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Abstract. The paper is dedicated to preferred conformations of mefenamic acid in DMSO. A complex approach based on NME experiments and GIAO quantum chemical calculations was applied to reveal the dominant conformation of the mefenamic acid molecule (2-[(2,3-dimethylphenyl)amino]benzoic acid). Unlike the nuclear Overhauser effect measurements, this method is fast and provides qualitative information on preferred conformations of small molecules in solutions. Obtained results agree well with the X-ray investigation data.

INTRODUCTION

Understanding conformational properties of chemical compounds is important in studies of structure, dynamics, and nucleation on the molecular level. This paper presents main results of studies of polymorphism and conformational properties of the mefenamic acid molecule (2-[(2,3-dimethylphenyl)amino]benzoic acid), which is a derivative of N-phenylantranly acid. This compound is used as medicine and belongs to the group of nonsteroidal anti-inflammatory drugs. Its therapeutic activity is due to inhibition of prostaglandin synthesis. Mefenamic acid may also be used in oncology treatment as a drug stimulating synthesis of endogenous interferon in humans.

A peculiarity of the molecular structure of mefenamic acid is coplanar disposition of the carboxyl group and nitrogen atom \cite{1}. The sum of the angles formed by bonds surrounding the nitrogen atom in mefenamic acid is close to 360°, and hence the nitrogen atoms can be assumed to be in the sp\textsuperscript{2} hybridization state. Mefenamic acid is a gray-to-white crystalline powder, almost insoluble in water and weakly soluble in ethanol.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mefenamic_acid_structure.png}
\caption{Structure of the 2-[(2,3-dimethylphenyl)amino]benzoic acid molecule for the polymorph MEF I (left) and MEF II (right).}
\end{figure}
Studies of mefenamic acid conducted in groups of McConnell and Lee [2], revealed that it can crystallize in two forms, called MEF I and MEF II. They are formed by different conformations of the mefenamic acid molecules (Fig. 1), having different orientations of the carboxyl group. MEF I is more stable since this conformation allows creating of an intramolecular hydrogen bond. Structures MEF I and MEF II differ also by the dihedral angle between the aromatic cores, which is responsible for minimization of the crystal lattice energy [3]. Polymorph MEF I is obtained by evaporation of solutions in acetone and ethanol, whereas MEF II can be produced by recrystallization of MEF I in dimethylformamide (DMF). The phase transition from MEF I to MEF II in the solid state is observed in the range from 160 to 190°; the transition temperature depends on the heating rate. This transition can also be induced by mechanical compression of the solid material. Generally, the crystallization process depends on the solution influence, as in the case of other drug compounds. Our hypothesis is that the molecular conformation existing in the saturated state, and eventually becomes like the configuration found in the crystal. On the other hand, a possibility of a complicated, multi-stage mechanism of arising of polymorphic forms is also possible, as it was found in a study of ibuprofen [5].

The aim of this work it determination of the most probable conformers of mefenamic acid dissolved in dimethyl sulfoxide using the methods of NMR spectroscopy and computer simulation. The obtained information in comparison with the X-ray data will help to determine possible nucleation mechanisms. The choice of the solution is justified by the fact that it is most often for growing crystals as an alternative to more toxic solvents. Two-dimensional NMR methods offer several approaches: nuclear Overhauser effect spectroscopy (2D NOESY, T-ROESY, QUIET-NOESY, etc.) [6,7] and analysis of residual dipolar couplings [8], as well as analysis of chemical shifts. The main difficulty in performing of 2D NMR experiments is their duration: they require sometimes tens of hours or a few days to complete. In this work we tested a fast method of evaluating the conformation distribution of mefenamic acid based on the analysis of experimental and calculated 13C NMR chemical shifts.

RESULTS AND DISCUSSION

First, low-energy conformers of mefenamic acid were calculated using the DFT B3LYP/6-311+G(2d,p) method; six conformations were found. The dihedral angle O=C–O–H was varied in the calculations. Then, the GIAO (gauge-independent / gauge-included atomic orbital) method within the density functional theory was used to calculate the shielding tensors $\sigma_i$ of carbon atoms. To find the 13C chemical shifts in different conformers of mefenamic acid, the shielding constant $\sigma_{TMS}$ of the standard was also calculated, and the chemical shifts were defined from the following expression:

$$\delta_{\text{calc}} = \sigma_{TMS} - \sigma_i$$

Obtained values are listed in Table 1.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Exp. ppm</th>
<th>Conf A</th>
<th>Conf B</th>
<th>Conf C</th>
<th>Conf D</th>
<th>Conf E</th>
<th>Conf F</th>
<th>$R^2$</th>
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<td>C1</td>
<td>170.18</td>
<td>185,3457</td>
<td>185,6209</td>
<td>183,0264</td>
<td>182,8695</td>
<td>181,2147</td>
<td>181,5865</td>
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<td>165,8452</td>
<td>168,0534</td>
<td>164,6410</td>
<td>167,5861</td>
<td>163,0400</td>
<td>165,3880</td>
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<td>C3</td>
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<td>154,3140</td>
<td>153,4562</td>
<td>154,4912</td>
<td>153,3650</td>
<td>154,8778</td>
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<tr>
<td>C4</td>
<td>137.85</td>
<td>154,6990</td>
<td>154,7384</td>
<td>154,5967</td>
<td>154,8435</td>
<td>154,6471</td>
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<tr>
<td>C5</td>
<td>134.17</td>
<td>149,1048</td>
<td>149,9630</td>
<td>148,0964</td>
<td>149,1040</td>
<td>148,8642</td>
<td>149,6239</td>
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<tr>
<td>C6</td>
<td>131.68</td>
<td>148,1066</td>
<td>148,0547</td>
<td>141,3965</td>
<td>141,5066</td>
<td>151,2061</td>
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<td>C7</td>
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<td>153,0697</td>
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<td>152,0946</td>
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<td>153,2470</td>
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<td>126.39</td>
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<td>142,2573</td>
<td>139,5667</td>
<td>142,1644</td>
<td>139,8882</td>
<td>142,2990</td>
<td>0.996</td>
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<tr>
<td>C9</td>
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<td>140,8332</td>
<td>139,4518</td>
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<tr>
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<td>140,3628</td>
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<tr>
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<td>125,4195</td>
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<td>127,3381</td>
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<td>120,7776</td>
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<td>32,3030</td>
<td>32,7159</td>
<td>32,2456</td>
<td>0.998</td>
</tr>
<tr>
<td>C15</td>
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<td>24,8105</td>
<td>24,1283</td>
<td>24,7095</td>
<td>23,9936</td>
<td>24,8138</td>
<td>0.998</td>
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</table>

TABLE 1. Experimental and calculated values of $^{13}$C NMR chemical shifts (ppm) of mefenamic acid with respect to TMS.
To determine which conformer of mefenamic acid shows the best correlation with the experiment, signal assignment was made with the aid of homo- and heteronuclear NMR spectroscopy (2D TOCSY, HSQC, and HMBC); see Figure 2 and 3. Correlations of the calculated and measured $^{13}$C NMR chemical shifts were built. The root mean square deviation was chosen as the main convergence criterion (Table 1). Conformer A was found to prevail among all six variants since its RMSD is 0.999; conformers C and F are also quite probable, while other forms show the RMDS value below 0.996.

![Conformers A, C, and E](image)

**FIGURE 2.** Most probable conformers of 2-[(2,3-dimethylphenyl)amino]benzoic acid: (a) Conf A, (b) Conf C, and (c) Conf E

![13C NMR spectrum](image)

**FIGURE 3.** $^{13}$C NMR spectrum of 2-[(2,3-dimethylphenyl)amino]benzoic acid

Comparison of the mefenamic acid structure determined by XRD with the conformers A and C found by quantum chemical calculation shows that the carboxyl group in them points to the same direction as in the MEF I polymorph. In the saturated solution, however, conformer E may also be present, which makes formation of MEF II quite possible. Thus, both polymorphs may appear in the saturated solution in DMSO. More detailed investigation of this problem will require a deep analysis using the 2D NOESY spectroscopy, which can provide quantitative information on the conformer populations in the DMSO solution.
CONCLUSION

An approach of predicting $^{13}$C NMR chemical shifts of different conformers of mfenamic acid was demonstrated. It showed a good qualitative agreement with X-ray data and allowed to evaluate the potential of the system mfenamic acid -- DMSO in studied of conformational polymorphism. This approach may be used in screening and candidate selecting in finding new polymorphic forms.

The considered method allows carrying out an express analysis for determination of the dominant conformer of a studied molecule; the crystallization peculiarities for the investigated compound can be predicted.

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