

included in Table 2, which gives the detailed geometry of the hydrogen bonding.

The hydrogen bonding is shown in the packing drawing of Fig. 2. Each molecule is linked by a very weak hydrogen bond (half of a bifurcated bond) from its O(5) hydroxyl to the O(4) hydroxyl oxygen of the translation-equivalent molecule in the adjacent cell along *c*, resulting in H-bonded molecular chains along *c*. The other branch of the bifurcated bond is intramolecular, between the O(5) hydroxyl and the carbonyl oxygen, O(1). Each of the H-bonded chains is further connected by O(4)—H(4)···O(5) H-bonds to the equivalent chain related to it by a 2<sub>1</sub> screw axis parallel to *c*. The result is the ribbon structure noted in the *Abstract*.

The computer programs used in this study and not specifically referenced are the same as those used for the corresponding calculations of Lisensky, Johnson & Levy (1976).

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## Conformation of cyclo(-L-Leu-L-Phe-Gly-D-Leu-D-Phe-Gly-) Dihydrate

BY M. BILAYET HOSSAIN AND DICK VAN DER HELM

*Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019, USA*

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**Abstract.** C<sub>34</sub>H<sub>46</sub>N<sub>6</sub>O<sub>6</sub>·2H<sub>2</sub>O, triclinic, *P* $\bar{1}$ , *a* = 8.599 (3), *b* = 16.825 (9), *c* = 6.136 (2) Å,  $\alpha$  = 92.38 (3),  $\beta$  = 95.85 (3),  $\gamma$  = 87.14 (4)°, *V* = 881.4 Å<sup>3</sup> at 138 ± 2 K and *a* = 8.701 (3), *b* = 16.948 (7), *c* = 6.172 (9) Å,  $\alpha$  = 93.65 (7),  $\beta$  = 96.29 (7),  $\gamma$  = 85.78 (3)°, *V* = 900.8 Å<sup>3</sup> at 295 K, *Z* = 1, *D<sub>x</sub>* = 1.236, *D<sub>m</sub>* = 1.241 Mg m<sup>-3</sup>. The molecule possesses a center of symmetry and contains two equivalent intramolecular NH···O hydrogen bonds (3.04 Å). The structure was obtained from 3639 reflections measured at 138 ± 2 K using Cu *K* $\bar{\alpha}$  radiation and refined to a final *R* factor of 0.068 for 2366 reflections used in the least-squares calculation (0.112 for all reflections).

**Introduction.** cyclo(-L-Leu-L-Phe-Gly-D-Leu-D-Phe-Gly-) is one of the series of cyclo-isomeric hexapeptides containing two residues of glycine, two of leucine and two of phenylalanine, synthesized by Bláha (1972). The peptide is a *meso* form and possesses a potential center of symmetry. The structure investigation was undertaken as part of our overall program to study the conformational features of cyclic peptides and the stereochemistry of cyclic isomers.

Crystals of the compound were obtained by evaporation from methanol solution at room temperature. The thin plate-shaped crystals showed large mosaic spreads ranging from 2 to 4°. A crystal 0.35 × 0.13 × 0.04 mm and with a mosaic spread of about 2° was selected for all measurements.

Photographic investigations showed the crystal to be triclinic. The intensity statistics:  $|\bar{E}| = 0.848$  and  $|\bar{E}^2 - 1| = 0.894$ , were not conclusive, but an *N*(*z*) test clearly indicated the centrosymmetric space group *P* $\bar{1}$ . Refinement in *P*1 yielded no improvement in the *R* value and only a 0.5% decrease in the value for  $\sum W||kF_o| - |F_c||^2$  over the refinement in *P* $\bar{1}$  and, therefore, all final results were evaluated according to the latter space group. The unit-cell parameters and intensity data were obtained at 138 K with a CAD-4 automatic diffractometer controlled by a PDP8/e computer and fitted with a low-temperature apparatus. The cell parameters were determined by a least-squares refinement of +2 $\theta$  and -2 $\theta$  values of 23 reflections measured with Cu *K* $\alpha_1$  ( $\lambda = 1.54051$  Å) radiation.

Intensities of all independent reflections with 2 $\theta$  ≤ 150° were measured employing the  $\theta$ -2 $\theta$  scan tech-

nique with variable scan rates and using Cu  $K\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ). The  $\omega$  scans of reflections showed a width of about  $2.5^\circ$ , while  $\theta-2\theta$  scans gave a width of  $1.0^\circ$ . The scan width of each reflection was, therefore, taken as  $(1.0 + 0.15 \tan \theta)^\circ$  and the receiving-aperture width as  $(5.7 + 0.86 \tan \theta) \text{ mm}$ . The tip of the beam tunnel attached to the detector was removed during the data collection in order to allow for the rather large mosaic spread of the crystal. The maximum scan time for a reflection was 90 s. For each reflection  $\frac{2}{3}$  of the time was spent scanning the peak ( $P$ ) and  $\frac{1}{3}$  of the time spent scanning each of the left and right backgrounds (LB and RB). The unscaled intensity was calculated as  $I = [P - 2(LB + RB)]$ . The intensity of the reflection  $3\bar{2}1$  was monitored after every 50 measurements and its net count did not alter significantly over the period of data collection (4 days). Three orientation-control reflections were checked after every 200 measurements and, in case of any angular change larger than  $0.1^\circ$ , a new orientation matrix was obtained from a list of 15 reflections. In all, 3639 reflections were measured, out of which 1231 were considered indistinguishable from background [ $I < 2\sigma(I)$ ]. Intensities were scaled by the use of the standard reflection, and Lorentz and polarization corrections were applied. Each structure amplitude was assigned a weight,  $w_F$ , obtained from counting statistics (Ealick & van der Helm, 1975).

The structure was determined by direct methods (Karle & Karle, 1966) using the program *MULTAN*

(Germain, Main & Woolfson, 1971) and was refined isotropically to an  $R$  factor [ $R = \sum (|kF_o| - |F_c|) / \sum |kF_o|$ ] of 0.140. The position of the water molecule was obtained from a difference Fourier map. Further refinements were carried out with anisotropic thermal parameters. All H atoms were located from a difference Fourier map and H positions were refined isotropically. The refinement was terminated when the maximum parameter shifts for non-hydrogen atoms were less than  $\frac{1}{3}$  of their corresponding standard deviations. The final  $R$  factor for 2366 reflections included in the least-squares calculations is 0.068, and is 0.112 for all reflections. The rather high discrepancy factor may be attributed to the mosaic spread of the crystal.

In all refinements, a block-diagonal least-squares program (Ahmed, 1966) was used in which the quantity  $\sum W_F (|kF_o| - |F_c|)^2$  was minimized. The scattering factors for O, N and C atoms were taken from *International Tables for X-ray Crystallography* (1974) and those for H atoms were taken from Stewart, Davidson & Simpson (1965).

The final positional parameters of all non-hydrogen atoms in the asymmetric unit are given in Table 1.\* The atoms are labelled following the convention of the IUPAC-IUB Commission on Biochemical Nomenclature (1970), and the complete labelling scheme is shown in Fig. 1. The parameter table contains only the atoms belonging to the L-leucine, the L-phenylalanine and one glycine residue (numbered 1, 2 and 3 respectively). The atom positions of the residues 4 (D-leucyl), 5 (D-phenylalanyl) and 6 (glycyl) can be obtained by inversion across  $(1, \frac{1}{2}, 0)$ .

Table 1. Positional parameters ( $\times 10^4$ ) for non-hydrogen atoms in an asymmetric unit

Standard deviations of last digits are in parentheses.

	x	y	z
O(1)	8721 (2)	7206 (1)	4605 (3)
O(2)	5998 (2)	4673 (1)	2943 (3)
O(3)	8974 (2)	4062 (1)	-1113 (3)
O(w)	7186 (2)	5497 (1)	-2106 (3)
N(1)	9838 (3)	6952 (1)	-837 (4)
N(2)	7536 (3)	6513 (1)	1700 (4)
N(3)	8507 (3)	4973 (1)	2723 (4)
C(1a)	9507 (3)	7455 (2)	1079 (5)
C(1)	8563 (3)	7041 (2)	2618 (5)
C(1 $\beta$ )	8608 (4)	8212 (2)	201 (5)
C(1 $\gamma$ )	7974 (4)	8793 (2)	1927 (6)
C(1 $\delta$ 1)	7082 (4)	9486 (2)	731 (7)
C(1 $\delta$ 2)	9227 (5)	9094 (2)	3586 (7)
C(2a)	6568 (3)	6067 (2)	2990 (5)
C(2)	6988 (3)	5181 (2)	2835 (5)
C(2 $\beta$ )	4821 (3)	6259 (2)	2375 (5)
C(2 $\gamma$ )	4399 (3)	7131 (2)	2731 (5)
C(2 $\delta$ 1)	3800 (4)	7596 (2)	985 (6)
C(2 $\epsilon$ 1)	3405 (4)	8404 (2)	1317 (6)
C(2 $\zeta$ )	3617 (4)	8750 (2)	3376 (7)
C(2 $\epsilon$ 2)	4215 (5)	8306 (2)	5124 (7)
C(2 $\delta$ 2)	4598 (4)	7495 (2)	4778 (6)
C(3a)	9099 (3)	4151 (2)	2857 (5)
C(3)	9374 (3)	3760 (2)	668 (5)

**Discussion.** The bond lengths and bond angles involving non-hydrogen atoms are shown in a schematic drawing of the peptide molecule in Fig. 1. Within experimental error, all peptide units are dimensionally

\* Lists of structure factors, anisotropic temperature factors and H-atom parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34600 (17 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

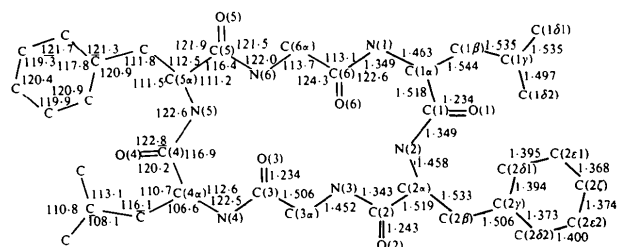


Fig. 1. Atom-labelling scheme along with bond lengths ( $\text{\AA}$ ) and bond angles ( $^\circ$ ). Estimated standard deviations of bond lengths range from 0.003 to 0.005  $\text{\AA}$  and those of angles range from 0.2 to 0.4 $^\circ$ .

equivalent. The average bond distances [ $N(i)-C(ia) = 1.458(6) \text{ \AA}$ ,  $C(i)-N(i+1) = 1.347(3) \text{ \AA}$ ,  $C(i)-O(i) = 1.237(4) \text{ \AA}$ ,  $C(ia)-C(i) = 1.514(6) \text{ \AA}$ ]\* and average bond angles compare well with those observed in other cyclic hexapeptides (Karle, Gibson & Karle, 1970; Hossain & van der Helm, 1978). The dimensions of the two side chains are normal with few exceptions. The average C-C distance in the phenyl ring of  $1.384 \text{ \AA}$  is slightly shorter than normal, while the bond length  $C(1\gamma)-C(1\delta_2)$  of  $1.497(5) \text{ \AA}$  ( $1.513 \text{ \AA}$  when corrected for a riding thermal motion) in the leucyl side chain is considerably shorter than the normally expected value. The last difference Fourier map, however, is essentially featureless in this region, with electron densities between  $0.0$  and  $0.2 \text{ e \AA}^{-3}$ , and gave no indication of disorder.

A stereoview of the molecule is shown in Fig. 2. Conformational angles of the molecular backbone are listed in Table 2. All the peptide units in the molecule are in the *trans* conformation with  $\omega$  values close to  $180^\circ$ . The molecule contains two centrosymmetrically related  $\beta$ -turns [ $\beta(I)$  turn with (L-Leu, L-Phe) at corners and  $\beta(I')$  turn with (D-Leu, D-Phe) at corners].† The conformational angles [ $\mp 58.9(3)$ ,  $\mp 32.0(3)^\circ$ ] and [ $\mp 115.1(3)$ ,  $\pm 35.6(3)^\circ$ ] for the  $\beta$ -turns are quite normal and fall within the calculated allowed regions (Venkatachalam, 1968). The  $\beta$ -turns are stabilized by two equivalent intramolecular  $NH \cdots O$  hydrogen bonds ( $N \cdots O$  distance of  $3.04 \text{ \AA}$ ). These bonds connect amide and carbonyl groups of the two glycyl residues. The 18-membered peptide ring is further stabilized by hydrogen bonding to water molecules (Fig. 2). Both phenylalanyl and leucyl side chains have extended conformations [torsion angles:  $N(1)-C(1\alpha)-C(1\beta)-C(1\gamma) 174.0(3)^\circ$ , and  $C(2)-C(2\alpha)-C(2\beta)-C(2\gamma) 175.0(3)^\circ$ ]. The phenyl ring is perfectly planar (r.m.s. deviation  $0.002 \text{ \AA}$ ) and it makes a dihedral

\* Figures within parentheses indicate r.m.s. deviations.

†  $\beta$ -turn designations are as defined by Hossain & van der Helm (1978).

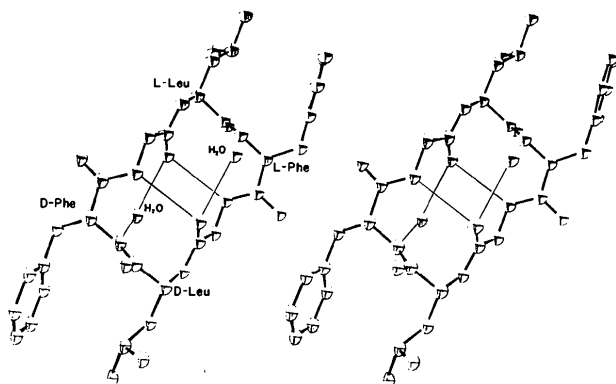


Fig. 2. Stereoview of a single molecule (Johnson, 1965). Hydrogen bonds are indicated by thin lines.

Table 2. Conformational angles ( $^\circ$ )

Standard deviations of the last digit are in parentheses. For  $\chi_C$ ,  $\chi_N$  and  $\tau'$ , the figures within parentheses are r.m.s. deviations.

$\phi$ ,  $\psi$ ,  $\omega$  are defined by IUPAC-IUB Commission on Biochemical Nomenclature (1970).  $\chi_C$ ,  $\chi_N$  are defined by Winkler & Dunitz (1971).  $\tau' = (\omega_1 + \omega_2)$ , mod  $2\pi$ . Angles for residues and units 4, 5 and 6 are equal in magnitude and opposite in sign to those of 1, 2 and 3 respectively.

	Residues		
	1	2	3
$\phi$	$-58.9(3)$	$-115.1(3)$	$+101.5(3)$
$\psi$	$-32.0(3)$	$+35.6(3)$	$+169.6(2)$
$\omega$	$+179.3(2)$	$+171.8(2)$	$+177.0(2)$
	Peptide unit		
	1-2	2-3	3-1'
$\chi_C$	$+1.9(3)$	$-4.3(3)$	$+2.2(3)$
$\chi_N$	$+4.4(12)$	$-0.5(14)$	$-6.4(17)$
$\tau'$	$+1.1(12)$	$-12.6(14)$	$-14.6(16)$

angle of  $61^\circ$  with the mean plane of the 18-membered peptide backbone. Adjacent peptide units are nearly perpendicular to each other (the dihedral angles between the planes through adjacent peptide units vary from  $69$  to  $85^\circ$ ). The conformational angles which are listed in Table 2 indicate small out-of-plane bending both at  $C(j)$  and  $N(j)$  atoms compared to those observed in the case of *cyclo(-L-Ala-L-Ala-Gly-Gly-L-Ala-Gly-)* or *cyclo(-L-Ala-L-Ala-Gly-L-Ala-Gly-Gly-)* (Hossain & van der Helm, 1978). It is interesting to note that for peptide unit 2,  $\chi_C$  is greater than  $\chi_N$ , indicating larger out-of-plane bending at atom  $C(j)$  than at atom  $N(j)$ , a situation contrary to that generally observed in peptides.

Table 3 contains the description of the hydrogen bonding in the structure. The hydrogen-bonding scheme uses all CO and NH groups of the peptide molecule and involves the  $H_2O$  molecule which forms three hydrogen bonds. Of the five unique hydrogen bonds, only one (bond B) directly connects peptide units in neighboring molecules. The packing diagram in Fig. 3 shows the contents of one unit cell along with the

Table 3. Hydrogen bonds

Bond	D	A	D-A	D-H	A-H	D-H-A
			(s.d.)	(s.d.)	(s.d.)	(s.d.)
			$0.003 \text{ \AA}$	$0.03 \text{ \AA}$	$0.03 \text{ \AA}$	$3^\circ$
A	N(3)	O(6 <sup>i</sup> )	$3.04 \text{ \AA}$	$0.90 \text{ \AA}$	$2.19 \text{ \AA}$	$157^\circ$
B	N(1)	O(1 <sup>ii</sup> )	2.90	0.97	1.97	163
C	O(W)	O(3 <sup>i</sup> )	2.85	0.99	1.87	169
D	O(W)	O(2 <sup>iii</sup> )	2.76	0.93	1.84	169
E	N(2)	O(W <sup>1</sup> )	2.84	0.98	1.99	144

Symmetry code

- (i)  $x, y, z$
- (ii)  $x, y, z - 1$
- (iii)  $1 - x, 1 - y, -z$

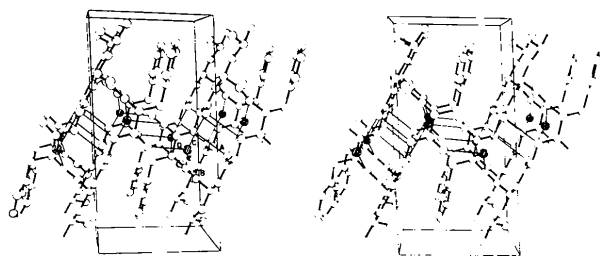


Fig. 3. Stereoview of the contents of one unit cell. Hydrogen bonds are indicated by thin lines. Water molecules are indicated by dashed circles. Directions of axes are  $a \rightarrow$ ,  $b \uparrow$ .

essential features of the hydrogen bonding. The plane of the peptide backbone lies nearly perpendicular to the  $c$  axis, while the long axis of the molecule extends along the diagonal of the  $ab$  face of the unit cell. The center of the molecule coincides with the crystallographic center of symmetry at  $(1, \frac{1}{2}, 0)$ . Molecules are stacked together along the  $c$  axis by strong hydrogen bonds (bond  $B$ ) between amide and carbonyl groups of leucyl residues belonging to two neighboring molecules. Neighboring molecules along the  $a$  axis are hydrogen bonded *via* water molecules (bond  $D$ ). There are no intermolecular linkages along the  $b$  axis.

In an earlier communication (Hossain & van der Helm, 1978), we reported the similarity in the intramolecular hydrogen-bonding scheme and conformational features of the cyclopeptides Gly<sub>4</sub>(D-Ala)<sub>2</sub> and L-Ala-L-Ala-Gly-L-Ala-Gly-Gly. The present molecule bears a noticeable resemblance to these two molecules. The conformational angles along with the intramolecular hydrogen-bond distances for the three molecules are illustrated schematically in Fig. 4. Although there are individual differences in conformational angles, the overall conformational features are remarkably similar, despite the fact that the three molecules contain quite different amino acid residues. It seems that, irrespective of amino acid contents, cyclic hexapeptides with internal water (water molecules involved in intramolecular linkages) have the preferred conformation with two  $\beta$ -turns [one  $\beta(1)$  and one  $\beta(1')$ ] and a centrosymmetric or pseudo-centrosymmetric backbone geometry containing two intramolecular NH...O hydrogen bonds. It may be mentioned that this conformation is similar to that of the most prevalent conformer of cyclohexakis(glycyl) (Karle & Karle, 1963) which does not contain any intramolecular water.

Another common feature in cyclic hexapeptides is the fact that the amino acid residues with larger side chains occupy the corners of the  $\beta$ -turns, and glycines, or amino acids with small side groups, form the extended chain. The structures of cyclo(-L-Ala-L-Pro-D-Phe)<sub>2</sub> (Brown & Teller, 1976) and cyclo(-Gly-Pro-D-Phe)<sub>2</sub> (Brown & Yang, 1979) are further examples of this observation.

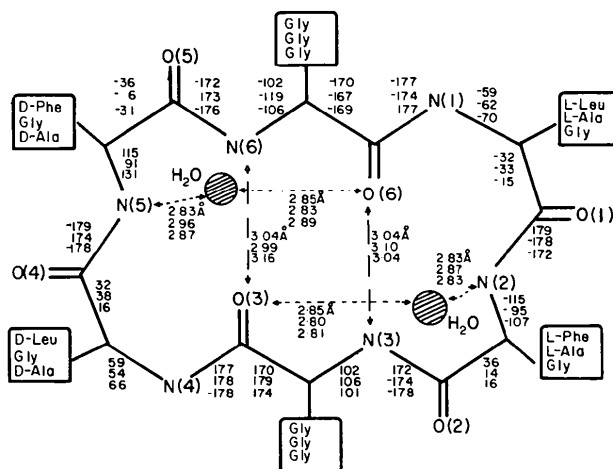


Fig. 4. Conformational angles in the 18-membered peptide ring. Upper values are for the present structure; middle values for cyclo(-L-Ala-L-Ala-Gly-L-Ala-Gly-Gly).2H<sub>2</sub>O; and lower values for cyclo[-Gly<sub>4</sub>(D-Ala)<sub>2</sub>].3H<sub>2</sub>O (Karle, Gibson & Karle, 1970).

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