Experimental and calculated $^1$H, $^{13}$C, $^{15}$N NMR spectra of famotidine

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Abstract

Famotidine, 3-[[2-[(aminoiminomethyl)amino]-4-thiazolyl]methyl][thio]-N-(aminosulfonyl), is a histamine $H_2$-receptor blocker that has been used mainly for the treatment of peptic ulcers and the Zollinger–Ellison syndrome. Its NMR spectra in different solvents were reported earlier; however, detailed interpretation has not been done thus far. In this work, experimental $^1$H, $^{13}$C and $^{15}$N NMR spectra of famotidine dissolved in DMSO-$d_6$ are shown. The assignment of observed chemical shifts is based on quantum chemical calculation at the Hartree–Fock/6-31G$^*$ level. The geometry optimization of the famotidine molecule with two internal hydrogen bonds, i.e. $\text{N(3)}-\text{H(23)}\cdots\text{N(9)}$ and $\text{N(3)}-\text{H(34)}\cdots\text{N(20)}$, is done by using the B3LYP method with the 6-31G$^*$ basis set. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

3-[[2-[(aminoiminomethyl)amino]-4-thiazolyl]-methyl][thio]-N-(aminosulfonyl), i.e. famotidine, whose chemical structure and atom numbering are shown in Fig. 1, is a histamine $H_2$-receptor antagonist that has been primarily used for the treatment of peptic ulcer and also for the Zollinger–Ellison syndrome [1]. Please note that in Fig. 1 intramolecular hydrogen bonding is not shown but it is discussed later in the paper. In the crystalline state, famotidine has two polymorphic forms that differ by the arrangement of intramolecular hydrogen bonds [2–6]. This drug can effectively bind metal ions in solution [7–10]; however, its metallocomplexes in the solid state are practically unknown. To our best knowledge, the only report concerns X-ray studies on Cu(II) complex [11]. Very recently, we have been successful in obtaining two Ni(II) complexes whose vibrational (Raman and IR) and NMR spectra show distinct differences from those measured for the Cu(II) complex [12].

In this work, we present $^1$H, $^{13}$C and $^{15}$N NMR spectra of famotidine measured at room temperature in deuterated dimethylsulfoxide-$d_6$, DMSO-$d_6$. In the solid state, the investigated famotidine has a crystal structure identical to that reported earlier in X-ray single-crystal diffraction studies [2], in which two intramolecular hydrogen bonds between guanidino nitrogen N(3) and thiazole nitrogen N(9) and between guanidino nitrogen N(3) and sulfamoyl nitrogen N(20) force famotidine to take the strongly folded conformation. Raman spectra of investigated
Fig. 1. Atom numbering of a molecule of famotidine without showing hydrogen bonding (see text for details).

Fig. 2. NMR spectra of famotidine in DMSO-\text{d}_6: (a) $^1$H; (b) $^{13}$C; and (c) $^{15}$N. See text for standards used to calculate chemical shifts.
famotidine in solid state and in DMSO solution show [5,6] that the structure of this drug is virtually the same, i.e. there is no indication that in solution famotidine forms a different structure than that in the solid state. Additionally, there is no vibrational and NMR evidence that there are more than one tautomer or distinct conformer in DMSO solution, although several other possible tautomers can be expected theoretically to exist in a solution [13–16]. Our interpretation of NMR spectra is based on Hartree–Fock theory by using the 6-31G* basis set. Calculations presented here are in fairly good agreement with the incomplete tentative assignment of $^1$H, $^{13}$C and $^{15}$N chemical shifts reported thus far [9,13,14,17,18]. In other words, to the best of our knowledge, this is the only work that offers full assignment of all $^1$H, $^{13}$C and $^{15}$N peaks appearing in the NMR spectra of famotidine with the structure described above.

2. Experimental

2.1. Compound

A famotidine sample of high purity and biological activity was supplied by ‘Polfa’ Pharmaceuticals from Starogard Gdański and used without further purification.

2.2. Spectral measurements

All NMR spectra were measured on a BRUKER model AMX 500-MHz spectrometer. For NMR experiments, famotidine was dissolved in DMSO-$d_6$, transferred to a standard NMR capillary and measured at room temperature. The $^1$H NMR spectra were measured with Me$_4$Si (TMS) as an internal standard. $^{13}$C NMR spectra were recorded with a resonance frequency of 125.77 MHz and the solvent peak at 39.5 ppm was used to calibrate the scale of chemical shifts. $^{15}$N NMR spectra were recorded with no proton decoupling in a 10-mm tube.

2.3. Calculations

Calculations of magnetic properties were carried out at the Hartree–Fock level with the 6-31G(d) basis following a geometry optimization using the B3LYP density functional theory method with the same basis set as recommended by Cheeseman et al. [19] to calculate the NMR spectra of organic ligands. The GAUSSIAN ‘98 program was used at the Academic Computer Center ‘Cyfronet' in Kraków. Shielding constants for each nucleus in the molecule were calculated by using peaks of standard compounds, i.e. TMS (32.91 ppm for $^1$H and 201.37 ppm for $^{13}$C) and nitromethane (270.17 ppm for $^{15}$N). Absolute shielding values for these two standards were calculated at the

<table>
<thead>
<tr>
<th>Atom number</th>
<th>Experimental δ (ppm)</th>
<th>Calculated δ (ppm)</th>
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<tbody>
<tr>
<td>C 13</td>
<td>28.4</td>
<td>25.0</td>
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<tr>
<td>C 12</td>
<td>31.8</td>
<td>28.2</td>
</tr>
<tr>
<td>C 10</td>
<td>36.7</td>
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<tr>
<td>C 7</td>
<td>105.7</td>
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<td>C 8</td>
<td>148.3</td>
<td>137.9</td>
</tr>
<tr>
<td>C 1</td>
<td>157.5</td>
<td>155.9</td>
</tr>
<tr>
<td>C 14</td>
<td>166.1</td>
<td>166.8</td>
</tr>
<tr>
<td>C 5</td>
<td>177.5</td>
<td>182.9</td>
</tr>
</tbody>
</table>
same level of quantum chemical calculation described above. Thus the calculated values were assigned to match the obtained experimental data and found to be in good agreement with those published for similar compounds [20].

3. Results and discussion

$^1$H, $^{13}$C and $^{15}$N NMR spectra of famotidine are shown in Fig. 2. The observed and calculated chemical shifts of $^1$H, $^{13}$C and $^{15}$N together with proposed assignments are listed in Tables 1–3. The integration of all peaks appearing in the $^1$H NMR spectrum (Fig. 2a) gives, in total, 15 protons, in perfect agreement with the famotidine chemical formula. Our calculations clearly show that the chemical shifts at 2.47, 2.70 and 3.62 ppm are attributed to aliphatic chain protons: H(30,31), H(29,28) and H(27,26), respectively. The first two signals are triplets, while the last one is a singlet that has to be expected for a $-\text{CH}_{2}\text{SCH}_{2}\text{CH}_{3}$ fragment of famotidine (see Fig. 1). The insert in Fig. 2a shows that the triplet at 2.47 ppm overlaps with the quintet at 2.50 ppm, the latter being readily assigned to the DMSO methyl groups. The integration of all these peaks shows two protons each at signal. As can be seen from Table 1 calculated chemical shifts for aliphatic chain protons are higher by about 0.2 ppm, only. The peak at 3.42 ppm is due to protons from trace water. Three peaks observed in the range between 6 and 7 ppm, i.e. at 6.56 (s;2), 6.59 (s;1), and 6.84 (s;4), (where s = singlet and the number stands for a number of protons obtained after peak area integration) have to be assigned to H(32,33), H(25), and H(21,22,23,24), respectively. Calculated values are higher for H(32,33) than for the thiazole proton, i.e. H(25), (Table 1); however, the assignment of these peaks is easy, since they differ in number of protons. Surprisingly, there is a very good agreement between experimental and calculated chemical shifts for guanidine protons, H(21,22,23,24), which are involved in one of the hydrogen bonds (vide supra). The highest chemical shifts are observed at 7.35 (s;1) and 8.26 (s;1) which are assigned to H(34) and H(35), respectively. In this case, calculated values are much higher than experimental values. This has to be due to the involvement of one of these protons in the intramolecular hydrogen bond. We were not able in our calculations to compensate this interaction for the assumed famotidine structure.

The $^{13}$C NMR (Fig. 2b, Table 2) spectrum of famotidine in DMSO-d$_6$ shows eight resonance signals. Previously, $^{13}$C NMR spectra were taken from D$_2$O solutions with different pH values [4,10,17]. It has to be mentioned that a full assignment of all carbon resonances has not been offered thus far. So, the lowest observed chemical shifts at 28.4, 31.8 and 36.7 ppm are attributed to the aliphatic carbons C(13), C(12) and C(10), respectively, as should be expected. However, we noticed that the calculated shifts were slightly lower than those observed, by around 10–15%, which was not unusual for calculations at the HF/6-31G$^*$ level. Additionally, observed shifts were at higher fields than those reported for propyl sulfide, CH$_3$CH$_2$CH$_2$SCH$_2$CH$_2$CH$_3$ [14], i.e. 34 ppm for carbons attached to a sulfur atom and 23 and 14 ppm for adjacent carbons in the chain. Obviously, the peak at 105.7 ppm has to be assigned to the methine carbon C(7) that is expected at around 100 ppm. This is additionally confirmed by our calculations. The signals at 148.3, 157.5, 166.1, and 177.5 ppm have to be assigned to C(8), C(1), C(14) and C(5), respectively. This is supported by our calculations, which show very good agreement with experimental data (see Table 2).

The $^{15}$N NMR spectrum of famotidine with no proton decoupling (Fig. 2c, Table 3) is almost identical (small differences in peak intensities) to that reported by Yanagisawa et al. [14] and consists of three triplets derived from four NH$_2$ groups (not distinguishable N(2) and N(3), and nonequivalent N(15) and N(20)) and three singlets of N(9), N(16) and N(4) nitrogens. The N(2) and N(3) triplets overlap

![Table 3](image-url)
giving peaks twice as strong as the other two triplet peaks indicating that both nitrogens are magnetically equivalent. Our calculations show that the assignment of the $^{15}$N chemical shifts has to be as follows: the singlets at 265, 210 and 156 ppm are due to N(9), N(16) and N(4), respectively, while the triplets at 110, 93 and 78 ppm are attributed to N(20) (sulfamoyl nitrogen), N(15) (amidine nitrogen), and N(2,3) (two nitrogen of the guanidino group), respectively. It has to be emphasized that our theoretical data are in excellent agreement with the presented experimental data; thus, there is no need, in our opinion, to perform either GHMQC or GHMBC experiments to distinguish between N(15) and N(20). The assignment proposed here is also in agreement with that proposed earlier by Yanagisawa et al. [14], which is used as a standard for $^{15}$N assignment of famotidine signals in the NMR spectrum. However, it has to be emphasized that the lecture on this paper, i.e. Ref. [14], has caused some confusion. The resonances at 110 and 93 ppm listed in the table inserted in Fig. 2, where the $^{15}$N NMR spectrum of famotidine is presented, were assigned to the amidine and sulfamoyl nitrogens, respectively. However, later in the text, a brief assignment of these two resonances (triplets without proton decoupling) was made vice versa, i.e. the peak at 110 ppm was assigned to thee sulfamoyl nitrogen, while that at 93 ppm was due to the amidine nitrogen. Thus, our calculations support the assignment discussed in the text on p. 1791 of Ref [14].

Acknowledgements

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References