CRYSTAL STRUCTURE OF L-PROLYL-L-METHIONINE MONOHYDRATE

V. M. PADMANABHAN AND V. S. YADAV

Neutron Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

ABSTRACT

L-prolyl-L-methionine monohydrate C$_{10}$H$_{19}$N$_{2}$O$_{5}$S.H$_{2}$O, crystallizes in the monoclinic space group P2$_{1}$ with Z = 2 and a = 19.381(9), b = 5.472(4), c = 6.411(5)Å, $\beta = 93.1(2)$, $D_{x} = 1.29(4)$, $D_{mol} = 1.29$ g cm$^{-3}$, and $\mu = 8.2$ cm$^{-1}$ (for Cu $K_{\alpha}$). The structure was solved by MULTAN and refined to an $R$ index of 0.088 for 812 diffraction data. $C^\alpha$ of the pyrrolidine ring is statistically situated on both sides of NC$^\beta$C$^\gamma$C$^\delta$ plane. The peptide group is significantly non-planar with a torsion angle $\omega$ of 168.5°. The side chain group atoms sulphur and terminal carbon of methionine show large thermal vibration parameters. The side chain conformation at C$^\alpha$-C$^\beta$, C$^\beta$-C$^\gamma$ and C$^\gamma$-S bond is gauche-trans-gauche.

INTRODUCTION

PEPTIDES containing proline residues have been the subject of intensive study$^1$ because of the widespread occurrence of pro-residues in proteins, the possibility of cis-trans isomerisation about x-pro bond and the variations in pyrrolidine geometries$^2$. As the cyclic aminoacid is regarded as a helix breaker, it will be also interesting to follow the effects in the neighbouring groups along the polypeptide chain, before and after the proline residue so as to come to some conclusions on protein conformation.

EXPERIMENTAL

Crystals of the title compound (Sigma Chemicals, USA) were grown from an aqueous solution. Elemental analysis indicated the presence of one water molecule. Preliminary Weissenberg photographs showed the crystal to be monoclinic. All measurements from a crystal 0.20×0.20×0.25 mm were made on the computer controlled Trombay four-circle diffractometer$^3$ with Ni-filtered CuK$_{\alpha}$ radiation ($\lambda = 1.5417\text{Å}$). The cell parameters were determined by least squares from the setting angles of 25 reflections. The intensities were

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>$B_{eq}$(Å$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_1$</td>
<td>3271(4)</td>
<td>3075(7)</td>
<td>3456(4)</td>
<td>2.73(12)</td>
</tr>
<tr>
<td>C$_1^\alpha$</td>
<td>3035(5)</td>
<td>2599(12)</td>
<td>5583(8)</td>
<td>2.78(12)</td>
</tr>
<tr>
<td>C$_2^\alpha$</td>
<td>3718(6)</td>
<td>2046(16)</td>
<td>6683(9)</td>
<td>4.39(14)</td>
</tr>
<tr>
<td>C$_3^\alpha$</td>
<td>4178(6)</td>
<td>945(12)</td>
<td>5367(12)</td>
<td>3.85(10)</td>
</tr>
<tr>
<td>C$_3^\gamma$</td>
<td>4249(10)</td>
<td>2907(16)</td>
<td>5722(13)</td>
<td>6.12(10)</td>
</tr>
<tr>
<td>C$_4^\gamma$</td>
<td>4014(6)</td>
<td>2477(12)</td>
<td>3369(12)</td>
<td>4.49(8)</td>
</tr>
<tr>
<td>C$_5$</td>
<td>2704(5)</td>
<td>4948(12)</td>
<td>6254(10)</td>
<td>2.91(6)</td>
</tr>
<tr>
<td>O$_1$</td>
<td>2853(4)</td>
<td>6865(12)</td>
<td>5399(9)</td>
<td>3.97(8)</td>
</tr>
<tr>
<td>N$_2$</td>
<td>2325(4)</td>
<td>4631(10)</td>
<td>8058(8)</td>
<td>3.23(10)</td>
</tr>
<tr>
<td>C$_6$</td>
<td>2085(5)</td>
<td>6738(12)</td>
<td>9113(6)</td>
<td>3.39(8)</td>
</tr>
<tr>
<td>C$_7$</td>
<td>2687(4)</td>
<td>2266(12)</td>
<td>185(6)</td>
<td>2.15(10)</td>
</tr>
<tr>
<td>O$_2$</td>
<td>2530(3)</td>
<td>403(8)</td>
<td>588(8)</td>
<td>3.44(8)</td>
</tr>
<tr>
<td>O$_3$</td>
<td>3238(3)</td>
<td>7254(10)</td>
<td>601(10)</td>
<td>3.55(10)</td>
</tr>
<tr>
<td>C$_8^\delta$</td>
<td>1606(5)</td>
<td>5962(7)</td>
<td>830(8)</td>
<td>3.94(6)</td>
</tr>
<tr>
<td>C$_9^\delta$</td>
<td>955(6)</td>
<td>42(7)</td>
<td>734(8)</td>
<td>6.34(10)</td>
</tr>
<tr>
<td>S</td>
<td>352(2)</td>
<td>438(1)</td>
<td>2050(7)</td>
<td>8.36(17)</td>
</tr>
<tr>
<td>C$_{10}^\delta$</td>
<td>768(8)</td>
<td>2128(10)</td>
<td>3685(7)</td>
<td>9.25(18)</td>
</tr>
<tr>
<td>O$_{w}$</td>
<td>5328(6)</td>
<td>2208(8)</td>
<td>9979(4)</td>
<td>5.03(16)</td>
</tr>
</tbody>
</table>

TABLE 1

The positional parameters (10$^4$) with e.s.d. in parenthesis and $B_{eq}$

...
RESULTS AND DISCUSSION

It was evident in the early stages of refinement that the C' atom of the pyrrolidine ring was disordered. There were deviations from the expected geometry of the pyrrolidine ring and large thermal parameters of C'. A difference synthesis excluding C' showed two maxima consistent with the pyrrolidine ring. With the occupancy factors as 0.6 and 0.4 for the two positions, the structure was refined. The R factor at the present stage of refinement with anisotropic temperature factor for all atoms (without hydrogens) was 0.088. The quantity minimise was \( \sum \omega(|F_o| - K|F_c|)^2 \) where \( \omega \) is the weight factor obtained from the counting statistics. The fractional coordinates and \( B_{eq} \) for the atoms are listed in table 1. (The \( F_o \) and \( F_c \) tables are available from the authors).

\[
\begin{array}{cccc}
\text{N}_1-C_1^e & 1.482 & C_6-O_2 & 1.338 \\
C_2^p-C_2^b & 1.547 & N_2-C_6 & 1.481 \\
C_2^f-C_2^r & 1.488 & C_6-C_7 & 1.558 \\
C_2^g-C_3^f & 1.402 & C_2-O_2 & 1.219 \\
C_3^r-C_3^b & 1.547 & C_7-O_3 & 1.239 \\
C_3^d-C_4^d & 1.565 & C_6-C_8^a & 1.543 \\
C_4^e-N_1 & 1.478 & C_8^a-C_8^e & 1.502 \\
C_5^p-C_5^e & 1.512 & C_8^e-S & 1.800 \\
C_5-O_1 & 1.226 & S-C_10^g & 1.778 \\
N_1-C_1^e-C_2^b & 103.5 & N_2-C_6-C_7 & 109.8 \\
C_1-C_2^p-C_3^b & 106.5 & C_6-C_7-O_2 & 118.3 \\
C_1-C_2^f-C_3^r & 104.8 & C_6-C_7-O_3 & 114.3 \\
C_1-C_2^g-C_3^f & 102.7 & N_2-C_6-C_8^a & 112.9 \\
C_2-C_1-C_2 & 105.6 & C_6-C_8^a-C_8^e & 114.8 \\
C_2-C_3^r-N_1 & 105.1 & C_8^e-C_8^e-C_8^d & 111.9 \\
C_3^d-C_3^e-N_1 & 101.3 & C_6-S-C_10^f & 101.3 \\
C_4^e-N_1-C_1^e & 108.8 & C_8^e-C_6-O_1 & 118.8 \\
N_1-C_1^e-C_5 & 105.9 & C_6-N_2-C_6 & 121.4 \\
\end{array}
\]

Table 2

| Bond lengths (\(\AA\)) and angles (\(\degree\)). The range of the e.s.d.'s are 0.006-0.010 \(\AA\) in bond distances and 0.2-0.6° in angles (for all data) |

Hydrogen-bond distances (A)

\[
\begin{array}{ccc}
\text{N}_1 & \ldots & \text{O}_3^{(x+1)} & 2.908 \\
\text{N}_1 & \ldots & \text{O}_3^{(y+1)} & 2.666 \\
\text{N}_2 & \ldots & \text{O}_2^{(x-1)} & 2.851 \\
\end{array}
\]

(The atom with superscript II is at \(x, 1/2+y, z\) and those with \((y-1), (z-1)\) and \((z+1)\) are in adjacent cells)

The molecular packing is illustrated in figure 1. The molecules linked by hydrogen bonds, form a spiral round the screw axis. Bond distances and bond angles are given in table 2. The lengths \(C_2^p-C_2^r\), 1.488 and \(C_2^f-C_3^r\), 1.402 (mean 1.445), \(C_3^p-C_4^d\), 1.547 and \(C_5^p-C_4^d\)

\[
\begin{array}{cccc}
\text{C}_1^e-N_1-C_1^e-C_5 & \phi^1 & 125.6 \\
N_1-C_2^p-C_5-N_2 & \psi_1 & 166.3 \\
N_1-C_2^f-C_5-O_1 & \psi_2 & -15.4 \\
C_1^e-C_5-N_2-C_6 & \omega & 168.3 \\
C_6-N_2-C_6-C_7 & \phi_1 & -70.5 \\
C_5-N_2-C_6-C_7 & \phi_2 & 171.8 \\
N_1-C_6-C_7-O_2 & \psi_3 & -28.9 \\
N_1-C_6-O_2-O_3 & \psi_3' & 160.5 \\
N_2-C_6-C_6-C_9^r & \chi_1 & -62.7 \\
C_6-C_6-C_6-C_9^r & \chi_2 & -168.4 \\
C_6-C_6-C_6-C_10^d & \chi_3 & -71.8 \\
\end{array}
\]

e.s.d. 0.2-0.6°

Table 3

Torsion angles (°)

Figure 1. Molecular packing viewed down b-axis. Circle around \(C_3^p\) indicates disordered atom. Broken line is the hydrogen bonding from water molecule.
### Table 4

Comparison of torsion angles (°) in methionine side chain

<table>
<thead>
<tr>
<th>Crystal</th>
<th>N₂-Cα-Cβ-Cγ</th>
<th>Cα-Cβ-Cε-S</th>
<th>Cβ-Cε-S-S-Cδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-methionine⁷ molecule A</td>
<td>-166.1</td>
<td>174.2</td>
<td>179.7°</td>
</tr>
<tr>
<td>L-methionine⁷ molecule B</td>
<td>-165.6</td>
<td>73.6</td>
<td>73.6</td>
</tr>
<tr>
<td>D-alanyl-L-methionine⁹</td>
<td>67.3</td>
<td>175.0</td>
<td>-173.8</td>
</tr>
<tr>
<td>L-methionyl-L-methionine</td>
<td>-167.2</td>
<td>-168.3</td>
<td>-178.4</td>
</tr>
<tr>
<td>first residue⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-methionyl-L-methionine</td>
<td>68.0</td>
<td>-170.5</td>
<td>-57.6</td>
</tr>
<tr>
<td>second residue⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-prolyl-L-methionine</td>
<td>-62.7</td>
<td>-168.4</td>
<td>-71.8</td>
</tr>
<tr>
<td>(this work)</td>
<td>χ₁</td>
<td>χ₂</td>
<td>χ₃</td>
</tr>
</tbody>
</table>

1.565 (mean 1.556), and C₂-O₃, 1.478Å in the pyrroldine ring differ from the expected values, but these deviations appear to have no influence on the Cα-Cβ-Cϵ and Cβ-Cα-Cγ angles. The disordered pyrrolidine ring exhibits two conformations which occur in the ratio 3:2. In the first conformation C₂ and C₃ are on the opposite side of the N Cα-Cβ-Cδ plane (equation 0.1769 x + 0.9660 y + 0.1883 z = 3.1656 = 0) and deviate from it by 1.22 and -0.59Å. In the second they are on the same side and C₃ deviates by 0.56 Å. The distance between C₃ and C₄ is 1.103Å. A similar behaviour of C₂ in proline residue has been reported in DL-proline manganese dibromide⁴.

The torsion angles are listed in table 3. Although ψ₁ is -70.5°, the other two torsion angles ψ₁(165.3°) and ω (168.3°) indicate that the main peptide chain is in extended conformation. The peptide group is significantly non-planar with a torsion angle ω of 168.3°. The negative value of N₁-Cα-Cß-O₁ (-15.4°) indicates that prolyl-methionine belongs to α-helix type of proline compounds whereas collagen type prolines⁶ exhibit positive ψ₂ values in the range +150 to 180°.

Methionine residue: The bond lengths and bond angles of the methionine residue of the present work are in reasonable agreement with the reported values⁷⁹. The temperature factors for the sulphur and the terminal methyl carbon are large (table 1), but this is not unusual. The torsion angles for the residue show some variations. In table 4 the torsion angles for the methionine side chain from several methionine peptides are listed. The side chain conformation according to minimum energy calculations⁶ should have χ₁, χ₂, χ₃ values of 60, 180, 60° or -60, 180, -60°. None of the side chains observed experimentally so far exhibited either of these conformations. However prolyl methionine has the torsion angles -62.7 – 168.4, -71.8°, indicating that prolyl-methionine favours the theoretical predictions. The conformation is gauche-trans-gauche.

The hydrogen bonds are listed in table 2. Water molecule acts both as a donor and acceptor. The other relevant groups in the molecule (peptide and amino nitrogens) utilize their hydrogen bonding potential.

Further refinement (with hydrogen atoms included) is in progress and will be reported in due course.

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6. Hospital, M., Courseille, C., Leproy, F., and Roques, B. P., Biopolymers, 1979, 19, 1147.