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^{1}H NMR Study on Putative Intramolecular Hydrogen Bonding for Histamine H_3-Receptor Agonists

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Abstract: Conformational stabilization by intramolecular hydrogen bonding of two histamine H_3-receptor agonists is studied by ^{1}H NMR. Stabilization of each individual conformation of the compounds by intramolecular hydrogen bonding is not strong at physiological pH and temperature. However, 52% – 61% of the molecules exist in conformations where an intramolecular hydrogen bond is possible.

Histamine is a biologically active amine acting on three distinct histamine receptors. The histamine H_3-receptor is a presynaptically located autoreceptor modulating the release and synthesis of histamine from histaminergic neurones. The H_3-receptor also controls the release of some other neurotransmitters at non-histaminergic neurones. Many therapeutic targets for H_3-receptor ligands have been suggested i.e. asthma, migraine, learning and memory degenerative disorders like Alzheimer's disease.[1,2]

Molecular modelling predicts intramolecular hydrogen bond formation for many histamine H_3-receptor agonists.[3,4] Intramolecular hydrogen bonding may be involved in the mechanism of activation of the H_3-receptor via a proton transfer process. A proton relay process between the ligand and the receptor protein is proposed as a mechanism of activation for both the serotonin 5-HT receptor[5] and histamine H_2-receptor.[6]

The prediction of conformational behaviour of the system in solution is difficult by computational methods. The aim of this work was to study the intramolecular hydrogen bond formation for two histamine H_3-receptor agonists in solution, namely R-(α)-methylhistamine[7] (**1**) and 2-S-amino-3-(1*H*-imidazol-4(5)-yl)propyl cyclohexylmethyl ether[8] (**2**) by temperature dependence of ^{1}H,^{1}H couplings.[9,10] The approach based on iterative fitting of data enables the characterization of the individual rotamers of chiral molecules and their thermodynamics. Three rapidly equilibrating rotamers of compounds **1** and **2** are illustrated in Fig. 1.

MATERIALS AND METHODS

The pH and pD of 0.03-0.04 mM H_2O and D_2O solutions of **1** and **2** were adjusted to 7.4 using 0.1 M HCl/DCl and NaOH/NaOD in order to get the monocationic state of the compounds.[11] The CD_3OD samples were converted to the monocationic form by adding first 0.5 equivalent of NaOH. After careful evaporation of the solvent, the residues were dissolved in CD_3OD and filtrated. ^{1}H NMR spectra in H_2O, D_2O and CD_3OD

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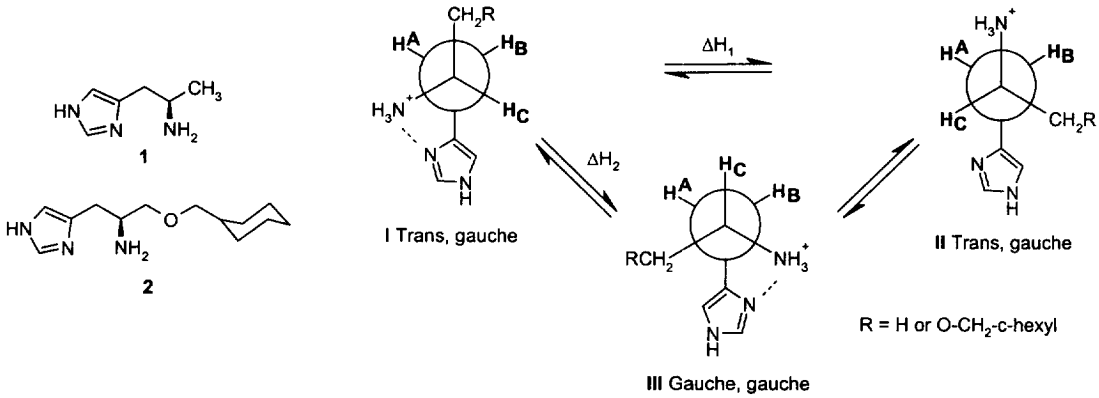

Figure 1. Structures and the three main rotamers of compounds **1** and **2**.

at 283, 298, 318 and 333K were recorded with a Bruker AM 400 WB spectrometer operating at 400.1 MHz. The spectral analyses were done using the program PERCHit.[12]

RESULTS AND DISCUSSION

Conformational analysis of the monocationic compounds, the predominant form at physiological pH, in H_2O, D_2O and CD_3OD was carried out by analysing the temperature dependence of the coupling constants $J(H_A,H_C)$ and $J(H_B,H_C)$. The coupling constants $J(H_A,H_C)$ and $J(H_B,H_C)$ of **1** in H_2O could not be solved due to chemical shift degeneracy. However, a replacement of the NH_3^+ hydrogens to deuterium in D_2O removed the degeneracy and allowed the analysis in D_2O at 333K. The couplings of the compound **2** could be solved in all the three solvents.

The similarity of the values of $J(H_A,H_C)$ and $J(H_B,H_C)$ indicates that none of the rotamers is clearly stabilized over the others. However, their sum decreased slightly when the temperature was increased (Table 1), which indicates an increase of the rotamer with small couplings $J(H_A,H_C)$ and $J(H_B,H_C)$, thus proving the existence of rotamer III.

If a three-site model is assumed (Fig. 1) the coupling constants depend on ten parameters: ΔH_1, ΔS_1, ΔH_2, ΔS_2, $J_I(H_A,H_C)$, $J_{II}(H_A,H_C)$, $J_{III}(H_A,H_C)$, $J_I(H_B,H_C)$, $J_{II}(H_B,H_C)$ and $J_{III}(H_B,H_C)$. The observed couplings were used as data for the program EQUILA[9] and the parameters were iteratively solved simultaneously for the two compounds. Their full refinement was not possible on the basis of the data and two simplified models are now reported. In **Model A** all the trans-couplings (J_t) and all the gauche-couplings (J_g) were kept equal; the optimized values were 13.1±0.1 and 2.8±0.1 Hz. In **Model B** J_t's were kept equal but J_g's were only constrained weakly to 2 Hz, which is a fair estimate of it, based on the molecular model and the Altona equation.[13] The results are given in Table 2. In model B, the optimized J_t was 12.7±0.1 Hz, the range of J_g was from 1.6 to 2.5 Hz. Several other models were tried, for example, including also the entropies. However, the resulting trends stayed the same. The picture thus obtained is semiquantitative and topological, allowing evaluation of some parameters rather accurately, for some parameters only their relative values are well-defined.

Table 1. The observed vicinal coupling constants based on total-line-shape analysis (Hz).[12,a]

	Compound 1[b]								
	H_2O			D_2O			CD_3OD		
T [K]	$J(H_A,H_C)$	$J(H_B,H_C)$	$J(H_A,H_C)$ + $J(H_B,H_C)$	$J(H_A,H_C)$	$J(H_B,H_C)$	$J(H_A,H_C)$ + $J(H_B,H_C)$	$J(H_A,H_C)$	$J(H_B,H_C)$	$J(H_A,H_C)$ + $J(H_B,H_C)$
283	–[c]	–	–	–	–	–	6.67	6.91	13.58
298	–	–	–	–	–	–	6.78	6.94	13.72
318	–	–	–	–	–	–	6.82	6.76	13.58
333	–	–	–	6.84	6.93	13.77	6.89	6.55	13.44
	Compound 2								
283	7.10	7.17	14.27	6.98	7.28	14.26	7.47	6.92	14.39
298	6.82	7.13	13.95	7.09	7.21	14.30	7.27	7.08	14.35
318	7.05	7.16	14.21	7.16	7.15	14.31	7.13	7.07	14.20
333	7.10	7.05	14.15	7.13	7.03	14.16	6.97	7.12	14.09

[a]The predicted standard deviations were ca. 0.02 Hz for compound **1**, for compound **2** they were 0.003-0.015 Hz (due to much higher first-order nature of the spectra). [b] The spectrum was very strongly second-order type and the std. deviations are obviously too small for this case. [c]Not analysable due to degenerated spectra.

The enthalpy difference ΔH_1 is ca. 0 kJ/mol for models A and B, this result is independent of the model. The enthalpy difference ΔH_2 depends on the model as in model A the ΔH_2 is ca. 2.4 – 4.4 kJ/mol lower than for model B. In both model A and B, the ΔH_2 for compound **2** is higher than for compound **1** (Table 2). The small differences between the three solvents are statistically significant, indicating small solvent and isotope effects on hydrogen-bonding. The latter is also observed for the chemical shift difference of the protons.

Table 2. Enthalpy parameters of rotamers calculated with the program EQUILA[9] for models A and B.

	Compound 1			
	Model A (rms = 0.13 Hz)		Model B (rms = 0.12 Hz)[a]	
Solvent	ΔH_1 [kJ/mol]	ΔH_2 [kJ/mol]	ΔH_1 [kJ/mol]	ΔH_2 [kJ/mol]
CD_3OD	-0.01±0.01[b]	1.44±0.11	-0.19±0.03	3.8±0.3
	Compound 2			
H_2O	-0.07±0.01	2.27±0.14	-0.21±0.03	6.1±0.4
D_2O	-0.05±0.01	2.49±0.15	-0.20±0.03	6.9±0.5
CD_3OD	0.11±0.01	2.57±0.15	-0.07±0.03	6.9±0.5

[a] In the model B, J_g's were constrained to 2 Hz by adding a constraining equation with such a weight that a 1.0 Hz deviation of each J_g from 2 Hz corresponded to 0.1 Hz deviation between the calculated and observed value of one data-point; a smaller weight led to unreasonable deviations for 2 Hz but not better rms. [b] Standard deviations predicted by the program. The std. deviations are far too small because they do not account for the uncertainties of the models.

The relative rotamer populations at 283 - 333K are given in Table 3. The population of the high energy state rotamer III and the total population of the rotamers I + III (intramolecular hydrogen bonding possible) is higher for compound **1** than for **2**. A slight excess of the compounds **1** and **2** exist in conformations where intramolecular hydrogen bond formation is possible (Table 3), although the small ΔH_1 indicates that the intramolecular hydrogen bond only weakly stabilizes rotamer I in solution.

Table 3. The rotamer populations calculated using the models A and B.

Compound 1						
	Model A			**Model B**		
T [K]	**I and II [%]**	**III [%]**	**I + III [%]**	**I and II [%]**	**III [%]**	**I + III [%]**
298	39.1	21.8	60.9	45.1	9.8	54.9
310	38.9	22.2	61.1	44.8	10.3	55.2
333	38.6	22.9	61.4	44.4	11.3	55.6
Compound 2						
298	42.1	15.7	57.9	48.3	3.4	51.7
310	41.9	16.2	58.1	48.1	3.7	51.9
333	41.4	17.1	58.6	47.8	4.4	52.2

CONCLUSIONS

The above analysis shows that the stabilization of each individual conformation by intramolecular hydrogen bonding is not strong for the compounds at physiological pH and temperature. However, 52% – 61% of the molecules exist in conformations where an intramolecular hydrogen bond is possible. The described method extends the possibility to determine hydrogen bond formation in solution, where common molecular modelling techniques encounter their limits. Moreover, this method is not restricted to the studied polar solvents, but can be extended to aprotic and apolar solvents as well. The approach offers a tool to analyze intramolecular hydrogen bonding in solution for chiral bioactive compounds. Therefore, it can be of assistance in drug design determining the role of intramolecular hydrogen bonding in receptor-ligand interactions and structure-activity relationship studies.

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