THEORETICAL STUDIES ON TAUTOMERIC EQUILIBRIA IN DEFERIPRONE

M. Sraka\textsuperscript{1}, K. Zborowski\textsuperscript{1}, T. Kiss\textsuperscript{3,4}, L. M. Proniewicz\textsuperscript{1,2}

\textsuperscript{1}Faculty of Chemistry, Jagiellonian University, 3 Ingardena Str., 30-060 Kraków, Poland
\textsuperscript{2}Regional Laboratory of Physicochemical Analysis and Structure Research, Jagiellonian University, 3 Ingardena Str., 30-060 Kraków, Poland
\textsuperscript{3}Biocoordination Chemistry Research Group of the Hungarian Academy of Sciences, University of Szeged, P.O. Box 440, H-6701 Szeged, Hungary
\textsuperscript{4}Department of Inorganic and Analytical Chemistry, University of Szeged, P.O. Box 440, H-6701 Szeged, Hungary

INTRODUCTION
Deferoxamine (1,2-dimethyl-3-hydroxy-pyridin-4-one), hereafter abbreviated as Hdmhp, is the first orally active iron chelator [1-2]. Because of that it is widely used in the treatment of thalassemia [3-4]. Thalassemia is a group of inherited disorders of hemoglobin metabolism in which the synthesis of globin chains is impaired [5]. There are several types of thalassemia. One of the most serious is \textit{β}-thalassemia. It is often treated by regular blood transfusions. Repeated transfusions induce excess of free iron ions in the blood [6] what is serious problem. Excess of iron ions in blood cannot be excreted via the kidney. They are accumulated mainly in the liver that can lead to a damage of this organ. Free iron ions can also react with oxygen and form free radicals [7]. Deferiprone molecules are able to bind iron ions into complexes that help in bringing down their concentrations in the blood [8]. However, this drug exhibits few side effects [9]. Thus, research on Hdmhp and related hydroxyperidines are still important. It also forms other complexes that can be potentially useful in medicine. For example, the deferiprone complex with vanadium(III) ion (V(dmhp)) can be used as a drug to normalize glucose levels in the diabetic rats [10]. Other examples are: deferiprone-aluminium (III) complex (Al(dmhp)) useful in therapy of neurological dysfunctions [11] or Ga(dmhp), which can be used as radiopharmaceutical for imaging [12]. The 3-hydroxy-4-pyridines are closely related to 3-hydroxy-4-pyrones like maltol, ethylmaltol or kojic acid. Such hydroxyperidines are widely known as ligands for many complexes of great biological activity [13].

Determination of the biologically active structures of studied compounds is essential for the research. Molecular structure of deferiprone, similarly to hydroxyperidines, depends upon pH. Thus, it can exist not only as neutral molecule but also as cation or anion [14]. Due to their structures neutral and cationic species exhibit tautomeric phenomena. The composition of tautomeric mixture composition can be calculated by theoretical methods [15].

METHODS
In this work we present results of some quantum mechanical calculations on structures of neutral deferiprone and its various charged species. RHF [16] and DFT (BLYP [17] and B1LYP [18] functionals) methods with the 6-311++G(d,p) basis set have been employed in our calculations, that have been performed using Gaussian 98 quantum chemical set of programs [19]. All possible tautomeric structures have been taken into account. Potential energy calculations have allowed to select the most energetically favourable tautomers. For that structures full geometry optimization and vibrational frequency calculations have been performed. In calculations performed for the most stable tautomers the CBS-4 [20] method has been additionally used. It calculates molecular energy with a very high accuracy [21].

Tautomeric equilibria constants among tautomers with the lowest energies have been evaluated from the equation (1) [22]

$$\Delta G_{AB} = -RT \ln K_{AB}$$

(1)

931
where R is the ideal gas constant and T is the absolute temperature. In all presented computations the pressure is set to 1 atmosphere and temperature is equal 298.15 K. $\Delta G_{38}$ is the free energy variations between two tautomers obtained from quantum chemical calculations. The whole procedure was described in details earlier [23] Obtained equilibrium geometries of different form of deferiprone and tautomeric equilibrium constants have been compared with the same data determined for maltol and other structurally similar hydroxyphyrines [23], see Fig.1.

![Maltol and Deferiprone](image)

**Fig. 1.** Structures of maltol and deferiprone.

**RESULTS**

Calculations for all possible neutral tautomers of deferiprone have been done. Three tautomers with the lowest energies have been used for further investigation. They are depicted in Fig. 2 and their energies are collected in Table 1. In case of HF and

![Neutral Tautomers of Deferiprone](image)

**Fig. 2.** Configurations of the neutral tautomers of deferiprone.

<table>
<thead>
<tr>
<th></th>
<th>RHF</th>
<th>BLYP</th>
<th>B1LYP</th>
<th>CBS-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.00 (0.00)</td>
<td>0.00</td>
<td>0.00 (0.00)</td>
<td>0.00</td>
</tr>
<tr>
<td>N2</td>
<td>57.40 (95.36)</td>
<td>16.96</td>
<td>25.40 (49.51)</td>
<td>15.15</td>
</tr>
<tr>
<td>N3</td>
<td>54.68 (28.26)</td>
<td>71.80</td>
<td>75.38 (47.99)</td>
<td>82.22</td>
</tr>
<tr>
<td>N1→N2</td>
<td>8.77E-11 (1.53E-17)</td>
<td>1.07E-3</td>
<td>3.55E-5 (9.56E-10)</td>
<td>1.46E-3</td>
</tr>
<tr>
<td>N1→N3</td>
<td>2.63E-10 (3.41E-5)</td>
<td>2.63E-13</td>
<td>6.23E-14 (2.40E-8)</td>
<td>2.20E-14</td>
</tr>
</tbody>
</table>

**Tab. 1.** Relative energies (in kJ/mol, ZPE included) and equilibrium constants for neutral tautomers of deferiprone. In brackets appropriate maltol values [23].

B1LYP methods, the values calculated for deferiprone can be compared with the data obtained earlier for maltol [23]. The BLYP and CBS-4 approximations were not used in our earlier studies. Performed calculations show that N1 enolic structure (see Fig.1) has always the lowest energy. This structure is
stabilized by intramolecular hydrogen bond formed between keto and hydroxyl groups. It do not have the C₃ symmetry since two closely located methyl groups. They are a bit twisted that causing in the small distortion of the ring. Because of that the strict planarity is not achieved. It is true for all studied deferiprone species. The second tautomer in the energy order is the N2 structure. It is similar to N1, but the labile proton is moved to the second oxygen atom (see Fig.1). The third in energy order is the keto N3 tautomer. Here, the heterocyclic ring is distorted because one of the methyl group which is strongly shifted out of the mean plane of the heterocyclic ring. The keto tautomer is favoured by the HF method. For this approximation the N3 structure has lower energy than N2.

The energy gap between N1 and N2 tautomers strongly depends on the theoretical method used in calculations. It is the largest for the HF method and it decreases about fifty percent for the B1LYP approximation. The same result is obtained for the maltol molecule, see Table 1. The lowest, and very similar in value, are the energy gaps between N1 and N2 for BLYP and CBS-4 methods. Calculated equilibrium constants show that, as for studied earlier hydroxypyrones, the N1 enol tautomer dominates in the gas phase of deferiprone.

Structures of the three cationic tautomers with the lowest energies are presented on Fig.3. Results of calculations are collected in Table 2. One can notice that energetically favourable is the C1 structure with the two hydroxyl groups. The conformation of these groups is the same as the one obtained for hydroxypyrones [23]. The next in the energy order is C2. Analogical structure was studied before for hydroxypyrones so appropriate values are compared in Table 2. The structure analogical to C3 has not been studied, yet. In this structure both labile protons bind to the –C–H groups. Thus formed –CH₂ groups are strongly twisted relatively one to another forcing a strong distortion of the whole heterocyclic ring.

Fig. 3. Configurations of the cationic tautomers of deferiprone.

| Tab. 2. Relative energies (in kJ/mol, ZPE included) and equilibrium constants for cationic tautomers of deferiprone. In brackets appropriate maltol enol values [23]. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | RHF             | BLYP            | B1LYP           | CBS-4           |
| C1              | 0.00 (0.00)     | 0.00            | 0.00 (0.00)     | 0.00            |
| C2              | 72.32 (249.75)  | 76.14           | 83.73 (266.55)  | 92.59           |
| C3              | 129.97          | 148.18          | 154.86          | 157.18          |
| C1→C2           | 2.14E-13 (1.76E-44) | 4.58E-14       | 2.14E-15 (2.00E-47) | 6.01E-17 |
| C1→C3           | 1.70E-23        | 1.09E-26        | 7.40E-28        | 2.90E-28        |

The energy gap between the tautomer with the lowest energy and the next one is always much bigger than for neutral tautomers of studied compound. On the other hand, this gap is much lower than for maltol and other hydroxypyrones. That is why we can conclude that in the case of deferiprone cation, like in related hydroxypyrones, protonation occurs at the oxygen atom of the keto
group (see the values of equilibrium constants in Table 2). No protonation is observed on the intraring nitrogen atom.

Finally, the structure of the deferiprone anion is presented in Fig. 4.

![Fig. 4. Configuration of the deferiprone anion.](image)

Because of the lack of a labile proton, tautomeric equilibria do not exist. The obtained structure is similar to the structures of hydroxypyrones' anions [23]. But, as mentioned earlier, the sterical hindrance between two methyl groups causes the lost of planarity of the deferiprone anion.

**ACKNOWLEDGEMENTS**

Calculations were performed at the Warsaw University's Interdisciplinary Centre for Mathematical and Computational Modeling ICM (project number G17-8). KZ is the recipient of the Jagiellonian University Rector Fund for outstanding researcher.

**REFERENCES**