Crystal and Molecular Structure of *cyclo*-L-Aspartyl-L-alanyl (3,6-Dioxo-5-methyl-2-piperazineacetic acid)

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The crystal and molecular structure of the cyclic dipeptide *cyclo*-L-aspartyl-L-alanyl (*c*-L-Asp-L-Ala) has been determined at 120 K using 1677 reflections with $I > 2.5\sigma I$. The space group is $P2_1$, with cell parameters $a = 7.573(3)$, $b = 8.650(2)$, $c = 6.151(1)$ Å and $\beta = 95.88(3)°$; final R-factor 0.032. The diketopiperazine (DKP) ring is essentially planar, with a slight tendency towards a boat conformation with both substituents quasi-axial. The aspartyl side-chain is folded above the DKP ring.

As part of a project on linear peptides, the cyclic dipeptide *cyclo*-L-Asp-L-Ala was crystallized. It belongs to a group of compounds which exhibit several interesting features, such as the geometry of the diketopiperazine (DKP) ring[1] and side-chain conformations. Numerous cyclic dipeptides have been investigated by X-ray diffraction but almost all have contained an aromatic or a proline residue. In order to obtain a better understanding of the factors affecting the geometry of such molecules, the crystal structure of the title compound has been determined.

Experimental

Large crystals of *c*-L-Asp-L-Ala were grown from an aqueous solution of L-aspartyl-L-alanine. The data collection procedure is summarized in Table 1. Cell parameters were determined by least-squares fit to the diffractometer settings for 25 general reflections. Standard deviations in the measured intensities were calulated as $\sigma I = [C_T + (0.02C_N)^2]^{1/2}$, where C_T is the total number of counts and C_N is the scan count minus the background count. The intensities were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved directly by MULTAN,[2] and all heavy atoms were refined isotropically by least-squares methods.

Table 1. Data collection.

Instrument	Nicolet P3
Radiation	Graphite Crystal Monochromated Mo$K\alpha$
Scanning mode	$\theta/2\theta$
Scan speed/° min^{-1}	3.0
Scan range/°	$2\theta_{\alpha 1} - 0.9$ to $2\theta_{\alpha 2} + 1.0$
Background count	For 35 % of scan time at scan limits
Temperature/K	120
2θ range/°	6.0–70.0
Crystal dimensions/mm	0.30×0.30×0.20
No. of refl. measured	1945
No. of unique refl. $I > 2.5\sigma I$	1677

The positions of all the hydrogen atoms were then obtained from a difference Fourier synthesis. All positonal parameters, anisotropic temperature factors for O, N and C, and isotropic temperature factors for H were refined by least-squares methods giving $R = 0.032$ and $R_W = 0.036$, with goodness of fit $S = [\Sigma w\Delta^2/(m-n)]^{1/2} = 2.08$. The final parameters are given in Table 2. Atomic scattering factors were taken from Ref. 3.

Crystal data

3,6-Dioxo-5-methyl-2-piperazineacetic acid (*cyclo*-L-Asp-L-Ala), $C_7H_{10}N_2O_4$; monoclinic, $a =$

Table 2. Fractional coordinates for *c*-L-Asp-L-Ala with standard deviations and equivalent isotropic temperature factors, B_{eq}, for non-hydrogen atoms.

Atom	*x*	*y*	*z*	B_{eq}/Å^2
OD1	0.3235(2)	0.7132(2)	0.9144(2)	2.0
OD2	0.4328(2)	0.7310(2)	0.5919(2)	1.7
O1	0.7290(1)	0.5000	0.4249(2)	1.3
O2	0.9164(1)	0.8249(2)	1.1584(2)	1.4
N1	0.7748(2)	0.6197(2)	0.9936(2)	1.1
N2	0.8618(2)	0.7082(2)	0.5889(2)	1.1
CA1	0.7093(2)	0.5236(2)	0.8079(2)	0.9
CB1	0.5059(2)	0.5058(2)	0.8006(2)	1.0
CG1	0.4102(2)	0.6594(2)	0.7776(2)	1.1
C1	0.7677(2)	0.5799(2)	0.5936(2)	0.9
CA2	0.9081(2)	0.8155(2)	0.7705(2)	1.0
CB2	0.8200(2)	0.9723(2)	0.7222(2)	1.4
C2	0.8652(2)	0.7520(2)	0.9891(2)	1.0
HOD2	0.383(4)	0.813(4)	0.581(4)	
HN1	0.747(3)	0.590(3)	1.123(3)	
HN2	0.887(2)	0.734(3)	0.458(3)	
HCA1	0.753(3)	0.422(3)	0.828(3)	
HC11	0.477(2)	0.462(3)	0.932(3)	
HC12	0.470(3)	0.436(3)	0.685(3)	
HCA2	1.037(2)	0.825(3)	0.783(3)	
HC21	0.850(3)	1.007(3)	0.585(3)	
HC22	0.850(3)	1.039(3)	0.829(4)	
HC23	0.697(3)	0.965(3)	0.709(3)	

7.573(3), b = 8.650(2), c = 6.151(1) Å, β = 95.88(3)°, V = 400.8(2) Å^3, M = 186.15, Z = 2, $F_{(000)}$ = 196, space group $P2_1$, D_C = 1.542 g cm^{-3}.

Description and discussion

An ORTEP[4] drawing of the molecule is shown in Fig. 1 with torsion angles indicated. Bond lengths and bond angles for heavy atoms are given in Fig. 2, which also depicts the geometry of the hydrogen bonds. The C–H distances vary between 0.88 and 0.97 Å (mean 0.94 Å) with e.s.d. 0.02 Å.

In the geometry of the DKP ring only small differences are observed compared with other cyclic dipeptides; however, the rather long C–O bonds and the short C1–N2 bond are noteworthy. The general agreement between this structure and that of the linear peptide L-Ala-L-Asp,[5] as reflected by bond angles and bond lengths, is rather poor. In particular, all the internal bond angles of the DKP ring are substantially larger (1.6–6.4°) than the corresponding angles in the linear peptide, and the C–O bonds are systematically longer in the cyclic structure.

The geometry of the aspartyl side-chain resembles that of unionized aspartyl residues in linear peptides.[5–8] The Cα–Cβ bond (1.541 Å) is significantly longer than in L-Ala-L-Asp (1.510 Å); in *c*-L-His-L-Asp[9] (zwitterion) this bond length is 1.530 Å.

An analysis of the thermal parameters for the heavy atoms showed that the central DKP ring could be treated as a rigid body. This resulted in a systematic increase in bond lengths which was less than the e.s.d., reflecting the low amplitude of thermal vibrations at 120 K. Refinement of the

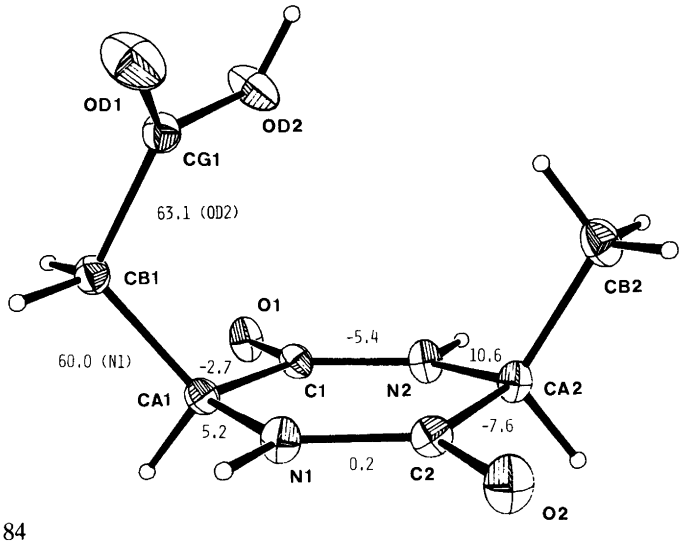

Fig. 1. ORTEP drawing of the molecule with numbering indicated. Ring and side-chain torsion angles are given with e.s.d.'s of 0.2°.

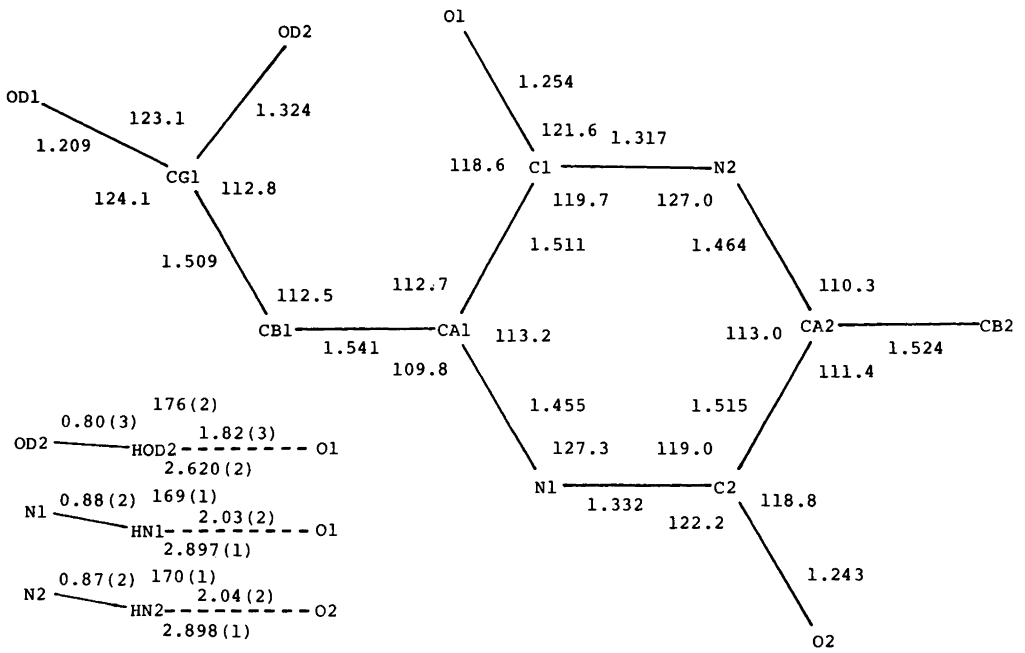

Fig. 2. Hydrogen bond geometry (e.s.d.'s indicated) and bond lengths and bond angles between heavy atoms (e.s.d.'s 0.02 Å and 0.1°, respectively).

data using 954 reflections with $\sin\theta/\lambda > 0.595$ ($2\theta > 50°$, $R = 0.029$, $R_W = 0.029$, $S = 1.19$) also failed to produce any significant shifts in bond lengths and bond angles: OD1–CG1 increased from 1.209 to 1.215 Å and C1–N2–CA2 decreased from 127.0 to 126.7°, the remaining shifts being $\leqq 0.003$ Å and 0.2°, respectively.

The DKP ring is fairly planar, with only a slight out-of-plane puckering for the two Cα-atoms. Data for least-squares planes through the DKP ring are given in Table 3. The relevant torsion angles are (in °) $\varphi_1 = 5.2$, $\psi_1 = -2.7$, $\omega_1 = -5.4$, $\varphi_2 = 10.6$, $\psi_2 = -7.6$ and $\omega_2 = -0.2$ (see also Fig. 1). The dihedral angle between two peptide planes is 172.9°. This gives a very flattened "flagpole boat" form, and in this context both substituents are quasi-axial. This is consistent with the geometry observed in most other crystal structures of cyclic dipeptides. Only in *c*-L-Ala-L-Ala[10] are both substituents clearly quasi-equatorial, as they are in all cyclic dipeptides containing a proline residue[11,12] which forces the ring into a non-planar conformation requiring a quasi-equatorial orientation of the β-carbons.

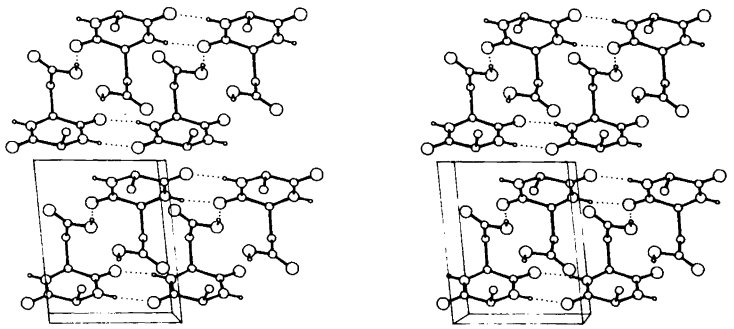

Fig. 3. Molecular packing of *c*-L-Asp-L-Ala in the crystal viewed along the *b* axis. Hydrogen bonds are dotted.

Table 3. Displacements (Å) from least-squares planes in the DKP ring. Atoms marked with an asterix are not included in the least-squares plane calculation.

N1	−0.025	N1	−0.010
N2	−0.043	N2	−0.010
C1	−0.003	C1	0.011
C2	−0.023	C2	0.011
CA1	0.044	CA1*	0.047
CA2	0.062	CA2*	0.108
O1*	−0.042	O1*	−0.036
O2*	−0.096	O2*	−0.052

Cyclic dipeptides containing an aromatic side-chain almost inevitably adopt a folded conformation with the aromatic ring facing the DKP ring. Other side-chains may also fold in this manner, as does the L-Asp residue in the present structure ($\chi_1^1 = 60.0°$). In *c*-L-His-L-Asp the χ_2^1 torsion angle is −70°, since in this case the L-His side-chain is folded above the ring and the carboxyl group is bent away. A similar arrangement is found in *c*-L-Leu-L-His,[13] with $\chi_1^1 = -56.1°$. In *c*-L-Ser-L-Ser,[14] however, both substituents are folded above the DKP ring with torsion angles χ_1^1 and χ_2^1 53.2 and 54.6°, respectively. In other cyclic dipeptides with L-Ser residues,[15,16] χ^1 is also close to + gauche, even when an aromatic ring is folded above the DKP ring.

It thus appears that a conformation with $\chi^1 \approx 60°$ is preferred for all kinds of side-chains. When it is not feasible to have both side-chains of a cyclic dipeptide in a folded conformation due to steric hindrance, an aromatic ring, if present, will normally occupy the favoured position above the DKP ring.

The unit cell contents and the crystal packing are shown in Fig. 3. In crystal stuctures of non-hydrated cyclic dipeptides,[1,10,17] a hydrogen-bonding system in which the DKP rings are hydrogen-bonded to two others to form ribbons is commonly observed. Hydrogen bonds to crystal water can add to this pattern,[9,13] or a completely different hydrogen-bonding system may result.[15] The molecules in the present structure form the regular ribbons via the DKP rings along the *c*-axis, with the L-Asp carboxyl group involved in a third bond giving a two-dimensional hydrogen bond network. The geometry of these three bonds is shown in Fig. 2. The resulting structure is compact, with a calculated density of 1.542 g cm^{-3} (at 120 K). This is exceeded in this group of compounds only by the density of the DKP structure itself,[1] viz. 1.592 g cm^{-3}.

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