Research Article
Study of a Conformational Equilibrium of Lisinopril by HPLC, NMR, and DFT

Sondes Bouabdallah,¹ Med Thaieb Ben Dhia,² and Med Rida Driss¹

¹ Laboratoire de Chimie Analytique Appliquée, Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisia
² Laboratoire de Chimie Organique Structurale, Synthèse et Etude Physico-Chimique (Equipe de Chimie de Coordination), Faculté des Sciences de Tunis, 1060 Tunis, Tunisia

Correspondence should be addressed to Sondes Bouabdallah; sondes.bouabdallah@laposte.net

Received 31 July 2013; Accepted 18 October 2013; Published 25 February 2014

Academic Editor: Richard G. Brereton

Copyright © 2014 Sondes Bouabdallah et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The isomerization of lisinopril has been investigated using chromatographic, NMR spectroscopic, and theoretical calculations. The NMR data, particularly the NOEDIFF experiments, show that the major species that was eluted first is the trans form. The proportion was 77% and 23% for the trans and cis, respectively. The thermodynamic parameters (∆H, ∆S, and ∆G) were determined by varying the temperature in the NMR experiments. The interpretations of the experimental data were further supported by DFT/B3LYP calculations.

1. Introduction

Lisinopril, N-(1-carboxy-3-phenylpropyl)-L-lysyl-L-proline, belongs to a class of antihypertensive agents which inhibit the angiotensin-converting enzyme (ACE) to control blood pressure [1]. The active parts of ACE inhibitors are peptide derivatives containing C-terminal proline residues. Like other proline-containing peptides, lisinopril exists as an equilibrium mixture of cis and trans isomers, with respect to the proline amide bond (Figure 1) [2, 3]. Under unstrained conditions most peptide bonds adopt the trans isomeric form, mainly because of the weaker steric repulsion between hydroxyl and carboxyl group effects in the molecule when compared to the cis. The trans form in lisinopril was shown to be the preferred isomer and biologically active [4–6]. The assignment separation of cis and trans form of lisinopril has been carried out by HPLC [5, 7–12], CZE [13–16], and NMR spectroscopy [2, 17–22]. The latter technique is a powerful tool and has been widely applied for structural and stereochemical characterization of amino acids, oligo- and polypeptides [23–26]. The cis-trans isomerization of peptide bonds is a slow process on the NMR time scale under normal conditions at ambient temperature due to the high barrier resulting from the C–N partial double bond character. NMR spectroscopy has therefore been successfully used to study the cis-trans isomerization process of lisinopril in solution [25, 27].

In this paper, we report on the isomerization of lisinopril using a combination of HPLC, NMR spectroscopy, and theoretical approaches. The effect of temperature on the cis-trans isomerization process of lisinopril was investigated in order to determine different thermodynamic parameters (∆H, ∆S, and ∆G).

2. Experimental

2.1. Samples. Lisinopril was kindly provided from Solvay Pharmaceuticals.

2.2. Reagents. Potassium dihydrogen phosphate, sodium hydroxide, and phosphoric acid were of RP quality from Pro-labo (France). Methanol, acetonitrile, and tetrahydrofuran (THF) were of HPLC grade from LabScan (Dublin, Ireland).

The mobile phase was prepared by first preparing a solution of 0.02 M KH₂PO₄, adjusting its pH to 2 with phosphoric acid and finally mixing the solution with an organic modifier (acetonitrile, methanol, and THF).
It appears that the separation of the two isomers of lisinopril can be achieved using a mobile phase consisting of a mixture of 20 mM phosphate buffer [pH 7]-acetonitrile (90/10; v/v), a column temperature of 279 K, and flow rate of 2 mL/min with retention time $t_{R1} = 3.49$ min and $t_{R2} = 4.55$ min. However, a higher temperature is required for the elution of lisinopril as a single sharp peak at 2.76 min (Figure 2).

This is because it was found that an elevated temperature led to deterioration in the separation of the two isomers. Moreover, at 328 K lisinopril was eluted as a narrow single peak due to the high isomerization rate of the two isomers. On the other hand, at low temperature the two isomers were resolved almost completely indicating that the interconversion rate had slowed down.

From ambient temperature chromatograms, the isomer trans/cis ratio was integrated to be 76/24. This result is similar to those reported earlier demonstrating that high temperature was useful for elution of proline-containing substances as a single peak [7,11,35]. Conversely, a low temperature is known to have a potential effect on the separation of isomers [5,9,36–39].

3.2. NMR Studies. The structure of lisinopril (Figure 3) shows 21 carbon atoms with two sets of two chemically equivalent carbons describing the ortho- and metapositions on the aromatic ring. So, we expect to observe 19 signals in $^{13}$C NMR spectra. However, the obtained spectra showed the doubling of all signals confirming the existence of the two isomers (Figure 4).

In addition, the $^{1}$H NMR spectra of lisinopril in CD$_3$CN/D$_2$O at 298 K (Figure 5(a)) show two sets of triplets of unequal intensities. The multiplicity of each signal set reflected first from the interaction of H58 with H25 and H26, giving the two signals in the 3.8–4.1 ppm region, and second from the interaction of H43 with H45 in the 4.1–4.4 ppm region. The same spectrum recorded at 333 K (Figure 5(b)) shows a better separation of the two signals at 4.1 ppm.

These isomers are assigned to a cis-trans equilibrium of the rotation around the amide bond. As described earlier, it is worth noting that this equilibrium appears to be slow on the NMR time scale at ambient temperatures [37,40]. Using the area of resonance signals of proton 58 (3.8–4.4 ppm), the isomer ratio was integrated to be 77/23 at 298 K. The result obtained in a separate experiment recorded at a probe temperature of 298 K is consistent with that determined by HPLC at the same temperature.

We conclude that the major conformer in the $^{1}$H NMR spectrum of lisinopril corresponds to the first eluted peak in the HPLC chromatogram at ambient temperature, which exists in a higher proportion. A similar study demonstrated this correspondence in the case of ramiprilat [4], enalaprilat [5], and perindopril [39] in different solvents.

3.1. HPLC Study. The study of the cis/trans equilibrium of lisinopril by HPLC demonstrates that chromatographic conditions such as flow rate, temperature, pH, and organic modifier have an important effect on peak shape and retention time of lisinopril.
Therefore, a low NOE ($\zeta = 8\%$) is observed at $H_{58}$ ($\delta = 3.16$ ppm) when $H_{43}$ ($\delta = 4.35$ ppm) is irradiated in the major conformer. Accordingly, a stronger NOE ($\zeta = 21\%$) is noted at $H_{58}$ ($\delta = 3.7$ ppm) when $H_{43}$ ($\delta = 3.31$ ppm) is irradiated in the minor conformer.

In addition, when $H_{48}$ ($\delta = 4.45$ ppm) is irradiated, we observe at $H_{58}$ a NOE ($\zeta = 34\%$) in the major conformer and a NOE ($\zeta = 10\%$) in the minor conformer (Figure 6).

This shows that the NOE in the minor conformer for the $H_{43}/H_{58}$ is more important than the NOE in the major conformer, which implies that in the major conformer, the distance between the nuclei is higher than the one in the minor conformer

$$r_{43–58(\text{min})} < r_{43–58(\text{maj})}$$

$$r_{48–58(\text{maj})} < r_{48–58(\text{min})}$$

(1)
Based on the relationship between NOE and internuclear distances, one can give the expression of distance in each conformer

\[
\begin{align*}
(r_{43-58})_{\text{min}} &= \left(\frac{8}{21}\right)^{1/6} (r_{43-58})_{\text{maj}} \\
(r_{48-58})_{\text{min}} &= \left(\frac{34}{10}\right)^{1/6} (r_{48-58})_{\text{maj}}
\end{align*}
\] (2)

Consequently, the examination of the molecular structure of each conformer of lisinopril confirmed that the distance \(r_{43-58}\) in the cis conformer is indeed smaller than the distance in the trans form. The \(^1\)H NMR intensities suggest that the major conformer is the trans form and the minor conformer is the cis form. The nuclei of the s-trans conformer are more deshielded than those of s-cis conformer, in agreement with literature results [41, 42].

3.3. Thermodynamic Study. At slow chemical exchange, the relative proportion of the two conformers at different temperatures in the range (279–333) K and the cis/trans equilibrium constant of lisinopril have been measured by relative integrals of the two resonance signals of the two states of isomerizations of lisinopril.

These signals are well resolved and allowed to determine accurately the equilibrium constants for the cis to trans interconversion at different temperatures and to measure the thermodynamic parameters: enthalpy (\(ΔH^o\)), entropy (\(ΔS^o\))
of the equilibrium on the basis of the van’t Hoff equation. The Gibbs free enthalpy (Δ𝐺°) is deduced at ambient temperature.

The plot of ln K versus the reciprocal of the absolute temperature is a straight line of equation ln K = 1212.91/T − 2.6359 (Figure 7). The correlation coefficient for this straight line is r = 0.995. The enthalpy was obtained via the slope and the entropy via the intercept of plot. The thermodynamic parameters obtained from experiment were Δ𝐻° = −10.36 kJ/mol, Δ𝑆° = 21.91 J/k·mol, and Δ𝐺°298K = −16.91 kJ/mol.

Remarkably, the equilibrium cis to trans isomerization was enthalpically and entropically favored in this condition. Consequently, the decrease in the temperature expected a displacement of the conformational equilibrium to the trans form over the cis isomer. This difference is in good agreement with other studies reported on a similar product such as enalapril [5].

Table 1: Selected bond lengths (Å) and angles (deg) for the cis and trans isomer of lisinopril.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>s-cis</th>
<th>s-trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lengths</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H56–O40</td>
<td>3.857</td>
<td>1.717</td>
</tr>
<tr>
<td>H56–O21</td>
<td>1.799</td>
<td>4.816</td>
</tr>
<tr>
<td>H23–O40</td>
<td>3.618</td>
<td>4.033</td>
</tr>
<tr>
<td>H56–N41</td>
<td>2.486</td>
<td>2.613</td>
</tr>
<tr>
<td>H56–N59</td>
<td>2.660</td>
<td>4.875</td>
</tr>
<tr>
<td>H23–N59</td>
<td>3.721</td>
<td>3.717</td>
</tr>
<tr>
<td>H23–N41</td>
<td>4.958</td>
<td>4.947</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bonds lengths</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H56–O55</td>
<td>0.988</td>
<td>0.995</td>
</tr>
<tr>
<td>O55–C53</td>
<td>1.338</td>
<td>1.338</td>
</tr>
<tr>
<td>H23–O42</td>
<td>0.972</td>
<td>0.972</td>
</tr>
<tr>
<td>O22–C20</td>
<td>1.346</td>
<td>1.556</td>
</tr>
<tr>
<td>H43–H58</td>
<td>4.058</td>
<td>4.471</td>
</tr>
<tr>
<td>H48–H58</td>
<td>6.612</td>
<td>6.255</td>
</tr>
<tr>
<td>H49–H58</td>
<td>5.982</td>
<td>5.934</td>
</tr>
<tr>
<td>Energy (au)</td>
<td>−359.2686</td>
<td>−359.2729</td>
</tr>
<tr>
<td>Energy (Kcal/mol)</td>
<td>−852953.828</td>
<td>−852956.552</td>
</tr>
<tr>
<td>μ (Debye)</td>
<td>3.930</td>
<td>10.646</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bond angle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C53–O55–H56</td>
<td>112.8</td>
<td>103.1</td>
</tr>
<tr>
<td>C18–C20–O21</td>
<td>125.0</td>
<td>124.8</td>
</tr>
<tr>
<td>N41–C39–O40</td>
<td>120.5</td>
<td>121.2</td>
</tr>
<tr>
<td>O54–C53–O56</td>
<td>121.6</td>
<td>122.2</td>
</tr>
<tr>
<td>Dihedral angle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H23–O22–C23–O24</td>
<td>172.5</td>
<td>174.2</td>
</tr>
<tr>
<td>O40–C39–C41–C50</td>
<td>2.3</td>
<td>176.6</td>
</tr>
<tr>
<td>O54–C35–O55–C53</td>
<td>−158.2</td>
<td>178.1</td>
</tr>
<tr>
<td>O40–H56–O55–C53</td>
<td>7.7</td>
<td>−41.8</td>
</tr>
<tr>
<td>O21–H56–O55–C53</td>
<td>156.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Charge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O40</td>
<td>−0.49</td>
<td>−0.53</td>
</tr>
<tr>
<td>H56</td>
<td>+0.47</td>
<td>+0.45</td>
</tr>
<tr>
<td>O55</td>
<td>−0.60</td>
<td>−0.50</td>
</tr>
<tr>
<td>H23</td>
<td>+0.42</td>
<td>+0.42</td>
</tr>
<tr>
<td>O22</td>
<td>−0.55</td>
<td>−0.56</td>
</tr>
</tbody>
</table>

3.4. Theoretical Calculations. In order to confirm the NMR data obtained for the cis/trans isomerization of lisinopril, the geometrics of the two conformers were fully optimized at the DFT/B3LYP level of theory using 6-31++G* basis set. The structures have been identified as local minima on the single potential energy surfaces (PES) (Figure 8). Optimized values of selected geometrical parameters are listed in Table 1. The potential energy difference between the two isomers of lisinopril was 11.397 kJ/mol indicating the stability of trans over the cis isomer. This difference is in good agreement with experimental data that the trans is the majorities form.

As shown in Table 1, in the trans conformer the interaction between H56–O40 (𝑑_H56-O40 = 1.717 Å) would be stronger than that in the cis (𝑑_H56-O40 = 3.857 Å), thus generating a sharp reduction of the valence angle C53–O55–H56 (θ = 103.1° in the trans form and θ = 2.3° in the cis form) and a strong variation on dihedral angle O40–C39–C41−C50 (φ = 176.6° in the trans form and 2.6° in the cis form), indicating possible existence of some hydrogen bond interactions which would be more favored in the trans than in cis isomers.

This stability of trans form over the cis form is further confirmed by the charge density between the same atoms (Δ𝑞_cis = 0.08, Δ𝑞_trans = 0.02). The strong interaction between O21–H56 atoms (𝑑_H56-O21 = 1.799 Å) in the s-cis conformer triggers a modification of the dihedral angle O21–H56–O55–C53 (φ = 156.8°) which may explain the low stability of the cis form. On the other hand, the low interaction between H56–O21 (𝑑_H56-O21 = 4.816 Å) in the s-trans conformer yields a change in the dihedral angle (φ = 3.9°) so the atoms H56 and O40 were far from each other and the trans was more stable.

It is worth noting that there are two hydrogen bonds, one between atoms H56–O40 and another between H55–O21, but the stability was determined by the first bond because...
the distance is shorter ($d_{O21-H56} = 1.799 \text{ Å}$). In addition, there are strong intramolecular interactions in the trans conformer ($\mu = 10.6 \text{ D}$) than in the cis form ($\mu = 3.9 \text{ D}$) indicating a higher stability of the conformer trans.

The distance between the nuclei in both conformers (cis and trans) was compared. It is revealed that in the cis conformer, H43 is close to H58 (4.058 Å), while H48 is at a much greater distance from H58 (6.612 Å). On the other hand, in the trans conformer H43 is further away from H58 (4.471 Å), while H48 is at much greater distance from H58 (6.225 Å). This is consistent with the results obtained with NOE difference experiments giving an enhancement of 8% and 21% in the first irradiation and 34% and 10% in the second irradiation.

4. Conclusions

Lisinopril exists individually as a mixture of cis-trans isomers in solution. The two isomers could be easily distinguished by HPLC, $^1$H NMR, $^1$C NMR, and $^1$H-$^1$H NOE spectra. The results of the various investigations by NMR and those of the theoretical study are in favor of assigning trans form to the major isomer present under the conditions used in the HPLC studies. Additionally, a relative to successful combination of
molecular modeling studies with experimental spectroscopic assays was used in order to elucidate the molecular bases.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments
The authors want to express their special thanks to Dr. M. A. M. K. Sanhoury of the Faculty of Sciences of Tunis for his valuable help and discussion of this work. They also thank the editors and anonymous referees for valuable comments and suggestions that greatly improved the paper.

References


