 (1H, m, H-benzyl, **a**), 4.9–5.0 (4H, m, 5'- CH_2 , **a** + **b**), 5.95 (1H, m, H4', **a**), 6.15 (1H, m, H4', **b**), 7.2–7.4 (10H, m, phenyl, **a** + **b**), 7.90 (2H, s, H6, **a** + **b**).

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