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An Unusual Hydrogen Bond in the Type IV β Turns of *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-]

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Abstract

The conformation of this cyclic octadepsipeptide provides the first example of a type IV β turn containing a hydrogen bond. Two such turns generate a rectangular-shaped molecule with D residues at the corners and containing pseudorotational symmetry. The hydrogen bonds are between the third and fourth residues in the type IV turns. Although these unusual 1→2 type hydrogen bonds are weak (3·13 and 3·15 Å), they appear to be the cause of severe distortion of the ω bonds within them (156, 153°). Analysis of the results suggests that other [-(DDLL)$_2$-] depsipeptides should favor conformations having comparable type IV turns with DD or LL residues at the corners. In this structure the bulky D-Hyi residues appear responsible for stabilizing the conformation in which the D residues rather than the L residues are at the corners. The 3→4 hydrogen bond further stabilizes the overall conformation. The orientation of the carbonyl groups of the molecule is not conducive to efficient metal-ion coordination and the observed conformation is consistent with the failure of the compound to complex cations or behave as an ionophore. Crystals of *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-] ($C_{40}H_{68}N_4O_{12}$, M_r = 937·01) are orthorhombic, space group $P2_12_12_1$, and have cell dimensions of a = 13·390 (1), b = 16·678 (1), c = 21·349 (1) Å (λ = 1·5418 Å, T = 291 K); R = 0·105 for observed data, 0·125 for all data.

Introduction

The title compound was synthesized as part of a study of natural and synthetic ion-transport antibiotics (Fonina, Savelov, Avotin', Ivanov & Ovchinnikov, 1976; Avotin', Fonina, Ivanov & Ovchinnikov, 1978). The cyclic trimer of D-Ile-Lac-Ile-D-Hyi is a valinomycin analog that selectively binds potassium. The octadepsipeptide formed by cyclization of dimers of D-Ile-Lac-Ile-D-Hyi was found to have little affinity for cations despite its cyclic arrangement of eight carbonyl groups. The investigation of the crystal structure of the uncomplexed form of the octadepsipeptide was undertaken in order to determine its molecular conformation. Crystals were grown from dimethylformamide solution. Crystallographic diffraction data were measured on a specimen crystal of dimensions 0·35 × 0·35 × 0·12 mm on a Syntex $P\bar{1}$ four-circle diffractometer at 291 K using Ni-filtered Cu $K\alpha$ radiation. Integrated relative intensities for 3379 independent reflections with $2\theta < 110°$ were measured in the θ–2θ scan mode; 2705 of these reflections were measured to be observed above background ($I > 2\sigma$).

The intensities were reduced to structure factor amplitudes, and phase angles sufficient for location of the nonhydrogen atoms were derived using the direct-methods program *QTAN* (Langs & DeTitta, 1977). Electron density peaks corresponding to 30 of the 68 H atoms were found in difference electron density maps. The other H atoms were placed in geometrically expected positions. The H atoms were included in structure factor calculations but not refined and were assigned isotropic thermal parameters of 4·0 Å^2.

The variance of each F was calculated according to the method of Stout & Jensen (1968) $\{\sigma^2(F) = k/4(\mathrm{Lp})I[\sigma^2(I) + (0{\cdot}06I)^2]$; $w(F) = 1/\sigma^2(F)\}$. Unobserved data were assigned weights of zero. The final values of the residual ($R = \sum||F_o| - |F_c||/\sum|F_o|$) were 0·105 for the observed data and 0·125 for all the measured data. Final positional parameters are listed in Table 1.*

Discussion

The crystallographically observed conformation is illustrated in Figs. 1 and 2 and has approximate twofold

* Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35346 (19 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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Table 1. *Atomic coordinates* ($\times 10^4$) *and isotropic thermal parameters* ($\times 10$) *of* *cyclo*[−(D-Ile-Lac-Ile-D-Hyi)$_2$-]

Standard deviations are in parentheses.

	x	y	z	B_{iso} (Å^2)		x	y	z	B_{iso} (Å^2)
C(1*A*)	13791 (5)	3191 (4)	5974 (3)	45 (3)	C(5*A*)	7244 (5)	6739 (4)	5506 (4)	51 (3)
C(1*B*)	14296 (6)	3258 (4)	6603 (4)	64 (4)	C(5*B*)	6484 (6)	6563 (5)	6023 (4)	63 (4)
C(1*G*1)	13637 (6)	3725 (6)	7081 (4)	71 (4)	C(5*G*1)	6885 (7)	6008 (5)	6512 (5)	90 (4)
C(1*G*2)	14562 (6)	2425 (5)	6858 (5)	75 (4)	C(5*G*2)	6121 (7)	7359 (6)	6300 (4)	80 (4)
C(1*D*1)	14197 (8)	4038 (6)	7630 (5)	96 (5)	C(5*D*1)	6062 (9)	5608 (6)	6894 (5)	128 (6)
C(1′)	13767 (5)	3990 (4)	5653 (3)	52 (3)	C(5′)	7425 (6)	5987 (4)	5100 (4)	61 (3)
N(1)	12763 (4)	2892 (3)	6002 (3)	46 (2)	N(5)	8196 (5)	7005 (3)	5749 (3)	53 (2)
O(1)	14243 (4)	4573 (3)	5809 (3)	79 (3)	O(5)	6866 (5)	5439 (4)	5089 (4)	137 (4)
C(2*A*)	12780 (6)	4807 (4)	5007 (3)	50 (3)	C(6*A*)	8699 (5)	5241 (4)	4658 (4)	46 (3)
C(2*B*)	12188 (7)	4724 (5)	4407 (4)	65 (4)	C(6*B*)	9516 (7)	5375 (5)	4174 (4)	67 (4)
C(2′)	12094 (5)	5075 (4)	5552 (4)	47 (3)	C(6′)	9136 (5)	4914 (4)	5273 (3)	43 (3)
O(2)	11788 (4)	4595 (2)	5930 (2)	55 (2)	O(6)	9330 (4)	5350 (3)	5705 (2)	58 (2)
O(2′)	13136 (4)	4013 (2)	5160 (2)	51 (2)	O(6′)	8279 (4)	6004 (2)	4803 (2)	53 (2)
C(3*A*)	11334 (5)	6246 (4)	6050 (3)	43 (3)	C(7*A*)	9664 (5)	3670 (4)	5797 (3)	43 (3)
C(3*B*)	11971 (6)	6623 (4)	6561 (4)	61 (3)	C(7*B*)	8859 (6)	3331 (4)	6243 (4)	56 (3)
C(3*G*1)	12681 (6)	7261 (5)	6301 (4)	61 (4)	C(7*G*1)	8139 (6)	2810 (4)	5896 (4)	62 (3)
C(3*G*2)	12543 (9)	5997 (6)	6933 (5)	93 (5)	C(7*G*2)	8379 (7)	4004 (5)	6625 (4)	70 (4)
C(3*D*)	13196 (8)	7751 (7)	6773 (6)	100 (5)	C(7*D*1)	7381 (8)	2385 (7)	6298 (5)	112 (6)
C(3′)	10687 (5)	6872 (4)	5737 (3)	45 (3)	C(7′)	10316 (5)	3017 (4)	5513 (4)	45 (3)
N(3)	11926 (4)	5858 (3)	5558 (3)	45 (2)	N(7)	9229 (4)	4114 (3)	5282 (3)	45 (2)
O(3)	10702 (4)	7022 (3)	5192 (2)	59 (2)	O(7)	10402 (4)	2847 (3)	4977 (2)	64 (2)
C(4*A*)	9583 (5)	7957 (4)	5907 (3)	46 (3)	C(8*A*)	11407 (6)	1935 (4)	5814 (4)	53 (3)
C(4*B*)	9543 (6)	8560 (5)	6424 (4)	81 (4)	C(8*B*)	11162 (6)	1229 (5)	6215 (4)	66 (4)
C(4*G*1)	9017 (9)	8296 (6)	6987 (5)	96 (5)	C(8*G*1)	11333 (7)	1378 (5)	6876 (4)	75 (4)
C(4*G*2)	10581 (9)	8838 (5)	6612 (5)	101 (5)	C(8*G*2)	10072 (8)	983 (5)	6084 (6)	110 (6)
C(4′)	8554 (5)	7740 (4)	5673 (3)	44 (3)	C(8′)	12539 (6)	2154 (4)	5843 (3)	48 (3)
O(4)	8059 (4)	8289 (3)	5429 (3)	67 (2)	O(8)	13126 (4)	1617 (3)	5731 (3)	60 (2)
O(4′)	10093 (3)	7247 (2)	6144 (2)	46 (2)	O(8′)	10811 (4)	2630 (3)	5994 (2)	51 (2)

symmetry. Bond lengths, valence angles and torsion angles (IUPAC–IUB Commission on Biochemical Nomenclature, 1970) of the two quasi-symmetric halves are compared in Fig. 3.

β bends or chain reversals are identified by calculating the distances between the i and $i + 3$ α carbon atoms. According to Lewis, Momany & Scheraga (1973), distances of generally less than 6·0 Å characterize a β bend. Since the distances between C(3*A*) and C(6*A*) and between C(7*A*) and C(2*A*) are 4·9 Å, the structure can be said to have two β bends with D-Hyi$_8$ and D-Ile$_1$ at the corners of one and D-Hyi$_4$ and D-Ile$_5$ at the corners of the other. The distances between C(5*A*) and C(8*A*) and between C(1*A*) and C(9*A*) are 9·7 Å. Since a value of 11 Å is expected for a fully extended peptide chain, the dipeptide sequence, -L-Lac-L-Ile-, may be considered to be in a semi-extended conformation. These conformational features are consistent with the φ and ψ torsion angles, illustrated in Fig. 4, which show that the L-Ile residues are in an extended conformation while the two L-Lac residues are in a partially extended conformation.

Examination of the torsion angles of the residues at the corners of the two bends indicates that the bends

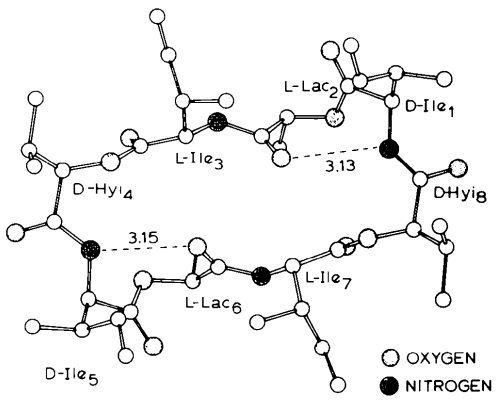

Fig. 1. Observed conformation and intramolecular 3→4 hydrogen bonds in *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-].

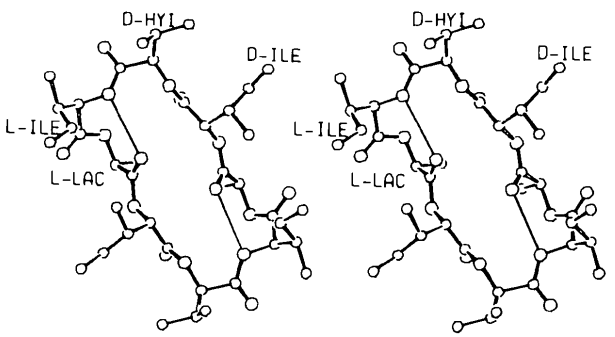

Fig. 2. Stereodiagram of *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-].

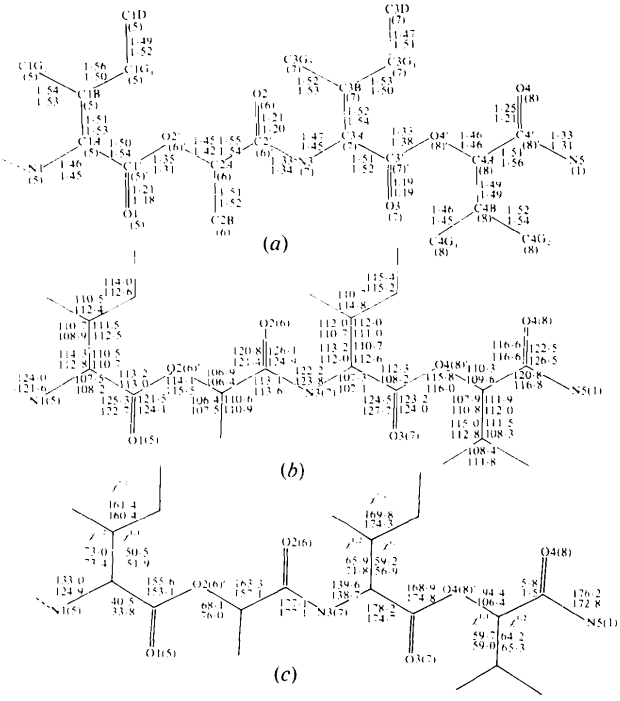

Fig. 3. Intramolecular dimensions of *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-]. The pseudosymmetrically related halves of the molecule are compared with the values for the residues labeled 1 through 4 in Fig. 1 over the values for residues 5 through 8. (*a*) Bond distances (Å); σ range = 0·008–0·010 Å. (*b*) Bond angles (°); σ range = 0·4–0·5°. (*c*) Torsion angles (°); σ range = 0·5–1·1°.

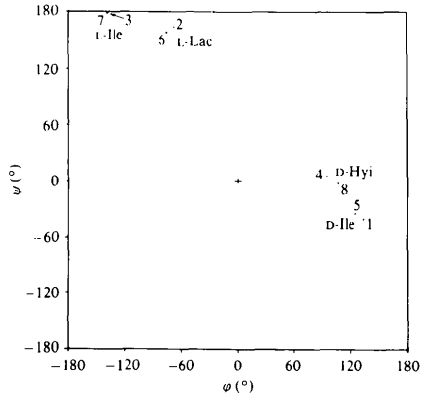

Fig. 4. φ–ψ plot for *cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-].

deviate significantly from the types of bends arising for a DD sequence, namely a type I′ or III′. Although it is more nearly like a type I′ β bend than a type III′, three of the torsion angles in one bend and one in the other deviate by more than 40° from that expected for a type I′ β bend. Therefore, according to the notation of Lewis, Momany & Scheraga (1973), it is appropriate to call this chain reversal type IV. While most chain reversals possess a hydrogen bond across the bend stabilizing the conformation, 4→1 hydrogen bonds are never observed in type IV bends. In this particular case a 4→1 hydrogen bond is impossible since the fourth residue is a hydroxy acid and therefore has no proton to donate. Furthermore, the carbonyl group which normally would be the acceptor of an intramolecular hydrogen bond has been rotated away from its idealized position.

A pair of intramolecular hydrogen bonds are observed in this structure but result from the donation of protons by the D-Ile amino groups to the carbonyl O atoms of the next residue, L-Lac. This produces a highly unusual 1→2 intramolecular hydrogen bond which is not associated with a chain reversal but rather with a 'kink' in the peptide backbone.

In addition to being the first example of a 1→2-type hydrogen bond this is the first observation of a hydrogen bond of any sort in a type IV β bend. Since these intramolecular hydrogen bonds occur between the third and fourth residues of a type IV β bend, it seems appropriate to call them 3→4 hydrogen bonds. While the observed N···O distances are long (3·13, 3·15 Å), the interaction is still strong enough to severely distort both the ω_1 and the ω_5 bonds by approximately 25° from planarity.

The molecules are linked into infinite chains extended in the **b** direction by two hydrogen bonds as shown in Fig. 5. The geometry of the intra- and intermolecular hydrogen bonds is summarized in Table 2.

It is of interest to consider why this molecule adopts such an unusual conformation and to determine if other pairs of β bends are consistent with the sequence of this cyclic octadepsipeptide. Only four combinations of residues can be placed at the corners of a β bend when the molecule has the (LLDD)$_2$ sequence.

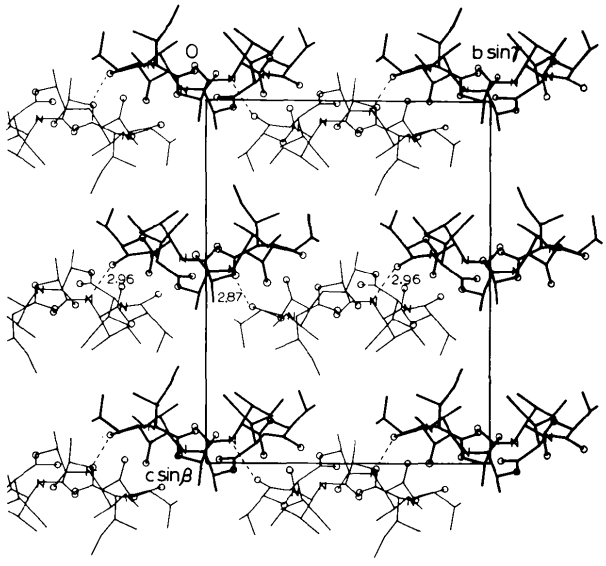

Fig. 5. A projection along [100] illustrating the intermolecular hydrogen bonding.

Table 2. *Hydrogen-bond geometry in cyclo*[-(D-Ile-Lac-Ile-D-Hyi)$_2$-]

Donor (*D*)	Acceptor (*A*)	*D*···*A*	*D*–H	H···*A*	∠*D*–H···*A*
Intermolecular					
N(3)	O(4)	2·96 Å	0·89 Å	2·09 Å	165°
N(7)	O(8)	2·89	0·97	1·94	163
Intramolecular					
N(1)′	O(2)	3·13	1·08*	2·21	141
N(5)	O(6)	3·15	1·08*	2·26	138

* Theoretical hydrogen positions.

The only chain reversal possible when either the LD or DL sequence resides at the corners of the bend is a type II bend. This kind of bend with the accompanying 4→1 hydrogen bond is observed in both the complexed and uncomplexed forms of valinomycin (Neupert-Laves & Dobler, 1975; Smith *et al.*, 1975; Karle, 1975). However, in order to link two such bends together in this octapeptide and maintain energetically favorable conformations of the connecting residues, a close contact of about 2 Å would be produced between a pair of carbonyl O atoms. Other alternative ways of linking the bend with the connecting residues in semi-extended or extended conformations produce conformations which are in the disallowed region of the φ–ψ plot. The only alternative conformation is to place the LL or DD sequence at the corners of the bend, in which case a type I or III bend is possible, stabilized by a 4→1 hydrogen bond. In this conformation, however, no hydrogen bonding is possible since the fourth residue is a hydroxy acid. The removal of this hydrogen-bonding constraint now permits conformations to exist which significantly deviate from type I or III bends and can furthermore allow the connecting residues to adopt an extended conformation. Conformations with the DD sequence at the corners are favored since bulky side chains can be accommodated with small steric interactions at the corner α carbon atoms. The 3→4 hydrogen bonds further stabilize the conformer in which the D residues are at the corners of the type IV β turns.

The inability of this cyclic octadepsipeptide to form complexes with metal ions can also be understood on the basis of the above discussion. In order to be an effective ionophore, the molecule must maintain intramolecular stability as a complex, provide a coordination sphere for the metal ion, and possess a hydrophobic exterior. In some cases these requirements are met by a single molecule surrounding the ion and in other cases the ion may be sandwiched between two or more molecules (Smith & Duax, 1976). It may be impossible for a cyclic octadepsipeptide with an LLDD sequence to meet all three of these requirements simultaneously. Hydrogen bonding across a type II β bend (requiring the DL or LD sequence at the corners of the bend) is either inconsistent with the placement of O atoms in positions to coordinate metal atoms or forces residues into unfavorable conformations. The crystallographically observed conformation (DD or LL sequence at the corners) has the carbonyl O atoms radially dispersed throughout the molecule and does not possess a hydrophobic exterior. In the observed conformation it would be almost impossible for a metal ion to be coordinated to more than two carbonyl O atoms at once. Consequently, three or more molecules would be needed to coordinate an ion fully and the resultant complex would have a hydrophilic surface. Thus the molecule's failure to act as an efficient ionophore is consistent with the crystallographically observed conformation.

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