

Structure of roxatidine acetate hydrochloride by ^1H , ^{13}C and ^{15}N NMR spectroscopy

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Abstract

The structure of roxatidine acetate hydrochloride was studied by multinuclear NMR in two solvents DMSO-*d*₆ and H₂O:D₂O (90:10). Using experiments like DQF-COSY, TOCSY, ^1H - ^{13}C gHSQC, gHMBC and ^1H - ^{15}N gHSQC a complete assignment of all resonances of this important H₂-receptor antagonist was possible. The ^1H , ^{13}C and ^{15}N chemical shift values are reported and their implication in the conformation of this drug in the two solvents is discussed.

Keywords: Roxatidine acetate hydrochloride, NMR, H₂-receptor antagonist, chemical shift

Introduction

Structural modifications of histamine have led to the development of a number of analogs, which possess varying H₂-receptor antagonist activity, with buriamide¹ as the earliest. Roxatidine acetate hydrochloride (2-acetoxy-N-(3-(m-(1-piperidinomethyl)-phenoxy)propyl)acetamide hydrochloride) is an H₂-receptor antagonist with strong anti ulcerative activity.^{2, 3} In contrast to the earlier H₂ antagonists, which possess a 2-thiabutyl connecting chain and a urea-like moiety, roxatidine acetate hydrochloride (Figure 1) has a propyloxy connecting chain and an acetamide moiety^{4,5}. X-ray^{4, 6} studies also show differences in conformation and binding to the H₂-receptor compared with cimetidine, ranitidine and famotidine. In the absence of any structural data for this receptor, a great deal of structure-activity relationship studies have been carried out to decipher the important features necessary for binding and activity⁷. The pharmacology² and biological^{8, 9} data of this molecule are well described in literature, and some ^1H NMR data, in CDCl₃ have been reported but without any resonance assignments². The complete assignment

including ^{13}C and ^{15}N chemical shift data has not as yet been published for this important molecule.

By using experiments like DQF-COSY, TOCSY, ^1H - ^{13}C gHSQC, gHMBC and ^1H - ^{15}N gHSQC we have been able to make a complete assignment of the ^1H , ^{13}C and ^{15}N resonances of this molecule in DMSO and water and draw some inferences on its conformation in the two solvents.

Results and Discussion

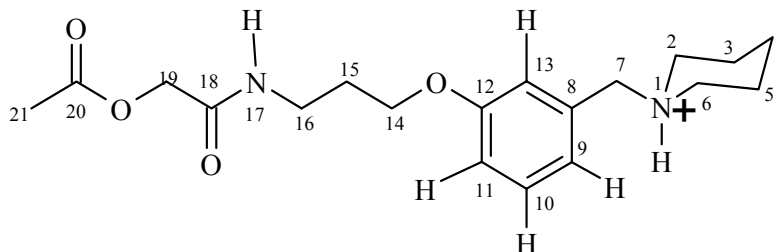
By applying a combination of 1D and 2D NMR experiments, we have been able to assign unambiguously all ^1H , ^{13}C and ^{15}N resonances of roxatidine acetate hydrochloride in the two solvents DMSO- d_6 and water. In the ^1H NMR spectrum of roxatidine (Table 1), a sub spectrum of the piperidine ring clearly indicates it in a chair conformation. The ten protons are distributed as five separate signals in the case of DMSO- d_6 and six separate signals in the case of water. The axial and the equatorial protons of the C2 and C6 carbons are deshielded corresponding to δ of 2.81/2.94 and 3.21/3.43 ppm (Table 1) in DMSO/ H_2O : D_2O (90:10) respectively.

Table 1. ^1H NMR chemical shifts (δ ppm), multiplicities and coupling constants (J Hz) for roxatidine acetate hydrochloride in DMSO- d_6 and water ^a

H at N or C number	DMSO- d_6	H at N or C number	$\text{H}_2\text{O}:\text{D}_2\text{O}$ (90:10)
1	10.55 (s); [0.0026] [‡]	1	8.84 (s); [0.0043] [‡]
2a, 6a	2.81-2.78 (m)	2a, 6a	2.94-3.00 (m)
2e, 6e, 16	3.21-3.26 (m)	2e, 6e, 16	3.43-3.48 (m)
3a, 5a, 3e, 5e, 15	1.76-1.89 (m)	3a, 5a	1.63-1.74 (m)
4a	1.30-1.41 (m)	3e, 5e	1.94 (d, 14.66)
4e	1.67 (d, 13.16)	4a	1.42-1.51 (m)
7	4.18 (d, 4.88)	4e	1.82 (d, 13.19)
9	7.10 (d, 7.80)	7	4.25 (s)
10	7.32 (t, 7.80)	9, 11	7.10-7.12 (m)
11	6.96 (dd, 8.29, 1.95)	10	7.46 (t, 7.92)
13	7.25 (s)	13	7.07 (s)
14	3.99 (t, 6.34)	14	4.12 (t, 5.86)
17	8.12 (t, 5.36)	15	2.02 (t, 6.16)
19	4.42 (s)	17	8.22 (t, 5.86)
21	2.07 (s)	19	4.57 (s)
		21	2.16 (s)

^a In parentheses are the multiplicities and coupling constants.

[‡]Temperature coefficient data in ppm/K.

**Figure 1**

A careful inspection of chemical shifts in the two solvents shows that apart from the small differences in proton chemical shifts and multiplicities, the C18 and C20 carbonyls and the N17 amide nitrogen have a differential chemical shift of 3.39 ppm and 10.67 ppm in the two solvents, respectively (Tables 2 and 3). The changes in the chemical shift for these atoms are independent of sample concentration. This observation, along with temperature coefficient data (Table 1), indicates that roxatidine acetate hydrochloride has different conformations in the two solvents. Preliminary molecular modeling studies show that the low temperature coefficient of the piperidine N⁺H results from H-bonding to the C=O group of either the acetate moiety or the amide functionality, with the first being more probable energetically.

Table 2. ¹³C NMR chemical shifts (δ ppm) for roxatidine acetate hydrochloride

C number	DMSO	H ₂ O:D ₂ O(90:10)
2	51.47	52.90
3	22.07	22.69
4	21.55	21.12
5	22.07	22.69
6	51.47	52.90
7	58.65	60.38
8	131.09	130.53
9	123.22	123.80
10	129.50	130.39
11	115.25	116.24
12	117.13	117.26
13	158.28	158.50
14	65.23	65.90
15	28.77	27.96
16	35.43	36.21
18	166.49	169.87
19	62.22	62.79
20	169.67	173.06
21	20.66	20.06

Table 3. ^{15}N NMR chemical shifts (δ ppm) for roxatidine acetate hydrochloride

N number	DMSO	H ₂ O:D ₂ O (90:10)
1	179.00	179.35
17	132.25	142.92

Conclusions

These data are extremely valuable for therapeutic molecules like roxatidine acetate hydrochloride, as they may be used to design more potent analogs of this class, besides being useful reference data for similar organic compounds.

Experimental Section

Materials

Roxatidine acetate hydrochloride was a gift sample from the Hoechst Research Center, Mumbai and was used without further purification. NMR solvents with 99.8% deuterium purity were procured from Merck, Germany.

NMR Spectroscopy

The NMR experiments were performed at 298 K on a Varian Mercury Plus Vx 400 MHz spectrometer using a multi-nuclei 5 mm autoswitchable probe and a dual channel 5 mm inverse detection probe, both equipped with pulse field z gradients. The sample concentration varied between 0.04 and 0.12 mmol ml⁻¹. ^1H , ^{13}C and ^{15}N NMR spectra were recorded in both DMSO- d_6 and 90:10 H₂O:D₂O solvents. All one- and two- dimensional NMR spectra were run using standard Varian software on a Sun workstation. Chemical shifts are given in the δ scale. The spectra in DMSO- d_6 were referenced to the residual solvent peak at 2.49 ppm for ^1H and 39.5 ppm for ^{13}C . For water experiments, DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid) was used as the internal standard and referenced to 0 ppm for proton and carbon. The ^{15}N chemical shifts in both the solvents were referenced using the method of *Wishart et al*¹⁰.

^1H chemical shift was obtained from 1D experiments and ^{13}C chemical shifts from the DEPT experiment with proton decoupling. For 1D ^1H experiments in DMSO- d_6 a spectral width of 6.4 KHz, 25 K data points, pulse width of 6.8 μs (45°) with a recycle delay of 1 s, acquisition time 2 s and a minimum of 8 scans was used. For experiments in water the presaturation pulse sequence (PRESAT) was employed to suppress the water signal in both 1D and 2D experiments. This was done by arraying the saturation frequencies with a delay of 1 sec at a saturation power of 6 to attain the maximum water suppression. The saturation frequencies thus obtained varied from -149.9 to -152.4 for different experiments. For 1D ^1H spectra in water, a spectral width of 4.8 KHz, 32 K data points, pulse width of 13.6 μs (90°), recycle delay of 50 ms, acquisition time 3.4 s and minimum 32 scans was used. In case of 1D ^{13}C experiments, a WALTZ-16 gated proton decoupling modulation was used throughout the experiments. Other parameters like

spectral width of 25 KHz, 60 K data points, pulse width of 5.2 μ s (45°) with a recycle delay of 2 s, acquisition time 2 s and minimum of 512 transients were used for both solvents.

Proton assignments were made using gCOSY or gDQF-COSY and HH-TOCSY experiments (min. 32 scans per t_1 , 200-256 x 2048 data matrix size).

The corresponding carbon atoms were assigned using the gradient version of HSQC (gHSQC, min. 32 scans per t_1 , 256-512 x 2048 data matrix) sequence. The quaternary carbons were assigned using the gradient version of HMBC (gHMBC, min. 8 scans per t_1 , 256-512 x 2048 data matrix size) experiment.

The nitrogen atoms were assigned using the gHSQC (min. 32 scans per t_1 , 512 x 2048 data matrix size) ^1H - ^{15}N experiment. A spectral width of 4 KHz was used in order to detect the piperidine and amide chemical shifts.

In the case of all 2D experiments linear prediction up to 1 K points was used during F1 processing and the final data matrix was zero filled to a 2 K x 2 K or 4 K x 4 K dimension.

The temperature coefficient ($-\Delta\delta/\Delta T$, ppm/K) of NH resonances gives clues about their involvement in H-bonding or solvent shielding. Values smaller than 0.003 ppm/K are indicative of H-bonding or solvent shielding. The temperature coefficient of NH chemical shifts was measured in the temperature range 298 to 328 K, with temperature increments of 10 K.

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References

1. Black, J.W.; Duncan, W.M.; Durant, G.J.; Ganellin, C.R.; Parsons, M.E. *Nature* **1972**, *236*, 385.
2. Shibata, K.; Itaya, T.; Yamakoshi, N.; Kurata, S.; Koizumi, N.; Tarutani, M.; Sakuma, H.; Konishi, K. *EP* 24510 and *US* 4293557, 1981.
3. Tarutani, M.; Sakuma, H.; Shiratsuchi, K.; Mieda, M. *Arzmeim.-Forsch.* **1985**, *35*, 703.
4. Yasuko, I.; Ishida, T.; Doi, M.; Inoue, M.; Shibata, K. *Chem. Pharm. Bull.* **1988**, *36*, 2295.
5. Takafumi, I.; Kazuhiko, I.; Katsunori, S.; Kyoko, H. *Br. J. Pharmacol.* **1997**, *122*, 1230.
6. Ishida, T.; Yasuko, I.; Shibata, M.; Doi, M.; Inoue, M.; Yanagisawa, I. *Mol. Pharm.* **1987**, *31*, 410.
7. Ganellin, C.R. *J. Med. Chem.* **1973**, *16*, 620.
8. Rendiae, S. *Croat. Med. J.* **1999**, *40*, 357.
9. Tsukimi, Y.; Kanatani, K.; Amagase, K.; Okabe, S. *J. Digest. Protec.* **2002**, *3*, 9.
10. Wishart, D.; Bigam, C.; Yao J.; Abildgaard, F.; Dyson, J.; Oldfield, E.; Markley, J.; Sykes, B. *J. Biomol. NMR* **1995**, *6*, 135.