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ORIGINAL PAPER

Refined Crystal Structure and Absolute Configuration of the Di-amino Acid Peptide Cyclo(L-Aspartyl-L-Aspartyl): Comparison with the DFT Calculated Structure

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Abstract The X-ray crystal structure of the di-amino acid peptide cyclo(L-Asp-L-Asp), $C_6H_{10}N_2O_4$, has been re-determined at 20 °C using CuKα radiation, $\lambda = 1.54180$ Å. The crystals are triclinic P1 with unit cell dimensions $a = 5.0829(3)$, $b = 5.0285(4)$, $c = 18.8765(10)$ Å, $\alpha = 88.95(2)°$, $\beta = 83.72(2)°$, $\gamma = 74.79(2)°$, unit cell volume 462.75(5) Å^3, and $Z = 2$ independent molecules A and B per asymmetric unit. Final R indices [$I > 2$sigma(I)] are R1 = 0.0492, wR2 = 0.1039 for 2,540 independent reflections; R1 = 0.0686 and wR2 = 0.1112 for all 3,193 data; Goodness of Fit, $S = 0.979$, and the Flack x parameter = 0.1(3). In both molecules the overall shape of the diketopiperazine (DKP) ring displays an almost identical slightly distorted boat conformation with pseudo symmetry C_{2v} (mm2). The two side chains of the cyclic peptide on opposite sides of both molecules differ in their conformations, one side being extended and the other coiled. The coiled chains are located away from the DKP ring plane while the extended chains lie approximately parallel to it. The crystal packing employs two strong hydrogen bonds, which traverse the entire crystal *via* translational repeats. The geometry of cyclo(L-Asp-L-Asp) derived from Ab initio calculations is compared with those of molecules A and B derived from the X-ray structure reported here. In this calculated model the DKP ring is in a pseudo twist boat conformation; both side chains are extended and lie approximately parallel to the DKP ring face as opposed to molecules A and B in the X-ray structure in each of which one side chain is approximately parallel and the other is folded away from the DKP ring face.

Keywords Cyclic dipeptide · Crystal structure · Absolute configuration · Ring geometry

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Introduction

Cyclic di-amino acid peptides also known as dioxopiperazines, piperazine-2,5-diones or diketopiperazines (DKPs) are amongst the "simplest" peptide derivatives commonly found in nature [1]. DKPs and their derivatives continue to be of long-standing interdisciplinary scientific interest with respect to potential pharmaceutical applications. Specific cyclic di-amino acid peptides exhibit antibiotic [2], inhibition of tumour growth and metastasis [3], and cardiovascular activity [4]. Many DKPs have emerged as by-products of fermentation and food processing [5, 6]. In addition many have also been found to occur naturally in certain groups of the plant and animal kingdom [7, 8]. The synthesis of DKPs and their analogues is a very active contemporary research area [9, 10]. DKPs may occur as by-products during peptide synthesis or during the degradation of peptides [11]. In fact DKPs are valuable chiral synthons, employed for example in Schöllkopf's versatile bislactim ether approach [12] and have found use as catalysts for enantioselective synthesis, for example in the asymmetric Strecker reaction [13]. Interestingly DKPs are being examined as model systems for studying self -assembly processes because they can give

rise to a variety of structures including entangled and elongated aggregates such as rods, ribbons, helices and tubules [14]. Cyclo(L-Asp-L-Asp) is an example of a cyclic di-amino acid peptide, which has a six membered ring, and the amide linkage adopts a *cis* conformation [15]. In contrast linear (L-Asp-L-Asp) is zwitterionic and has a single amide function which adopts the *trans* conformation.

X-Ray structures for a number of symmetrically substituted DKPs have been previously reported showing (near) planar ring conformations of cyclo(Gly-Gly) [16] and cyclo(L-Ser-L-Ser) [17]. A number of boat (ring) conformations have also been established, for example cyclo(L-Met-L-Met) [18]. Other spectroscopic techniques such as NMR [19, 20] and CD [21] have also been used to determine the structure and conformation of DKPs in the solution state, and microwave spectroscopy has been used to investigate the boat conformation of cyclo(Gly-Gly) [22] in the gaseous state. Theoretical spectroscopic investigations have also played a part in structural analysis [23, 24]. Copper II complexes of cyclo(L-Asp-L-Asp) have been reported, with the aim of obtaining a good model for the enzyme superoxide dismutase, which acts as an anti-oxidant. Anti-oxidant activity was measured by spectroscopic and voltammetric techniques [25]. In addition it has been reported that DKP tetrapeptides based on cyclo(L-Asp-L-Asp) can form microcapsules in an acidic environment [26]. The synthesis [26] and X-ray structure of cyclo (L-Asp-L-Asp) [27] have previously been reported. Tayhas et al. [28] also discuss the engineering of hydrogen bonded layers ('tapes') in mixed crystal structures by co-crystallization. For the purposes of the present study, requiring precise molecular geometry, it was decided to carry out a re-determination of the crystal structure [27] using a more complete measured set of independent intensities (94% against 36%) and correspondingly improved data/parameter ratio (3193/326 against 1215/369).

Ab Initio Calculations

Ab initio calculations for cyclo(L-Asp-L-Asp) have been reported [29] using the *Gaussian 98* program [30], under the Linux environment on a PC workstation using the hybrid SCF-DFT method B3-LYP, which incorporates Becke's three parameter hybrid functional [31] and the Lee, Yang and Parr correlation functional [32]. These calculations were performed using the cc-pVDZ basis set [33] and geometry optimisation was conducted assuming C_2 symmetry. The geometry of this calculated form of cyclo(L-Asp-L-Asp) is compared with those of molecules A and B derived from the X-ray structure reported here.

Experimental

Materials

Cyclo(L-Asp-L-Asp) was obtained from Bachem Ltd (Saffron Walden, Essex, UK) and used without further purification. The physico-chemical properties of the compound were in accord with those reported in the scientific literature.

X-Ray Crystallography

Crystals were grown by extremely slow evaporation from a methanol/ethanol (1:1) solution, initially at 4 °C for 3 weeks, followed by further evaporation for 2 days at room temperature. A suitable crystal $0.23 \times 0.32 \times 0.45$ mm^3 was mounted on an Enraf–Nonius CAD-4 automated 4-circle diffractometer equipped with a graphite monochromator. X-Ray intensity measurements were recorded at room temperature employing CuKα radiation, $\lambda = 1.54180$ Å. CAD-4 Software [34] was used for cell determination and refinement and data reduction. Accurate cell parameters were determined from 25 reflections ($25 < \theta < 28$ deg.). For data collection ω-2θ scans were used under computer control. Intensities of 3,550 reflections were measured for $\theta < 74.17°$.

The crystals are triclinic, P1, with unit cell: $a = 5.0829(3)$ Å, $b = 5.0285(4)$ Å, $c = 18.8765(10)$ Å, $\alpha = 88.95(2)°$, $\beta = 83.72(2)°$, and $\gamma = 74.79(2)°$. The unit cell volume = 462.75(5) Å^3, with $Z = 2$ molecules per asymmetric unit (i.e. symmetry independent) with a corresponding density (calculated) =1.652 mg/m^3 and absorption coefficient 1.252 mm^{-1}. A total of 3,193 independent reflections were measured (94.1% completeness) with R(int) = 0.0326. The crystal showed no significant variations in the intensities of three check reflections during the course of data collection. Lorenz and polarization corrections were made and an empirical correction for absorption effects was also applied [35]. The structure was solved by Direct Methods using the program SHELXS-96 [36, 37] and refined using SHELXL-97 [37, 38] both implemented in the WinGX system of programs [39]. Non hydrogen atoms were refined anisotropically by full-matrix least squares methods. Hydrogens were added either using peaks in difference electron density maps or geometrically using the program algorithms, and refined positionally with riding isotropic temperature factors. Geometrical calculations were made with the programs PARST and PLATON [40] as implemented in WinGX. The program MERCURY 1.4.1 [41] was used to prepare Fig. 1a; ORTEP [42] and RASTER3D [43], as implemented in WinGX, were used to prepare Figs. 2b, c, 3b, c and 4; RASMOL [44] was used to produce Figs. 1b, 2a and 3a. In the final refinement cycles there were 3,193 data to 349 parameters resulting in a Goodness-of-fit on F^2 of 0.991 and final R indices [$I > 2$sigma(I)] of R1 = 0.0496,

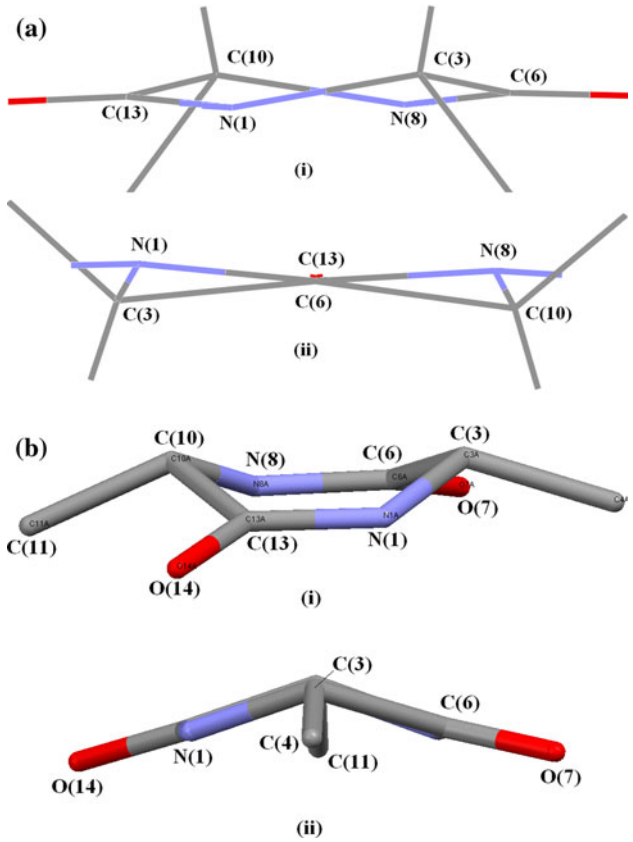

Fig. 1 **a** DKP ring in the calculated model [29] was conducted assuming $C_2(2)$ symmetry and is in fact in a twist boat conformation with pseudo $D_2(222)$ symmetry. The two twofold symmetry axes of the twist boat ring are shown (1) perpendicular to the N(1)–C(3) bond and (2) in the perpendicular direction along O(7)…O(14). The twist boat ring description refers only to the shape of the ring and ignores the different atom types of the ring atoms and substituents. Drawn with MERCURY [41]. **b** The DKP ring in both crystal structure molecules A and B is in an almost identical boat conformation with pseudo symmetry C_{2v} (mm2). The two *m* symmetries of the boat are shown: (1) perpendicular to the N(1)–C(13) bond and (2) in the perpendicular direction along C(3)…C(10). These ring descriptions refer only to the shape of the ring and ignore different atom types of the ring atoms and substituents. Drawn with RASMOL [44]

wR2 = 0.1061. The absolute structure parameter =0.1(3) [45] indicating the correct choice of chirality, and the largest and smallest difference electron density regions were 0.193 and −0.248 e. $Å^{-3}$, respectively. Crystal data are summarized in Table 1. Hydrogen bond geometry in Table 2 corresponds to an overall hydrogen bonded motif formed in a layer pattern. The carboxylic acid groups hydrogen bond to form tapes, and additionally the *cis* amide groups also hydrogen bond to form layers. Figure 4 shows a hydrogen bonded A, B pair of molecules.

X-Ray Structure

Figure 5 shows the chemical scheme and atom numbering. Figure 2a shows the calculated least energy boat conformation of cyclo(L-Asp -L-Asp) [29] and Fig. 2b and c are ORTEP/RASTER [42, 43] views of molecules A and B in the crystal with 50% probability thermal ellipsoids. The three views have been selected for ease of comparison of the geometry of the three molecules and show both similarities and differences as discussed below.

Molecular Geometry

Bond lengths in the crystal structure are determined approximately to ±0.006Å, bond angles to ±0.4° and torsion angles to ±0.6°. Both bond lengths and bond angles compare well with those found in organic compounds [46] exhibit no unusual values, and exhibit no significant differences between molecules A and B. The presence of two symmetry independent molecules in the asymmetric unit of a crystal structure provides an ideal opportunity to look for variations in the molecular geometry. This is extended here to include the calculated geometry of the molecule. Table 3 presents details of the three molecular geometries. It is set out for ease of comparison between the molecules. Data for the side *X* side and *Y* side chains, Fig. 5, are given in progression towards the ring i.e. starting at the atoms furthest from the ring. Data for the ring are given starting at N(1) going clockwise.

Side Chain Bond Lengths

In terms of side chain bond lengths the bond O(24)–C(18) (labelled # in Table 3) in the *Y* side chain, in both molecules A and B, is *slightly shorter* (∼0.05Å) than the corresponding bond in the *X* side chains, O(22)–C(15) (also labelled # in Table 3) in both molecules A and B, and in both calculated side chains. No other differences in bond length are noteworthy.

Side Chain Bond Angles

In terms of bond angles, in the *X* side chain C(4)–C(3)–C(6) (labeled * in Table 3) is about 5 degrees *less* in the calculated geometry than in the observed molecules A and B. Similar but less significant differences are observed for the *corresponding bond angle* in the *Y* side chain C(11)–C(10–C(13) (also labeled * in Table 3), although it is not obvious as to why there should be a reduction in the calculated bond angles in this region. There are no other noteworthy differences in bond angles.

Side Chains: Conformations and Dispositions with Respect to the DKP Ring

Torsion angles in the calculated model (column 1) and in Molecules A and B (columns 2 and 3) are listed in Table 3.

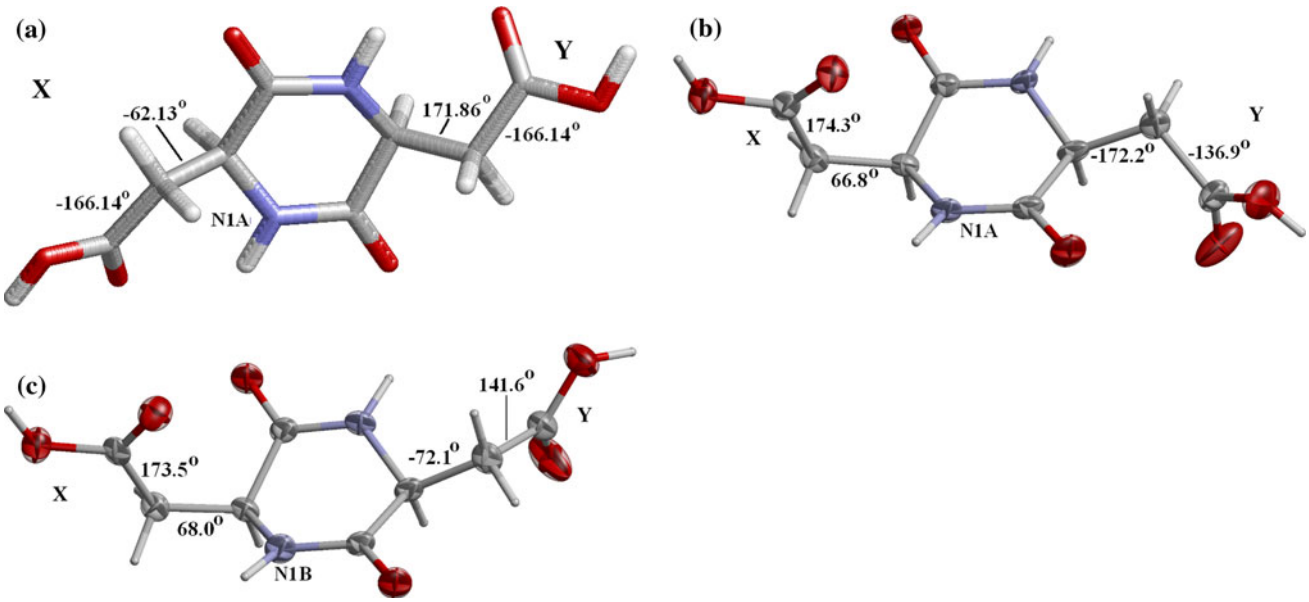

Fig. 2 Cyclo (L-Asp-L-Asp): **a** Gaussian calculated least energy twist boat model drawn with RASMOL [43]; **b** and **c**, ORTEP/RASTER [42, 43] views of molecules A and B respectively in the crystal structure showing 50% thermal motion ellipsoids. The views of the molecules are selected to aid the detailed comparison given in the text

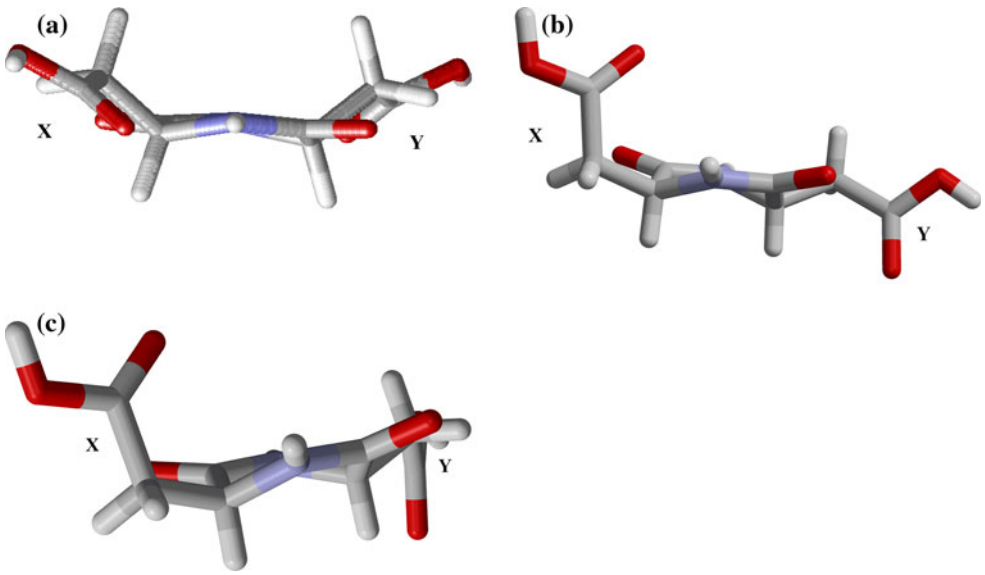

Fig. 3 **a** Gaussian calculated model of cyclo (L-Asp-L-Asp) viewed along N(1)…N(8). Drawn with RASMOL[44]. Both the *X* and the *Y* side chains (Fig. 5) are quite extended and lie approximately parallel to the DKP ring face. (This is opposed to molecules A and B in the X-ray structure in which one side chain is approximately parallel and the other is folded beneath the DKP ring face). **b** Molecule A: Viewed down N(1)…N(8). Drawn with ORTEP/RASTER [42, 43]. The *X* and *Y* side chains (Fig. 5) are labelled. The *X* side chain is extended to a similar extent to that in the calculated model (Fig. 3a) but in the opposite sense and is folded beneath the DKP ring. The *Y* side chain is in a less extended conformation and lies approximately parallel to the face of the ring. **c** Molecule B: Viewed down N(1)…N(8). Drawn with ORTEP/RASTER [42, 43]. The *X* and *Y* side chains (Fig. 5) are labelled. The *X* side chain is extended to a similar extent to that in the calculated model (Fig. 3a) but in the opposite sense and is folded beneath the DKP ring. The *Y* chain is partially coiled but remains predominantly parallel to the face of the ring as in the calculated model (see Table 3 and Fig. 3c)

The conformation of side chain *X* (Fig. 5) is defined by the torsion angle τ_1, and its disposition with respect to the DKP ring by the torsion angle τ_3.

Torsion angles in the *X* side chain correspond well for molecules A and B. About the linkage bond C(4)–C(3) (labelled % in Table 3) the calculated side chain rotates in

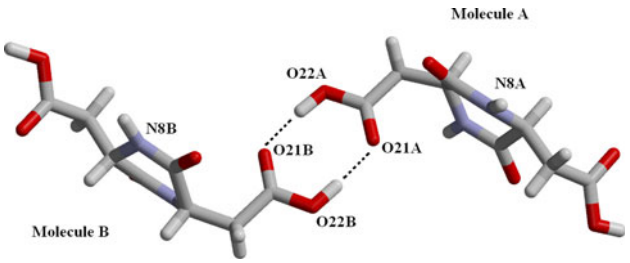

Fig. 4 Hydrogen bonded pair of molecules A and B. Drawn with ORTEP/RASTER [42, 43]

Table 1 Crystal data and structure refinement for cyclo(L-Asp-L-Asp)

Identification code	Cyclo(L-Asp-L-Asp)
Empirical formula	$C_8\ H_{10}\ N_2\ O_6$
Formula weight	230.18
Temperature	20 °C
Wavelength	1.54180 Å
Crystal system	Triclinic
Space group	P1
Unit cell dimensions	$a = 5.0829(3)$ Å $\alpha = 88.95(2)°$
	$b = 5.0285(4)$ Å $\beta = 83.72(2)°$
	$c = 18.8765(10)$ Å $\gamma = 74.79(2)°$
Volume	462.75(5) Å^3
Z	2 molecules per asymmetric unit
Density (calculated)	1.652 mg/m^3
Absorption coefficient	1.252 mm^{-1}
$F(000)$	240
Crystal size	0.23 × 0.32 × 0.45 mm^3
Theta range for data collection	7.08–74.17°.
Index ranges	$-6 \le h \le 6$, $-6 \le k \le 6$, $-23 \le l \le 23$
Reflections collected	3,550
Independent reflections	3,193 [R(int) = 0.0326]
Completeness to theta = 74.17°	94.1%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3193/3/326
Goodness-of-fit on F^2	0.979
Final R indices [$I > 2$sigma(I)]	R1 = 0.0492, wR2 = 0.1039
R indices (all data)	R1 = 0.0686, wR2 = 0.1111
Absolute structure parameter	0.1(3)
Largest diff. peak and hole	0.216 and −0.254 e.Å^{-3}

Table 2 Hydrogen bonds for cylo(L-Asp-L-Asp) [Å and °]

D–H…A	d(D–H)	d(H…A)	d(D…A)	<(DHA)
O(24A)–H(26A)… O(23B)#1	0.82	1.89	2.673(5)	158.2
N(1A)–H(2A)…O(7A)#2	0.86(7)	2.04(7)	2.873(4)	162(6)
O(22A)–H(25A)…O(21B)	0.96(8)	1.79(8)	2.744(5)	169(7)
N(8A)–H(9A)…O(14A)#3	0.87(6)	2.02(6)	2.873(5)	166(5)
O(24B)–H(26B)… O(23A)#4	1.00(7)	1.71(7)	2.646(5)	155(6)
N(1B)–H(2B)…O(7B)#2	0.87(7)	2.07(7)	2.893(5)	157(6)
O(22B)–H(25B)…O(21A)	0.95(6)	1.83(6)	2.774(5)	171(5)
N(8B)–H(9B)…O(14B)#3	0.98(6)	1.89(6)	2.863(4)	169(5)

Symmetry transformations used to generate equivalent atoms: #1 x + 1,y−1,z−1 #2 x + 1,y−1,z #3 x−1,y + 1,z #4 x−1, y + 1,z + 1

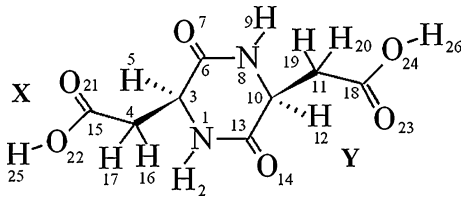

Fig. 5 Cyclo (L-Asp-L-Asp): Chemical scheme and atom numbering. For reference purposes the two side chains of the molecule are labeled *X* [C(15) side] and *Y* [C(18) side]

the opposite direction to that in both molecules A and B ($\sim -60°$ calculated as opposed to $\sim +60°$ observed) and is therefore coiled away from the diketopiperazine ring as shown in Fig. 3a. The imposed two-fold symmetry of the calculated model results in the values of the torsion angles τ_1 to τ_4 in chain *X* corresponding with those of τ_5 to τ_8 in chain *Y*, as can be seen in Table 3 column 1. The value of $\tau_1 = \tau_5 = 166.41°$ in the calculated model corresponds to an extended *X* and *Y* chain conformation, and $\tau_3 = \tau_7 = -62.23°$ indicates that the *X* and *Y* chains are turned away from the DKP ring surface in the same sense, Fig. 3a. In the experimental molecules A and B τ_1 to τ_4 in chain *X* have similar values (columns 2 and 3), with τ_1 corresponding to an extended conformation in both. The effect of the coiled conformation of τ_3 on the disposition of the *X* side chain in both molecules A and B can be seen in Fig. 3b and c.

The conformation of side chain *Y* (Fig. 5) is defined by the torsion angle τ_5, and its disposition with respect to the DKP ring by the torsion angle τ_7 (Table 3). The *Y* side chain has the same extended conformation in the calculated molecule and in molecule A, but is coiled in molecule B. The side chain orientations can be seen in Fig. 3a, b, and c. The distribution of torsion angles τ_5 to τ_8 in chain *Y* of molecules A and B are markedly different (Table 3 columns 2 and 3) both from each other and from the values for chain *X* as described above. In molecule A the overall conformation of chain *Y* is more extended than in molecule B, which is partially coiled. These differences can be seen by inspection of Fig. 3b and c.

Table 3 Comparison of calculated and experimental geometry for cyclo(L-Asp-L-Asp)

	Calculated	Experimental (esd's in parentheses)	
		Molecule A	Molecule B
Bond lengths			
Side chain *X* (Fig. 5)			
r(O22–H25)	0.9757	0.96(8)	0.95(6)
r(O22–C15)	1.3471	1.324(5)	1.324(5)
r(C15–O21)	1.2153	1.207(5)	1.216(5)
r(C4–C15)	1.5099	1.495(6)	1.499(6)
r(C4–H17)	1.0989	0.97	0.97
r(H16–C4)	1.1041	0.97	0.97
r(C4–C3)	1.5339	1.511(6)	1.510(6)
Side chain *Y* (Fig. 5)			
r(O24–H26)	0.9757	0.82	1.00(7)
r(O24–C18)	1.3471	1.295(6)	1.299(5)
r(C18–O23)	1.2153	1.227(6)	1.212(6)
r(C11–C18)	1.5099	1.508(6)	1.501(6)
r(C11–H20)	1.0989	0.97	0.97
r(H19–C11)	1.1041	0.97	0.97
r(C11–C10)	1.5339	1.513(6)	1.516(6)
Ring atoms			
r(N1–C3)	1.4566	1.460(5)	1.462(5)
r(N1–H2)+	1.0194	0.86(7)	0.87(7)
r(C3–C6)	1.533	1.515(5)	1.518(5)
r(C3–H5)	1.1077	0.98	0.98
r(C6–N8)	1.3583	1.332(6)	1.329(6)
r(C6–O7)	1.2235	1.241(5)	1.289(5)
r(N8–C10)	1.4566	1.456(5)	1.465(5)
r(N8–H9)+	1.0194	0.87(6)	0.98(6)
r(C10–C13)	1.533	1.525(5)	1.513(5)
r(C10–H12)	1.1077	0.98(2)	0.98(2)
r(C13–N1)	1.3583	1.325(6)	1.331(6)
r(C13–O4)	1.2235	1.245(5)	1.251(5)
Bond angles			
Side chain *X* (Fig. 5)			
θ(H25–O22–C15)	106.3	116(5)	113(4)
θ(O22–C15–C4)	111.92	112.2(4)	112.0(4)
θ(O21–C15–O22)	122.53	123.7(4)	123.8(4)
θ(C4–C15–O21)	125.54	124.1(4)	124.1(4)
θ(C15–C4–C3)	113.06	115.4(4)	115.2(4)
θ(C15–C4–H16)	107.72	108.4	108.5
θ(C15–C4–H17)	110.06	108.4	108.5
θ(H16–C4–C3)	110.18	108.4	108.5
θ(H17–C4–C3)	109.07	108.4	108.5
θ(H17–C4–H16)	106.55	107.5	107.5
θ(C4–C3–C6)*	108.96	113.8(3)	114.1(4)
θ(C4–C3–N1)	111.24	112.6(3)	112.6(3)
θ(H5–C3–C4)	107.56	106.3	106.4
Side chain *Y* (Fig. 5)			
θ(H26–O24–C18)	106.3	103(3)	118(3)
θ(O24–C18–C11)	111.92	115.4(4)	113.6(4)
θ(O24–C18–O23)	122.53	123.7(4)	123.8(4)
θ(O23–C18–C11)	125.54	121.0(4)	122.5(4)
θ(C18–C11–C10)	113.06	111.5(4)	112.1(4)
θ(C18–C11–H19)	107.72	115(3)	108(3)
θ(C18–C11–H20)	110.06	108(3)	106(3)
θ(H19–C11–C10)	110.18	106(3)	112(3)
θ(H20–C11–C10)	109.07	112(3)	108(3)
θ(H20–C11–H19)	106.55	106(4)	110(4)
θ(C11–C10–C13)*	108.96	110.1(4)	111.3(4)
θ(C11–C10–N8)	111.24	110.1(4)	109.9(4)
θ(C11–C10–H12)	107.56	110(3)	110(3)
Ring atoms			
θ(N1–C3–C6)	113.53	110.7(2)	110.3(4)
θ(N1–C3–H5)	109.69	102(3)	110(3)
θ(H5–C3–C6)	105.55	107(2)	102(2)
θ(C3–C6–N8)	116.74	116.7(3)	116.5(4)
θ(C3–C6–O7)	120.15	120.9(4)	120.4(4)
θ(O7–C6–N8)	123.11	122.4(4)	123.0(4)
θ(C6–N8–C10)	126.9	123.4(4)	123.0(3)
θ(H9–N8–C10)	115.05	111(3)	123(3)
θ(C6–N8–H9)	115.56	123(3)	111(3)
θ(N8–C10–C13)	113.53	109.5(3)	110.4(3)
θ(N8–C10–H12)	109.69	111(3)	110(3)
θ(H12–C10–C13)	105.55	106(2)	105(3)
θ(C10–C13–N1)	116.74	116.7(4)	116.1(4)
θ(C10–C13–O14)	120.15	119.7(4)	121.2(4)
θ(O14–C13–N1)	123.11	123.6(4)	122.6(4)
θ(C13–N1–C3)	126.9	123.4(3)	123.3(4)
θ(C13–N1–H2)	115.56	120(3)	121(3)
θ(H2–N1–C3)	115.05	116(4)	116(3)
Torsion angles			
Side chain *X* (Fig. 5)			
τ(H25–O22–C15–O21)	0.95	1(3)	−2(3)
τ(H25–O22–C15–C4)	−178.1	−179(3)	178(3)
τ(O21–C15–C4–H16)	−107.14	−129(3)	−133(3)
τ(O21–C15–C4–H17)	137.08	116(3)	116(3)
τ(O21–C15–C4–C3)	14.84	−5.9(6)	−6.4(6)
τ(O22–C15–C4–H16)	71.88	51(3)	47(3)
τ(O22–C15–C4–H17)	−43.9	−64(3)	−64(3)
τ(O22–C15–C4–C3)*	−166.14	174.3(3)	173.5(3)
τ(C15–C4–C3–N1)%*	−62.23	66.8(5)	68.0(5)
τ(C15–C4–C3–C6)	171.86	−60.3(5)	−58.8(5)
τ(C15–C4–C3–H5)	57.91	−174(2)	−171(3)

Table 3 continued

	Calculated	Experimental (esd's in parentheses)	
		Molecule A	Molecule B
τ(H16–C4–C3–N1)	58.36	−171(3)	−168(3)
τ(H17–C4–C3–C6)	49.07	176(3)	176(3)
τ(H16–C4–C3–H5)	178.5	−51(4)	−47(4)
τ(H17–C4–C3–N1)	174.98	−57(3)	−57(3)
τ(H16–C4–C3–C6)	−67.55	62(3)	65(3)
τ(H17–C4–C3–H5)	−64.88	−62(4)	64(4)
Side chain *Y* (Fig. 5)			
τ(H26–O24–C18–O23)	0.95	13(3)	−16(3)
τ(H26–O24–C18–C11)	−178.1	−165(3)	162(3)\
τ(O23–C18–C11–H19)	−107.14	165(3)	−165(3)
τ(O23–C18–C11–H20)	137.08	−78(3)	77(3)
τ(O23–C18–C11–C10)x	14.84	45.3(6)	−40.5(6)
τ(O24–C18–C11–H19)	71.88	−17(3)	17(3)
τ(O24–C18–C11–H20)	−43.9	100(3)	−101(3)
τ(O24–C18–C11–C10)*	−166.14	−136.9(4)	141.6(4)
τ(C18–C11–C10–N8)	−62.23	−172.2(4)	−72.1(5)
τ(C18–C11–C10–C13)*	171.86	66.8(5)	165.2(4)
τ(C18–C11–C10–H12)	57.91	−49(3)	49(3)
τ(H19–C11–C10–N8)	58.36	63(3)	50(3)
τ(H20–C11–C10–C13)	49.07	−172(3)	49(3)
τ(H19–C11–C10–H12)	178.5	−174(4)	171(4)
τ(H20–C11–C10–N8)	174.98	−52(3)	171(3)
τ(H19–C11–C10–C13)	−67.55	−58(3)	−73(3)
τ(H20–C11–C10–H12)	−64.88	71(4)	−68(4)
Ring atoms			
τ(C13–N1–C3–C6)	−22.67	−34.1(5)	−36.9(6)
τ(C13–N1–C3–H5)	95.13	85(3)	75(3)
τ(C13–N1–C3–C4)	−146	−162.9(4)	−165.7(4)
τ(H2–N1–C3–C6)	176.19	153(4)	146(4)
τ(H2–N1–C3–H5)	−66.01	−89(5)	−103(5)
τ(H2–N1–C3–C4)	52.86	25(4)	17(4)
τ(N1–C3–C6–N8)	11.81	30.3(5)	32.3(5)
τ(N1–C3–C6–O7)	−168.05	−151.8(4)	−151.3(4)
τ(H5–C3–C6–N8)	−108.36	−92(3)	−84(3)
τ(H5–C3–C6–O7)	71.78	86(3)	92(3)
τ(C4–C3–C6–N8)	136.38	158.5(4)	160.3(4)
τ(C4–C3–C6–O7)	−43.48	−23.6(6)	−23.2(6)
τ(C3–C6–N8–C10)	10.29	5.5(6)	4.1(6)
τ(C3–C6–N8–H9)	171.35	164(4)	167(3)
τ(O7–C6–N8–C10)	−169.86	172.3(4)	−172.3(4)
τ(O7–C6–N8–H9)	−8.8	−14(4)	−10(3)
τ(C6–N8–C10–C13)	−22.67	−37.9(6)	−37.4(6)
τ(C6–N8–C10–H12)	95.13	78(3)	78(3)
τ(C6–N8–C10–C11)	−146	−159.4(4)	−160.7(4)
τ(H9–N8–C10–C13)	176.19	161(4)	162(4)
τ(H9–N8–C10–H12)	−66.01	−82(5)	−82(5)

Table 3 continued

	Calculated	Experimental (esd's in parentheses)	
		Molecule A	Molecule B
τ(H9–N8–C10–C11)	52.86	40(4)	39(4)
τ(N8–C10–C13–N1)	11.81	33.8(5)	32.8(6)
τ(N8–C10–C13–O14)	−168.05	−147.5(4)	−148.1(4)
τ(H12–C10–C13–N1)	−108.36	−86(3)	−85(3)
τ(H12–C10–C13–O14)	71.78	93(3)	94(3)
τ(C11–C10–C13–N1)	136.38	154.9(4)	155.2(4)
τ(C11–C10–C13–O14)	−43.48	−26.4(6)	−25.6(6)
τ(C10–C13–N1–C3)	10.29	1.3(6)	3.6(6)
τ(C10–C13–N1–H2)	171.35	174(3)	−179(4)
τ(O14–C13–N1–C3)	−169.86	−177.3(4)	−175.5(4)
τ(O14–C13–N1–H2)	−8.8	−5(4)	2(4)

Bond distances (r) are quoted in Å, bond angles (θ) and torsion angles (τ) are in degrees

Key values are denote with *. The calculated conformation of side chain *X* is coiled, as are those of both molecules A and B in the crystal structure, which correspond within experimental error. The calculated conformation is coiled in the opposite direction. See Figs. 2 and 3

This side chain has markedly different conformations in experimental molecules A and B, being extended in A and coiled in B, Fig. 3b and c. In the calculated molecule it is also extended, Fig. 2a

In the two crystallographically independent molecules A and B and in the calculated conformation the diketopiperazine ring takes on the same boat conformation with corresponding atoms forming the boat. The calculated boat conformation is quantitatively less close to the ideal symmetry C_{2v} (mm2) than in the experimental conformations. The *m* planes are defined by O(7)…O(14) and N(1)…N(8)

Diketopiperazine Ring

The DKP ring in the calculated model [29] was assumed to have $C_2(2)$ symmetry and is in fact in a twist boat conformation with pseudo $D_2(222)$ symmetry [47]. The two twofold symmetry axes of the twist boat ring as shown in Fig. 1a are (1) perpendicular to the N(1)–C(3) bond and (2) in the perpendicular direction along O(7)…O(14). Note: the twist boat ring description refers only to the shape of the ring and ignores the different atom types of the ring atoms and its substituents.

The DKP rings in both crystal structure molecules A and B are very similar to each other and exhibit almost identical boat conformations with pseudo symmetry C_{2v} (mm2) [47]. This is a widely different conformation from that of the calculated model which is in a twist boat conformation. The two *m* symmetries of the boat are shown in Fig. 1b: (1) perpendicular to the N(1)–C(13) bond and (2) in the perpendicular direction along C(3)…C(10). These ring descriptions refer only to the shape of the ring and ignore different atom types of the ring atoms and its substituents. (Ladd, M.F.C. and Palmer, R.A. (2003) "Structure

Determination by X-ray Crystallography" 4th Edition (Klewer-Plenum, NY) Tables 7.24 and 7.25.)

Previous X-Ray Structure

A crystal structure for cyclo (L-Asp-L-Asp) was previously published by Tayhas et al. 1998 [27, 28] which is of much lower quality than that published here. The diffraction data employed in the present structure was measured to a maximum θ value of 74.17°, 0.8012 Å resolution comprising 3550 data; the previous data was cut off at $\theta = 57.14$ deg., 0.9177 Å resolution and comprising only 1,215 data. Use of such a restricted data set would be difficult to justify, would result in over refinement, and would be flagged with an A alert by contemporary validating checking software. Furthermore the previous structure determination has importantly failed to establish the absolute configuration of the molecule, which is determined unequivocally in the present work.

Supplementary Material

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 256275. Copies of available material can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: teched @ chemcrys.cam.ac.uk).

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