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PROTONATION STUDIES OF SCHIFF BASES USING ^{13}C AND ^{15}N-NMR SPECTROSCOPY

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Abstract: ^{13}C and ^{15}N-NMR spectroscopy provides clear evidence that the Schiff bases of 3-hydroxy-4-pyridinecarboxaldehyde exist as a mixture of hydroxyimino and oxoenamino tautomers in acid media. The study of the stabilities of the tautomeric forms has been approached using density functional calculations (B3LYP/6-31G**). The X-ray molecular structure of (*E*)-4-[(4-bromophenylimino)methyl]-3-hydroxypyridinium tetrafluoroborate has been solved.

• INTRODUCTION

Vitamine B_6 forms Schiff bases (imines) between the pyridoxal-5'-phosphate (PLP) cofactor and the substrates as intermediates in the metabolism of amines and aminoacids (Figure 1).[1]

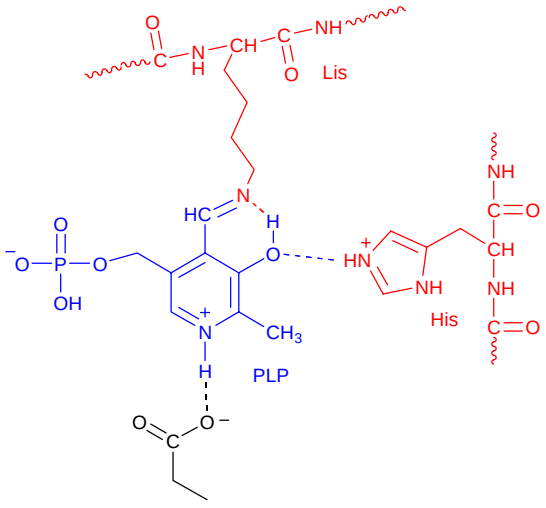


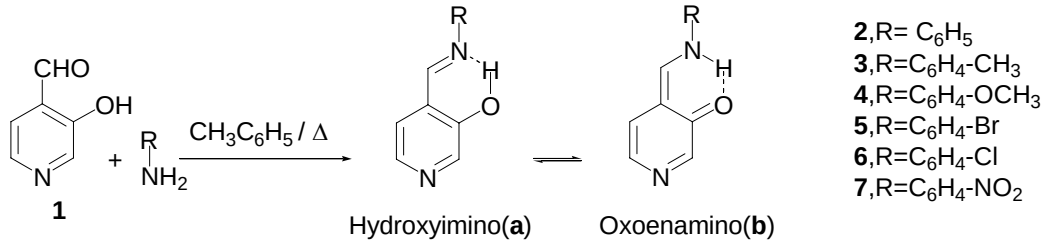
Figure 1: Active site of 2-amino-3-ketobutyrate-CoA ligase isolated from beef liver.[1]

Our research for systems that sustain phenomena such as inter- and intra-molecular hydrogen bonds, led us to select Schiff bases derived of 3-hydroxy-4-pyridinecarboxaldehyde (**1**).[2] These systems can exist in equilibrium, through proton exchange, between hydroxyimino and oxoenamino tautomers. The exact location of the proton depends on variety of factors, including the nature of substituents, solvent, temperature or other energetic stimuli.[3]

• RESULTS AND DISCUSSION

FORMATION OF IMINES AND PROTONATION

Imines (**2**-**7**) were synthesised by reaction of 3-hydroxy-4-pyridine-carboxaldehyde (**1**) with *p*-substituted anilines (Scheme 1). In all cases we obtained only the *E* isomer and the tautomeric form in neutral media is the hydroxyimino form **a**.[4-6]



Scheme 1: Synthesis of 3-hydroxy-4-pyridine-carboxaldehyde imines (**2**-**7**).

Protonation was achieved in solution by treating the imines (**2**-**7**) with trifluoroacetic acid (TFA) and the salts of **4** and **5** were prepared as the tetrafluoroborates in ether solution. A priori protonation can take place in three sites, pyridine nitrogen (tautomers **c** and **d**), imine nitrogen (tautomers **e** and **f**), and, less probably, on the hydroxyl group (**g**), as shown in Figure 2.

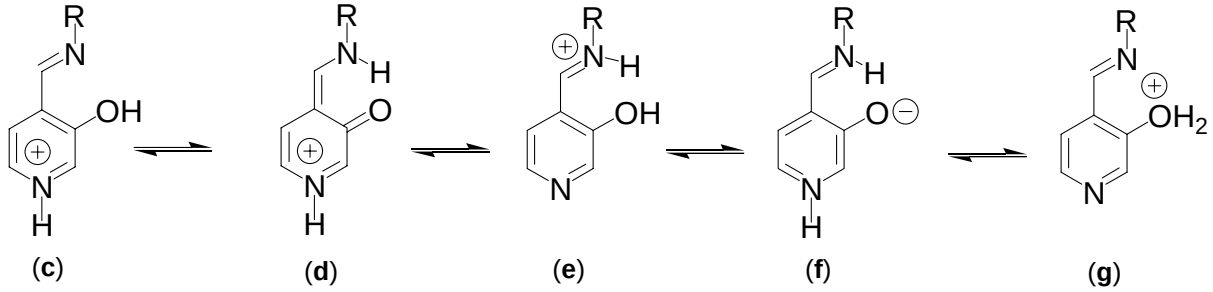


Figure 2: Possible protonation sites of the imines (**2**-**7**).

THEORETICAL CALCULATIONS OF NEUTRAL AND PROTONATED IMINES

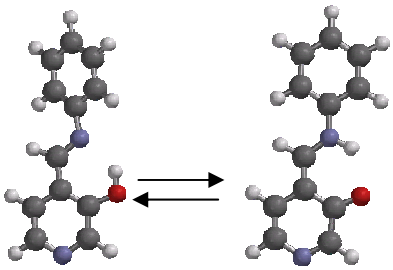
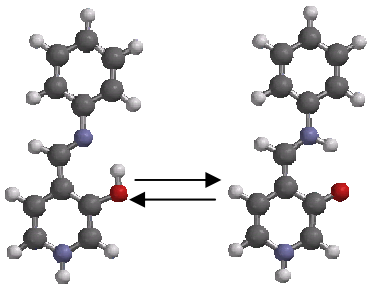
DFT computational studies were carried out to predict the stability of the different tautomers, and in all cases for the neutral molecule, the hydroxyimino tautomer (**a**) was found to be the most stable one in 20-22 kJ/mol^{-1}. In acid media the oxo tautomer (**c**) is only 4-7 kJ/mol^{-1} higher in energy than the hydroxyimino one (**d**). Thus, the protonation of the pyridine nitrogen reduces the energy difference between the two tautomers, so the two forms could be present (Table 1).

^{1}H, ^{13}C and ^{15}N NMR STUDIES IN NEUTRAL AND ACID MEDIA

All derivatives have been characterized by multinuclear ^{1}H, ^{13}C, ^{15}N-NMR spectroscopy in neutral solvents[4] and in acid media. The difference between the ^{1}H-NMR chemical shifts are the expected ones for a protonated pyridine ring, the main difference is shown for H5 (~ +1ppm) and for the iminic proton (~ +0.6 ppm). The results of ^{13}C-NMR are reported in Figure 3, in this case the most affected signals are C5, C6 and C1'. Note that all the coupling constants suffer an increment of their values in acid media.[7]

The most relevant results in solution have been obtained by ^{15}N-NMR, in neutral medium ($CDCl_3$) the chemical shift values of imine nitrogen around -70 ppm account only for the presence of the hydroxyimino tautomer (Figure 4).

Table 1: Energy differences (kJmol^{-1}) between hydroxyimino and oxoenamino tautomers using the hybrid B3LYP/6-31G** level with basis sets of Gaussian type functions using Spartan '02.

Compound	Neutral Media		Acid media	
	 Hydroxyimino (**a**)	Oxoenamino (**b**)	 Hydroxyimino (**c**)	Oxoenamino (**d**)
2	0.00	20.55	0.00	4.00
3	0.00	20.28	0.00	3.84
4	0.00	22.43	0.00	6.67
5	0.00	22.1	0.00	5.93
6	0.00	22.18	0.00	6.23
7	0.00	21.98	0.00	5.89

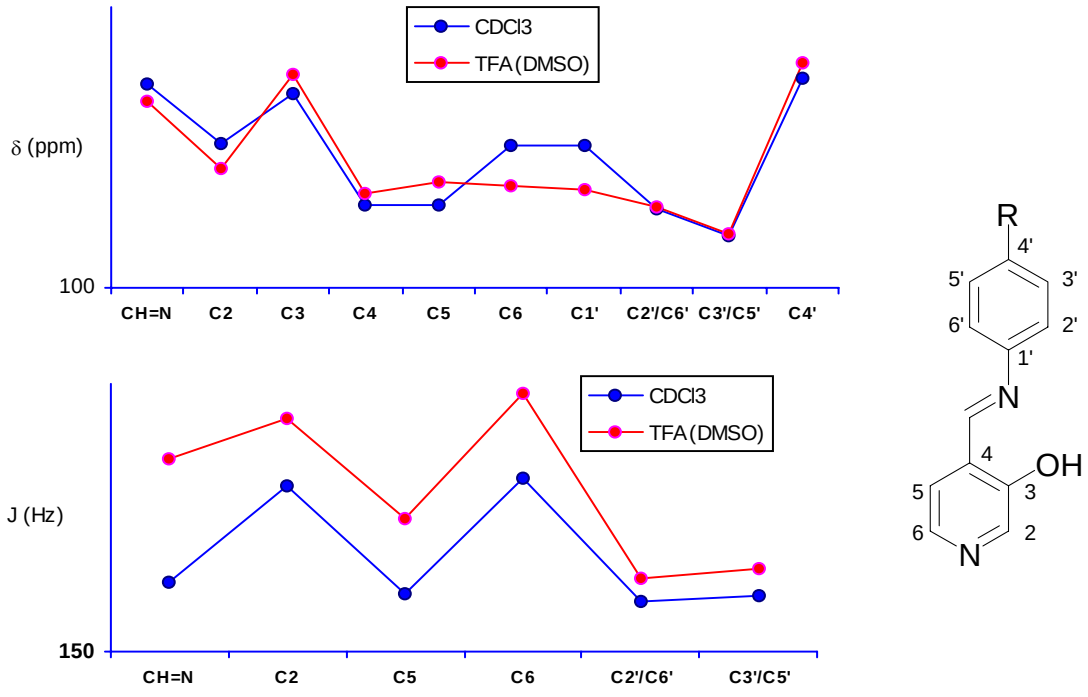


Figure 3: Chemical shifts (δ ppm) and coupling constants (*J* Hz) of Schiff bases (**2**-**7**) in neutral and acid media.

However in acid media (trifluoroacetic acid solution) the values shift to about -180 ppm and -170 ppm for the pyridine and imine nitrogens respectively (Figure 4).

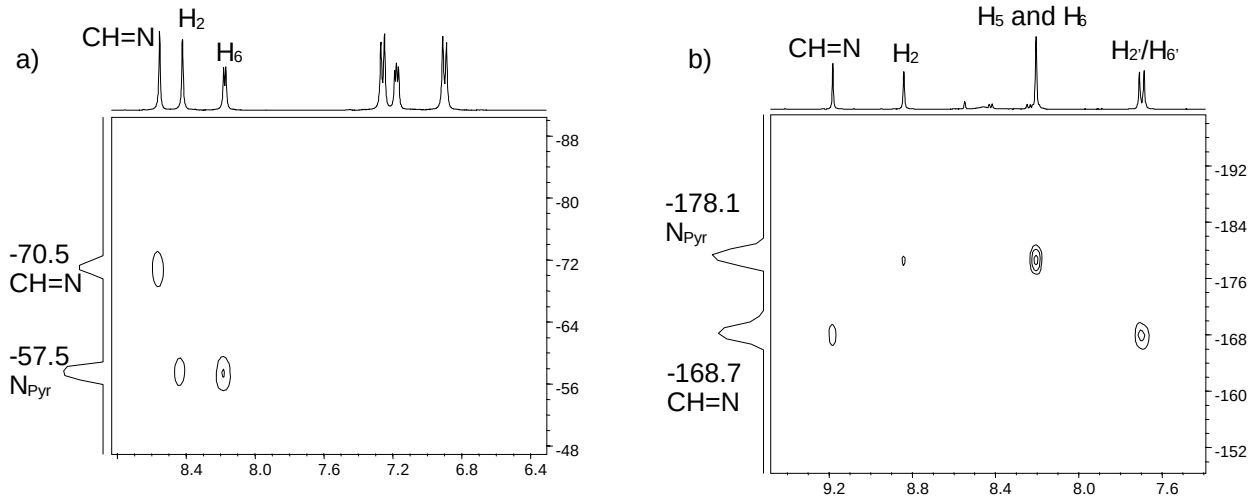
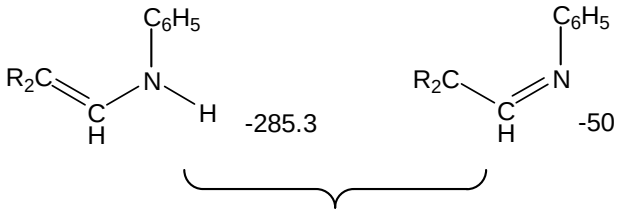


Figure 4: gs-HMBC ^{15}N-NMR of **4** in solution (a) $CDCl_3$, (b) in CF_3COOH.

These results prove that the pyridine nitrogen has been protonated and by comparison of the imine nitrogen chemical shifts with the values reported in the literature (Figure 5)[8], we come to the conclusion that there is an 1:1 equilibrium between the hydroxyimino (**c**) and oxoenamino (**d**) tautomers.



average δ= -167.3

Figure 5: ^{15}N-NMR chemical shifts values reported in the literature.

The tetrafluoroborate salts of **4** and **5** were prepared and studied in solution and solid state NMR. These salts exist only in the hydroxyimino form. We show in Figure 6 the gs-HMBC ^{15}N-NMR in solution of **4** in DMSO-d_6 and the X-ray molecular structure of **5** in Figure 7.

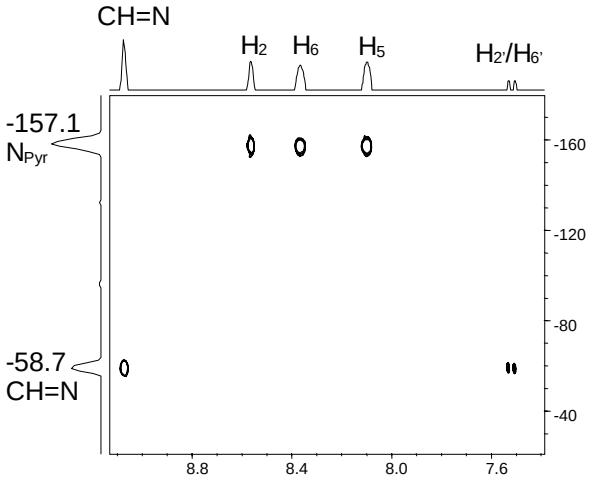


Figure 6: gs-HMBC ^{15}N-NMR of (*E*)-4-((4-methoxyphenylimino)methyl)-3-hydroxypyridinium tetrafluoroborate.

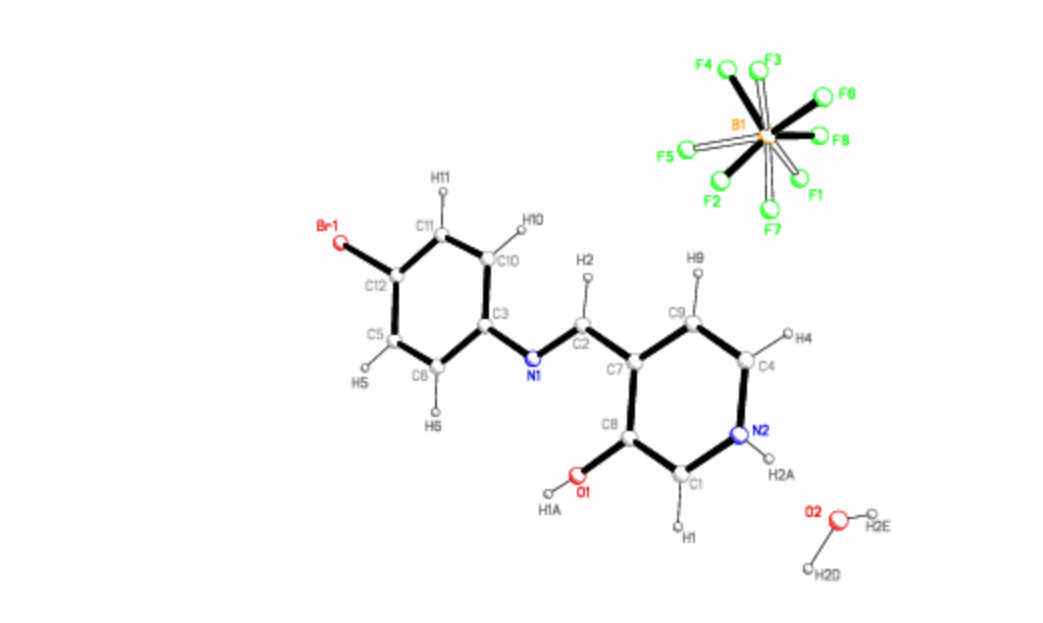


Figure 7: X-Ray molecular structure of (*E*)-4-((4-bromophenylimino)methyl)-3-hydroxypyridinium tetrafluoroborate.

- **CONCLUSIONS**

In solution, only when the imines are dissolved in an acid excess media, the energy gap decreases enough and hydroxyimino-oxoenamino forms are found in equilibrium. In solid state all compounds exist only in the hydroxyimino form and protonation takes place on the pyridine nitrogen atom.

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