Vol.9 No.2
Spring 1998

J. Sci. I. R. Iran

MOLECULAR MODELING AND NMR STUDY OF HISTIDINE AND ITS ANALOGUES AS PYRIDOXAL 5´-PHOSPHATE DEPENDENT HISTIDINE DECARBOXYLASE INHIBITORS

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Abstract

Molecular modeling analysis of charge density and heat of formation by PM3 method as well as ^{13}C, ^{1}H NMR and 2D-NMR measurements of histidine (substrate) and some of its derivatives as histidine decarboxylase inhibitors were performed. It was established that the atom, usually nitrogen, which forms internal aldimine with pyridoxal 5´-phosphate (PLP), (coenzyme), has negative and almost equal charge in histidine and other inhibitors. The preferred conformation of compounds with higher inhibitory activity is calculated to be in such a form that the amino group and imidazole ring have a dihedral angle of 60°. In this conformation, molecules are able to form internal hydrogen bonds. In contrast, their derivatives with lower activity have a different conformation. NMR measurements support our theoretical calculations.

Introduction

The biogenic amine, histamine, is known to be involved in allergic reactions [1], inflammation (via H_1 receptors) and gastric secretion [2-3] (via H_2 receptors). The most recent elucidation of the histaminergic neuron system and the identification of a specific presynaptic subtype of the histamine receptor (H_3) in the brain provide support for its proposed role in neuroregulation [4-5]. Occasional indications can be found in the literature of high histamine levels in some tumors [6-7].

Histidine decarboxylase (HDC, l-histidine decarboxylase, EC 4.1.1.22) catalyzes the decarboxylation of l-histidine to histamine [8-14]. Thus, HDC plays an important role in the *in vivo* formation of histamine.

Pyridoxal-5´-phosphate (PLP) acts as a coenzyme in HDC active site and forms an internal aldimine with specific LYS residue from HDC structure. l -histidine (substrate) [10] replaces the internal aldimine to form an external aldimine with PLP using its amino group.

Histidine decarboxylase inhibitors may have a promising potential for developing effective and safe agents in the prevention of histamine overproduction

Keywords: Histidine; HDC; NMR; PLP; PM3

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conditions [15-19].

The effects of various compounds on HDC activity have been reported in the literature. Among these, α-methylhistidine has been shown to be a strong and specific inhibitor [19, 21], inhibiting rat HDC activity greater than 90% at 10 mM [18]. Kollonitsch *et al.* [23] reported that (s)-α-fluoromethyl histidine is an irreversible inhibitor of histidine decarboxylase, and subsequent studies [16, 22, 25] on the mammalian enzyme and HDC from *morganella morganii* [24, 25] suggest that it acts as a suicide substrate (IC50~1μM; Ki = 8.3μM).

Dopa, cysteine and methionine inhibit HDC activity at about 91, 77 and 69% respectively, at 10 mM concentration [18]. Tryptophane and its 5-hydroxy derivative inhibit HDC at a relatively weak about 27% at 10 mM concentration. α-Hydrazinohistidine [16], 1-histidinemethyl ester, 2-hydrazino 3-(4-imidazolyl) propionic acid and α-methyldopa [26] have to some extent an inhibitory effect.

In the present work, histidine and some of the HDC inhibitors are studied by quantum chemical calculations and NMR measurements [30].

Materials and Methods

Theoretical Calculations

The quantum mechanical semi-empirical PM3 method, because of its superiority to the previous methods [27], using Mopac program was selected for the conformational and charge density calculations on VAX 3100 computer. The structures of the compounds (Fig. 1) were built by using the pcmodel [31] program and a preliminary energy minimization was performed within this molecular mechanic program. A full geometry optimization was carried out using the 1995 version of the Mopac program approximation.

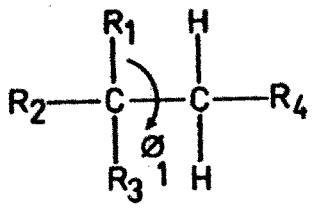
NMR Measurements

l-histidine, l-histidinemethyl ester, α-methylhistidine, α-hydrazinohistidine, (s)-α-fluoromethylhistidine, l-dopa, l-methionine, l-cysteine, l-tryptophane and 5-hydroxy l-tryptophane were purchased from Sigma and used without further purification.

purification.

^{1}H NMk spectra were recorded at 400 MHz on a Varian unity plus spectrometer. Measurements were carried out for 2 mM solutions of compounds in D_2O at room temperature. In all cases, 5 mM sample tubes were used and spectra were recorded immediately after sample preparation.

Typical conditions for one-dimensional ^{1}H NMR spectra included a repetition delay of 1s, a pulse flip angle of 45°, spectral width of 4000 Hz and a 16 K data



No.	R_1	R_2	R_3	R_4
1	H	COOH	NH_2**	Imidazole
2	CH_3			
3	$NHNH_2$			
4	CH_2F			
5*	CHF_2			
6	H	$COOCH_3$		
7	$NHNH_2$**	COOH	H	
8	H		NH_2	3,4-Dihydroxyphenyl
9	CH_3			
10	H			Indole
11				5-Hydroxyindole
12				SH
13				CH_2SHCH_3
14*				Benzimidazole

Figure 1. Histidine and l-histidine decarboxylase inhibitors

*These compounds are considered as probable inhibitors

**The atom which is probably responsible for forming the external aldimine with PLP[10]

acquisition table. HOD signal at 4.67 ppm was used as reference. Zero-filling to 32 K was generally applied, leading to a digital resolution of at least 0.25 Hz/Pt in the transformed spectra.

^{13}C NMR spectra were recorded at 100 MHz on a Varian unity plus 400 spectrometer for samples in 5 mM sample tubes at room temperature in D_2O. Spectral width of 25,000 Hz and a repetition delay of 5s were used. Broad-band proton irradiation was achieved using the standard composite pulse-decoupling. T_1 measurements for both ^1H and ^{13}C were applied using the fast inversion recovery technique.

Two-dimensional J-resolved (Homo2dj, Hetero2dj), Phase-sensitive Nuclear Overhauser Effect (NOESY) [29] and Double-Quantum Filtered Correlated Spectroscopy (DQFCOSY) [28] were recorded on a Varian 400 spectrometer. 2D NMR data were collected without sample spinning.

The NOESY and DQFCOSY spectra were obtained using repetition times of 1s, with minimum spectral widths for the region of interest. A mixing time of 3s was used in the NOESY experiment.

NOESY experiments were run in absorption mode utilizing the States-Habekorn method [32]. Phase-cycling permitted quadrature detection in t_1. Spectra were acquired using 1024 × 256 real data points. Prior to Fourier transformation, the data matrix was multiplied by a Gaussian function (gf) in both t_1 and t_2 dimensions for the DQFCOSY and NOESY spectra. Zero filling was also applied in both dimensions and final two-dimensional matrixes were left unsymmetrized.

Results and Discussion

Histidine as substrate for PLP-dependent l-histidine decarboxylase and HDC inhibitors (Fig. 1) were studied using the quantum chemical calculations.

Table 1 reports the values of the heat of formation in kcal/mol and charge density on nitrogen atom (N—Cα) for each compound. Previous works have shown that histidine [10] and some of the HDC inhibitors [25] react with the internal aldimine, via a nucleophilic, usually a nitrogen atom in their structure, to form external aldimine with PLP (coenzyme).

Our results show that the value of the charge density of nitrogen atom is negative and appears to be nearly equal in all of the compounds considered (Table 1). It can be concluded that the inhibitors might be able to form Schiff base with PLP in the same manner as histidine.

We suggest that the compound containing the benzimidazole ring (compound 14 in Fig. 1), which has similar charge distribution on nitrogen atom (Table 1), might be able to inactivate HDC.

The results reported by Snell *et al.* [25] demonstrate that the inactivation of HDC by (s)-α fluoromethylhistidine is a mechanism-based process which after decarboxylation and β-elimination of fluoric ion (F^-) the new product is formed. Therefore, i nucleophilic methylene group attacks the internal aldimir of the enzyme.

We studied the compounds formed during th inactivation of the HDC by (s)-α-fluoromethylhistidin and its difluoro derivative (Table 2). The PM3 calculation show that these compounds are not stable enough (heat c formation = 39.67, -4.73 kcal/mol).

It has been also found that the net atomic charge on th carbon atoms in these compounds (Table 2) is similar t the charge density on the nitrogen atoms in the other (Table 1), while the values of charge density on nitroge atoms in compounds No. 15 and 16 (Table 2) are different

The conformational calculations for histidine an HDC inhibitors (Fig. 1) were performed using the PM semi-empirical approximation method. Table 3 present the conformation of the compounds around φ torsiona angle. It can be concluded that all of the compounds have the similar preferred conformation according to the theoretical calculations.

For compounds containing protons on both C_α and C_β NMR measurements were carried out. In Table 3, the experimental NMR coupling constants (^3JH-C_α-C_β-H) are

Table 1. Heat of formation and charge density on nitrogen in histidine and some of the l-histidine decarboxylase inhibitors calculated using the Mopac program

Compound	Heat of formation (kcal/mol)	Charge density on N*
1	-62.520974	-0.0398
2	-68.088798	-0.0383
3	-33.166413	-0.752
4	-109.730852	-0.0656
5	-158.416685	-0.030
6	-55.93302	-0.032
7	-39.295111	-0.0628
8	-159.18298	-0.0313
9	-164.376837	-0.033
10	-53.27919	-0.0303
11	-98.048036	-0.028
12	-88.715586	-0.032
13	-99.871331	-0.0245
14	-47.272622	-0.036

*Atom responsible for forming external aldimine with coenzyme

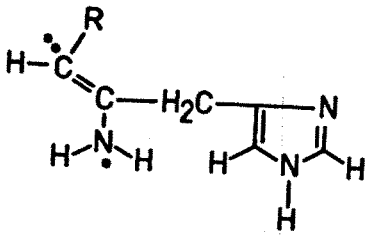


Table 2. Heat of formation and charge density on nitrogen and carbon in the compounds formed during the inactivation of HDS by (s)-α-fluoromethylhistidine and (s)-α-difluoromethylhistidine calculated using the PM3 method

No.	R	Heat of formation (kcal/mol)	Charge density	
			N*	C**
15	H	39.66406	0.0534	-0.2609
16	F	-4.725149	0.0704	-0.1054

given for histidine and some of the HDC inhibitors.

Figure 2 shows the homo2dJ spectrum from which coupling constants for cysteine have been obtained. These constants were used in the Karplus equation (1) to determine the NMR predicted torsional angles shown in Table 3.

$$^3J = A\cos^2\theta + B\cos\theta + C \qquad (1)$$

The results of the conformational calculations by NMR are in accordance with theoretical results.

Based on the theoretical calculations, histidine (substrate) and its analogues (Fig. 1) which are potent HDC inhibitors [18-20] appear to have the preferred conformational torsion of 60° for imidazole —COOH bond (Fig. 3). In this conformation, these compounds are able to form intramolecular hydrogen bonds, while compounds with lower inhibitory activity [18] (compounds No. 10 and 11) have a different conformation in which the carboxyl group and aromatic ring have the torsional angle

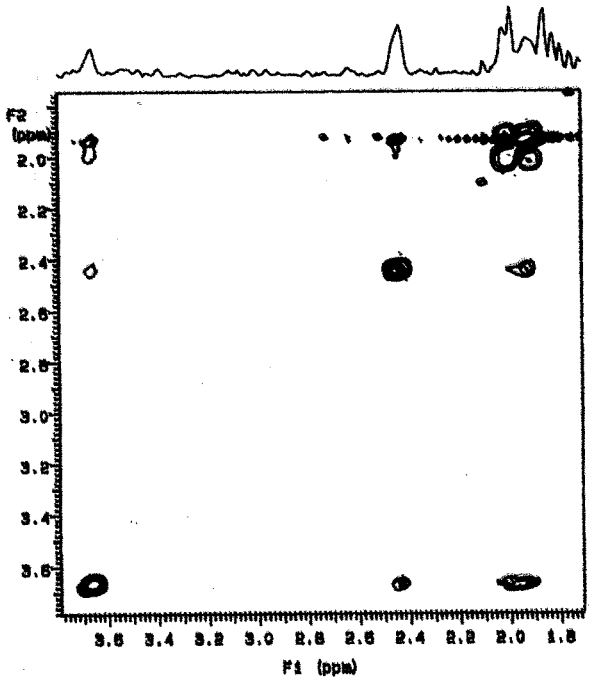


Figure 2. Homonuclear 2DJ-resolved spectrum for l- cysteine shown in stack plot mode

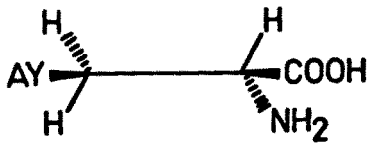


Figure 3. Predicted geometry for histidine and its analogues calculated by theoretical and experimental methods

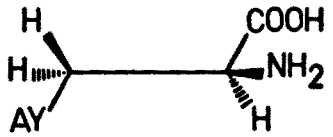


Figure 4. Predicted geometry for l-tryptophane and its 5-hydroxy derivative calculated by theoretical and experimental methods

of 180° (Fig. 4).

Compounds No. 8, 9 and 14 have the similar preferred conformation to the compounds with higher inhibitory activity. It can be concluded that compound No. 14 (Fig. 1), which has the same conformation as the histidine analogues, may also exhibit HDC inhibitory activity.

According to the PM3 conformational prediction, methionine has the preferred conformation similar to the compounds with lower HDC inhibitory activity, while NMR (NOESY) results show different geometry in which the molecule does not have the extended conformation. Methionine has relatively higher inhibitory activity [18], so it may be concluded that the geometry predicted by NMR is more probable (Fig. 5).

Figure 6 shows the NOESY spectrum of methionine, the cross peaks are due to the NOE between protons which have couplings through space. The phase of the cross peaks are opposite to that of the diagonals. To estimate internuclear distances from NOESY crosspeak integrals, we graded the crosspeaks into strong, medium and weak and inferred that a weak crosspeak is close to 4-5 angstroms, a strong crosspeak is less than 2.5 angstroms and a medium one shows about 3-4 angstroms distance between protons.

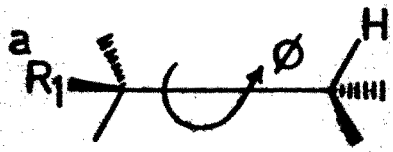


Table 3. Predicted geometry for histidine and some of the HDC inhibitors

Compound	ϕ[b]	^{3}JHH'(Hz)[c]	NMR[d]
1	-47.59	4.75	53.4
2	-69.07	—	—
3	-43.17	—	—
4	-67.8	—	—
5	-66.18	—	—
6	-53.07	6.8	42.9
7	-84.39	—	—
8	-56.43	5.2	51.2
9	-57.11	—	—
10	-64.59	5.0	52.2
11	-71.04	4.75	53.2
12	-69.67	5.6	56.0
13	-64.96	7.2	51.2
14	-83.40	—	—

[a]Refers to Figure 1
[b]R_1-C-C-H torsional angle calculated by computational method
[c]^{3}J H-C-C-H R_1 = H
[d]Magnitudes of torsional angles calculated from the Karplus equation $^3J = A\cos^2\theta + B\cos\theta + C$:
^3J = (CH - CH)^{31} $\theta<90°$ A = 12.5, B = 0, C = -0.3
$90°<\theta<180°$ A = 14.5, B = 0, C = -0.3

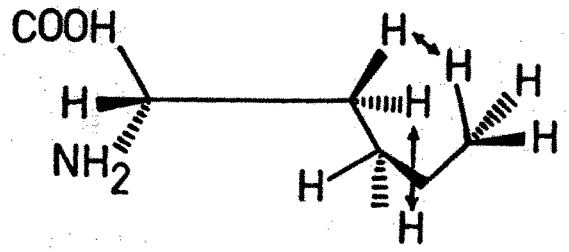


Figure 5. Predicted geometry for l-methionine calculated by NMR (NOESY)

Acknowledgements

This work was part of a Ph.D. thesis in the Faculty of Pharmacy, Tehran University of Medical Sciences. The authors are grateful to Dr. A.M. Sobhani for debugging some of the software and to Tarbiat Modarres University for its assistance.

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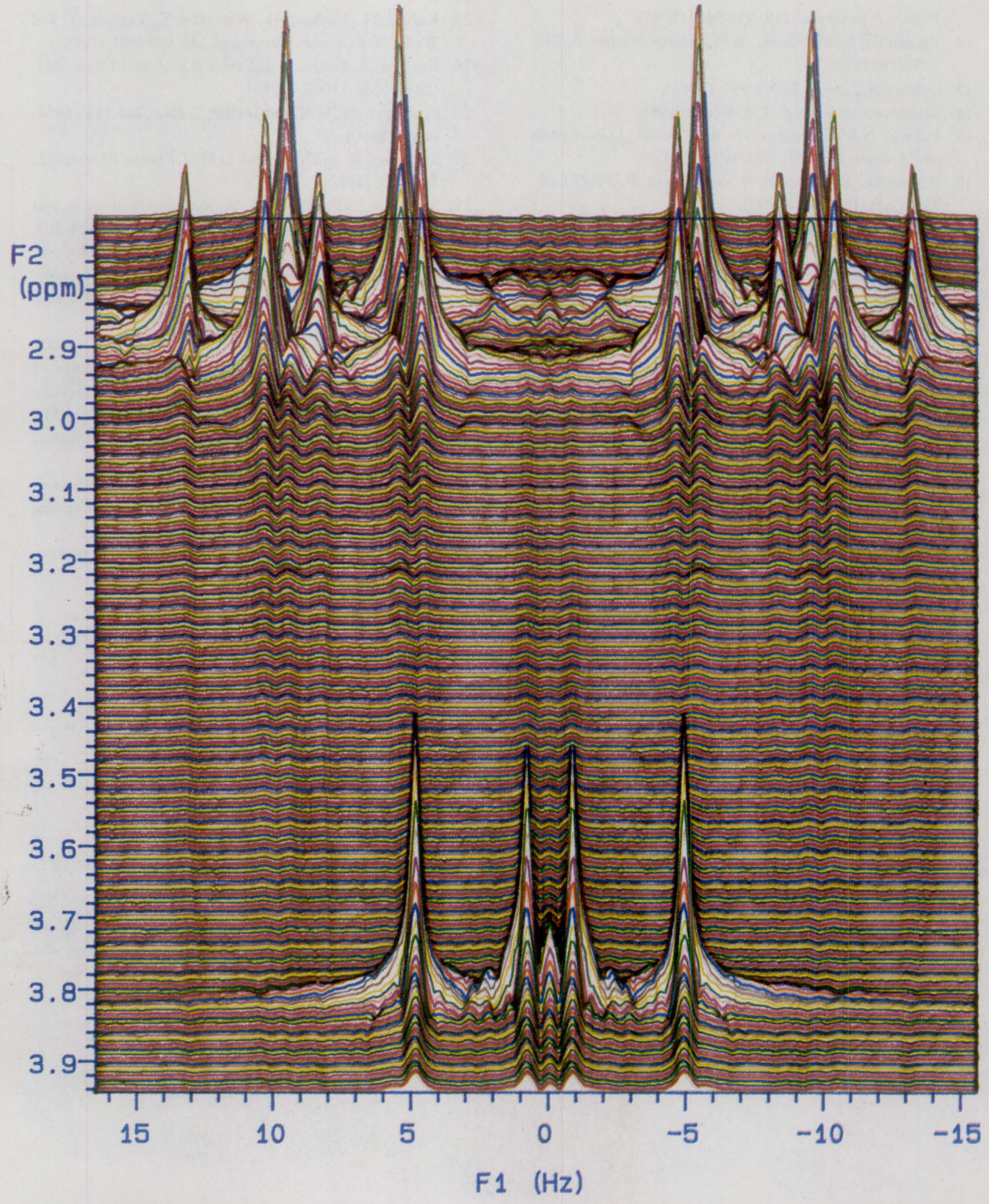

Figure 6. The NOESY spectrum of l-methionine in D_2O with mixing time of 3s

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