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Conformation of Cyclic Octapeptides. VI. Structure of *cyclo*-Bis-(-L-alanyl-glycyl-L-prolyl-L-phenylalanyl-) Tetrahydrate

BY KRISHNA K. BHANDARY

Oral Biology Department and Dental Research Institute, School of Dental Medicine, University at Buffalo, Buffalo, New York 14214, *USA and Biophysics Department, Roswell Park Memorial Institute, Buffalo, New York* 14263, *USA*

AND KENNETH D. KOPPLE

L-940, Smith Kline & French Laboratories, PO Box 1539, *King of Prussia, Pennsylvania* 19406-0939, *USA*

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Abstract. $C_{38}H_{48}N_8O_8.4H_2O$, $M_r = 816{\cdot}9$, monoclinic, $P2_1$, $a = 10{\cdot}381$ (1), $b = 13{\cdot}273$ (1), $c = 15{\cdot}742$ (1) Å, $\beta = 101{\cdot}83$ (1)°, $V = 2123{\cdot}1$ Å^3, $Z = 2$, $D_x = 1{\cdot}278$ g cm^{-3}, λ(Cu $K\alpha$) = 1·5418 Å, $\mu = 7{\cdot}6$ cm^{-1}, $F(000) = 872$, $R = 0{\cdot}035$, $wR = 0{\cdot}045$ for 3497 reflections [$I > 2\sigma(I)$], 4552 unique reflections measured. The synthetic cyclic octapeptide crystallizes from water/methanol solution as a tetrahydrate and the crystals are isomorphous to those of the disulfide-bridged cystine analog *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-) [Kopple, Wang, Cheng & Bhandary (1988). *J. Am. Chem. Soc.* **110**, 4168–4176]. The coordinates of the Cys analog were taken as the starting coordinates for full-matrix least-squares refinement. The cyclic octapeptide ring has two β turns encompassing the residues Pro-L-Phe, one type I and the other type II, with all peptide links *trans*. The conformation of the cyclic octapeptide backbone is similar to the Cys analog; all backbone dihedral angles in the two molecules agree to within 6°. This suggests that the disulfide bridge of the Cys analog does not impose any conformational constraint on the octapeptide ring backbone.

Introduction. Cyclic oligopeptides retain significant backbone conformational freedom even though they are conformationally constrained. Information obtained from multiple crystal structures, or crystal data in conjunction with NMR data, can provide indications of this conformational flexibility. We have used this combination to examine the conformation of diastereomeric octapeptides having the sequence *cyclo*-bis(-L- or -D-Ala-Gly-L-Pro-L- or -D-Phe-). On the basis of NMR measurements of Me_2SO solutions, two of these, the L-Ala/L-Phe isomer and the D-Ala/D-Phe isomer, were judged to have conformationally stable all-*trans* backbones in solution (Kopple, Parameswaran & Yonan, 1984). The crystal structure of the D-Ala/D-Phe peptide (Kopple, Bhandary, Kartha, Wang & Parameswaran, 1986) was found to have a backbone conformation similar to a likely solution conformation. The D-Ala/L-Phe isomer has a predominant all-*trans* conformer in solution, but does not exhibit spectroscopic evidence from which a single conformation may be clearly deduced. Rotating-frame spin-lattice-relaxation studies suggest that it has more flexibility than the D/D isomer. In fact, two forms of the D/L isomer have been crystallized (Kopple, Kartha, Bhandary & Romanowska, 1985) and they show different backbone conformations. The L-Ala/D-Phe isomer, which in solution contains a large proportion of *cis* Gly-Pro peptide bonds, crystallizes with two molecules in the asymmetric unit, each having exact twofold symmetry and with *cis* Gly-Pro linkages. The two molecules have different conformations at the Ala-Gly linkages (Bhandary, Kopple & Parameswaran, 1989). The Cys analog of this series of octapeptides, *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-) (Kopple, Wang, Cheng & Bhandary, 1988), has a backbone conformation in which the two β turns at Pro-Phe differ. The cyclic ring contains both type I and type II turns, although at room temperature and above in solution the molecule has C_2 symmetry in the NMR time average. In this paper we report the crystal structure and conformation of the L/L isomer, *cyclo*-bis(-L-Ala-Gly-L-Pro-L-Phe-), which crystallizes in the monoclinic cell that is isomorphous to the Cys analog.

Experimental. A crystal of size 0·25 × 0·30 × 0·45 mm, grown by slow evaporation of a solution of the octapeptide in water/methanol and sealed in a capillary with the mother liquor, was used for preliminary examination and intensity data collection on an Enraf–Nonius CAD-4 automated diffractom-

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eter, Ni-filtered Cu $K\alpha$ radiation, ω–2θ scans and integrated counts with $\theta < 75°$, $-13 < h < 13$, $0 < k < 16$, $0 < l < 19$. 4814 reflections measured, 4552 independent, 3497 with $I > 2\sigma(I)$. 436 equivalent (unique) reflections were merged ($R_{merge} = 0{\cdot}02$). Lattice parameters determined using 25 reflections ($20 < \theta < 27°$). Three standard reflections ($\bar{6}00$, 060, 007) measured at intervals of 2 h showed a 7% decrease in intensity during data collection and a linear decay correction was applied. Lp corrections and empirical absorption (transmission: min. 82·7, max. 99·8%) corrections (North, Phillips & Mathews, 1968) based on a series of ψ scans. Initial coordinates for the octapeptide were taken from the structure of *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-). Four water molecules located from difference Fourier map. Full-matrix least-squares refinement (on F) for 58 non-H atoms with anisotropic thermal parameters. H-atom positions located from difference Fourier map were included in the refinement with isotropic thermal parameters. $\sum w(|F_o| - |F_c|)^2$ minimized, $w = 4F^2/\sigma(F^2)$, $(\Delta/\sigma)_{max} = 0{\cdot}01$, $\Delta\rho_{max} = 0{\cdot}12$, $\Delta\rho_{min} = -0{\cdot}14$ e Å^{-3}. Final $R = 0{\cdot}035$, $wR = 0{\cdot}045$ for 3497 reflections with $I \geq 2\sigma(I)$, $S = 1{\cdot}465$. Extinction coefficient of the type described by Zachariasen (1963) ($8{\cdot}977 \times 10^{-6}$) was applied. Atomic scattering factors were from *International Tables for X-ray Crystallography* (1974, Vol. IV, Table 2.2B). All calculations were performed on a MicroVAX II computer using the *SDP/VAX* software package (Frenz, 1978).

Discussion. The final atomic parameters are given in Table 1.* Bond lengths and angles are listed in Table 2. Backbone bond distances averaged over eight residues are 1·457 (4) Å for N_i—C_iA, 1·514 (4) Å for C_iA'—C_i', 1·227 (4) Å for C_i'—O_i and 1·338 (4) Å for C_i'—N_{i+1}, and the characteristic backbone bond angles are 122·0 (3)° for C_{i-1}'—N_i—C_iA, 112·8 (3)° for N_i—C_iA—C_i', 120·4 (3)° for C_iA—C_i'—O_i, 117·0 (3)° for C_iA—C_i'—N_{i+1} and 122·5 (3)° for O_i—C_i'—N_{i+1}. These values agree very well with those reported for octapeptide analogs (Kopple, Bhandary, Kartha, Wang & Parameswaran, 1986; Kopple, Kartha, Bhandary & Romanowska, 1985) and other cyclic peptides (Karle, 1979).

The molecular conformation of the octapeptide is shown in Fig. 1 and the torsional angles are listed in Table 2. The backbone conformation is similar to the backbones found for *cyclo*-bis(-D-Ala-Gly-L-Pro-D-Phe-) (Kopple, Kartha, Bhandary & Romanowska,

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53755 (28 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Postional parameters and their estimated standard deviations*

Anisotropically refined atoms are given in the form of the equivalent isotropic displacement parameter defined as: $B_{eq} = (4/3)[a^2B(1,1) + b^2B(2,2) + c^2B(3,3) + ab(\cos\gamma)B(1,2) + ac(\cos\beta)B(1,3) + bc(\cos\alpha)B(2,3)]$.

	x	y	z	B_{eq}(Å^2)
N1	−0·0450 (3)	0·2598	0·2039 (2)	4·17 (5)
C1*A*	0·0140 (3)	0·2401 (3)	0·1290 (2)	4·34 (7)
C1*B*	0·1410 (5)	0·2994 (4)	0·1338 (3)	7·0 (1)
C1′	0·0338 (3)	0·1286 (3)	0·1148 (2)	4·20 (7)
O1	0·0877 (3)	0·1027 (2)	0·0550 (1)	5·81 (6)
N2	−0·0127 (3)	0·0632 (2)	0·1642 (2)	4·10 (5)
C2*A*	−0·0129 (3)	−0·0439 (3)	0·1502 (2)	4·04 (6)
C2′	0·1036 (3)	−0·0971 (3)	0·2066 (2)	3·53 (6)
O2	0·1958 (2)	−0·0512 (2)	0·2509 (1)	4·27 (5)
N3	0·0989 (2)	−0·1982 (2)	0·2044 (2)	3·76 (5)
C3*A*	0·1896 (3)	−0·2582 (3)	0·2677 (2)	4·02 (6)
C3*B*	0·1395 (4)	−0·3653 (3)	0·2500 (4)	6·8 (1)
C3*G*	0·0361 (6)	−0·3638 (4)	0·1775 (4)	10·3 (2)
C3*D*	−0·0048 (4)	−0·2605 (3)	0·1514 (3)	5·35 (9)
C3′	0·3342 (3)	−0·2489 (3)	0·2654 (2)	3·78 (6)
O3	0·4163 (2)	−0·2751 (2)	0·3292 (1)	5·69 (6)
N4	0·3654 (2)	−0·2137 (2)	0·1925 (1)	3·33 (5)
C4*A*	0·5028 (3)	−0·2066 (3)	0·1843 (2)	3·50 (6)
C4*B*	0·5160 (3)	−0·2375 (3)	0·0922 (2)	4·14 (6)
C4*G*	0·4678 (3)	−0·3432 (3)	0·0712 (2)	3·78 (6)
C4*D*1	0·3435 (4)	−0·3609 (3)	0·0272 (3)	6·6 (1)
C4*E*1	0·2964 (5)	−0·4583 (4)	0·0108 (4)	9·3 (1)
C4*Z*	0·3773 (4)	−0·5382 (4)	0·0386 (3)	6·9 (1)
C4*E*2	0·5035 (4)	−0·5221 (3)	0·0807 (3)	5·69 (9)
C4*D*2	0·5473 (3)	−0·4251 (3)	0·0971 (2)	4·71 (7)
C4′	0·5618 (3)	−0·1022 (3)	0·2046 (2)	3·79 (6)
O4	0·6801 (2)	−0·0905 (2)	0·2058 (2)	5·68 (6)
N5	0·4816 (2)	−0·0288 (2)	0·2185 (2)	4·03 (5)
C5*A*	0·5205 (3)	0·0774 (3)	0·2235 (2)	4·17 (7)
C5*B*	0·4410 (5)	0·1360 (3)	0·1472 (2)	6·1 (1)
C5′	0·5102 (3)	0·1260 (3)	0·3090 (2)	3·67 (6)
O5	0·6031 (2)	0·1737 (2)	0·3506 (1)	5·23 (5)
N6	0·3951 (2)	0·1200 (2)	0·3340 (2)	4·13 (5)
C6*A*	0·3727 (3)	0·1719 (3)	0·4110 (2)	4·00 (6)
C6′	0·2277 (3)	0·1831 (3)	0·4057 (2)	3·56 (6)
O6	0·1490 (2)	0·1682 (2)	0·3365 (1)	4·47 (5)
N7	0·1881 (2)	0·2134 (2)	0·4768 (2)	3·79 (5)
C7*A*	0·0467 (3)	0·2267 (2)	0·4749 (2)	3·48 (6)
C7*B*	0·0448 (3)	0·2695 (3)	0·5648 (2)	4·38 (7)
C7*G*	0·1713 (3)	0·2294 (3)	0·6216 (2)	4·91 (8)
C7*D*	0·2698 (3)	0·2314 (3)	0·5640 (2)	4·82 (8)
C7′	−0·0126 (3)	0·2983 (2)	0·4020 (2)	3·36 (5)
O7	0·0431 (2)	0·3759 (2)	0·3872 (1)	4·49 (5)
N8	−0·1316 (2)	0·2706 (2)	0·3559 (1)	3·31 (5)
C8*A*	−0·2049 (3)	0·3271 (2)	0·2826 (2)	3·56 (6)
C8*B*	−0·2333 (4)	0·4377 (3)	0·3052 (2)	4·71 (7)
C8*G*	−0·3176 (4)	0·4424 (3)	0·3707 (2)	4·95 (7)
C8*D*1	−0·4559 (4)	0·4470 (4)	0·3446 (3)	6·7 (1)
C8*E*1	−0·5338 (5)	0·4465 (4)	0·4064 (4)	8·3 (1)
C8*Z*	−0·4758 (6)	0·4388 (4)	0·4922 (3)	8·9 (1)
C8*E*2	−0·3435 (5)	0·4355 (4)	0·5195 (3)	8·3 (1)
C8*D*2	−0·2633 (5)	0·4394 (3)	0·4588 (3)	7·0 (1)
C8′	−0·1487 (3)	0·3189 (3)	0·2011 (2)	4·01 (6)
O8	−0·2055 (3)	0·3621 (2)	0·1348 (1)	6·72 (6)
O*W*1	0·2964 (2)	−0·3267 (2)	0·4691 (2)	5·35 (5)
O*W*2	−0·1290 (3)	0·0315 (3)	0·3164 (2)	6·59 (7)
O*W*3	0·2151 (3)	−0·0926 (2)	0·0405 (1)	5·95 (6)
O*W*4	0·1595 (4)	0·5124 (3)	0·5129 (2)	9·07 (8)

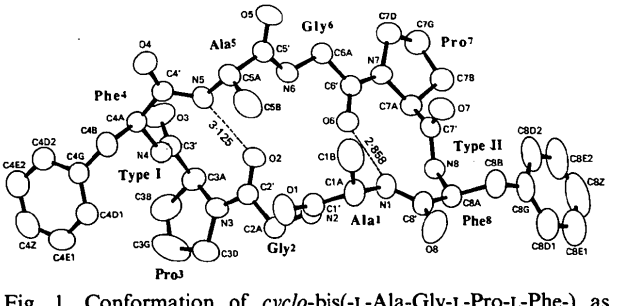

Fig. 1. Conformation of *cyclo*-bis(-L-Ala-Gly-L-Pro-L-Phe-) as observed in the crystal structure. Distances are in Å.

Table 2. *Bond distances* (Å), *bond angles* (°) *and torsion angles* (°) *in cyclo-bis(-Ala-Gly-Pro-Phe-Ala-Gly-Pro-Phe-)*

	$i=$	Ala 1	Gly 2	Pro 3	Phe 4	Ala 5	Gly 6	Pro 7	Phe 8
Bond distances*									
N_i—C_iA		1·459	1·439	1·459	1·461	1·465	1·453	1·473	1·454
C_iA—C_i'		1·517	1·519	1·514	1·522	1·517	1·497	1·519	1·517
C_i'—O_i		1·239	1·225	1·227	1·234	1·225	1·237	1·227	1·224
C_i'—N_{i+1}		1·320	1·344	1·340	1·329	1·335	1·331	1·349	1·322
C_iA—C_iB		1·524		1·521	1·540	1·524		1·529	1·553
C_iB—C_iG				1·398	1·503			1·525	1·485
C_iG—C_iD				1·469				1·498	
C_iD—N_i				1·474				1·479	
C_iG—C_iD1					1·355				1·411
C_iD1—C_iE1					1·387				1·387
C_iE1—C_iZ					1·367				1·366
C_iZ—C_iE2					1·359				1·353
C_iE2—C_iD2					1·372				1·391
C_iD2—C_iG					1·376				1·385
Bond angles*									
C_{i-1}'—N_i—C_iA		123·6	123·3	120·8	120·7	122·6	121·6	119·9	124·0
N_i—C_iA—C_i'		112·7	113·2	116·3	113·5	112·8	109·4	109·8	113·9
C_iB—C_iA—N_i		111·7		103·7	110·1	110·5		103·2	113·5
C_iB—C_iA—C_i'		111·7		112·0	109·4	110·9		112·8	112·7
C_iA—C_i'—O_i		118·6	122·6	119·0	118·3	120·2	120·5	122·3	118·9
C_iA—C_i'—N_{i+1}		118·6	115·4	117·7	117·6	117·8	117·6	114·9	117·7
O_i—C_i'—N_{i+1}		122·8	122·1	123·4	124·1	121·8	121·8	122·8	123·2
C_{i-1}'—N_i—C_iD				126·4				127·8	
C_iA—N_i—C_iD				112·0				112·3	
C_iA—C_iB—C_iG				108·2	111·4			103·9	111·5
C_iB—C_iG—C_iD				111·8				104·6	
C_iG—C_iD—N_i				103·3				103·1	
C_iB—C_iG—C_iD1					120·9				120·6
C_iB—C_iG—C_iD2					121·3				121·1
C_iD1—C_iG—C_iD2					117·7				118·3
C_iG—C_iD1—C_iE1					121·3				120·0
C_iD1—C_iE1—C_iZ					119·6				119·5
C_iE1—C_iZ—C_iE2					120·1				122·0
C_iZ—C_iE2—C_iD2					119·2				119·5
C_iE2—C_iD2—C_iG					122·1				120·7
Torsion angles									
C_{i-1}'—N_i—C_iA—C_i'	φ	−129·8	96·8	−64·0	−96·6	−121·8	159·9	−55·1	73·1
N_i—C_iA—C_i'—N_{i+1}	ψ	5·7	171·6	−20·0	−6·5	−54·3	168·9	138·0	2·5
C_iA—C_i'—N_{i+1}—$C_{i+1}A$	ω	172·8	−167·6	−177·5	−169·0	−174·5	179·3	−179·0	176·1
C_iD—N_i—C_iA—C_iB	χ^0			1·8				7·8	
N_i—C_iA—C_iB—C_iG	χ^1			4·6	−58·7			−26·8	−62·9
C_iA—C_iB—C_iG—C_iD	χ^2			−9·5	95·8			36·7	−91·2
C_iB—C_iG—C_iD—N_i	χ^3			10·1				−31·2	
C_iG—C_iD—N_i—C_iA	χ^4			−7·0				14·7	

* Average e.s.d.'s in bond distances, bond angles and torsion angles are 0·005 Å, 0·3 and 0·4°, respectively.

1985), *cyclo*-bis(-D-Ala-Gly-L-Pro-L-Phe-) (Kopple, Bhandary, Kartha, Wang & Parameswaran, 1986) and *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-) in that two roughly planar halves delineated by Pro-Phe β turns form a dihedral angle. The angle between the two 'best planes' through the α-carbons of Ala(1)-Gly(2)-Pro(3)-Phe(4)-Ala(5) and Ala(5)-Gly(6)-Pro(7)-Phe(8)-Ala(1) being 80·2° which is close to that found for the D-Ala/D-Phe isomer (94°) and the Cys analog (81°), but contrasts with the 148° in the D-Ala/L-Phe isomer. All the peptide links are *trans*, with ω for Gly(2) deviating by 13° from the ideal value of 180°. Although the octapeptide contains C_2 sequence symmetry, the cyclic backbone conformation is asymmetric, with major differences in the torsional angles around Ala-Gly and Pro-Phe links (ψ_{Ala} 5·7 and −54·3°; φ_{Gly} 96·8 and 159·9°; ψ_{Pro} −20·0 and 138·0°; φ_{Phe} −96·6 and 73·1°). These differences in torsional angles indicate that the Ala-Gly and the Pro-Phe peptide bond planes are in different orientations relative to their local α-carbon framework. Thus orientations of the Ala-Gly planes differ by about 60° while those of the Pro-Phe planes differ by about 160°.

There is a type I β turn at Gly(2)-Pro(3)-Phe(4)-Ala(5) and a type II β turn at Gly(6)-Pro(7)-Phe(8)-Ala(1). Both turns have dihedral angles (Table 2) close to the standard values reported by Venkatachalam (1968) and include 4→1 hydrogen bonds (Table 3). Although type II β turns with residues of like configuration at $i+1$ and $i+2$ positions on the turn are of higher energy than type I β turns, there are a number of peptide crystals in which type II turns have been observed with L-configuration residues in the corners of the turn. Apart from the Pro(7)-Phe(8) turn in the present structure and Pro(7)-Phe(8) in the isomorphous Cys analog, other examples in the literature include Pro-Ala in isobutyryl-Pro-Ala-

Table 3. *Hydrogen-bonding and short contact distances below 3·2 Å in the crystal structure of cyclo-bis(-L-Ala-Gly-L-Pro-L-Phe-)*

D	H	*A*	Symmetry*	*D*—*A*	H—*A*	*D*—H—*A*
N1	HN1	O6	(i) (000)	2·858 (3)	2·09 (4)	150 (4)
N2	HN2	O*W*2	(i) (000)	2·927 (4)	2·30 (4)	141 (4)
N4	HN4	O*W*3	(i) (000)	3·035 (3)	2·17 (4)	153 (3)
N5	HN5	O2	(i) (000)	3·125 (3)	2·19 (4)	153 (3)
N6	HN6	O2	(i) (000)	3·170 (4)	2·48 (5)	151 (5)
N8	HN8	O5	(i) (−100)	3·026 (3)	2·29 (5)	126 (4)
O*W*1	H1*W*1	O5	(ii) (1−11)	2·817 (3)	2·07 (4)	142 (4)
O*W*1	H2*W*1	O3	(i) (000)	2·827 (3)	1·88 (7)	159 (6)
O*W*2	H1*W*2	O*W*4	(ii) (0−11)	2·781 (4)	1·75 (7)	159 (6)
O*W*3	H1*W*3	O8	(ii) (0−10)	2·806 (3)	2·05 (5)	169 (5)
O*W*3	H2*W*3	O1	(i) (000)	2·940 (4)	1·92 (5)	163 (4)
O*W*4	H1*W*4	O7	(i) (000)	2·771 (4)	1·75 (5)	171 (4)
O*W*4	H2*W*4	O*W*1	(i) (010)	2·731 (5)	1·82 (7)	163 (6)
N2	HN2	O6	(i) (000)	3·201 (3)	2·76 (4)	120 (4)†
N4	HN4	O2	(i) (000)	3·043 (3)	2·87 (4)	91 (3)†
O*W*2	H2*W*2	O4	(i) (−100)	2·858 (4)	2·72 (5)	90 (4)†

* Symmetry code: (i) x, y, z; (ii) $-x, \frac{1}{2}+y, -z$.
† Short contacts.

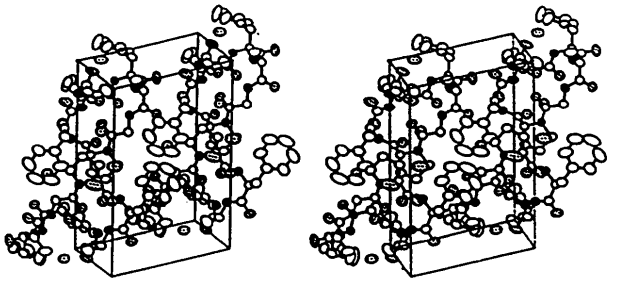

Fig. 2. Stereoview of the crystal packing. The hatched circles indicate carbonyl O atoms, the solid circles indicate N atoms and the stippled circles represent water O atoms. **a** is horizontal, **b** is in the plane of the paper and **c** is vertical.

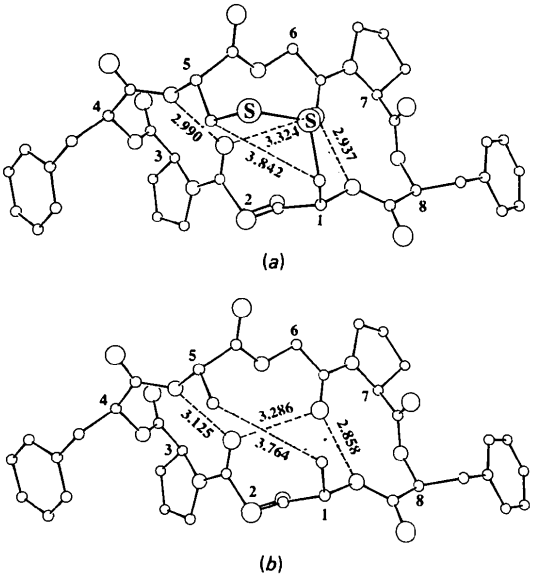

Fig. 3. Comparison of the conformation of (*a*) *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-) and (*b*) *cyclo*-bis(-L-Ala-Gly-L-Pro-L-Phe-). The inter-ring (Å) distances in the two structures are shown and the numbers indicate the α-carbon atoms in the two structures.

isopropylamide (Aubry, Protas, Boussard & Marraud, 1977), Phe-Ala in *cyclo*(-Pro-Val-Phe-Phe-Ala-Gly-) (Karle & Chiang, 1984) and Phe-Gln in pressinoic acid (Langs, Smith, Stezowski & Hughes, 1986). Intermolecular and intramolecular hydrogen bonding may contribute to the stability of such turns.

Hydrogen-bonding distances and short contacts below 3·2 Å are given in Table 3, and the stereoscopic view of crystal packing is shown in Fig. 2. All the amide N atoms and carbonyl O atoms of the octapeptide form hydrogen bonds. Direct intermolecular hydrogen bonding between peptide molecules occurs through the amide of Phe(8) and the carbonyl O atom of Ala(5) of a symmetry-related molecule. Water molecules bridge peptide molecules by hydrogen bonding to the carbonyl O atoms and/or amides of symmetry-related peptide molecules. All waters and all C=O and N—H units are involved in these interactions.

The similarity in backbone conformation of *cyclo*-bis(-L-Ala-Gly-L-Pro-L-Phe-) to the Cys analog *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-) is striking (see Fig. 3). Distances between atoms within the ring in the two structures are comparable and all of the backbone dihedral angles are within 6° when the two molecules are compared and all of the side-chain angles are within 10°. The disulfide bridge fits well, in what must be presumed to be a low-energy conformation, on to this form of the cyclic octapeptide ring.

The cyclic octapeptide backbone found here also approximates to that found in amanitin analogs which like *cyclo*-bis(-L-Cys-Gly-L-Pro-L-Phe-) are also potentially constrained by bridging side chains (Kostansek, Lipscomb, Yocum & Thiessen, 1978; Shoham, Rees, Lipscomb, Zanotti & Wieland, 1984). This result for a monocyclic molecule indicates an intrinsically stable cyclic octapeptide backbone type. The different orientations of CONH planes in the Ala-Gly and Pro-Phe pairs, coupled with the C_2 symmetry observed in the NMR average (Kopple, Parameswaran & Yonan, 1984), indicate probable sites of internal motion in that backbone.

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Structures of Geometric Isomers of a Cyclic Dipeptide Derivative: *cyclo*(-*N*-Acetyl-L-phenylalanyl-*p*-chloro-2,3-dehydrophenylalanyl-)

BY CHUNG-GI SHIN AND YASUCHIKA YONEZAWA

Laboratory of Organic Chemistry, Kanagawa University, Kanagawa-Ku, Yokohama 221, *Japan*

KATSUHIKO UENO

Research Institute for Polymers and Textiles, Tsukuba, Ibaraki 305, *Japan*

AND JUJI YOSHIMURA

Department of Fundamental Science, Iwaki Meisei University, Iwaki, Fukushima 970, *Japan*

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Abstract. (3*S*)-4-Acetyl-3-benzyl-6-[(*Z*)-*p*-chlorobenzylidene]-2,5-piperazinedione (*Z*), $C_{20}H_{17}ClN_2O_3$, M_r = 368·8, orthorhombic, $P2_12_12_1$, a = 7·182 (1), b = 9·813 (1), c = 25·501 (3) Å, V = 1797·2 (4) Å^3, Z = 4, D_m = 1·36, D_x = 1·363 Mg m^{-3}, λ(Cu $K\alpha$) = 1·5418 Å, μ(Cu $K\alpha$) = 1·960 mm^{-1}, F(000) = 768, T = 298 K, R = 0·062 for 1730 independent observed reflexions. (3*S*)-4-Acetyl-3-benzyl-6-[(*E*)-*p*-chlorobenzylidene]-2,5-piperazinedione (*E*), $C_{20}H_{17}ClN_2O_3$, M_r = 368·8, orthorhombic, $P2_12_12_1$, a = 6·295 (1), b = 16·592 (3), c = 16·678 (2) Å, V = 1742·0 (4) Å^3, Z = 4, D_m = 1·41, D_x = 1·406 Mg m^{-3}, λ(Cu $K\alpha$) = 1·5418 Å, μ(Cu $K\alpha$) = 2·022 mm^{-1}, F(000) = 768, T = 298 K, R = 0·051 for 1298 independent observed reflexions. Both molecules are in the folded conformation with the aromatic rings of the benzyl groups facing the 2,5-piperazinedione (PDO) ring [χ^1 = 52·8 (5) and 68·9 (8)° for (*Z*) and (*E*), respectively]. The PDO rings are in boat (*Z*) and twist-boat (*E*) conformations with the benzyl side chains in the flagpole positions. The dihedral angles of the two amido groups in the PDO rings are −31·1 (2) (*Z*) and −25·3 (3)° (*E*), respectively. The *p*-chlorophenyl moiety and ethylene bond of the benzylidene group are nearly coplanar in (*Z*), but not coplanar in (*E*). The conformational angles around the C—C single bond connecting these planes are 4·0 (6) (*Z*) and −46·4 (9)° (*E*), respectively.

Introduction. It has been shown that a cyclic dipeptide having one or two *cis* substituents at the 3- and/or 6-positions, especially in the case of an aromatic one, favors the flagpole boat conformation in solution (Kopple & Marr, 1967) and in the solid state (Lin & Webb, 1973) because of the direct interaction between the 2,5-piperazinedione (PDO) and aromatic rings on stacking. The conformation is changeable to planar or bowsprit boat due to the interference between bulky substituents, *trans* orientation of substituents (Benedetti, Marsh & Goodman, 1976) or the special nature of the side chain as in a proline dipeptide (Karle, Ottenheym & Witkop, 1974). It has been proposed, from circular dichroism (CD) experiments in the region of 190–250 nm, that both the π–π^* and n–π^* transitions in the Cotton effect reflect the conformation of the

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