Enthalpies of Combustion and Formation of Histidine Stereoisomers

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1.Introduction

The aim of this study was a calorimetric characterization of L-, D-, and DL-histidine isomers.

Histidine is a heterocyclic amino acid containing an imidazole ring. The imidazole ring is aromatic, as it contains six π electrons. Histidine is a proteinogenic amino acid, and it is able to form π stacking interactions. Consequently, the side chain can have a role in stabilizing the folded structures of proteins. Histidine is an essential amino acid that is not synthesized de novo in humans and is needed for growth and tissue repair [1].

The imidazole ring allows histidine to act as a coordinating ligand in metal proteins, including certain enzymes. It is important in hemoglobin, as well. Histidine is involved in stabilizing oxyhemoglobin and destabilizing CO-bound hemoglobin. Histidine is important for maintenance of myelin membranes that protect nerve cells and is metabolized to the neurotransmitter histamine [2].

Two divergent values of the enthalpy of formation of L-histidine are found in the literature: −441.8 ± 2.6 kJ·mol⁻¹ [3] and −466.7 ± 2.8 kJ·mol⁻¹ [4]. Vasilev et al. [3] were aware that their data differ from previously reported values [4] and considered that the difference is due to the inadequate purity of the sample of the later authors. No data about the enthalpy of formation of D-histidine and for the racemic are found in the literature. The main purpose of this work is to determine these parameters and to compare them with those calculated by the group additivity method. The paper brings more information about the thermal behavior of the investigated compounds in function of the isomer type.

2.Experimental

2.1. Materials. D-histidine and DL-histidine were obtained commercially from Sigma-Aldrich, mass fraction purities ≥98% and 99%, respectively, and L-histidine, assay >99%,
from Fluka with $M = 155.15 \text{ g mol}^{-1}$. Samples were dried in a vacuum oven for 3 hours at 90°C and preserved in a desiccator before use, in order to eliminate adsorbed water.

The purity of samples was tested by DSC and polarimetry.

Our DSC data confirm the purity of over 99% for the DL- and D-isomers (99.06 and 99.25%, respectively) while for the D-histidine, the purity was 98.86%. The data are shown in Table 1 of Supplementary materials.

Specific rotations $[\alpha]_{D}^{25}$ of the investigated compounds were determined on solutions in deionized water for checking the amino acid optical purity. A 341 PerkinElmer polarimeter was used in the D line of sodium, with glass cells (1 cm path length), at 25°C. The data are shown in Table 1 of Supplementary materials.

Like in the case of other amino acids, the only impurities amounting at least 0.1% (other than water) certified by the manufacturer consist of other amino acids, with similar values of the massic heat of combustion.

2.2. Methods

2.2.1. Combustion Calorimetry. The combustion experiments were performed using a Parr Instruments model 6200 microprocessor controlled isoperibol oxygen bomb calorimeter. Temperatures are measured with a high-precision electronic thermometer using a specially designed thermometer sensor sealed in a stainless steel probe which is fixed in the calorimeter cover. Measurements were taken with 0.0001 K resolution. The jacket temperature is held constant for isoperibol operation. The semimicro kit handling samples from 25 to 200 mg was used because of the small amounts of the studied compounds. High-purity oxygen 99.998% was used for combustion. Calorific grade benzoic acid supplied by Parr, with heat of combustion 26,454 J·g$^{-1}$, was used for the standardization of the combustion calorimeter. The determined calorimeter constant was $e_{\text{calor}} = 2326.9 \pm 1.9$ J·K$^{-1}$.

The samples were pressed into pellets of 3 mm diameter. The pellets were weighed with a Mettler–Toledo analytical balance, model XP6 with an accuracy of $\pm 2 \times 10^{-6}$ g.

The final solution from the bomb was analyzed for the presence of nitric acid (about 20% from the total nitrogen) by titration with solution of Na$_2$CO$_3$ 0.1 mol·L$^{-1}$. The heat due to nitric acid formation was obtained using the value of the enthalpy of formation of nitric acid solution, $\Delta_{f}H_{\text{HNO}_3, aq} = -58.8$ kJ·mol$^{-1}$ [6].

2.2.2. Thermal Analysis. For the thermal characterization of histidines, a simultaneous thermogravimetry (TG) and differential scanning calorimetry (DSC) TGA/DSC Setaram Setsys Evolution 17 analyzer was employed. Thermal properties (temperatures, enthalpies, and mass losses) associated with melting and/or decomposition processes of the histidine stereoisomers were measured in the temperature ranging from 20 to 600°C with a scanning rate of 10°C·min$^{-1}$ in alumina crucibles, using Ar flow. Standard metallic substances of 99.999% purity (In, Sn, Pb, Zn, and Al) were used for the calibration in temperature. The melting onset temperatures and heats of fusion of standard materials were used for temperature correction and energy calibration. The sample mass for simultaneous TG-DSC measurements was about 1-2 mg. The error of TG measurement is $\pm 0.15\%$. All thermal analysis (TG-DSC) data were processed using Calisto software.

3. Results

3.1. Combustion Energy. At least 6 runs were retained for each isomer. Some runs were rejected because of doubt about combustion completeness. In runs used in data calculation, there was no evidence of soot formation in the bomb. The data regarding the combustion measurements for the three isomers are given in Tables 2–4 in Supplementary materials. The assigned uncertainties are twice the standard error of the mean. $\Delta H$ (fuse) and $\Delta U$ (ign) were calculated from the mass of cotton and $\Delta_{u}$ (cotton) = 16240 $\pm$ 20 J·g$^{-1}$ [7] and from the mass of the fire and $\Delta_{u}$ (Ni–Cr) = 5.86 kJ·g$^{-1}$ (certified by the fabricant), respectively. The values obtained experimentally for the combustion energy were reported to the standard state ($T$ = 298.15 K and $p = 101.325$ kPa). Corrections were performed using Washburn methodology [8].

In order to calculate the enthalpies of formation, the following values were used: $\Delta H_{298}$ CO$_2$([g] = $-$393.51 $\pm$ 0.13 kJ·mol$^{-1}$ and $\Delta_{f}H_{\text{H}_2\text{O}(l)} = -285.83 \pm 0.042$ kJ·mol$^{-1}$ [9]. In Table 2 are presented our data for the solid-state enthalpies of formation, together with literature values [3, 4].

The values of the enthalpies of formation of the L-enantiomer are quasi-identical with that of racemic, while that of D-histidine is more negative (within the cumulated experimental errors).

3.2. DSC. Figure 2 presents the DSC curves of L-, D-, and DL-histidine isomers. A single-peak sharp is recorded for all three stereoisomers.

The temperature ranges in which the decomposition processes (decomposition prevails due to the high temperature) of the three stereoisomers take place are similar (274–290°C).

Our peak temperatures of the enantiomers (Table 3) are in reasonable agreement with the values reported by Olafsson and Bryan [10] (288°C), Weiss et al. [11] (272°C), and Anandan et al. [12] (275°C) as well as with that included in the Handbook of Chemistry and Physics ([5, p. C445] (287°C), but not with the value of Wesolowski and Erecinska [13] (250°C). The “melting” points usually found for amino acids are irrelevant since they decompose, so that the temperatures may vary according to the morphology of the sample and to the experimental conditions used by the researchers [13].

3.3. Thermogravimetry. Figure 2 shows the temperatures and weight losses in the TG and DTG curves. Thermogravimetric records for samples show a first-step fast weight loss of 17-18% starting above 240°C, followed by a continuous mass decrease of the sample. At 600°C, a mass reduction of over 53% is observed for both L- and
D-enantiomers and the racemic (Table 4). The difference between the initial mass of samples and the mass loss due to decomposition was consistent with the mass of solid residue.

Table 5 shows comparative data of thermal analysis on histidine reported by different authors.

4. Discussion

The formation enthalpies in solid state experimentally found were compared with those obtained by means of the group additivity method, with parameters recommended by Domalski and Hearing [14]. The value of the solid-state group parameter corresponding to an imine nitrogen atom bound to a carbon atom (N\textsubscript{I}-(C)) is missing. A value of 67 kJ mol\(^{-1}\) was assigned to this parameter, taking into account the values of the same parameter for the liquid and gaseous states. Generally, because of the presence of an \(\alpha\)-amino acid moiety, a zwitterion contribution is considered. A comparison between experimental and calculated values in solid state is shown in Table 2.

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**Table 1:** Polarimetric data of histidine stereoisomers.

<table>
<thead>
<tr>
<th>Isomeric histidines</th>
<th>Concentration (g (10^{-2}) mL(^{-1}))</th>
<th>([\alpha]_{25}^\circ)</th>
<th>([\alpha]_{25}^\circ) [literature [5]]</th>
<th>([\alpha]_{25}^\circ) [calculated](^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-histidine</td>
<td>1.128</td>
<td>-0.044</td>
<td>39.0</td>
<td>-39.01 (25°C)</td>
</tr>
<tr>
<td>D-histidine</td>
<td>2.66</td>
<td>+0.103</td>
<td>38.72</td>
<td>+39.80 (23°C)</td>
</tr>
<tr>
<td>DL-histidine</td>
<td>1.128</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\)Uncertainty included the uncertainties of the enthalpies of formation of the reaction products H\(_2\)O and CO\(_2\). \(^b\)Estimated value by means of the group additivity method, with parameters recommended by Domalski and Hearing [14].

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**Table 2:** Enthalpies of combustion and formation in solid state of isomeric histidines.

<table>
<thead>
<tr>
<th>Isomeric histidines</th>
<th>(-\Delta_H^\text{c}) (kJ mol(^{-1}))</th>
<th>(-\Delta_cH^\circ) (kJ mol(^{-1}))</th>
<th>(-\Delta_fH^\circ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-histidine</td>
<td>3196.2 ± 2.4</td>
<td>3195.6 ± 2.4</td>
<td>451.7 ± 3.4</td>
</tr>
<tr>
<td>D-histidine</td>
<td>3199.2 ± 2.3</td>
<td>3198.6 ± 2.3</td>
<td>448.7 ± 3.3</td>
</tr>
<tr>
<td>DL-histidine</td>
<td>3196.4 ± 2.3</td>
<td>3195.8 ± 2.3</td>
<td>451.5 ± 3.3</td>
</tr>
</tbody>
</table>

**Table 3:** DSC data of isomeric histidines (10°C min\(^{-1}\)).

<table>
<thead>
<tr>
<th>Isomeric histidines</th>
<th>(T_{on\ set}) (°C)</th>
<th>(T_{max}) (°C)</th>
<th>(T_{end\ set}) (°C)</th>
<th>(\Delta H) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-histidine</td>
<td>277.1</td>
<td>282.1</td>
<td>287.6</td>
<td>72.6 (melting-decomposition)</td>
</tr>
<tr>
<td>D-histidine</td>
<td>272.8</td>
<td>282.4</td>
<td>290.3</td>
<td>70.2 (melting-decomposition)</td>
</tr>
<tr>
<td>DL-histidine</td>
<td>274.2</td>
<td>280.4</td>
<td>286.6</td>
<td>75.4 (melting-decomposition)</td>
</tr>
</tbody>
</table>

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**Figure 1:** DSC curves for L-, D-, and DL-histidines (10°C min\(^{-1}\)).

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D-enantiomers and the racemic (Table 4). The difference between the initial mass of samples and the mass loss due to decomposition was consistent with the mass of solid residue.

Table 5 shows comparative data of thermal analysis on histidine reported by different authors.

4. Discussion

The formation enthalpies in solid state experimentally found were compared with those obtained by means of the group additivity method, with parameters recommended by Domalski and Hearing [14]. The value of the solid-state group parameter corresponding to an imine nitrogen atom bound to a carbon atom (N\textsubscript{I}-(C)) is missing. A value of 67 kJ mol\(^{-1}\) was assigned to this parameter, taking into account the values of the same parameter for the liquid and gaseous states. Generally, because of the presence of an \(\alpha\)-amino acid moiety, a zwitterion contribution is considered. A comparison between experimental and calculated values in solid state is shown in Table 2.
k_+ he calculated enthalpy of formation \[14\] agrees fairly with our experimental values. Only four hydrogen bonds per molecule were reported, less than for other amino acids. One of them is intramolecular in the case of enantiomers \[15,16\] while all four are intermolecular for DL \[17\]. k_+ three of them are taken into account in the group additivity calculations.

The enthalpy of formation in the ideal gas state of the histidine stereoisomers was calculated by means of the same quantity in the crystalline state and of the standard enthalpy of sublimation (Table 6). Gaffney et al. \[18\] have derived a value of 142 $\pm$ 8 kJ·mol$^{-1}$ from vapor pressure measurements in the temperature range 392–492 K. A positive correction to the standard state of 6 $\pm$ 2 kJ·mol$^{-1}$ is obtained by means of the estimation methods, recommended by Chickos et al., for phase-change enthalpies and heat capacities \[19,20\]. A much larger value of 182 kJ·mol$^{-1}$ is predicted by Badelin et al. \[21\] by quantum chemical computations.

The processes revealed by DSC and TG are essentially, if not exclusively due to decomposition. Bryan and Olafsson \[22\] state that the main decomposition reaction is decarboxylation, possibly preceded by deamination. Weiss et al. \[11\] consider that a single main reaction occurs, taking into account that a sharp peak is obtained during the DSC run. The weight loss in the case of decarboxylation reaction was the only one that would be about 28.4% (higher than our experimental value, about 18%). The calculated thermal effect of the decarboxylation reaction, by means of the group additivity method \[14\], is about 79 kJ·mol$^{-1}$ (in standard conditions) comparable with our experimental values (Table 6).

![Figure 2: TG-DTG curves for L-, D-, and DL-histidines.](image)

**Table 4**: Thermogravimetric data of isomeric histidines (10°C min$^{-1}$).

<table>
<thead>
<tr>
<th>Isomers histidines</th>
<th>$\Delta m$ (%)/$\Delta t$ (°C)</th>
<th>$\Delta m$ (%)/$\Delta t$ (°C)</th>
<th>$\Delta m$ (%)/$\Delta t$ (°C)</th>
<th>$\Delta m$ (%)/(600°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-histidine</td>
<td>−17.98/244.1–300.1</td>
<td>−21.6/301.3–403.5</td>
<td>−11.36/402.5–594.1</td>
<td>−52.37</td>
</tr>
<tr>
<td>D-histidine</td>
<td>−18.82/246.6–301.6</td>
<td>−21.17/301.6–402.0</td>
<td>−10.63/402.0–594.2</td>
<td>−48.76</td>
</tr>
<tr>
<td>DL-histidine</td>
<td>−17.53/241.0–298.6</td>
<td>−21.79/298.6–400.0</td>
<td>−10.97/400.0–595.0</td>
<td>−48.96</td>
</tr>
</tbody>
</table>

**Table 5**: Comparative values of melting-decomposition parameters of L-histidine reported by different researchers.

<table>
<thead>
<tr>
<th>$T_{on set}$ (°C)</th>
<th>$T_{max}$ (°C)</th>
<th>$T_{end set}$ (°C)</th>
<th>Weight loss (%)</th>
<th>$\Delta H$ (kJ·mol$^{-1}$)</th>
<th>Method</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>277.1</td>
<td>282.1</td>
<td>287.6</td>
<td>17.98</td>
<td>72.6</td>
<td>TG/DSC</td>
<td>This work</td>
</tr>
<tr>
<td>288</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DSC</td>
<td>[10]</td>
</tr>
<tr>
<td>275</td>
<td>296</td>
<td></td>
<td>13</td>
<td>82</td>
<td>TG/DTA</td>
<td>[12]</td>
</tr>
<tr>
<td>287</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[5]</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TG/DTA</td>
<td>[13]</td>
</tr>
</tbody>
</table>

As it may be easily seen, the discrepancies with respect to the calculated value are mainly due to the large uncertainties in the evaluation of the standard enthalpy of sublimation. A small molecule (possibly CO) is evolved during the first step and other gaseous molecules (H$_2$O and NH$_3$) at higher temperatures.
Weiss et al. [11] obtained totally different results by means of mass spectroscopy, i.e., histidine ejects 1 mol H₂O in the following reaction:

\[
\text{His} = C_6H_7N_3O_2 \rightarrow H_2O + C_6H_7N_3O
\] (1)

This observation was confirmed in their opinion by a weight loss of 13–15%, in contradiction with our value of about 18% and incompatible with decarboxylation. The above authors consider that inner cyclization seems likely. However, the proposed structure of the residue would be in this case C₆H₇N₃O (139 Da), so that a difference of 2 Da is arising.

**Data Availability**

All data supporting the results reported in the manuscript are included either in the manuscript itself or in the Supplementary materials.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Acknowledgments**

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**Supplementary Materials**

Results of the histidine isomers containing combustion experiments. (Supplementary Materials)

**References**


