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The Crystal Structure and Conformation of the Cyclic Dipeptide *cyclo*(-L-Seryl-L-histidyl-) Monohydrate

BY MICHEL COTRAIT

Laboratoire de Cristallographie, associé au CNRS, Université de Bordeaux I, 351 *cours de la Libération,* 33405 *Talence, France*

AND MARIUS PTAK

Centre de Biophysique Moléculaire (CNRS) et Université d'Orléans, 45045 *Orléans CEDEX, France*

(*Received* 26 *April* 1977; *accepted* 8 *August* 1977)

$C_9H_{12}N_4O_3.H_2O$ is orthorhombic, space group $P2_12_12_1$ with $a = 8{\cdot}162$ (3), $b = 23{\cdot}191$ (7), $c = 5{\cdot}756$ (4) Å, $Z = 4$. The final $R = 0{\cdot}036$. The diketopiperazine ring is planar. The two side chains are folded above the central ring: $\chi_1^1 = 69{\cdot}8°$ for the seryl and $\chi_2^1 = 62{\cdot}2°$ for the histidyl residues. The conformation is different from that of *cyclo*(-L-threonyl-L-histidyl-) but looks more like the conformation of *cyclo*(-L-seryl-L-tyrosyl-). Such a doubly folded conformation was found to be the most stable from empirical calculations and, from NMR studies, is partly populated in aqueous solutions.

Introduction

As part of a study of short peptides with polar side chains, we present the structure of the cyclic dipeptide *cyclo*(-L-Ser-L-His-) monohydrate (Ser = seryl, His = histidyl). Recently, we determined the structure of *cyclo*(-L-Thr-L-His-) dihydrate (Thr = threonyl) (Cotrait, Ptak, Busetta & Heitz, 1976), which allows comparison of their conformations.

The seryl and histidyl residues associated with an aspartyl residue are functional in the active site of many serine proteases, *e.g.* α-chymotrypsin (Birktoft & Blow, 1972). The catalytic properties of some synthetic peptides (Kapoor, 1972) considered as model enzymes might also involve interactions between such residues. Cyclic dipeptides are used here as models to investigate the side-chain–side-chain interactions between two neighbouring residues. Our crystallographic studies were made in parallel with NMR studies in solution (Ptak, Heitz & Dreux, 1977) and empirical calculations of intramolecular energy (Genest & Ptak, 1976), in order to give a complete view of the conformational properties of short peptides containing functional polar residues.

Experimental

cyclo(-L-Ser-L-His-) was prepared by cyclization of *Z*-dipeptide methyl esters by catalytic hydrogenation. It crystallized in the neutral form from water–acetone solutions as thin colourless needles.

The cell constants were deduced from Eulerian angle measurements for 15 reflections made with a Siemens four-circle diffractometer. The calculated density is 1·476 g cm^{-3} if one water molecule per dipeptide molecule is assumed (formula $C_9H_{12}N_4O_3.H_2O$). This is appreciably higher than that for *cyclo*(-L-Thr-L-His-) dihydrate (1·32 g cm^{-3}). The intensities of 1150 reflections ($\theta < 70°$) were collected by θ–2θ scans and the five-point measurement technique with Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å). No absorption corrections were applied.

The structure was solved by direct methods with a program similar to that described by Germain, Main & Woolfson (1971). An E map with the set of phases having the highest figure of merit displayed most of the atoms. Missing atoms and the O atom of the water molecule were located by a Fourier synthesis. The structure was refined first with isotropic, then aniso-

Table 1. *Fractional atomic coordinates and standard deviations* ($\times 10^4$; *for* H $\times 10^3$)

	x	y	z
O_w	9388 (3)	2883 (1)	655 (3)
O_1^γ	1812 (2)	3866 (1)	6342 (3)
C_1^β	1706 (3)	3285 (1)	5642 (4)
C_1^α	3268 (3)	3092 (1)	4412 (4)
N_1	4630 (3)	3054 (1)	6052 (3)
C_2	6082 (3)	3313 (1)	5871 (5)
O_2	7171 (2)	3241 (1)	7323 (4)
C_2^α	6420 (3)	3683 (1)	3773 (4)
N_2	5048 (2)	3734 (1)	2157 (3)
C_1	3608 (3)	3487 (1)	2371 (4)
O_1	2478 (2)	3563 (1)	931 (3)
C_2^β	7043 (3)	4284 (1)	4529 (5)
C_2^γ	5840 (3)	4648 (1)	5807 (4)
$C_2^{\delta 2}$	5213 (3)	5160 (1)	5118 (5)
$N_2^{\varepsilon 2}$	4251 (3)	5355 (1)	6901 (4)
$C_2^{\varepsilon 1}$	4322 (3)	4956 (1)	8608 (5)
$N_2^{\delta 2}$	5274 (2)	4521 (1)	8037 (3)
H_1^γ	228	389	788
$H_1^{\beta 1}$	151	302	718
$H_1^{\beta 2}$	68	323	445
H_1^α	306	266	369
H_1	445	280	753
H_2^α	741	347	277
H_2	520	400	72
$H_2^{\beta 1}$	808	423	569
$H_2^{\beta 2}$	742	451	295
$H_2^{\delta 2}$	543	539	349
$H_2^{\varepsilon 2}$	358	574	694
$H_2^{\varepsilon 1}$	365	498	1025
H_1^w	1023	316	27
H_2^w	874	303	−63

Table 2. *Bond lengths* (Å) *and angles* (°) *with standard deviations*

O_1^γ–C_1^β	1·411 (3)	C_1–C_1^α	1·515 (3)
C_1^β–C_1^α	1·525 (4)	C_2^α–C_2^β	1·545 (3)
C_1^α–N_1	1·461 (4)	C_2^β–C_2^γ	1·490 (3)
N_1–C_2	1·332 (3)	C_2^γ–$C_2^{\delta 2}$	1·353 (3)
C_2–O_2	1·232 (3)	$C_2^{\delta 2}$–$N_2^{\varepsilon 2}$	1·369 (3)
C_2–C_2^α	1·508 (3)	$N_2^{\varepsilon 2}$–$C_2^{\varepsilon 1}$	1·351 (3)
C_2^α–N_2	1·461 (3)	$C_2^{\varepsilon 1}$–$N_2^{\delta 1}$	1·314 (3)
N_2–C_1	1·313 (3)	$N_2^{\delta 1}$–C_2^γ	1·395 (3)
C_1–O_1	1·253 (3)		
O_1^γ–C_1^β–C_1^α	111·2 (3)	N_2–C_1–C_1^α	120·1 (3)
C_1^β–C_1^α–N_1	110·7 (3)	O_1–C_1–C_1^α	117·6 (3)
C_1^α–N_1–C_2	126·8 (3)	C_1–C_1^α–N_1	113·4 (3)
N_1–C_2–O_2	121·8 (3)	C_1–C_1^α–C_1^β	109·7 (3)
N_1–C_2–C_2^α	118·8 (3)	C_2^α–C_2^β–C_2^γ	115·7 (3)
O_2–C_2–C_2^α	119·3 (3)	C_2^β–C_2^γ–$C_2^{\delta 2}$	127·0 (3)
C_2–C_2^α–N_2	114·5 (3)	C_2^β–C_2^γ–$N_2^{\delta 1}$	123·6 (3)
C_2–C_2^α–C_2^β	110·4 (3)	$C_2^{\delta 2}$–C_2^γ–$N_2^{\delta 1}$	109·2 (3)
N_2–C_2^α–C_2^β	111·0 (3)	C_2^γ–$C_2^{\delta 2}$–$N_2^{\varepsilon 2}$	106·7 (3)
C_2^α–N_2–C_1	126·2 (3)	$C_2^{\delta 2}$–$N_2^{\varepsilon 2}$–$C_2^{\varepsilon 1}$	107·1 (3)
N_2–C_1–O_1	122·3 (3)	$N_2^{\varepsilon 2}$–$C_2^{\varepsilon 1}$–$N_2^{\delta 1}$	111·7 (3)
		$C_2^{\varepsilon 1}$–$N_2^{\delta 1}$–C_2^γ	105·3 (3)

tropic thermal factors. The average thermal factor calculated by Wilson's (1949) method is 2·7 Å^2.

The H atoms were located by a difference synthesis. However, C–H, N–H and O–H distances were fixed at 1·09, 1·04 and 0·96 Å respectively, according to neutron diffraction data, and refinement was resumed only with C, N and O atoms and B_i factors of H atoms. Further refinement led to a final $R = 0{\cdot}036$.* Atomic parameters are listed in Table 1; partial projections of the structure along x and z are shown in Fig. 1.

Results

Distances and angles

Bond lengths and angles not involving H atoms are listed in Table 2. Fig. 2 shows the standard labelling of atoms and the torsion angles. In *cyclo*(-L-Ser-L-His-) (I), only small differences are observed in the geometry of the diketopiperazine (DKP) ring compared with other cyclic dipeptides, *e.g. cyclo*(-L-Thr-L-His-) (II), *cyclo*(-Gly-L-Tyr-) (III) and *cyclo*(-L-Ser-L-Tyr-) (IV) (Lin & Webb, 1973). Distances and angles do not significantly differ from those usually found for unprotonated histidyl residues.

Molecular conformation

The conformation is defined by two sets of φ, ψ and ω angles for the DKP ring and by the χ_1^1 (L-Ser), χ_2^1 and $\chi_2^{2,1}$ (L-His) angles for the side chains (Fig. 2). This conformation viewed as a projection on the DKP mean plane (equation: $-0{\cdot}3392X + 0{\cdot}7898Y + 0{\cdot}5111Z = 6{\cdot}0910$) is shown in Fig. 3. The DKP ring is nearly planar; deviations (Å) are C_1^α −0·035, N_1 0·000, C_2 0·019, O_2 0·013, C_2^α −0·013, N_2 −0·015, C_1 −0·007, O_1 0·022.

Torsional angles for the DKP ring take the following values: φ_1 −1·8°, ψ_1 +3·0°, ω_1 −1·7°, φ_2 −0·6°, ψ_2 +1·1°, ω_2 +0·7°. Intra- and intermolecular interactions, therefore, do not induce any distortion of this ring. This is not the case for (II), (III), and (IV), especially for (III) where the DKP ring is described as a flag-pole boat with $\varphi_1 = 19°$ and $\varphi_2 = 16°$.

The imidazole ring is planar (equation: $0{\cdot}7874X + 0{\cdot}4732Y + 0{\cdot}3949Z = 10{\cdot}176$), atomic deviations being <0·008 Å. For C_2^β, the deviation is somewhat higher: 0·08 Å.

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32978 (14 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

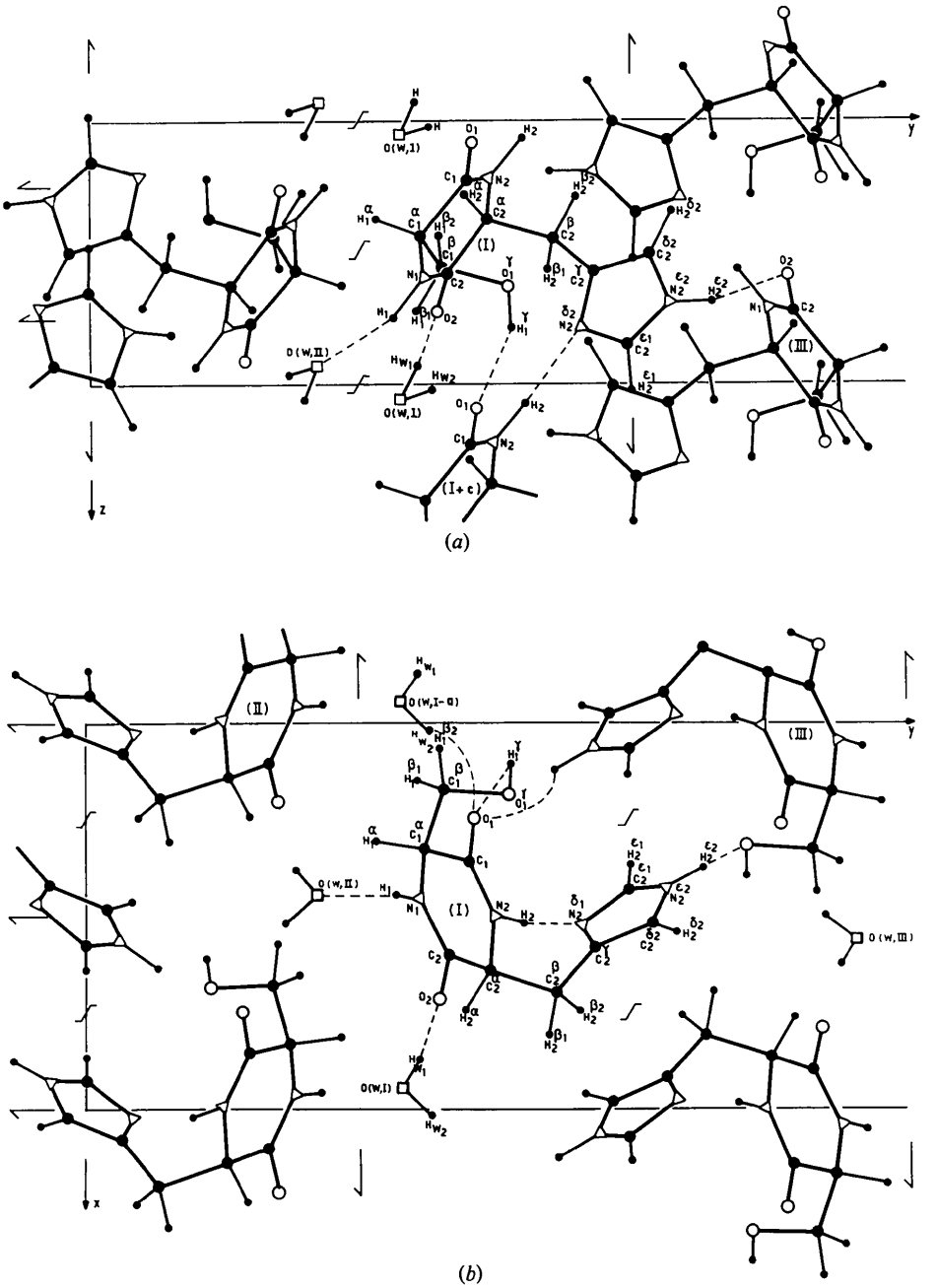

Fig. 1. Partial projections of the structure along (*a*) the *Ox* and (*b*) the *Oz* axes.

The L-Ser side chain is folded above the DKP ring, with $\chi_1^1 = 69{\cdot}8°$. Such a folded conformation was also found in (IV) with $\chi_1^1 = 72°$ and in (II) for the L-Thr side chain ($\chi_1^1 = 69{\cdot}5$).* As discussed later, this conformation appears to be an intrinsically stable form for the hydroxylated side chains in cyclic dipeptides. The L-His side chain is also folded ($\chi_2^1 = 62{\cdot}2°$), the imidazole ring facing the DKP ring ($\chi_2^{2,1} = 63{\cdot}3°$). Such a conformation is close to the L-Tyr side-chain conformation in (III) and (IV), where $\chi_2^1 = 55°$ in both molecules and $\chi_2^{2,1} = 71$ and 72° respectively, but differs from the open form observed in (II). Let us recall that in (II) there is an intramolecular water molecule inserted between the two polar side chains;

* The χ_1^1 (L-THr) value given in Cotrait, Ptak, Busetta & Heitz (1976) should be 69·5°. The conclusion concerning the conformation of (II) is unchanged.

this molecule is hydrogen bonded to O^{γ}_{1} (L-Thr) and the N^{δ}_{2} (L-His) respectively.

In amino acids and dipeptides having an unprotonated imidazole ring, χ^1 is close to either 180 or 300° while $\chi^{2,1}$ is generally close to 60° (Barrans, Bellocq, Cotrait & Richard, 1976). In the present structure, the L-His side chain shows the same behaviour as aromatic side chains such as Tyr, Phe or Trp in the solid state (Cotrait & Barrans, 1973) and in solution (Deslauriers, Grzonka, Schaumburg, Shiba & Walter, 1975). Moreover, the folding of the two L-Ser and L-His side chains does not induce a direct interaction between them [*e.g.* a hydrogen bond between the N^{δ}_{2} (L-His) and the O^{γ}_{1} (L-Ser) atoms]. As seen below, both atoms are engaged in intermolecular hydrogen bonds.

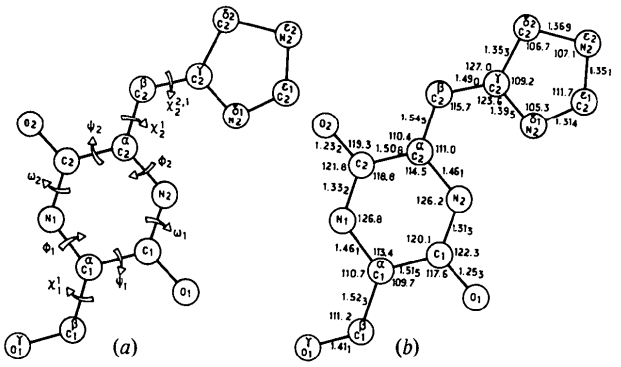

Fig. 2. (*a*) Conformational angles as defined for peptides. (*b*) Bond lengths (Å) and valency angles (°).

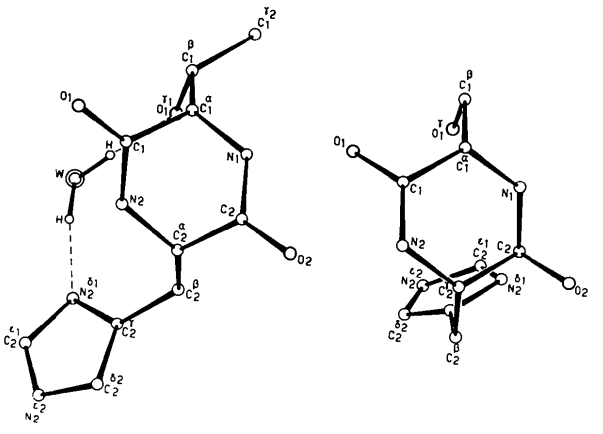

Fig. 3. Projections of *cyclo*(-L-Thr-L-His-) and *cyclo*(-L-Ser-L-His-) molecules on their DKP mean planes

Hydrogen bonds

Hydrogen bonds are listed in Table 3 and shown in Fig. 1(*a*) and (*b*). There is no regular hydrogen-bond network connecting the DKP rings as is usually observed in cyclic dipeptides [*e.g.* (II), (III) and (IV)]. The cohesion is mainly determined by side-chains–DKP-ring intermolecular interactions with an additional contribution of the water molecule interacting with DKP rings. The hydrogen bonding is rather limited inasmuch as O^{γ}_{1} (L-Ser) is not engaged in a hydrogen bond as a proton acceptor. This agrees with the thermal motion of O^{γ}_{1} ($B_i = 4{\cdot}1$ Å^2), which is somewhat higher than for the other C, N and O atoms.

None of the hydrogen bonds appear to be particularly strong:

– both NH···O bonds are rather weak (distances >2·92 Å and large deviations from linearity) (Ramakrishnan & Prasad, 1971);

– the three OH···O bonds are quite weak, especially the last one (Novak, 1974);

– only the NH···N bond seems to be of medium strength and nearly linear.

Van der Waals and dipolar interactions contribute significantly to the crystal cohesion in addition to a rather poor hydrogen-bond network. The relatively high density of the present crystal may be correlated with the absence of a bulky methyl group in the L-Ser side chain compared with the L-Thr side chain in (II) and to a very compact molecular conformation where the L-His side chain is folded above the central DKP ring.

Crystal structure, NMR and conformational analysis

Empirical calculations of intramolecular energy provide a basic set of conformations, some of which

Table 3. *Hydrogen bonds in the crystal*

Bond number	A–H···B	$d(A$–$B)$	d(H···B)	$\alpha(A$–H···$B)$
1	O(W,I − c)–H$_2$(W,I − c)···O$_2$(I)	2·76$_4$ Å	1·80$_7$ Å	167·9°
2	$N^{\varepsilon 2}_{2}$(I)–H$^{\varepsilon 2}_{2}$(I)···O$_1$(III)	2·93$_4$	1·92$_3$	161·0
3	O^{γ}_{1}(I)–H$^{\gamma}_{1}$(I)···O$_1$(I + c)	2·78$_7$	1·92$_0$	148·1
4	N$_2$(I)–H$_2$(I)···N$^{\delta 1}_{2}$(1 − c)	2·99$_8$	1·96$_2$	174·6
5	N$_1$(I)–H$_1$(I)···O(W,II)	2·89$_1$	1·89$_9$	157·0
6	O(W,I)–H$_1$(W,I)···O$_1$(I + a)	2·97$_8$	2·09$_4$	151·3

will be found in the crystal state and in solution, possibly with distortions induced by intermolecular interactions. The differences observed in the solid state between (I) and (II) do not necessarily express fundamental differences in their conformational behaviour as suggested by NMR studies and empirical calculations.

In calculations of intramolecular energy (Genest & Ptak, 1976), the most stable conformations for these two isolated molecules are doubly folded forms characterized, on the assumption of a planar DKP ring, by: χ_1^1 near 60° for L-Ser and L-Thr side chains, χ_2^1 near 60° and $\chi_2^{2,1}$ close to either 60 or 300° for L-His side chains. Slightly less stable conformations are found when the L-His side chain is unfolded: χ_2^1 near 300° and $\chi_2^{2,1}$ near ±30° define an open form in which the $N_2^{\delta_1}$ imidazole atom interacts with the NH group (L-His). Such a conformation allows the insertion of a water molecule between the two polar side chains [as found in the crystalline dihydrate (II)] by rotating the imidazole ring around C^{β}–C^{γ} (L-His) by 70°.

The most stable conformation determined by empirical calculations is found in the crystal (I), where a single water molecule, inserted between dipeptide molecules, is needed to assure the best packing. The bulky γ methyl group in the L-Thr residue probably inhibits such an arrangement in the crystal structure of (II) and another conformation is then selected: a water molecule is inserted between two side chains, expelling the L-His side chain towards the exterior of the molecule.

In aqueous solutions, ^1H and ^{13}C NMR studies (Ptak, Heitz & Dreux, 1977) reveal the intrinsic stability of the folded (χ^1 near 60°) L-Thr and L-Ser side chains. Nevertheless, in neutral *cyclo*(-L-Ser-L-His-), the rotational freedom of the L-Ser side chain remains somewhat higher. On the other hand, the folded form (χ_2^1 near 60°) of the L-His side chain is not unique; it is in fast equilibrium with unfolded forms (χ_2^1 near 180 or 300°). Two main effects can contribute to this special behaviour in solution:

–firstly, a distortion of the DKP ring favours the folding of L-Thr and L-Ser side chains. Steric hindrance then reduces the possibility of the L-His side chain being completely locked in a folded conformation;

– secondly, there is a strong hydration effect on the imidazole moiety which perturbs the interactions between the two side chains. A water bridge, connecting the OH group (L-Thr or L-Ser) and the imidazole ring (L-His), could exist in fast equilibrium with other possible kinds of interactions. At present, there is no direct proof for this and we plan to investigate such a possibility.

Conclusion

In cyclic dipeptides, no direct hydrogen bonding between a hydroxylated side chain (L-Thr or L-Ser) and the L-His side chain is observed. This does not preclude the existence of side-chain–side-chain interaction in larger peptides in which the peptide backbone can be different and does not dictate so strongly the side-chain conformation. In all cases, this type of interaction is required to allow proton transfer between residues (which has a bearing on catalytic properties). The synthesis of larger peptides incorporating Ser, His and Asp residues has been started.

The conformational properties of cyclic dipeptides found in the crystal, in solution and in vacuum by calculation are self-consistent. Theoretical calculations establish the fundamental conformational properties of such molecules. It should be of interest to investigate the effect of specific hydration on the conformations of polar residues. The crystal structure and the molecular conformation of cyclic dipeptides containing acid residues are also under investigation.

We thank Michel Dreux for the synthesis of the cyclic dipeptides, and Monique Genest, Françoise Vovelle and Dr R. E. Marsh for helpful discussions.

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